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Short communication

Exploring the contribution of bacteriophages to antibiotic resistance☆

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ABSTRACT

Bacteriophages (phages) are the most abundant and diverse biological entities in our planet. They infect susceptible bacterial hosts into which they either multiply or persist. In the latter case, phages can confer new functions to their hosts as a result of gene transfer, thus contributing to their adaptation (short-term) and evolution (long-term). In this regard, the role of phages on the dissemination of antibiotic resistance genes (ARGs) among bacterial hosts in natural environments has not yet been clearly resolved. Here, we carry out a comprehensive analysis of thirty-three viromes from different habitats to investigate whether phages harbor ARGs. Our results demonstrate that while human-associated viromes do not or rarely carry ARGs, viromes from non-human sources (*e.g.* pig feces, raw sewage, and freshwater and marine environments) contain a large reservoir of ARGs, thus pointing out that phages could play a part on the spread of antibiotic resistance. Given this, the role of phages should not be underestimated and it should be considered when designing strategies to tackle the global crisis of antibiotic resistance.

1. Introduction

The emergence of antibiotic resistance among bacterial populations threatens the success of antibiotic therapy. The use and misuse of antibiotics over recent decades and the presence of antibiotic residues, even at subinhibitory concentrations, in different environments could have accelerated this phenomenon by exerting a chronic selective pressure. Bacterial populations susceptible to antibiotics become resistant either through genetic mutation or through horizontal transfer of antibiotic resistance genes (ARGs) from other bacterial strains, either distantly or closely related. This latter process is mediated by mobile genetic elements (MGEs), such as insertion sequences, transposons, integrative conjugative elements, plasmids and bacteriophages (phages), which are involved in bacterial acquisition and recombination of foreign DNA (Frost et al., 2005; Brown-Jaque et al., 2015). It is therefore essential to estimate how often MGEs encode ARGs (Martínez et al., 2015), and to understand the mechanisms and conditions under which these elements promote horizontal gene transfer (HGT) in nature. Although a recent study provided compelling evidences that ARGs are rarely encoded in phage genomes (Enault et al., 2016), the contribution of phages on the spread of ARGs in environmental settings has not been fully explored. Phages are the most abundant and diverse biological entities in the biosphere and they have the potential to transfer genetic material between bacterial hosts (*i.e.* transduction), thereby favoring their adaptation to local conditions and eventually driving their evolution (Balcazar, 2014). The recruitment of novel genes carried by phages or the alteration of expression patterns of already existing genes can confer beneficial phenotypic traits that allow adaptation to adverse conditions (Wang and Wood, 2016). Phage-mediated genetic exchange can occur not only between closely related bacterial species but also between distantly related species, and even highly conserved 16S rRNA gene sequences have been found within the genome of a broad-host-range transducing phage (Beumer and Robinson, 2005). In this study, we carried out a comprehensive analysis of several viromes to assess whether or not phage genomes encode ARGs and if so, to explore their diversity and abundance.

2. Material and methods

Thirty-three viromes from diverse habitats, including those sampled from human feces, pig feces, raw sewage, and freshwater and marine environments were obtained from public repositories (Supplementary Table S1). Metagenomic reads from each virome

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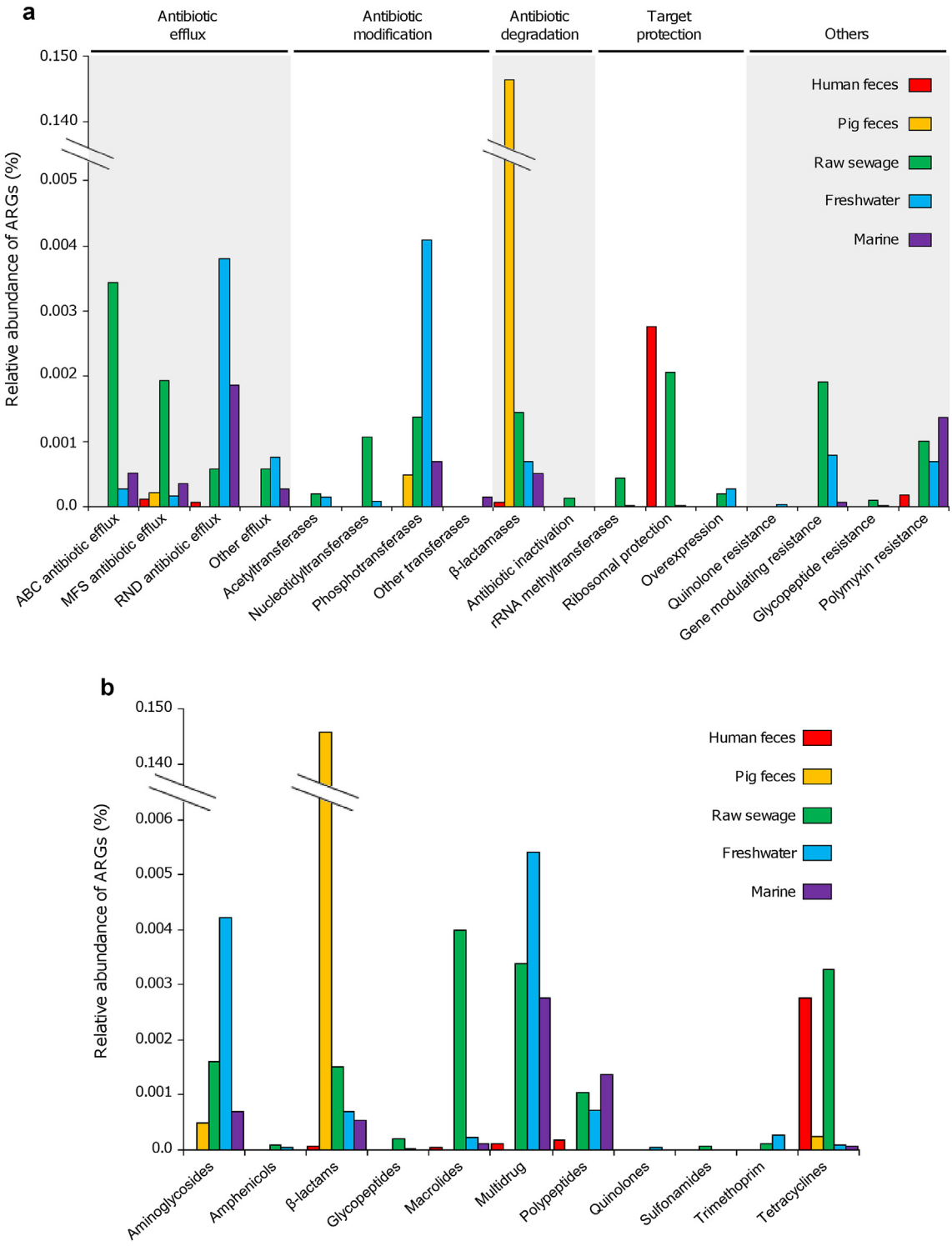

Fig. 1. Relative abundance of antibiotic resistance genes (ARGs) in viromes from different habitats, which are distributed according to their mechanisms of action (a) and the class of antibiotics that they confer resistance to (b). Counts were normalized to the total number of reads per virome. ABC, ATP-binding cassette; MFS, major facilitator superfamily; RND, resistance-nodulation-cell division. Multidrug indicates resistance at least three classes of antibiotics.

were then compared against an in-house database containing sequences from the Comprehensive Antibiotic Resistance Database (CARD) (McArthur et al., 2013), the Antibiotic Resistance Genes Database (ARDB) (Liu and Pop, 2009), and the Bush, Palzkill and Jacoby collection of curated β-lactamase proteins (http://www.lahey.org/studies/). A conservative threshold (*i.e.* BLASTX hits with ≥80% identity over at least 25 amino acids) was used to identify putative ARGs. ARGs were grouped according to their mechanism of resistance and the class of antibiotics that they confer resistance to. The abundance of ARGs was normalized to the total number of reads per virome. The presence of 16S rRNA gene sequences was determined using METAXA2 (Bengtsson-Palme