Silencing the silent pandemic: eliminating antimicrobial resistance by using bacteriophages

Mao Ye[1], Jian-Qiang Su[2], Xin-Li An[2] & Yong-Guan Zhu[2,3*]

[1]Institute of Soil Science, Chinese Academy of Sciences, Nanjing 210008, China;
[2]Key Lab of Urban Environment and Health, Institute of Urban Environment, Chinese Academy of Sciences, Xiamen 361021, China;
[3]State Key Lab of Urban and Regional Ecology, Research Center for Eco-environmental Sciences, Chinese Academy of Sciences, Beijing 100085, China

As a result of the global spread of antibiotic-resistant bacteria (ARB) and antibiotic resistance genes (ARGs) among humans, animals, and environments, antibiotic resistance has become a silent pandemic that threatens public health worldwide (Larsson and Flach, 2022). The risk of ARB transfer has further aggravated and become the most pressing issue to be addressed now and in the coming years. The increasing use of disinfectants during the COVID-19 pandemic has further worsened the situation because disinfectants can facilitate the proliferation of ARB (Lu and Guo, 2021). In response to the escalating threats caused by ARB during the COVID-19 pandemic, health ministers from the Group of Seven (G7) countries held a meeting at Oxford University on June 4, 2021, and issued a joint statement highlighting the current challenges posed by the spread of antibiotic resistance (https://www.g7uk.org/g7-health-ministers-meeting-communique-oxford-4-june-2021/). Anthropogenic activities, such as clinical antibiotic application, intensive animal farming, and landfills, have been widely recognized to pose the greatest risks of antibiotic resistance dissemination (Zhu et al., 2013; Zhu et al., 2017). Approximately 4.95 million (3.62–6.57) deaths associated with bacterial antibiotic resistance occurred in 2019 (Antimicrobial Resistance Collaborators, 2022). Moreover, human life losses of more than approximately 10 million and economic losses of more than 100 trillion dollars would be incurred by 2050 if the antibiotic resistance crisis is left unresolved (O'Neill, 2016). In addition to public health, antibiotic resistance threatens ecosystem health. Consequently, developing safe and effective strategies to combat this worsening silent pandemic by reducing the presence of ARBs and ARGs in the environment and accelerating the implementation of the "ONE EARTH, ONE HEALTH" action plan is urgently needed.

In China, great progress has been made in reducing the risk of antibiotic resistance. Since the imposition of antibiotic prohibition regulations by the Chinese Ministry of Agriculture and Rural Development in 2015 and 2020, the abuse of antibiotics has been banned in veterinary settings and animal farming. At the same time, the regulation of antibiotic prescription for therapeutic purposes in clinical settings has been tightened, thus further curtailing the dissemination of ARB in the environment. In addition to the regulation of antibiotic usage, risk control and remediation technologies have been developed, such as biochar application in ARB and ARG hotspot soils to alleviate the migration of ARB and ARG from soils to the edible part of crops; composting methods to dissipate ARB and ARG during organic fertilizer preparation; and advanced oxidizing techniques and bioelectrochemical systems to remove antibiotics, ARB, and ARGs simultaneously in wastewater treatment (Figure 1) (Chen et al., 2021; Shao et al., 2022; Zhang et al., 2021).

*Corresponding author (email: ygzhu@rcees.ac.cn)

However, ARGs were widely present in the bacterial genome long before the anthropogenic use of antibiotics, and not all ARGs pose serious threats to planetary health. Zhang et al. (2021) reported that only 8.9% out of 73% of the ARG-harboring genomes in gut microbiomes from fecal microbiota transplantation donors are of high risk. Consequently, ranking ARG risks and developing strategies targeting the elimination of ARGs with the highest risks are important. Thus far, most of these technologies have limited applications due to their lack of specificity, long treatment times, and high costs. Here, we propose that these limitations may be overcome by the use of novel bacteriophage (phage) therapy.

Phages are a group of bacterial viruses that feed on bacteria by infecting and lysing host bacteria. They are one of the most abundant living entities on Earth, and their numbers are 10–100 times higher than those of bacteria. Phage therapy involves the isolation and screening of lytic phages that can infect and kill pathogens conferring antibiotic resistance; the enriched pure cultures of isolated phages are inoculated into pathogen-harboring environments. The major role of phages in curtailing antibiotic resistance includes the following: (i) lytic phages directly infect and kill antibiotic-resistant pathogens and decrease the overall abundance of the pathogenic bacteria in the environment, and (ii) ARGs within ARB cells (intracellular ARGs) are released into the environment (extracellular ARGs) and become ready for biodegradation (Reardon, 2014). Phage therapy has the advantage of high targetability over traditional antibiotic treatment because most phages can only infect and lyse one or a small group of bacteria, resulting in the minor perturbation of the overall microbial community. Moreover, the initial substantial inoculum amount is not essential for phage therapy because of the self-replicating characteristic and the reliance on host bacteria shown by phages, as well as the sensitive response of phages to the abundance of host bacteria in the environment. From an evolutionary perspective, the increase in phage diversity due to phage combined therapy commonly leads to the acceleration of the antibiotic resistance rate and the increase in the abundance of host pathogens. This situation results in tradeoffs in host metabolism and ultimately decreases the growth rate and resource utilization capacity of pathogenic bacteria, thereby reducing the overall antibiotic resistance risks in the environment. Therefore, compared with other ARB-controlling methods, phage therapy is more cost-effective and favorable for maintaining the overall diversity and functioning of microbial communities. A growing body of evidence shows that phage therapy is of high priority for reducing ARB risks in the environment (Ye et al., 2019). Currently, methods that combine phage therapy and biochar have been found to be highly effective in specifically inactivating ARB and reducing the abundance of ARBs and ARGs in soil-plant sys-

tems, thus indicating the flexibility of phage therapy (Ye et al., 2018).

In addition to lytic phage application, the temperate phage-delivered clustered regularly interspaced short palindromic repeat (CRISPR)/Cas system has been investigated as a promising strategy for tackling ARB. In prokaryotes, the CRISPR/Cas system acts as the adaptive prokaryotic immune system and protective barrier that function in adaptive immunity to mobile genetic elements (Yosef et al., 2015). CRISPR/Cas consists of CRISPR genes (including the leader, repeat, and spacer sequences) and a set of genes encoding Cas proteins. Given that it can eliminate ARGs radically, the CRISPR/Cas9 system has shown tremendous potential in halting the proliferation of ARGs and ARBs. Phages are well known to be the main sources of auxiliary metabolic genes in environments, thus contributing to the horizontal transfer of these accessory genes among microbial communities. Host bacteria with the Cas9 system can withstand infection by phages that encode ARGs, thereby inhibiting phage-mediated ARB dissemination. In a previous study, a pheromone-responsive conjugative plasmid that contains Cas9 and ARGs was constructed and introduced into the coat protein of the model phage. This genetically engineered phage was then used to infect ARG-harboring host bacteria. During this process, the CRISPR/Cas9 system acted as a constitutively expressed module encoded in a pheromone-responsive conjugative plasmid for the selective removal of ARGs, thereby turning the ARB into antibiotic-sensitive bacteria owing to the loss of ARGs and weakening the risk of ARB transmission (Rodrigues et al., 2019). As a result, the CRISPR/Cas9 system has been regarded as the emerging strategy for combating ARB due to its high editing efficiency and simple operative requirement.

The following fields are of promising importance in future studies on mitigating ARB risks by using phages. First, additional culturable phage species are needed to extend the usage of phage therapy in controlling antibiotic resistance risks. Consequently, constructing a platform for phage resource sharing on regional and/or international scales is important and will require substantial cooperative work among different groups. The most pivotal of this work involves the isolation of phage strains that can infect and lyse diverse pathogens conferring antibiotic resistance. The phage-based cocktail strategy that applies a variety of host-specific phages can be developed only with an extended phage resource pool that includes host-specific, polyvalent, and genetically modified phages to guarantee the efficacy and persistence of the phage lysis of antibiotic resistance hosts. Furthermore, taking advantage of the high efficiency of the genetic engineering CRISPR/Cas9 system and further promoting the eradication of ARGs in host bacteria by enhancing the delivery efficiency of CRISPR/Cas9-harboring phages in host bacteria are promising approaches. Host
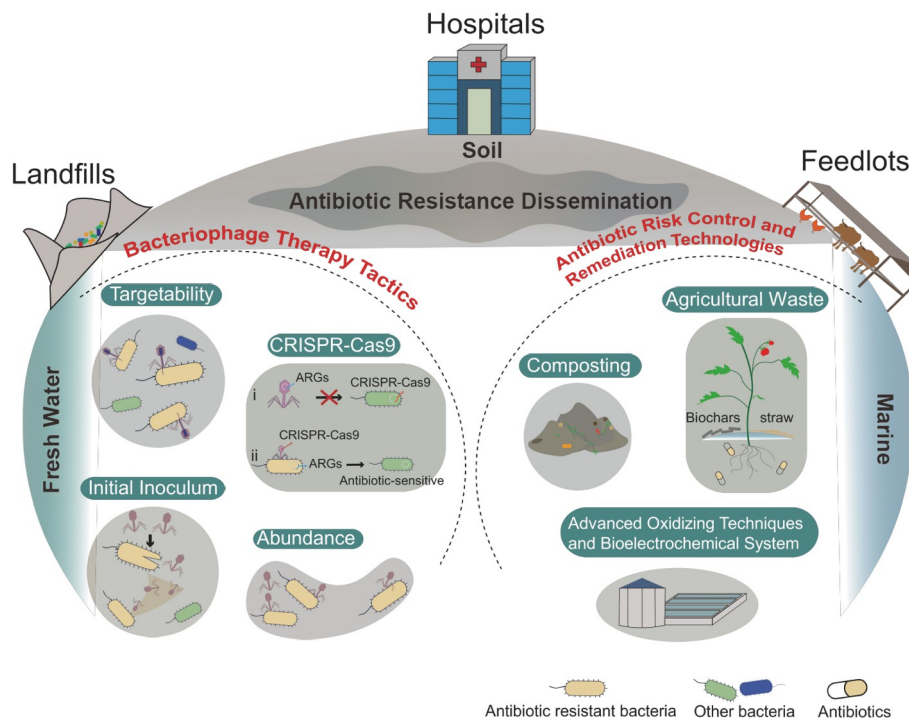
Figure 1   Anthropogenic activities are exacerbating the silent pandemic of antibiotic resistance worldwide. Consequently, there is an urgent need to develop clustered, regularly interspaced short palindromic repeat (CRISPR)/Cas-based bacteriophage therapies and other strategies to combat this crisis.

bacteria have evolved different mechanisms, including anti-CRISPR systems, to prevent phage infection and lysis to counteract the predatory pressure exerted by phages. This situation could decrease the efficacy of phage therapy in controlling antibiotic resistance risk and remains a major challenge in phage therapy application (Dupuis et al., 2013). Second, exploring the underlying microbial and molecular mechanisms when using phage-related strategies is important. Uncovering the responses of overall microbial communities to phage disturbance, including community assembly mechanisms, community structure and diversity, metabolic versatility, and inter- or intrakingdom microbial interactions, has become possible with the advent of metagenomics, metaviromics, metatranscriptomics, proteomics, and metabolomics. With the above mechanisms and strategies in mind, we still need to develop proper equipment to support the on-site application of phage therapy. Importantly, the field application of phage therapy remains difficult because targeted technical guidelines and administrative policies are still missing. Consequently, we need to develop ecological risk assessment models and standards and thus facilitate the establishment of site-flexible technical guidelines for the application of phage therapy in ARB/ARG-contaminated environments. The full use of phages to combat the silent pandemic of antibiotic resistance in the coming decades is possible only with collaborative efforts among the academe, industry, administration, and other relevant stakeholders.

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