Review

The role of aquatic ecosystems as reservoirs of antibiotic resistance

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Although antibiotic resistance has become a major threat to human health worldwide, this phenomenon has been largely overlooked in studies in environmental settings. Aquatic environments may provide an ideal setting for the acquisition and dissemination of antibiotic resistance, because they are frequently impacted by anthropogenic activities. This review focuses primarily on the emergence and dissemination of antibiotic resistance in the aquatic environment, with a special emphasis on the role of antibiotic resistance genes.

Antibiotic resistance: emergence and impact

The development of antibiotics has been one of the major achievements of the 20th century and millions of human lives have been saved since the 1940s when the first antibiotics, penicillin and streptomycin, were introduced. Antibiotics are used to treat a wide range of bacterial infections and are indispensable in medical treatment such as intensive care, organ transplantation, chemotherapy, care of preterm babies, and surgical procedures, which could not be performed effectively without the availability of effective antibiotics.

However, antibiotic resistance has become a global public health concern because the organisms that cause infections are becoming resistant to the most commonly prescribed antibiotic treatments, resulting in prolonged illness and greater risk of death [1]. Another worrying aspect is that antibiotic resistance has developed over time, from resistance to single classes of antibiotics to multidrug resistance and extreme drug resistance [2], increasing the challenge for the development of more effective antibiotics. According to recent data from the European Centre for Disease Prevention and Control and the European Medicines Agency, every year approximately 25 000 European citizens (5.1 per 100 000 inhabitants) die from infections caused by bacteria that have developed resistance towards antimicrobials (http://www.ecdc.europa.eu/). In the USA, nosocomial infections are responsible for 12 000 deaths (4.0 per 100 000 inhabitants) each year [3], and it is estimated that more than 70% of bacteria that cause these infections are resistant to at least one of the antibiotics commonly used to treat them [4].

Susceptible bacteria may become resistant to antibiotics through multiple and complex mechanisms, such as (i) exclusion of the antibiotic by the cell membrane; (ii) intracellular modification and/or deactivation of the antibiotic; (iii) reduction in sensitivity of the cellular target; (iv) extrusion from the cell; and (v) intracellular sequestration [5]. These mechanisms can evolve through mutation and selection or by acquiring from other bacteria the genetic information that encodes resistance. The last event may be mediated by horizontal gene transfer (HGT), which is largely, although not exclusively, responsible for the development of antibiotic-resistant bacteria through various processes such as conjugation (by bacterial plasmids and conjugative transposons), transformation (by acquisition of free naked DNA from the environment), and transduction (by bacteriophages).

Despite the fact that antibiotic resistance is a major and growing public health concern, the surveillance for the expansion of this phenomenon in environmental settings is remarkably limited. One possible explanation could be the fact that antibiotic concentrations in nonclinical settings are generally very low. However, recent studies have revealed that selection of resistant bacteria can occur at extremely low antibiotic concentrations [6], similar to those concentrations found in some aquatic and soil environments [7,8], showing that even subinhibitory concentrations of antibiotics may promote antibiotic resistance. Moreover, the overuse and misuse of antimicrobial agents in human and veterinary medicine, animal farming, industrial settings, and their subsequent release in wastewater treatment plants (WWTPs) have contributed to the emergence and dissemination of resistant bacteria into the environment, including bacteria causing infections in both humans and animals [9–11]. Given this, aquatic environments including surface water and groundwater bodies provide ideal settings for the horizontal exchange of mobile genetic elements (MGEs) encoding antibiotic resistance [5,12]. This review will, therefore, focus on the emergence and dissemination of antibiotic resistance in the aquatic environment, with a special emphasis on the role of antibiotic resistance genes (ARGs).

Link between clinical and environmental resistance

Several studies suggest that antibiotic resistance occurs in nature and has an ancient origin, which is not linked to the

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Keywords: aquatic environments; bacteria; antibiotic resistance; antibiotic resistance genes; mobile genetic elements; bacteriophages.

0966-842X/$ – see front matter
 http://dx.doi.org/10.1016/j.tim.2013.11.001

anthropogenic use of antibiotics [13,14]. In the environment, bacteria have developed an ability to synthesize bioactive molecules to either cooperate with or antagonize other members of the community and, as a result, they have developed defense systems to protect themselves against the molecules of others. These molecules are encoded by genetic elements which constitute the resistome [15]. Moreover, the new high-throughput sequencing tools have revealed that an 'intrinsic resistome' exists, including innumerable sequences normally belonging to bacterial metabolic networks that can eventually participate in resistance to antimicrobial agents. These pre-resistance genes can evolve to new resistance mechanisms if they reach an environment with a high concentration of antibiotics. In this regard, antibiotics may act as selector agents of mechanisms of resistance but also as accelerator agents of the evolution of resistance [16]. Several metagenomic studies have been performed to explore antibiotic resistance diversity in different environments. In fact, one study demonstrated that uncultured soil bacteria from a Wisconsin oak savannah harbored unknown aminoglycoside and tetracycline resistance genes significantly diverse from previously sequenced genes [17]. Another study found genes encoding for β-lactamases in one Alaskan soil, where the anthropogenic activities are minimal. These β-lactamases were more closely related to ancestral β-lactamases than those isolated in clinical settings but they were still capable of conferring resistance on *Escherichia coli* [18].

Collectively, these studies suggest that the environment represents a huge reservoir of ARGs; however, only within the past 10 years has there been evidence of the mobilization of environmental resistance genes into clinical human pathogens. Some studies, for instance, have shown the similarity of the gene encoding for CTX-M, an extended spectrum β-lactamase (ESBL) often located in clinical pathogens, with chromosomally encoded β-lactamases from *Kluyvera* spp., a typical environmental bacterium [19–21]. It has also been demonstrated that genes encoding quinolone resistance may be present in environmental bacteria. The origin of the *qnrA* gene, which confers low levels of resistance to quinolones, was identified in the chromosome of *Shewanella algae*, a Gram-negative species widely distributed in marine and freshwater environments [22]. The reservoirs of the *qnrB* and *qnrS* genes remain unknown, although it seems that these genes may be closely related to chromosome-encoded Qnr-like determinants in some species of the *Vibrionaceae* family [23]. More recently, a platform has been developed to facilitate the rapid and efficient functional characterization of metagenomic libraries, namely Parallel Annotation and Reassembly of Functional Metagenomic Selections (PARFuMS). Applying this platform to a collection of soil-derived cultures, ARGs of all major mechanistic classes were found in nonpathogenic soil bacteria with perfect nucleotide identity to ARGs from many diverse human pathogens. Moreover, the ARGs were located within long sequences flanked by MGEs, suggesting a recent HGT event and probably the mechanism through which this exchange occurred [24].

Now that the link between clinical and environmental resistance has been demonstrated, it is essential to study how antibiotic resistance evolves in the environment (Box 1). Moreover, owing to the introduction of antibiotics into the environment from human and veterinary applications, the environment turns into a reactor where bacteria from different origins, antibiotics, disinfectants, and heavy metals are mixed, contributing to the evolution and dissemination of antibiotic resistance [25]. Wright *et al.* [26] quantified the abundance of an MGE, the class 1 integrase (*intI1*) gene, in total community DNA extracted from contaminated and reference riverine and estuarine microhabitats, and in metal- or antibiotic-amended freshwater microcosms. The authors found that the *intI1* gene was more abundant in all contaminant-exposed bacterial communities indicating that relative gene transfer potential is higher in these communities. Likewise, Rosewarne *et al.* [27] demonstrated that the abundance of *intI1* was increased as a result of ecosystem perturbation, indicated by a strong positive correlation with heavy metals such as zinc, mercury, lead, and copper. Both studies suggest that the presence of some pollutants, such as heavy metals, could co-select for antibiotic resistance.

Although a high number of ARGs have been found in environmental settings influenced by anthropogenic activities, these increases remain partially understood. There are two main hypotheses to explain these observations. The first suggests that antibiotics released in the environment exert a selection pressure on bacteria, selecting the resistant populations and thus increasing the amount of ARGs. The second hypothesis proposes that ARGs from other sources, such as human and animal origin, are transported mostly through runoff processes into the aquatic environment [28]. A study carried out in the South Platte River basin supports the hypothesis of transport, because the molecular signatures between pristine and impacted sites were different and because ARGs were detected with greater frequency in suspended sediments than in streambed sediments, where the antibiotic concentrations were higher [29]. Conversely, a metagenomic study was performed to investigate how microbial communities respond to the presence of wastewater discharges. The results revealed high levels of ARGs as well as elements for HGT, which suggest that those discharges carrying antibiotic residues promote ARGs and the exchange of MGEs [30]. Another study revealed that repeated applications of manure increase the abundance of ARGs in soil, suggesting that HGT is an important factor in the dissemination of ARGs, because bacteria from manure may not be well adapted to the soil environment [31]. Ultimately, the natural environment, either pristine or polluted, possesses a pool of ARGs that should be taken into account to better understand the evolution and dissemination of antibiotic resistance.

Acquisition and dissemination of ARGs

Several studies have demonstrated that ARGs are spread by MGEs, including plasmids, insertion sequences, insertion sequence common region elements, transposons, integrons, genomic islands, integrating conjugative elements, and bacteriophages (Table 1, [32–38]), which are involved in bacterial acquisition and recombination of foreign DNA [39]. Plasmids, circular double-stranded DNA molecules