Origin and evolution of antibiotic resistance: the common mechanisms of emergence and spread in water bodies

Agnese Lupo, Sébastien Coyne and Thomas Ulrich Berendonk

1 Institute of Hydrobiology, Department of Hydrosciences, Technical University Dresden, Dresden, Germany
2 Department of Biomolecular Chemistry, Leibniz Institute for Natural Product Research and Infection Biology – Hans Knöll Institute, Jena, Germany

INTRODUCTION

Evolution of bacterial antibiotic resistances, and its spread and emergence, represent one of the most threatening health care problems with worldwide proportions (Hawkey, 2008). The rise of new resistances and of multi-drug resistances urgently asks for a better understanding of the factors and hot spots involved in its diffusion and development. All the known antibiotic resistance mechanisms, acquired by opportunistic and pathogenic bacteria, evolve by means of Darwinian forces, i.e., mutations occurring in pre-existing genes of the bacterial chromosome positively selected by environmental forces (Gullberg et al., 2011; Zhang et al., 2011). Mutations within the chromosome can be responsible for the decreased affinity of antibiotics to their targets. Furthermore, some resistance mechanisms (e.g., efflux pumps, chromosomal AmpC β-lactamases) are finely regulated in their expression and at a basal level confer a naturally reduced susceptibility to the drugs. Mutations in the genomic architectures regulating such mechanisms result in their over-expression and high level of antibiotic resistance (Jacoby, 2009; Coyne et al., 2011). However, adaptation to the selective pressure of antibiotics accelerates acquisition of antibiotic resistance genes by lateral transfer from donor species (Wiedenbeck and Cohan, 2011).

The environment, and especially freshwater, constitutes a reactor where the evolution and the rise of new resistances occur. In water bodies such as waste water effluents, lakes, and rivers or streams, bacteria from different sources, e.g., urban, industrial, and agricultural waste, probably selected by intensive antibiotic usage, are collected and mixed with environmental species. This may cause two effects on the development of antibiotic resistances: first, the contamination of water by antibiotics or other pollutants lead to the rise of resistances due to selection processes, for instance, of strains over-expressing broad range defensive mechanisms, such as efflux pumps. Second, since environmental species are provided with intrinsic antibiotic resistance mechanisms, the mixture with allochthonous species is likely to cause genetic exchange. In this context, the role of phages and integrons for the spread of resistance mechanisms appears significant. Allochthonous species could acquire new resistances from environmental donors and introduce the newly acquired resistance mechanisms into the clinics. This is illustrated by clinically relevant resistance mechanisms, such as the fluoroquinolones resistance genes qnr. Freshwater appears to play an important role in the emergence and in the spread of antibiotic resistances, highlighting the necessity for strategies of water quality improvement. We assume that further knowledge is needed to better understand the role of the environment as reservoir of antibiotic resistances and to elucidate the link between environmental pollution by anthropogenic pressures and emergence of antibiotic resistances. Only an integrated vision of these two aspects can provide elements to assess the risk of spread of antibiotic resistances via water bodies and suggest, in this context, solutions for this urgent health issue.

Keywords: water, freshwater, antibiotic resistance, environment, gene transfer

Aminov (2011) has reviewed the role of horizontal gene transfer mechanisms in environmental microbiota. Although many more studies are necessary to completely understand the role of horizontal gene transfer in the environment, experimental evidences have demonstrated that transduction has an important role in genetic exchanges among environmental microbiota, especially in freshwater. Horizontal gene transfer events are responsible for the acquisition of heterologous resistance mechanisms among species and from antibiotic producers to commensal and pathogen bacteria. Hospitals, human community, farms, aquacultures, and agriculture are reactors where the usage of antibiotics selects for resistant bacteria and promotes the gene exchange. Recently, much more attention has focused on the role of the environment and of connected ecological habitats, water bodies such as rivers, streams, waste water effluents, and lakes, that have been suggested to be important in facilitating the transport and transfer of the antibiotic resistance genes (Aminov and Mackie, 2007; Baquero et al., 2008). Low-cost pharmaceuticals, preventative medication with broad spectrum antibiotics together with the overuse of those drugs contribute significantly to the emergence of bacterial drug resistances (Depledge, 2011). The combination of all these factors together with an inadequate waste-management
of the pharmaceuticals seem to be responsible for the alarming pollution of the environmental habitats such as agricultural soils and rivers, which probably contribute to the selection of antibiotic resistant bacteria and speed up the emergence of new resistances. Furthermore, rivers often receive bacteria from different sources, e.g., waste water treatment plants or water originating from urban effluent, industrial, or agricultural activities, thus constituting potential compartments where environmental, human, and/or animal related bacteria can coexist, at least temporarily (Baquero et al., 2008). This mixing can result in two main risks: (i) many environmental bacterial species are provided with intrinsic antibiotic resistance genes, constituting part of the so-called resistome. These bacteria represent a reservoir of drug resistance mechanisms and may act as donors for human related bacteria which, in turn, could introduce new acquired resistance mechanisms in the clinics (Wright, 2010); (ii) due to the intensive usage of antibiotics in medicine, agriculture, and aquaculture, human or animal related bacteria are more likely to be selected for antibiotic resistances within polluted environments directly by the presence of antibiotics and indirectly through co-selection by other pollutants (Martinez, 2009). Thus, when antibiotic resistant bacteria contaminate rivers, their resistance mechanisms can spread in the environment through bacteria, and/or mobile genetic elements. The localization of antibiotic resistance genes on diverse genetic structures such as integrons, which are platforms for gene aggregation, and mobile genetic elements (e.g., transposons and plasmids), together with the presence of phages, enhance their spread, influencing the course of their evolution (Wright et al., 2008). In particular, integrons are supposed to have a crucial role in the development of multi-drug resistances (Cambray et al., 2010). Recent studies suggest that the spread of resistant bacteria in natural fresh water systems can reach drinking water supplies and thus enter the human food chain (Walsh et al., 2011). These factors lead to an unlimited spread of antibiotic resistances and indicate that water sanitation or a better management of the respective water quality is crucial for a better control of the spread of antibiotic resistances.

This review aims to describe the current knowledge on the origins of antibiotic resistances mechanisms and environmental reservoirs of antibiotic resistances. Mechanisms, originating and spreading in bacterial populations naturally occurring in the water habitats, will be highlighted. The consequences of horizontal gene transfer by transduction and gene recombination events mediated by integrons in water habitats will be underlined. The Table 1 summarizes the mechanisms of resistances cited in the review.

**CHROMOSOMALLY ENCODED CEPHALOSPORINASE**

Bacteria, especially Gram-negative, can also resist to β-lactams by the production of hydrolytic enzymes, β-lactamas. Detection of antibiotic resistant bacteria, in freshwater, harboring β-lactamases has been reported from several geographical areas. Clinical occurrence of β-lactamases encoding genes has been extensively reviewed (Bonnet, 2004; Pitout et al., 2005; Coque et al., 2008; Bush, 2010) and recently much attention to their propagation in the environment has been paid (see hereafter the section on acquired β-lactamases). According to Ambler (1980) β-lactamases are classified in four classes, simplistically ranging from A to D. Bush and Jacoby have proposed a categorization of the β-lactamases according to their hydrolytic and inhibitory profiles. For an exhaustive knowledge of β-lactamases nomenclature and biochemical characteristics, we suggest the recent review by Bush and Fisher (2011). Jacoby (2009) has reviewed the distribution, origins, and enzymatic action of AmpC β-lactamas, belonging to the class C. The author has observed that ampC genes are located on the chromosome of bacteria belonging to different and phylogenetically distant species. ampC genes are widely distributed in bacterial species of environmental origins. Water borne species such as *Aeromonas spp.*, *Pseudomonas aeruginosa*, *Pseudomonas fluorescens*, *Ochrobactrum anthropi*, as well as several Enterobacteriaceae, commonly found in water habitats, like *Enterobacter spp.*, *Morganella morgani*, and *Hafnia alvei* harbor chromosomal ampC genes. Typically, ampC genes are regulated
Table 1 | Overview of some mechanisms of bacterial antibiotic resistances occurring in water habitats.

| Antibiotic | Resistance mechanism | Host | Source |
|------------|----------------------|------|--------|
| β-Lactams | PBP2 mutations       | Proteus mirabilis |        |
|           | FBP5 mutations       | Enterococcus faecium |        |
|           | ampC regulators mutations | Gram-negative species |        |
|           | ampC promoter region mutations | Escherichia coli | Recreational beaches, drinking water |
|           | Acquired AmpC        | E. coli | Recreational beaches, drinking water, river, biofilm of water supplies |
|           | Acquired CTX-M       | E. coli | River, sediment, birds |
|           | Acquired KPC         | Klebsiella pneumoniae | Hospital waste water effluent |
|           | Acquired VIM         | Brevundimonas diminuta, Rhizobium radiobacter, Pseudomonas monteilii, Pseudomonas aeruginosa, Ochrobactrum anthropi, Enterobacter ludwigi, Pseudomonas pseudoalcaligenes | Hospital waste water effluent |
|           | Acquired IMP         | Pseudomonas fluorescens | Waste water |
|           | Acquired OXA-23      | Acinetobacter baumannii | River, hospital waste water effluent |
|           | Acquired OXA-48      | Serratia marcescens | River |
|           | Acquired NDM-1       | P. aeruginosa, Achromobacter spp., Kingella denitrificans | Tap water |
| Fluoroquinolones | QRDR (quinolones resistance determining region) mutations | P. aeruginosa | Hospital and urban waste water effluent |
|           | QnrS                 | Aeromonas spp., E. coli | River and lake, urban effluent |
|           | QnrS2                | Aeromonas allosaccarophila | Lake |
|           | QnrVC4               | E. coli | River |
|           | QepA efflux          | E. coli | Waste water effluent |
|           | OqxAB efflux         | Metagenome | River sediment, water from farm environment |
|           | Vancomycin modification of the peptidoglycan | Enterococci spp. | Farm water |
|           | Chloramphenicol and florfenicol | FloR efflux | Waste water effluents, biofilm |
|           | Tetracyclines and MDR | Tet efflux | Aquacultures streams |
|           | MDR                  | Over-expression of RND efflux pumps | Farms, sediment |

*Observed in clinics but likely occurring in environmental and water habitats.

and their expression is induced in the presence of β-lactams. The regulation of ampC expression is quite complex and has been reviewed for P. aeruginosa by Lister et al. (2009). Mutations in the transcription factor AmpR, a LysR-type transcriptional regulator, in the inner membrane permease AmpG, or in the cytosolic amidaspe AmpD, have been found to confer a constitutive expression of the cephalosporinase gene, even in the absence of antibiotics.

Mataseje et al. (2009) described the over-expression of ampC, by mutations in the promoter region, in Escherichia coli strains isolated from recreational beaches and drinking water (Table 1). As for other β-lactamases, chromosomal cephalosporinases have been described to evolve by point mutation, hydrolyzing a broader spectrum of β-lactams (Jacoby, 2009). Of particular concern is the plasmidic location of several ampC genes, which likely originated from the chromosomal cephalosporinase of environmental species. Details will be discussed in the section on acquired β-lactamases. AmpC enzymes are indistinguishable from the D-peptidases, involved in the cell wall biosynthesis (Jacoby, 2009). Thus, these enzymes probably adapted to confer β-lactams resistance from the natural physiological function, likely by gene duplication and mutation events (Sandegren and Andersson, 2009). Genes encoding AmpC enzymes are largely distributed on the chromosomes of many bacterial species of environmental origins. The intrinsic function of AmpC remains unknown, but the conservation of this enzyme in several unrelated species and the complex regulation of its structural gene highly suggests a physiological role. Deciphering this role could provide useful information on the evolutionary processes and driving forces that have lead to the selection of β-lactamases.

DNA GYRASE AND TOPO-ISOMERASE

Generally, mutations in the quinolone resistance determining region (QRDR) of gyrA, gyrB, parC, and parE genes coding for the bacterial DNA gyrase and the topo-isomerase IV respectively, are responsible for the onset of bacterial resistance to fluoroquinolones. This mechanism is known to occur in water
environments. Schwartz et al. (2006) have detected ciprofloxacin resistant *P. aeruginosa* in six different treatment plants from four cities in Germany receiving the waste water from hospitals and cities. Molecular investigations demonstrated the occurrence of mutations in *gyrA* and *parC* genes. Further, the study demonstrated the spread of the ciprofloxacin resistant *P. aeruginosa* also in the waste water receiving river (Table 1). Alcaide et al. (2010) have reported about the *gyrA* and *parC* mutation conferring fluoroquinolones resistances in a variety of *Aeromonas* spp. isolated from freshwater. The authors found that the mutations in *gyrA* and *parC*, which are responsible for fluoroquinolones resistance, in recently described *Aeromonas* spp. such as *Aeromonas media*, *Aeromonas veronii*, and *Aeromonas popoffi* are similar to the one described in *Aeromonas hydrophila*, *Aeromonas sobria*, *Aeromonas caviae*, and *Aeromonas salmonicida*. In Portugal, Figueira et al. (2011) reported about mutations in *gyrA* and *parC* mostly linked to *Aeromonas punctata* and *A. media* isolated from an urban effluent. The same authors have recently characterized *E. coli* strains, isolated from a waste water effluent, that harbored mutations in *gyrA* and *parC* genes, likely responsible for the observed ciprofloxacin resistance (Table 1).

**EFFLUX PUMPS**

The role of efflux pumps in conferring antibiotic resistance and multi-drug resistances in bacteria has been extensively studied and reviewed (Poole, 2004; Piddock, 2006; Martinez, 2009; Nikaido and Pages, 2011). Efflux systems conferring drug resistance typically belong to five main families: the ATP-binding cassette (ABC) transporter, the major facilitator superfamily (MFS), the small multi-drug resistance (SMR), the multi-drug and toxic-compound extrusion (MATE), and the resistance nodulation division (RND) families, the latter present only in Gram-negative bacteria and chromosomally located. The structural genes for these systems can be located on transferable genetic elements and constitute the main acquired mechanisms for drug resistance (e.g., the Tet and the CmlA/FloR efflux systems families for tetracycline and chloramphenicol resistance, respectively). However, bacteria are intrinsically provided with chromosomally encoded efflux systems that are believed to participate in the cell homeostasis, by extruding endo and/or exogenous toxic compounds, heavy metals, virulence factors, quorum sensing signal, etc. In Gram-negative bacteria, RND systems exhibit a wide substrate spectrum, which is not restricted to drugs of different classes. Nikaido and Pages (2011) have recently reviewed the role of these efflux pumps in a wide range of pathogenic and opportunistic bacterial species such as *E. coli*, *Klebsiella pneumoniae*, *Enterobacter* spp., *P. aeruginosa*, *Acinetobacter baumannii*, and the emergent opportunistic *Stenotrophomonas maltophilia*. Typically, the expression of RND efflux pumps is finely regulated by a dedicated regulator (Coyne et al., 2011). A more complex regulation network, linking efflux to membrane permeability and other cellular functions, is likely to occur in these bacteria, as described for the *mar* regulon in *E. coli* (reviewed by Grkovic et al., 2002). Some RND efflux genes are not expressed in absence of inducing signal, whereas others exhibit a basal level of expression, and therefore contribute to intrinsic resistance (Coyne et al., 2011). Point mutations in a regulator or in the promoter sequence of RND efflux genes can be responsible for their over-expression and, in turn, for enhanced resistance. Similarly, the acquisition of an insertion sequence, carrying a strong and constitutive promoter, upstream of the regulator or the promoter sequence of RND efflux genes, can also mediate their over-expression and cause drug resistance. These systems have been mostly studied in the context of antibiotic resistance; therefore only little information concerning the natural and physiological mechanisms inducing the expression of RND efflux genes exist. Recently, studies have identified the role of oxidative and nitrosative stress in the activation of MexXY and MexEF–OprN, respectively (Fetar et al., 2011; Fraud and Poole, 2011). These stress signals are likely to occur in the environment and might represent natural inducers of the efflux systems expression. The natural role of efflux systems has been extensively reviewed by Martinez et al. (2009) who concluded that the intrinsic role of efflux in the bacterial physiology has lead to the conservation of the genes coding for efflux pumps among species of the same genus. For example, if the over-expression of *mdfA* confers MDR to *E. coli*, a basal expression is involved in the Na+ (K+)/H+ antiport, that allows the pH homeostasis of the cell (Lewinson and Bibi, 2001). Efflux pumps conferring resistance to antibiotics, such as the AcrAB–ToIC from *Salmonella* spp. has also been shown to efflux bile salts, therefore conferring a selective advantage which allowed colonizing and surviving in human or animal intestines (Lacroix et al., 1996). Mosqueda and Ramos (2000) described the contribution of efflux pumps in the cellular extrusion of toluene, an organic solvent, in *Pseudomonas putida*. This species, able to grow on the liquid interface of water and toluene and to survive in highly contaminated environments, extrudes the solvent by the TtgABC pump. The genes coding for this RND efflux pump usually exhibit a basal expression level but are induced by the presence of toluene in the medium. In water sediment, Groh et al. (2007) demonstrated that a MexF-like pump from *Shewanella oneidensis*, further than contributing to resistance to tetracycline and chloramphenicol, confers an increased fitness in anoxic environments. The underlying mechanism is unclear but could involve the extrusion of toxic compounds. A well documented role, for some efflux pumps, is their involvement in the cell to cell communication. This function has been demonstrated for MexAB–OprM in *P. aeruginosa* (Evans et al., 1998), BpeAB–OprB in *Burkholderia pseudomallei* (Chan and Chua, 2005) and AcrAB–ToIC in *Enterobacteriaceae* (Rahmati et al., 2002). These RND pumps, further than extruding homoserine lactones, are also able to confer MDR. Moreover, several reports have shown that efflux pumps, notably from the RND family, are involved in mechanisms leading to bacterial virulence. For example, Piddock (2006) highlighted the crucial role of efflux pumps in extruding abiotic substances such as flavonoids during plant colonization and in establishing virulence. In antibiotic producing bacteria, efflux pumps play a crucial role as a self defense mechanism by extruding the bioactive secondary metabolites. For instance, an efflux-mediated self-resistance has been developed in the oxytetracycline-producing *Streptomyces rimosus* (Petkovic et al., 2006). Bacteria living in the same habitat, being exposed to the produced antibiotics, could either adapt their intrinsic mechanisms, e.g., by the over-expression of an efflux pump, or acquire by horizontal gene transfer the resistance mechanism from the producers. The first option would require a point mutation to
over-express a pre-existing efflux system able to pump out the toxic compound, whereas the second pathway would involve the mobilization and transfer of the gene coding for the self-protecting mechanism. Thus, efflux pumps had an ecological role much before they conferred drug resistances in clinics, as they constitute a selective advantage in presence of competing microorganisms. The massive usage of these drugs has further selected optimized mechanisms and enhanced their spread. The role of mobile and mobilizing genetic elements, such as insertion sequences, integrons, transposons, and plasmids, were critical for a successful and rapid spread. Nikaido and Pages (2011) have observed that the rise of resistance due to efflux pumps mechanisms in clinics is tightly linked to the sub-inhibitory concentration of the antibiotics during clinical therapies. Consequently, the appearance of this kind of resistance favors the emergence of other mechanisms such as reduced membrane permeability to drugs, increase of point mutation in the drug target genes or activation of enzymatic resistance mechanisms. It would be of interest to investigate this aspect of resistance development in environmental habitats, where the concentration of antibiotics varies dependent on the degree of pollution and where other selective forces are present. Especially, heavy metals, naturally present in the soil, and solvents produced as consequences of metabolic activities, have been demonstrated to be substrates of several efflux pumps conferring multi-drug resistance (Silver and Phung, 1996; Moken et al., 1997). Concerning heavy metals, pumps have the additional role to defend bacteria from a toxic excess and to maintain the proper intra-cellular concentration for co-factors and enzymes (Teitzel et al., 2006). The presence of these compounds in freshwater could therefore select for the over-expression of an intrinsic efflux pump. Some heavy metals efflux genes, notably from the SMR family, are located on R plasmids containing antibiotic resistance genes, and heavy metals may favor the co-selection of these two features. In the environment, maintenance and propagation of antibiotic resistance genes might have been promoted by heavy metals selection (Martinez et al., 2009). Moreover, a causal relationship between pollution of the water environment by antibiotics or other pollutants and the selection of bacteria expressing or over-expressing efflux pumps appears conceivable. Hernandez et al. (2011a) have recently demonstrated in vitro how triclosan, a detergent antibiotic used in cosmetic, binds the regulator SmeT of the SmeDEF pump in S. maltophilia, leading to the over-expression of the pump and consequent multi-drug resistance. This observation is of major concern since S. maltophilia is an aquatic species that can be responsible for nosocomial infection.

Until now, it remains unclear how the efflux pumps contribute to the emergence of resistant bacteria in the environment. It has been demonstrated that an efflux pump over-expression could be coupled with a reduced bacterial fitness. However, this is not a general rule. Sanchez et al. (2002) investigated the fitness of two P. aeruginosa mutants over-expressing the MexAB–OprM and MexCD–OprJ efflux pumps, both conferring multi-drug resistance. The authors demonstrated in vitro that the MexAB–OprM over-expressing mutant showed a significantly decreased survival in water compared to the wild type strain, while no significant differences were observed for the second efflux pump mutant. In addition, the production of biofilm in both mutants was not affected if not promoted in the MexCD–OprJ mutant. Production of biofilm implies a higher probability of survival in natural water ecosystem and would thus constitute a beneficial characteristic. Selection in polluted environments of opportunistic species such as P. aeruginosa, S. maltophilia, or A. baumannii, over-expressing efflux systems could contribute to the spread of these bacteria and their introduction into clinics. It would be interesting to focus on the above described mechanisms also in water environments, to gain a better understanding of their physiological function and their role in the emergence of bacterial drug resistance.

ACQUISITION OF GENES IN WATER HABITATS AND DEVELOPMENT OF ANTIBIOTIC RESISTANCES

Acquisition of heterologous genes by lateral transfer largely facilitate the adaptive evolution of bacteria, especially under strong selective pressures. Transfer of exogenous DNA in bacteria may be mediated by plasmids, phages, transposons, genomic islands, or capture of free DNA by transformation. Sengelov and Sorensen (1998) have found that in environments such as bulk water, plasmid transfer from a donor to a recipient cell occur, even at a low frequency. Taylor et al. (2011) have observed that several factors could, not only influence, but also promote gene transfer among bacteria in water environment. One such factor is filter feeding organisms that collect bacteria belonging to different species and concentrate them at high density in a reduced space, facilitating gene exchange. Biofilm matrix in water habitats also creates favorable conditions both for plasmid exchange and transformation process (Molin and Tolker-Nielsen, 2003). Interestingly, Meibom et al. (2005) have demonstrated how chitin present in the crustacean exoskeletons is able to activate the competence status of Vibrio cholerae, and thus enhance transformation by acquisition of exogenous DNA. Although they are not classified as mobile genetic elements, integrons are platforms for genes aggregation, and thus contribute to MDR development. Furthermore, the abundance of integrons in bacterial communities of water habitats seems to be associated with the degree of water bodies’ pollution (Wright et al., 2008). Many findings support the crucial role of genetic transfer in water habitats mediated by phages (Ripp and Miller, 1995).

INTEGRONS

Several studies have highlighted the crucial role of integrons, particularly class 1 integrons, in the evolution of antibiotic resistances in clinics (Cambray et al., 2010). Indeed, class 1 integrons are not only platforms for genes aggregation, leading to the establishment of multi-drug resistance, but their localization on mobile genetic elements such as plasmids and transposons favor the spread of several genes in a unique transfer event. Recently, studies on environmental microbial communities have demonstrated that integrons of class 1 are largely present in the environment. Gillings et al. (2008) have provided evidences that the clinical class 1 integrons originated from environmental bacterial communities. The authors observed that class 1 integrons isolated from environmental samples do not carry any antibiotic resistance gene and harbored the qac gene cassettes, which is responsible for the bacterial resistance to quaternary ammoniums by efflux. Clinical class 1 integrons would have arisen from environmental ones by integration on a Tn402-like transposon, which then
disseminated in human commensals and pathogens. The presence of the qac gene has conferred a selective advantage to adapt in clinical environments, where bacteria are often challenged by disinfectants. The establishment of class 1 integrons in clinical strains has later on enabled the acquisition of antibiotic resistances positively selected by the usage of drugs. This hypothesis is also supported by the fact that clinical class 1 integrons demonstrated similar structures among them, in terms of integrases and recombination site, inferring a common ancestor. Gaze et al. (2005) have demonstrated how pollution of water bodies and their sediments with quaternary ammonium compounds, directly select for bacteria harboring qacE gene cassettes, located on the class 1 integrons. Furthermore, evidence of selection of bacteria harboring class 1 integrons in water bodies contaminated by industrial waste has been provided by Wright et al. (2008). The authors demonstrated that the contamination of freshwater with heavy metals correlated positively with a higher abundance of class 1 integrons in the bacterial community. More recently, Gaze et al. (2011) showed in sewage sludge and pig slurry that the prevalence of class 1 integrons and of qac genes was higher in bacteria exposed to detergents and/or antibiotic residues. All these studies demonstrate that pollution of water bodies with different agents increases the risk of selection and spread of integron structures. These genetic structures may be acquired by bacterial species that play role as shuttle between environment and clinics, constituting gene vectors for further dissemination in nosocomial bacteria.

**PHAGES**

Phages are major constituents of environmental ecosystems, in particular freshwater (Weinbauer, 2004; Srinivasiah et al., 2008). Their abundance is usually higher than bacterial abundance and, since a significant fraction of the prokaryotic community is infected with phages in aquatic systems, phages are likely to play an important role in horizontal gene transfer. Parsley et al. (2010) have proven the presence of β-lactamases genes in the viral metagenome of an activated sludge, confirming that transduction events may be responsible for the propagation of antibiotic resistance genes in these environments. Interestingly, Colomer-Lluch et al. (2011) demonstrated the presence of blaTEM and blaCTX-M, the most common genes conferring β-lactams resistance in Enterobacteriaceae, and mecA, responsible for methicillin resistance in Staphylococcus aureus, in phage DNA isolated from a waste water treatment plant and the natural water of the receiving river.

The presence of mecA in the phage fraction of natural freshwater is of great sanitary concern because of the threat represented by methicillin resistant *Staphylococcus aureus* (MRSA) infections, both in hospitals and communities (Campanile et al., 2011). This finding is also of interest for the understanding of the propagation of this gene. mecA codes for a protein with a low affinity to penicillin (PBP2a), conferring methicillin resistance. This gene is located on a mobile genomic element, the staphylococcal cassette chromosome (SCCmec), and has been reported only from the *Staphylococcus* genus from clinics. Baba et al. (2009) have characterized a methicillin resistance gene complex, mecIRA2, which could be the progenitor of SCCmec observed in clinical MRSA, from a strain of *Macroccocus caseolyticus* (closely related to *S. aureus*), isolated from animal meat. Interestingly, Tsukishita et al. (2010) found a mecA gene in *S. fleurettii* chromosomally located and not associated to the SCCmec element. Thus, the authors advanced the hypothesis that *S. fleurettii*, an animal related species, is the progenitor of this resistance mechanism. The mecA gene has been reported rarely from natural water, but Schwartz et al. (2003) detected mecA in hospital waste waters. Later, Bockelmann et al. (2009) have reported the sporadic presence of mecA in a ground water recharge system. Kassem et al. (2008) described the presence of the mecA gene in 18 *Proteus vulgaris*, four *M. morganii*, and three *Enterococcus faecalis* isolated from surface water. A ca. 250 bp-sequence of mecA from one representative isolate of *P. vulgaris*, *M. morganii*, and *E. faecalis* was found to exhibit 100% similarity with the *S. aureus* mecA gene. However, this result, which is the first report of MecA in non-staphylococcal organisms, has never been confirmed by other studies or investigated further. Acquisition by transduction of heterologous genes, particularly of antibiotic resistance genes, might represent an important mechanism of horizontal gene transfer in water bodies. Considering the high concentration of phages in such environments (Weinbauer, 2004; Srinivasiah et al., 2008), transduction constitutes probably one of the main gene transfer mechanisms and of genome evolution for bacteria in water habitats. More studies are needed to understand the impact of phage communities on bacterial evolution and antibiotic resistance spread within the water bodies.

**ORIGINS OF ACQUIRED ANTIBIOTIC RESISTANCE MECHANISMS**

Recently, D’Costa et al. (2011) have reported a metagenomic analysis of the Beringian permafrost, which is 30,000 years old. They showed molecular evidences of the ancient origins of antibiotic resistances, detecting β-lactamases genes, vanX-like, component of the vancomycin resistance operon, and tetM, coding for a protein protecting the ribosomal target from tetracycline. Sequence analysis revealed that the β-lactamases genes recovered from the permafrost demonstrated an amino-acid homology (53–84%) to known β-lactamases from β-lactams producing Streptomyces. The tetM sequences revealed a high similarity to the genes coding for the ribosomal protection protein of actinomycetes. The vanX sequence showed a similarity to the vanX gene recovered in pathogenic vancomycin resistant enterococci (VRE) and to the vanX gene from *Amycolatopsis orientalis*. This environmental species, belonging to the actinobacteria phylum, is a natural producer of vancomycin, and very likely the progenitor of the van genes operons, responsible for resistance to vancomycin. The integration of the van operons on transposons and on conjugative plasmids has enhanced their spread (Courvalin, 2006). Reports of VRE in freshwater have been provided by several authors (Talebli et al., 2008; Lata et al., 2009; Luczkiwicz et al., 2010). Interestingly, Schwartz et al. (2003) detected vanA genes in the biofilm of drinking water supplies, in the absence of enterococci, demonstrating the lateral transfer of this gene. Notably, the progenitors of these resistance genes are soil bacteria thus most likely, a shuttle has been responsible for the introduction of these genes into the commensal bacterial community and afterward into the pathogenic species.
FLUOROQUINOLONES RESISTANCE BY TARGET PROTECTION

Resistance mechanisms originating from bacterial population of water bodies are less well documented than from soil organisms. However, the significance of water bodies as natural source for resistance mechanisms is similar compared to the soil. For example, a well known example is provided by the acquired fluoroquinolones resistance genes of the qnr family. qnr genes encode proteins binding the bacterial DNA gyrase, thus preventing the interaction of the antibiotic with its target. Generally, the presence of these acquired genes does not confer a high level of fluoroquinolones resistance, but provides a selective advantage in the presence of these drugs, even at low concentrations (Rodriguez-Martinez et al., 2011). Further, this protecting mechanism and the associated low level resistance may favor the emergence of strains with higher resistances to fluoroquinolones by mutations in the QRDR, quinolones resistance determining region, and/or by over-expressing efflux systems. Several aquatic bacterial species have been proposed as progenitors for these genes families. Poirel et al. (2005b) reported evidences that the qnrA gene located on plasmids and found in clinical isolates of fluoroquinolones resistant Enterobacteriaceae, is derived from the chromosome of *Shewanella algae*, a bacterial species present in marine and freshwater. The authors advanced the hypothesis that the gene jumped from the environmental species to Enterobacteriaceae probably under pressure of antibiotic usage. Beaber et al. (2004) have demonstrated that the presence of fluoroquinolones induces the SOS bacterial repair system, which in turn promotes horizontal gene transfer. Poirel et al. (2005a) conducted further investigations in order to understand the origin of this antibiotic resistance mechanism. Their study highlighted that the chromosomes of water borne bacteria, *Vibrio vulnificus*, *Vibrio parahaemolyticus*, and *Photobacterium profundum* harbored qnr-like genes with homology (40–67% identity) to the plasmidic qnrA, qnrB, and qnrS genes described in clinical Enterobacteriaceae.

Interestingly, qnrA has been observed frequently associated with the insertion sequence ISCR1, a genetic element able to mobilize adjacent genes. Toleman et al. (2006) hypothesized that the ISCR1 mediated mobilization of qnrA, as well as a further localization on a class 1 integron to form a so-called complex integron structure. The authors formulated that this complex integron structure is responsible for the successful dissemination of qnrA gene. Arsène and Lecerçq (2007) investigated the intrinsic resistance of *E. faecalis* to fluoroquinolones and found that this species is provided with a chromosomal qnr-like gene, which contributes to resistance against fluoroquinolones. Soon afterward, Sánchez et al. (2008) discovered that the aquatic bacterium *S. maltophilia* is a sink of qnr genes and the chromosomally located Smaqr gene identified in this species is able to confer resistance to fluoroquinolones in heterologous species. In 2010, Velasco et al. (2010) reported qnr-like genes from *Serratia marcescens*, an environmental species. These genes, called Smaqr, were largely present in the chromosome of the genus. Recently, Jacoby et al. (2011) have highlighted that the *Citrobacter* spp. chromosome constitutes a reservoir for the qnrB fluoroquinolones resistance gene. The presence of qnr genes in the chromosome of phylogenetically distant bacterial species (*Shewanella, Stenotrophomonas, Vibrio, Enterococcus, Serratia, Citrobacter*), suggests an ancestral role of this antibiotic resistance mechanism. Hernandez et al. (2011b) postulated a regulatory role for the Qnr proteins. Indeed, by interacting with the DNA gyrase, Qnr may protect the DNA gyrase against toxic DNA substances and indirectly modulate gene expression in response to environmental changes. Moreover, a beneficial role of these protecting mechanisms has been shown for *qnrA3*, which confers a fitness advantage to the bacteria, favoring its dissemination. The fitness advantage was found abolished when *qnrA3* was carried by large multi-drug resistance plasmids (Michon et al., 2011). The activation of *qnrB* expression by the SOS-response system could also have an implication in the conservation of such mechanism. As ciprofloxacin induces the SOS-response system, it activates its corresponding resistance mechanisms (Da Re et al., 2009). Several studies have reported *qnr* genes in heterologous species from water habitats. Cattoir et al. (2008) recovered from the Seine River *A. punctata* and *A. media* harboring *qnrS2*. Similarly, Picado et al. (2008) detected *qnrS* genes in *Aeromonas allosaccarophila* from the Lugano Lake, in Switzerland. A *qnrVC*4 allele was isolated from aquatic environments in *A. punctata* by Xia et al. (2010). All these reports demonstrate that the *Aeromonas* genus represents a reservoir for fluoroquinolones resistance mediated by Qnr. Our own studies characterized a *qnrS* determinant in *E. coli* belonging to ST131 isolated from freshwater of a Ukrainian River (Lupo et al., submitted). Similarly, Dhanji et al. (2011) isolated *E. coli* strains belonging to ST131 harboring a *qnrS* allele from the Thames River (Table 1). These findings reflect a spread of these resistance mechanisms by geographical and clonal means and highlight the potential of rivers in the dissemination of international resistant clones.

ACQUIRED EFFLUX MECHANISMS

Another acquired fluoroquinolones resistance mechanisms is represented by efflux mechanisms. The *qepA* gene, initially characterized on a conjugative plasmid from a clinical isolate of *E. coli* (Perichon et al., 2007), encodes a MFS efflux pump. It has been recently recovered from the metagenome of river sediments impacted by improperly managed urban waste waters (Cummings et al., 2011). Environmental reports of this gene are rare; however, Deng et al. (2011) have highlighted the possible spread of this gene by animal and human related bacterial strains in water compartments. Similarly, the detection of the OqxAB efflux pump, conferring resistance to fluoroquinolones, olaquindox, and chloramphenicol, remains rare in environmental samples. The *oqxAB*, found on a conjugative plasmid in *E. coli* strains, represents the only example of transferable RND efflux pumps, so far (Hansen et al., 2004). Recently, Zhao et al. (2010) have reported an *E. coli* strain, isolated from a water pond in a farm environment, harboring the *oqxAB* gene (Table 1).

Resistance by acquired efflux mechanisms to other drug classes than fluoroquinolones has been extensively reported in the literature (Poole, 2004; Piddock, 2006; Nikaido and Pages, 2011). Studies conducted in water habitats such as aquaculture, impacted by anthropogenic activities, and notably by the application of antibiotics, demonstrated the risk of selection of acquired efflux pumps. Fernandez-Alarcon et al. (2010) reported the presence of different Gram-negative species from aquacultures in Chile expressing the floR gene, which codes for a chloramphenicol and florfenicol
exporter, drugs intensively used in veterinary medicine. Alarmingly, those strains resistant to florfenicol also demonstrated a multi-drug resistance, suggesting a process of co-selection. Gordon et al. (2008) characterized the floR gene, in *Aeromonas bestiario*um strains from freshwater streams in France, located on a 25-kb-plasmid harboring also the tetracycline efflux gene *tet(Y)*, *strB-strA*, conferring resistance to streptomycin, and *sul2* conferring resistance to sulfonamides (Table 1). Interestingly, this plasmid contained sequences with high nucleotide homologies to other genetic elements recognized in different aquatic bacterial species such as *V. cholerae* and *Photobacterium damselae*. This demonstrated the contribution of horizontal gene transfer in the spread of these resistances in aquatic habitats. Furthermore, genes encoding tetracycline efflux mechanisms have been found to circulate between farm environments and ground water (Aminov et al., 2002). Propagation of tetracycline resistance genes, by efflux or by ribosomal protection, has been linked to the extensive usage of this drug class in animal feeding, and although the usage of this antibiotic has been restricted, tetracycline resistance genes seem to persist in the food chain and in the environment.

**THE ENZYMATIC β-LACTAMS RESISTANCE**

Resistance to β-lactams has spread worldwide. The low toxicity of these molecules and the broad spectrum of action of some of them make β-lactams the most prescribed antibiotic drug class and propagation of resistance constitutes therefore a major clinical concern. Studies have highlighted that the rise of the bacterial resistance against β-lactams is related to the usage of the drug in clinics, both because of selection of resistant bacteria and by promoting the mobilization of the genes responsible for such resistances (Bush and Fisher, 2011). Similarly, the presence of antibiotics in water environments could promote the selection of antibiotic resistant strains. Detecting and measuring the concentration of antibiotics or intermediary products from their metabolism could promote the selection of resistance against antibiotics (Bush and Fisher, 2011). The crucial molecular vector of its spread has been recognized by Naas et al. (2008), who characterized the location of *bla*KPC gene on
a Tn-3-like transposon, the Tn4401, probably responsible for the original mobilization of this gene. The transposon contains several sequences encoding transposases or insertion sequences derived from environmental bacterial species, but the ancestral host of this enzyme has not been identified, so far. Recently Chagas et al. (2011) have detected K. pneumoniae producing KPC in an effluent receiving hospital waste water, highlighting an environmental vector for the dissemination of these enzymes (Table 1). VIM enzymes have been rarely reported from environmental isolates. Scotta et al. (2011) have detected this enzyme has not been identified, so far. Recently Chagas et al. (2011) have isolated a strain of Pseudomonas monteilii, P. aeruginosa, O. anthropi, and Enterobacter ludwigi strains producing VIM enzymes, again from an effluent receiving the waste water of a hospital. Previously, Quinteira et al. (2005) isolated a strain of Pseudomonas pseudoalcaligenes harboring blaVIM from a hospital wastewater effluent (Table 1). Probably, the presence of VIM producer species in the environment is due to nosocomial selective conditions and contamination by wastewater from hospitals. However, the detection of blaVIM in different environmental species from freshwater highlights the potential of water as a reservoir for these genes and as a vector facilitating their spread. Concerning IMP enzymes, so far, a unique report has been provided by Pellegrini et al. (2009), in a strain of P. fluorescens recovered from waste water (Table 1). A carbapenemase activity is also exhibited by several class D β-lactamases, among which the families of OXA-23, OXA-40, OXA-58, and OXA-51 are associated to A. baumannii (Poirel et al., 2010). This opportunistic pathogen, provided with an intrinsic but silent blaOXA1-like gene, is widely distributed in nature. The origin of blaOXA-40 and blaOXA-58-like genes remains unknown but Poirel et al. (2008) have characterized a blaOXA-23-like chromosomally located in Acinetobacter radiore sistens, suggesting that this species is the progenitor for OXA-23. Moreover, A. baumannii isolates carrying blaOXA-23 have been detected in river (Girlich et al., 2010) and wastewater from hospitals (Ferreira et al., 2011, Table 1). OXA-48 represents another class D carbapenemase that dramatically spreads among Enterobacteriaceae. This latter enzyme is supposed to originate from the chromosome of the water borne species S. oneidensis (Poirel et al., 2004). Recently, S. marcescens strains harboring blaOXA-48 have been isolated from a river in Morocco (Potron et al., 2011), demonstrating the risks for their dissemination in water habitats (Table 1). The recent emergence and dramatic spread of NDM-1 enzyme in clinical isolates of A. baumannii and Enterobacteriaceae, has focused major attentions. Usually, strains harboring this broad spectrum carbapenemase gene demonstrate a multi-drug resistant phenotype and a wide set of virulence genes (Walsh et al., 2011). The carriage of bacteria harboring blaNDM-1 by healthy individuals has lead researchers to investigate the source of this gene. Walsh et al. (2011) recently demonstrated the presence of different bacterial species (P. aeruginosa, Achromobacter spp., and Kingella denitrificans) harboring blaNDM-1 in tap water used as drinking water in India (Table 1). This finding is closing the transmission circle and explains the fast and successful dissemination of this gene. Several genes encoding carbapenemase enzymes have been found chromosomally located in bacterial species of environmental origin and water related, for instance the sme gene on the chromosome of S. marcescens, and the sfe gene on the S. fonticola chromosome (Naas and Nordmann, 1994; Henrques et al., 2004). The water borne S. maltophilia also harbors a gene coding for the L1 carbapenemase. Avison et al. (2001) have elucidated that this gene is located on a plasmid-like element considered intrinsic to S. maltophilia.

Table 1

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CONCLUSION

The emergence of antibiotic resistance is the consequence of a complex interaction of factors involved in the evolution and spread of resistance mechanisms. The over-usage of antibiotics in clinics has been believed to be the principal element involved in the rise of new resistances. Recently, many more evidences suggest that environmental habitats especially water bodies such as rivers and streams are ideal vectors for the antibiotic resistance dissemination. Here, the propagation of bacteria harboring antibiotic resistance genes can occur spatially along the river. Furthermore, the dispersion of these bacteria in the environment favors the interaction with the autochthonous microbiota, creating new scenarios for the evolution of antibiotic resistances.

In strong contrast to clinics, there are no data available on the epidemiology of antibiotic resistances in the environment, especially for geographically based data. This in turn makes extremely difficult to make any predictions on the risk of spread and emergence of new antibiotic resistances. For this reason we assume that a better knowledge on the environmental reservoir of resistances is fundamental to predict the emergences of new resistances of clinical concern.

It should be noticed that the pollution of water can select antibiotic resistant bacteria. This process, involving notably co-selection events, could cause an ecological imbalance leading to the dominance of resistant bacteria and global disturbance of the ecosystems. This latter point highlights the necessity of collecting information and data on the status of the sampling sites when performing a study on antibiotic resistance in freshwater. The development of antibiotic resistances occurs very likely naturally in the environment, but factors like pollution, especially of water bodies, could force the speed of its evolution. A good status of water quality could limit this phenomenon. Despite this, the status of the surface water quality in many countries is still poor. We argue for the implementation of feasible methodologies to characterize quality parameters and detect antibiotic resistance in water bodies, and thus to establish adapted and pragmatic measures to improve water resources. Amelioration of water status is
of major concern: it can contribute to a direct and local decreased risk for the health of populations living in the vicinity of the freshwater, and lead to more global effects by avoiding that water bodies could constitute reactors for antibiotic resistance emergence and evolution.

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