Bacteriophages: Underestimated vehicles of antibiotic resistance genes in the soil

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Bacteriophages (phages), the most abundant biological entities on Earth, have a significant effect on the composition and dynamics of microbial communities, biogeochemical cycles of global ecosystems, and bacterial evolution. A variety of antibiotic resistance genes (ARGs) have been identified in phage genomes in different soil samples. Phages can mediate the transfer of ARGs between bacteria via transduction. Recent studies have suggested that anthropogenic activities promote phage-mediated horizontal gene transfer events. Therefore, the role of phages in the dissemination of ARGs, which are a potential threat to human health, may be underestimated. However, the contribution of phages to the transfer of ARGs is still poorly understood. Considering the growing and wide concerns of antibiotic resistance, phages should be considered a research focus in the mobile resistome. This review aimed to provide an overview of phages as vehicles of ARGs in soil. Here, we summarized the current knowledge on the diversity and abundance of ARGs in soilborne phages and analyzed the contribution of phages to the horizontal transfer of ARGs. Finally, research deficiencies and future perspectives were discussed. This study provides a reference for preventing and controlling ARG pollution in agricultural systems.

KEYWORDS bacteriophages, antibiotic resistance genes, mobile antibiotic resistome, horizontal gene transfer, soil

Introduction

With the extensive use of antibiotics in clinics and agriculture, the problem of antibiotic resistance has become increasingly prominent and has become a major public health risk faced by human society in the 21st century (Kavagutti et al., 2019; Pu et al., 2020). According to the latest report by the Lancet, there were an estimated 4.95 million deaths associated with bacterial antimicrobial resistance in 2019, including 1.27 million deaths attributable to bacterial antimicrobial resistance (Murray et al., 2022). If it is not effectively controlled, it is expected that this number will be as high as 10 million by 2050 (Willyard, 2017; Amarasiri et al., 2020). Recently, it has been shown that antibiotic resistance genes (ARGs) carried by clinically drug-resistant pathogens originate from the environment (Forsberg et al., 2012; Yang et al., 2013; Fang et al., 2015; Huijbers et al., 2019). Therefore, ARGs have been widely considered as a new...
Phages as bacterial viruses, are the most abundant and diverse entities in the biosphere, with a total population of approximately $10^{30}$–$10^{33}$, which is 10 times that of bacteria (Balcazar, 2014; Dion et al., 2020). The complete biological structure of a phage consists of a nucleic acid core (DNA or RNA) surrounded by an outer shell of a protein coat (capsid) with an estimated size of 20–200 nm (Jebri et al., 2021). Additionally, most phages belong to the order Caudovirales, which includes five families: Myoviridae, Siphoviridae, Podoviridae, Ackermannviridae, and Herelleviridae (Adams et al., 2017; Walker et al., 2019; Jebri et al., 2021). Phages are divided into two categories according to their life cycle: temperate and virulent phages. Temperate phages can be inserted into the host genome as a prophage and replicate with the host; this process is known as the lysogenic cycle (Ghosh et al., 2008). When external conditions change (such as UV radiation and heavy metal exposure), the prophage enters the lytic cycle using host nutrients to synthesize the offspring phage and kill the host (Ghosh et al., 2008; Zhong et al., 2021). Phages have a significant effect on the composition and diversity of microbial communities, biogeochemical cycles of global ecosystems, and bacterial evolution (Magana de Almeida Kumlken et al., 2021; Lobo and Faciola, 2021).

Phages exist in various environments, among which the soil is an important habitat (Williamson et al., 2007; Ghosh et al., 2008). It is estimated that the number of phage particles in soil accounts for 10% of the total number of viruses globally (Vos et al., 2009; Cobian et al., 2016). High biodiversity and rich soil nutrition provide a suitable living environment for phages (Gomez and Buckling, 2011). Phages have been detected in various types of soil samples, including farmland, forests, wetlands, croplands, sand dunes, and even extreme environments, such as deserts and Antarctic soil (Williamson et al., 2007; Gomez and Buckling, 2011; Wang, 2019; Bezuidt et al., 2020; Chen et al., 2021). The abundance of phages in the different soil types is summarized in Table 1. Among the various soil samples that have been investigated by epi-fluorescence microscopy, the abundances of phages in farmlands and forests are the highest, reaching $10^9$/g, whereas the abundance of phages in deserts is nearly 3–6 orders of magnitude lower (Zablocki et al., 2016; Williamson et al., 2017; Trubl et al., 2018). With the continuous progress of metagenome sequencing and bioinformatics technology, the research of the “dark matter” of the microbial world—the Environmental virome (mainly composed of phages) has developed rapidly (Monaco and Kwon, 2017; Ofir and Sorek, 2018; Han et al., 2022). A viral metagenomic analysis of soil has suggested that viruses (phages) affect the microbial ecology (Ashelford et al., 2003; Dinsdale et al., 2008; Parsley et al., 2010; Cobian et al., 2016; Kavagutti et al., 2019). A recent study showed that phages play an important role in phosphorus metabolism, carbon metabolism, soil organic matter degradation, and polysaccharide binding after analyzing the permafrost thaw gradient (Emerson et al., 2018; Trubl et al., 2018; Emerson, 2019; Han et al., 2022). Additionally, soil properties and the bacterial community significantly affects the structure of the viral community (Chen et al., 2016, 2021). However, our understanding of soil phages is still relatively backward due to the high soil heterogeneity and the limitation of publicly available viral databases, especially regarding the contribution of phages to the soil antibiotic resistome (Larrañaga et al., 2018; Sun et al., 2018; Zhao et al., 2019).

**Phages in soil**

Phages exist in various environments, among which the soil is an important habitat (Williamson et al., 2007; Ghosh et al., 2008). It is estimated that the number of phage particles in soil accounts for 10% of the total number of viruses globally (Vos et al., 2009; Cobian et al., 2016). High biodiversity and rich soil nutrition provide a suitable living environment for phages (Gomez and Buckling, 2011). Phages have been detected in various types of soil samples, including farmland, forests, wetlands, croplands, sand dunes, and even extreme environments, such as deserts and Antarctic soil (Williamson et al., 2007; Gomez and Buckling, 2011; Wang, 2019; Bezuidt et al., 2020; Chen et al., 2021). The abundance of phages in the different soil types is summarized in Table 1. Among the various soil samples that have been investigated by epi-fluorescence microscopy, the abundances of phages in farmlands and forests are the highest, reaching $10^9$/g, whereas the abundance of phages in deserts is nearly 3–6 orders of magnitude lower (Zablocki et al., 2016; Williamson et al., 2017; Trubl et al., 2018). With the continuous progress of metagenome sequencing and bioinformatics technology, the research of the “dark matter” of the microbial world—the Environmental virome (mainly composed of phages) has developed rapidly (Monaco and Kwon, 2017; Ofir and Sorek, 2018; Han et al., 2022). A viral metagenomic analysis of soil has suggested that viruses (phages) affect the microbial ecology (Ashelford et al., 2003; Dinsdale et al., 2008; Parsley et al., 2010; Cobian et al., 2016; Kavagutti et al., 2019). A recent study showed that phages play an important role in phosphorus metabolism, carbon metabolism, soil organic matter degradation, and polysaccharide binding after analyzing the permafrost thaw gradient (Emerson et al., 2018; Trubl et al., 2018; Emerson, 2019; Han et al., 2022). Additionally, soil properties and the bacterial community significantly affects the structure of the viral community (Chen et al., 2016, 2021). However, our understanding of soil phages is still relatively backward due to the high soil heterogeneity and the limitation of publicly available viral databases, especially regarding the contribution of phages to the soil antibiotic resistome (Larrañaga et al., 2018; Sun et al., 2018; Zhao et al., 2019).

**Abbreviations:** HGT, Horizontal gene transfer; ARGs, Antibiotic resistance genes; AMGs, Auxiliary metabolic genes; qPCR, Quantitative polymerase chain reaction; SOM, Organic matter in soil; pARGs, Antibiotic resistance genes carried by phages.
The occurrence of soilborne pARGs

Research on ARGs in soilborne phages began relatively late compared with that in the aquatic environment. Ross and Topp (2015) quantified a set of ARGs from soilborne phages using quantitative polymerase chain reaction (qPCR) technology. In the following years, the occurrence and abundance of soilborne pARGs from different regions were investigated using qPCR technology (Anand et al., 2016; Larrañaga et al., 2018; Sun et al., 2018; Zhao et al., 2019). Recently, metagenomics has become a powerful tool to study the diversity and abundance of pARGs in soil (Enault et al., 2017). The diversity and abundance of soilborne pARGs are summarized in Table 2.

Detection methods of pARGs

The main methods for detecting pARGs are based on PCR technology, such as qPCR and droplet digital PCR (ddPCR), which can obtain absolute quantification information of specific ARG subtypes (Ross and Topp, 2015; Hu et al., 2022). Furthermore, recent metagenomic approaches provide a valuable tool for exploring the composition of the viral community, ARGs, and the correlations between them in different environmental settings (Chen et al., 2021). With the development of sequencing technology, more pARGs in soil will be unearthed, providing unprecedented opportunities to elucidate the mechanisms of mobile antibiotic resistance in soil (Chen et al., 2021). PCR technology and metagenomic sequencing have their own advantages. As a classical method for the absolute quantitative detection of ARGs in environmental samples and pure strains, PCR technology has the advantages of accuracy and rapidity (Hu et al., 2019a). In contrast, metagenomic sequencing does not depend on the microbial culture and screening process, and has the advantages of high specificity, sensitivity, and throughput (Hu et al., 2013; Hu et al., 2019a). Metagenomic sequencing facilitates the detection of more ARGs, which is conducive to the comprehensive understanding of the diversity of phage-mediated ARGs. The continuous innovation of sequencing technology can capture the phylogenetic and genetic diversity, fully understand the types, abundance, and transmission routes of phage-mediated ARGs, and predict the possible risk of antibiotic resistance and other potential threats to humans (Parsley et al., 2010; Bengtsson-Palme, 2017).

The diversity of soilborne pARGs

ARG subtypes that confer resistance to 13 antibiotics (β-lactams, tetracycline, quinolone, aminoglycosides, sulfonamide, streptomycin, chloramphenicol, trimethoprim, MLSB, vancomycin, rifamycin, pleuromutilin, and mupirocin) were identified in phage genomes from various soil sources (Table 2). Among these, tet genes are the main types of soilborne pARGs. Tetracyclines are one of the most commonly used antibiotics in the livestock breeding industry, and their resistance genes are ubiquitous in agricultural soil. For example, Sun et al. (2018) detected six genes conferring resistance to tetracyclines in the phage fraction of greenhouse soil, including tetC, tetE, tetG, tetM, tetO, and tetX. In addition, large amounts of the bla gene have been identified in soilborne phages, including blaTEM, blaCTX-M, and blaVH1. Hu et al. (2022) analyzed a total of 25 ARG subtypes using ddPCR technology and demonstrated that the detection rate of soilborne pARGs with the application of organic fertilizer was 76%, whereas the rates of soilborne pARGs with the application of non-fertilizer and chemical fertilizer were only 68 and 72%, respectively. Chen et al. (2021) analyzed the viral DNA of soil samples using the Illumina NovaSeq 6000 platform and detected 16 unique ARG subtypes, including catB, macB, vatB, dfrB2, dfrB6, and so on. Another study detected 144 ARGs in soil viruses, which were divided into 12 categories according to the types of antibiotic resistance (Wang, 2019).

The abundance of soilborne pARGs

Generally, the abundance of pARGs varies greatly between different soil types and ARG subtypes, ranging from 10^1 to 10^10 copies/g (Wang, 2019). A study of soilborne pARGs resistant to tetracycline found that the order of accumulative

| Soil source | Virus (phage) abundance (gDW⁻¹) | Method | References |
|-------------|---------------------------------|--------|------------|
| Farmland    | 10⁷                             | Epifluorescence microscopy | Chen et al. (2014) |
|             | 10⁶ - 10⁷                       | Epifluorescence microscopy | Li et al. (2019) and Williamson et al. (2005) |
| Desert      | 64,520 vOTU³                    | Illumina sequencing | Chen et al. (2021) |
| Forest      | 10⁹                             | Epifluorescence direct counts | Gonzalez-Martin et al. (2013) |
|             | 10⁸                             | Epifluorescence direct counting | Williamson et al. (2007) |
| Wetland     | 10⁸                             | Epifluorescence microscopy | Williamsom et al. (2005) |
|             | 10⁷                             | Epifluorescence microscopy | Helsley et al. (2014) |
| Antarctica  | 10⁷                             | Epifluorescence direct counting | Williamson et al. (2005, 2007) |
|             | 11–33 vOTU                      | Ion Proton sequencing | Adriamenssens et al. (2017) |

³dGW⁻¹ (per gram dry weight of soil).
⁴vOTU (viral operational taxonomic units).

TABLE 1 The viral abundance in different soil types.
Table 2: Subtypes and abundance of antibiotic resistant genes (ARGs) in soilborne phage genomes.

| ARGs type   | ARGs subtype | Soil sample source | Location | ARGs abundance | Method          | References                      |
|-------------|--------------|---------------------|----------|----------------|-----------------|---------------------------------|
| β-lactams   | β-lactams    | Animal farm         | India    | 3.09%          | PCR             | Anand et al. (2016)             |
|             | β-lactams    | Soil matrices       | Spain    | 10^6–10^7 GC/g | qPCR            | Larrañaga et al. (2018)         |
|             | β-lactams    | Animal farm         | India    | 3.6%           | PCR             | Anand et al. (2016), Chen et al. (2017) |
|             | β-lactams    | Raw manure          | Canada   | 10^5–10^6 GC/ng DNA | Model (qPCR-based) | Ross and Topp (2015) |
| Tetracycline| tetA         | Animal farm         | India    | 12.7%          | PCR             | Anand et al. (2016)             |
|             | tetW         | India               |          | 9.1%           |                 | Anand et al. (2016)             |
|             | tetC         | Greenhouse          | China    | 10^7–10^8 copies/g | qPCR           | Sun et al. (2018)               |
|             | tetE         | China               |          | 10^7–10^8 copies/g | qPCR           | Sun et al. (2018)               |
|             | tetG         | Soil matrices       | Spain    | 10^5–10^6 GC/g | qPCR            | Sun et al. (2018)               |
|             | tetM         | Soil matrices       | Spain    | 10^7–10^8 copies/g | qPCR           | Sun et al. (2018)               |
|             | tetO         | Soil matrices       | Spain    | 10^7–10^8 copies/g | qPCR           | Sun et al. (2018)               |
|             | tetX         | Soil matrices       | Spain    | 10^7–10^8 copies/g | qPCR           | Sun et al. (2018)               |
|             | tetT         | Organic fertilizer  | China    | /              | Illumina sequencing | Chen et al. (2021)            |
|             | tetQ         | Farmland soil       | China    | /              | Illumina sequencing | Wang (2019)                     |
|             | tetN         |                     |          |                |                 |                                 |
|             | tetV         |                     |          |                |                 |                                 |
|             | tetL         |                     |          |                |                 |                                 |
|             | tetM         |                     |          |                |                 |                                 |
|             | qnrA         | Soil matrices       | Spain    | 10^5–10^6 GC/g | qPCR            | Larrañaga et al. (2018)         |
|             | aqxAB        | Farmland            | China    | /              | Illumina sequencing | Wang (2019)                     |
| Aminoglycosides| aadA | Raw manure          | Canada   | 10^5 GC/ng DNA | Model (qPCR-based) | Ross and Topp (2015)         |
|             | armA         | Soil matrices       | Spain    | 10^5–10^6 GC/g | qPCR            | Larrañaga et al. (2018)         |
|             | aac(3)-1     | Organic fertilizer  | China    | /              | Illumina sequencing | Chen et al. (2021)            |
|             | aadD(9)      |                     |          |                |                 |                                 |
|             | aac(2)′-1    | Farmland            | China    | /              | Illumina sequencing | Wang (2019)                     |
|             | aadE         |                     |          |                |                 |                                 |
| Sulfamethazine| sul1        | Raw manure          | Canada   | 10^5 GC/ng DNA | Model (qPCR-based) | Ross and Topp (2015)         |
|             | sul2         | Soil matrices       | Spain    | 10^5 GC/g      | qPCR            | Larrañaga et al. (2018)         |
| Streptomycin| strA         | Raw manure          | Canada   | 10^5 GC/ng DNA | Model (qPCR-based) | Ross and Topp (2015)         |
|             | strB         | Soil matrices       | Spain    | 10^5–10^6 GC/g | qPCR            | Larrañaga et al. (2018)         |
|             | vgaD         | Farmland            | China    | /              | Illumina sequencing | Wang (2019)                     |
|             | vatB         |                     |          |                |                 |                                 |
|             | vatF         |                     |          |                |                 |                                 |
| Chloramphenicol| cmIA        | Dairy farm          | China    | 3.5 × 10^5–1.1 × 10^6 copies/g | Real-time PCR | Zhao et al. (2019)            |
|             | catB         | Organic fertilizer  | China    | /              | Illumina sequencing | Chen et al. (2021)            |
| Trimethoprim| dfrA1        | CA                  |          |                |                 |                                 |
|             | dfrB2        |                     |          |                |                 |                                 |
|             | dfrB6        |                     |          |                |                 |                                 |
|             | dfrA12       |                     |          |                |                 |                                 |
|             | dfrA20       |                     |          |                |                 |                                 |
| MLSB*       | vatB         | Farmland            | China    | /              | Illumina sequencing | Wang (2019)                     |
|             | macB         |                     |          |                |                 |                                 |
|             | lmrC         |                     |          |                |                 |                                 |
|             | carA         |                     |          |                |                 |                                 |

(Continued)
pARG abundance is as follows: efflux pump ARGs (tetC + tetE + tetG) > ribosome protection ARGs (tetM + tetO) > enzymatic modification ARG (tetX; Sun et al., 2018). As shown in Table 2, human activities, such as the application of organic fertilizer, affect the abundance of ARGs carried by soil phages. Another study also found that the relative abundance of pARGs in the soil receiving organic fertilizer treatment is significantly higher than that in soil receiving chemical fertilizer (p < 0.05; Wang, 2019). Hu et al. (2022) also found that soil receiving organic fertilizer has the highest total abundance of pARGs, reaching 1.6 × 10^9 copies/g, and soil treated with non-fertilizer has the lowest pARG abundance (6.0 × 10^5 copies/g).

**Characteristics of horizontal gene transfer in pARGs**

The mechanisms of the horizontal transfer of ARGs are conjugation, natural transformation, and transduction (von Wintersdorff et al., 2016). Among them, plasmid-mediated conjugation has been identified as the main mechanism of HGT (Svara and Rankin, 2011). Some studies have found that ARGs resistant to amoxicillin and sulfanilamide are located in plasmids, with frequencies ranging from 10^-4 to 10^-3 (Musovic et al., 2010; Zhang et al., 2017). In addition, many plasmids have a wide-range of hosts, enabling ARGs to transfer among different biological species (Binh et al., 2008; Musovic et al., 2010). Transformation refers to the process in which the recipient bacteria directly obtain extracellular fragments of the donor bacteria to obtain new genetic traits (Hu et al., 2019a; Ding et al., 2021). However, the poor stability of extracellular DNA and low proportion of bacteria with natural transformation abilities make excludes transformation as the main mechanism of the horizontal transfer of ARGs (Nielson et al., 2007). Recently, phage-mediated transduction has been considered as another mechanism for the horizontal transfer of ARGs (Subirats et al., 2016; Calero-Cáceres et al., 2019; Debroas and Siguet, 2019; Jebri et al., 2021).

Phage-mediated transduction of ARGs has been documented for several bacterial species, including Streptococcus pyogenes, Enterococci, Escherichia coli, and Salmonella sp., and the transduced ARGs are mainly resistant to erythromycin, tetracycline, gentamicin, and β-lactam (Ubukata et al., 1975; Hyder and Streitfeld, 1978; Schmiegier and Schicklmaier, 1999; Mazaheri et al., 2011; Billard-Pomares et al., 2014). Transduction can be divided into generalized or specialized transduction according to classical microbiology (Figures 1A,B). Generalized transduction that achieves a genetic trait is carried by two types of phage particles (temperate and virulent phages) from a donor cell to a recipient cell during lytic pathways (Sander and Schmiegier, 2001). Phages can transfer any gene from one bacterium to another during this process (Penadés et al., 2014). Transduction particles wrap host DNA (including ARGs) by mispackaging, and their frequency is very low (10^-6–10^-4; Maganha De Almeida Kuumlien et al., 2021). Another model of genetic material mediated by phages is specialized transduction, which can only be carried out by temperate phages during the late lysogenic cycle (Debroas and Siguret, 2019). More specifically, specialized transduction particles carry fixed genes of the bacterial chromosome located adjacent to the prophage (including ARGs) attachment site with a low occurrence frequency (10^-6; Balcazar, 2014). The frequency of gene transfer due to “misloading” of host DNA fragments is very low, whether by general transduction or specialized transduction (Poté et al., 2003; Muniesa et al., 2013). The third recently discovered transduction model, lateral transduction, may be the most effective way for bacteria to obtain ARGs (Figure 1C; Chen et al., 2018; Fillol-Salom et al., 2021). Kenzaka et al. (2010) accurately measured the transduction frequency between E. coli mediated by phages using cycling primed in situ amplification-fluorescent in situ hybridization (CPRINS-FISH). The results revealed that the frequency of DNA transfer is 10^-6–10^-4, indicating that the phage-mediated exchange of genes occurs at an unexpectedly high frequency (Kenzaka et al., 2007, 2010). Phage-mediated HGT has many distinct characteristics, which can be summarized into the following three aspects.

**High occurrence frequency**

A previous study showed that the transduction frequency of phages in aquatic environments is several orders of magnitude higher than expected (nearly 1%; Muniesa et al., 2013). Recently, Chen et al. (2018) found that lateral transduction in the process of HGT is mediated by the temperate phage of Staphylococcus aureus, proving that phages can transfer genes with extremely high

**TABLE 2 Continued**

| ARGs type  | ARGs subtype | Soil sample source | Location | ARGs abundance | Method | References |
|------------|--------------|-------------------|----------|----------------|--------|------------|
| Vancomycin | vanD, vanS   |                   |          |                |        |            |
| Rifamycin  | rpoB2, rplB  |                   |          |                |        |            |
| Pleuromutilin | TacA       |                   |          |                |        |            |
| Mupirocin  | mupB         |                   |          |                |        |            |

MLSB (Macrolide-Lincosamide-Streptogramin B).

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**References**

Binh et al., 2008; Musovic et al., 2010; Musovic et al., 2010; Maganha De Almeida Kuumlien et al., 2021.
frequency. The mechanism of this newly discovered transduction is DNA packaging initiated in situ from integrated prophages. Several hundred kilobases of the \textit{S. aureus} genome, are packaged in phage heads at very high frequencies. \textit{In situ} replication before DNA packaging creates multiple prophage genomes, such that lateral-transducing particles form during normal phage maturation, transforming parts of the \textit{S. aureus} chromosome into hypermobile regions of gene transfer (Chen et al., 2018). With the deepening of our understanding of phages and their transduction mechanisms, the frequency of phage-mediated HGT in the environment may be much higher than expected. Overall, phages act as powerful agents to transfer bacterial chromosomal DNA to another organism via genetic transduction (Fillol-Salom et al., 2021).

No direct contact between recipient and donor cells

Conjugation has been considered to play a major role in HGT and the consequent spread of ARGs and requires cell-to-cell contact (Muniesa et al., 2013). However, phage transduction does not need to meet the above conditions. Additionally, ARGs can be transferred by phages at different time points (Muniesa et al., 2013). Researchers have found that viral DNA can be transferred from hot springs (−82°C) to ice (−0°C), which strongly supports this view (Sano et al., 2004). Paez-Espino et al. (2016) identified that the virus can infect organisms from different phyla by linking the virus to the microbial host through the CRISPR spacer and transfer RNA matches. Furthermore, phage-mediated HGT is not restricted to microorganisms of the same species, but occurs between different species, genera, or even phyla (Muniesa et al., 2013).

Persistence and broad time-scale

The genetic material of the phage is tightly surrounded by a protein coat called a capsid. Owing to their structure, phages thrive successfully in the environment and are resistant to natural and anthropogenic stressors such as enzymes, radiation, and antimicrobial substances (Muniesa et al., 2013; Göller et al., 2020). Durán et al. (2002) conducted an inactivation experiment of phages in rivers and showed that phages were more resistant than fecal coliforms and enterococci. As mentioned above, phage DNA will not be damaged in different extreme environments, and this conclusion is supported by the premise that the protein capsid is not broken (Sano et al., 2004). Overall, phages have stronger persistence and a broader time scale in the environment, making them more suitable as carriers for ARG transfer (Calero-Cáceres and Muniesa, 2016).
The contribution of phages in the horizontal transfer of ARGs

Phages act as vectors for genetic exchange, facilitate reproduction (short-term) or promote microbial evolution (long-term). When phages containing ARGs move between different bacteria, it leads to the horizontal spread of ARGs and even the emergence of drug-resistant bacteria in soil (Kenzaka et al., 2007). Understanding the horizontal transfer mechanism of pARGs is important when investigating the mobile resistome, which is conducive to controlling the spread of ARGs.

Research methods for phage-mediated transduction of ARGs

A summary of the research methods for phage-mediated transduction of ARGs is shown in Figure 2. Before the advent of new generation sequencing technology, phage-mediated ARG transduction was mainly studied through cultivation-based methods (Bowring et al., 2022). Firstly, a specific phage containing ARGs is used to infect antibiotic sensitive bacteria (such as E. coli). After co-culturing, the lysate is diluted and plated on drug-free and drug-containing plates under appropriate conditions. The frequency of transduction is calculated by dividing the number of colonies on the drug-containing plate by that on the drug-free plate (Zeph et al., 1988; Mazaheri et al., 2011; Figure 2A1). Another research method uses the phage community as a whole to conduct in vitro transduction experiments. First, the bacteria and phages are isolated from environmental samples. After co-culturing, the plate count of the antibiotic-resistant bacteria (ARB) is carried out to investigate the rate of ARG transfer (Modi et al., 2013; Figure 2A2). With the emergence of molecular biotechnology, research on the horizontal transfer of ARGs is more profound and rigorous (Ross and Topp, 2015). Bacteria and phages are obtained from environmental samples by means of centrifugal filtration, and their DNA is extracted. Then, the abundance of ARGs is analyzed through PCR or 16s sequencing (Harrington et al., 2012; Larrañaga et al., 2018; Watson et al., 2018; Zhao et al., 2019; Figure 2B1). In recent years, genome sequencing technology has overcome the limitations of bacterial culture and microbial loss caused by isolation. As shown in Figure 2B2, the
transfer of ARGs is studied through the extraction of total environmental DNA, followed by the sequencing and analysis of the resistome and mobilome. The origin of ARGs and the role of phages in ARG transmission are explored through phylogenetic diversity and phage-host linkage analysis, respectively. In addition, the results of metagenomic or (virome) analysis can simultaneously reveal the microbial (viral) composition and function in soil, which contribute to the reliable risk assessment of ARG transmission through the source-tracking method and correlation analysis (Lekunberri et al., 2017; Chen et al., 2021).

**Horizontal transfer of ARGs mediated by phage**

Many experimental studies have provided evidence that phages participate in the horizontal transfer of ARGs. Ross and Topp (2015) conducted transduction experiments using phages isolated from the soil to infect *E. coli* K-12. They found that a certain selective pressure (such as antibiotics) could promote the horizontal transfer of phage-mediated soilborne ARGs. Another study used phages-mediated ARGs to perform subculture experiments with *E. coli* as the host cells. By measuring the ARGs in the phage DNA before and after culture, it was proven that phages can reinfect the host and are important carriers for the transfer and transmission of ARGs in the environment (Larrañaga et al., 2018). Researchers have successfully transferred the *bla* gene in phage DNA into the *E. coli* genome using electroporation technology, which also suggests that phages are vehicles that transfer ARGs effectively (Colomer-Lluch et al., 2011).

However, our knowledge of phage-mediated HGT in soil lags far behind from what is known in other environmental media (e.g., water, animal gut) because of the high heterogeneity and rich biodiversity of the soil. A virome study showed that mouse intestinal phages were enriched with ARGs due to antibiotic treatment; these ARG-carrying phages infected intestinal bacteria, increasing the number of antibiotic-resistant bacteria (Modi et al., 2013). Shousha et al. (2015) found that phages isolated from chickens transduced the resistance of *E. coli* to various antibiotics (including ampicillin, tetracycline, and chloramphenicol). Another study demonstrated that phages released from *S. aureus* enable their hosts to acquire streptomycin-resistant genes from adjacent cells (Haaber et al., 2016).

**Factors affecting transduction of ARGs in soil**

**Antibiotics**

Only a few studies were conducted to explore the relationship between antibiotics and the transfer of pARGs. A study on the gut virome showed that antibiotic treatment lead to the enrichment of pARGs. In addition, they assessed the proportion of resistant bacteria before and after phage infection with naive microbiota from mice gut as host by *in vitro* experiments, and found that the basal frequency (before infection) was lower than that microbiota infected with phages, especially in antibiotic-treated mice (Modi et al., 2013). These results demonstrated that antibiotic treatment enhanced the ability of phages to transmit resistance.

**Physicochemical characteristics of soil**

Numerous studies have demonstrated a positive correlation between the transfer of ARGs and the physicochemical characteristics of soil (Ji et al., 2012; Debroas and Sigüère, 2019). Environmental factors, such as temperature, affected the dissemination of ARGs by exchange community boundaries (Iyer et al., 2013; Martí et al., 2014). Heavy metals could exert pressure on ARGs, thereby promoting the transfer of ARGs (He et al., 2020). Another study found that the concentration of Cu and Zn in soil was significantly positively correlated with the transduction of pARGs (Yang et al., 2021). Organic matter and heavy metals (Zn, Cr, and Cu) had a greater effect on pARGs than others factors, such as Hg, As, available phosphorus, available potassium, and total nitrogen (Hu et al., 2022). The concentrations of NH₃-N and total phosphorus are positively correlated with two types of pARGs conferring resistant to tetracyclines and macrolide-lincosamide-streptogramin B (Yang et al., 2021). Random forest modeling and partial least squares path modeling results indicated that pH is a key factor affecting the occurrence of pARGs during the fermentation process (Xu et al., 2022).

**Bacterial community**

Soil bacteria and phages are crucial reservoirs of ARGs in the natural environment (Sun et al., 2018; Guo et al., 2021). The ARGs in phages are generally positively correlated with that of their bacterial hosts (Yang et al., 2021). Virus-host linkage analyses revealed that the phage-mediated ARGs are closely related to five bacterial phyla, including Firmicutes, Bacteroidetes, Proteobacteria, Crenarchaeota, and Planctomycetes (Chen et al., 2021). Yang et al. (2021) investigated the association between bacterial communities and pARGs and found that the bacterial community contributes to 16.7% of the variation in the pARG profiles. The results showed that *Terrisorobacter*, *Desulfovibrio*, and *Acinetobacter* are the main drivers impacting pARGs (Yang et al., 2021). Several recent studies have confirmed that the bacterial community is the main driver impacting the pARG profiles. Using the variance partitioning analysis (VPA), our previous study also found that the bacterial community contributes the most to pARG variation (10.81%; Hu et al., 2022).

**Others**

Recently, some studies have found that nano-metal oxide particles have an effect on the horizontal transfer of ARGs (Hu et al., 2019b; Otinov et al., 2020; Li and Zhang, 2022). Han et al. (2020) conducted an experiment using phage gM13 and *E.coli* exposed to nano-TiO₂, and the results showed that nano-TiO₂ increases the transduction frequency up to 4.5 fold compared to the control. TiO₂ photoexcitation can drastically improve phage
transduction efficiency 20.4-fold (Xiao et al., 2021). The transfer rate of ARGs mediated by nano-$\text{Al}_2\text{O}_3$, via a transduction-like pathway was $10^4$ times higher than that of the control. (Ding et al., 2021). The possible mechanisms of these nanomaterials promoting transduction are presumed to the increase of phage attachment on host cell surface, and cell membrane permeability. In the contrast, excessive UV irradiation led to a decrease in transduction efficiency (Xiao et al., 2021). Additionally, ionic liquid, as an environmental friendly compound, facilitates the horizontal transfer of ARGs (Wang et al., 2015a,b).

**Future perspectives**

Phages play an important role in the antibiotic resistome of soil, and their contributions to the spread of ARGs should not be underestimated. However, there are still many uncertainties regarding the distribution characteristics of ARGs in the phage genome and the mechanism of phage-mediated HGT at different environmental sites. Therefore, additional studies are required to elucidate this and to subsequently support the inclusion of phages in monitoring, evaluation, and surveillance programs aimed at the emergence and spread of ARGs (Balcázar, 2020). The following gaps in research should be addressed:

1. The influence mechanism of the physicochemical properties and biological communities of soil on ARG transduction via phages is unclear. Although phages can mediate the spread of ARGs in agricultural soil and increase the number of antibiotic resistant bacteria under selective pressures, whether they can improve the antibiotic resistance of other indigenous bacteria and related mechanisms needs to be elucidated.
2. The research methodology on soilborne pARGs needs to be improved. Existing studies have only focused on ARGs in free phages, ignoring prophages in bacterial genomes, which leads to the inevitable underestimation of the contribution of phages to the environmental antibiotic resistome. In addition, the removal of bacterial contamination during phage DNA extraction is a challenge in the quantitative study of phage metagenomes in the soil (Göller et al., 2020). Although the development of sequencing technologies will undoubtedly help solve these problems, these approaches should be standardized to avoid misleading and incorrect conclusions.

**Conclusion**

Phages are extremely abundant in soil and a large number of ARGs are present in their genomes. ARGs can be horizontally transferred through transduction at high frequencies, including specialized, generalized, and lateral transduction. Transduction can occur without contact between donor and recipient bacteria and is not limited by time and space. Moreover, there is increasing evidence that human activities, especially the application of organic fertilizers, lead to the enrichment and transmission of ARGs in soilborne phages. Therefore, the role of phages in the transfer of ARGs should not be ignored or underestimated. However, the contribution of phages to the transfer of ARGs has been relatively poorly studied because of the high heterogeneity and complexity of soils. With the advancement of sequencing technology, recent related research has been greatly developed. Additional studies are needed to elucidate the mechanisms and influencing factors contributing to the dissemination of ARGs via phages.

**Author contributions**

YZ: writing — original draft preparation, visualization, and conceptualization. YG: writing — original draft preparation and conceptualization. TQ: writing — review and editing and funding acquisition. MG: writing — review and editing. XW: supervision, project administration, writing — review and editing, and funding acquisition. All authors have approved this work for publication.

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**Conflict of interest**

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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