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Commentary

How do bacteriophages promote antibiotic resistance in the environment?

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Antibiotic resistance has become a major global health concern because the increasing prevalence of this phenomenon is compromising the effectiveness of antimicrobial therapy. Around 700 000 deaths worldwide are attributed annually to antibiotic-resistant infections (http://amr-review.org). Bacteria can acquire antibiotic resistance through chromosomal mutations or the acquisition of genetic material from other bacteria or the environment via horizontal gene transfer. This latter process is largely driven by mobile genetic elements, such as plasmids, transposons or bacteriophages, which play an essential role in the evolution and ecology of bacterial communities by controlling the intra-species and interspecies exchange of genetic information [1]. While the transfer of these mobile genetic elements may occur through transformation or transduction, conjugation is considered the most efficient mechanism to exchange genetic material among bacteria [2]. Because antibiotic resistance genes (ARGs) are acquired and frequently spread by conjugation through conjugative plasmids and transposons, the contribution of these elements to antibiotic resistance has been extensively studied in hospital and community settings [3,4]. However, little is known about the role of bacteriophages as vehicles for ARGs in environmental settings. Recent findings based on cutting-edge genomic technologies suggest that, in these settings, bacteriophages play a more important role in the mobilization of ARGs than previously expected.

Bacteriophages (phages) are viruses that infect bacteria and have the ability to transfer genetic material between bacteria via transduction. Broadly speaking, they may be grouped according to their life cycle: lytic phages and lysogenic (temperate) phages. After lytic phages infect their bacterial host, the phage genome is replicated and assembled into phage particles that are released through cell lysis. Temperate phages integrate their genetic material into the bacterial chromosome as prophages, persisting in a latent or dormant state without causing cell lysis [5].

Phages have the potential to transfer genetic material between hosts using either generalized or specialized transduction (Fig. 1). Generalized transduction is the process by which bacterial DNA fragments are randomly packaged into the capsid during the lytic cycle, whereas specialized transduction is limited to temperate phages that integrate their genomes into the host chromosome at specific locations. Some temperate phages may encode factors that increase the fitness and survival of their hosts in a process known as lysogenic conversion. As a consequence, phages have emerged as prime suspects in bacterial adaptation and evolution by facilitating the exchange of genetic material. These characteristics make them suitable vehicles for acquisition, maintenance and spread of antibiotic resistance determinants. A recent study demonstrated a relatively high prevalence of integron–integrase genes (i.e. *intI1*, *intI2* and *intI3*) and genes conferring resistance to tetracyclines and β-lactam antibiotics (i.e. *tetA*, *tetW*, bla_{OXA} and bla_{TEM} genes) in phages isolated from different environmental sources [6]. A high prevalence of the $bla_{CTX\text{-}M\text{-}15}$ gene, which encodes resistance to extended-spectrum β-lactam antibiotics, was also detected in *Escherichia coli* phages from sewage samples [7]. Interestingly, a comparative study revealed that the abundance of genes conferring resistance to β-lactams (bla_{TEM}, bla_{NDM} and bla_{KPC}) and glycopeptides (*van*A) was increased in phage and plasmid DNA but not in the bacterial DNA fraction from samples collected downstream of wastewater treatment plant discharges [8]. Although previous studies have shown that human-associated viromes rarely carry genes conferring resistance to antibiotics [9], a recent study

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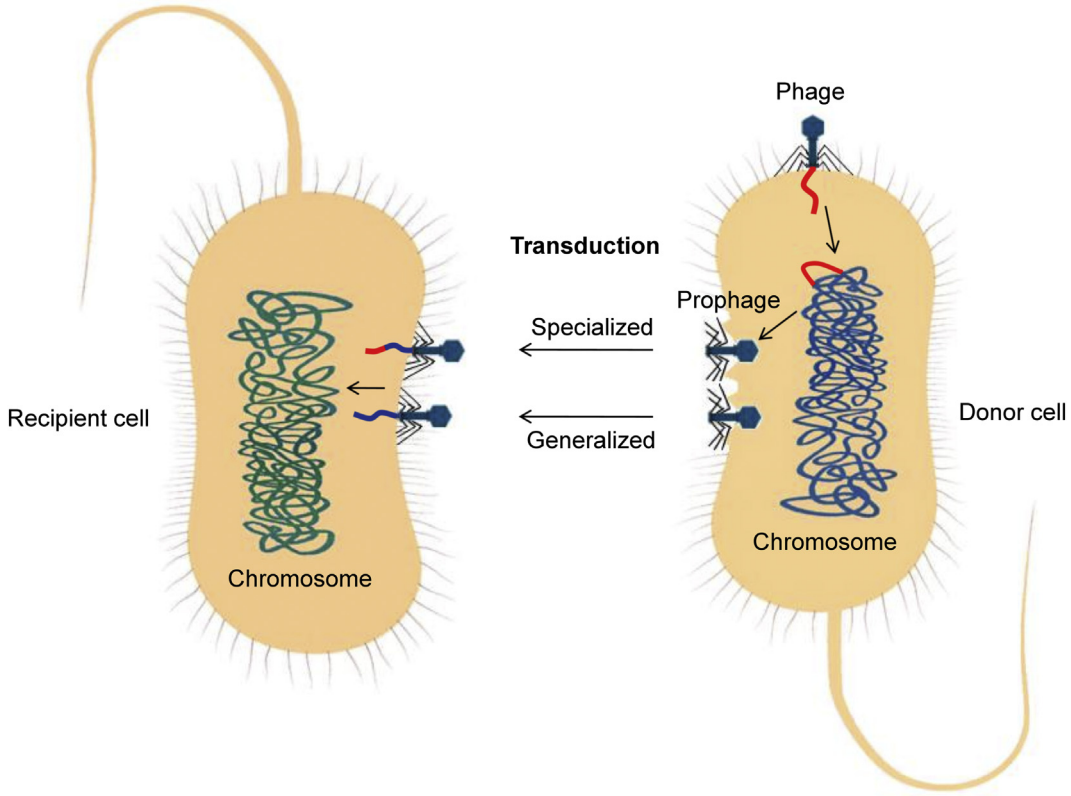

Fig. 1. Generalized and specialized transduction processes.

revealed that viromes from both anthropogenically impacted and non-impacted aquatic environments contain a large reservoir of resistance genes, including those conferring multidrug resistance to at least three different antibiotics [10]. Similar results have been observed in aquatic viromes from the Lambro River (Italy), whose relative abundance of reads associated with ARGs ranged from 0.48% to 1.92% [11]. Likewise, an extensive study of viromes from different aquarium systems revealed the presence of genes conferring resistance to several antibiotic classes, with trimethoprim being the most common [12]. Considering that environmental settings are frequently exposed to antibiotic residues and resistant organisms from anthropogenic sources, such impacts may trigger ecological interactions between phages and their hosts. In fact, phages can provide their bacterial hosts with a substantial advantage under challenging conditions. A comparative study showed that the virome of antibiotic-treated mice was highly enriched for genes conferring resistance not only to the administered antibiotic but also to other antibiotics [13].

Although transduction is a mechanism of horizontal gene transfer associated with phages, a recent study has demonstrated that certain phages (lytic phages) may also be able to promote transformation [14], whose process involves the uptake of naked DNA and recombination. Homologous recombination and DNA-repair processes normally limit the feasibility and success of transformation to DNA from closely related bacteria; however, ARGs may be potentially spread via broad-host-range plasmids without the need for recombination [15]. It should be noted that many bacteria carry plasmids, extrachromosomal genetic elements that frequently encode antibiotic resistance, which can be released during phage infection and can subsequently be acquired by other bacteria through transformation.

Altogether, these studies demonstrate that phages might play an important role in the acquisition and spread of ARGs into the environment. Several studies have also demonstrated that municipal wastewater treatment plants are hot spots for antibiotic-resistant bacteria and their ARGs, which are eventually released into receiving water bodies [16,17]; therefore, this problem can be amplified because phages can survive wastewater treatment processes better than bacteria [18,19]. Given this, further studies are required to elucidate the extent to which phages contribute to the mobilization of ARGs in environmental settings. A better understanding of the factors and mechanisms that promote environmental antibiotic resistance dissemination via phages will allow us to reach these goals.

Transparency declaration

No conflicts of interest have been declared.

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