Review

The role of aquatic ecosystems as reservoirs of antibiotic resistance

Elisabet Marti, Eleni Variatza, and Jose Luis Balcazar

Catalan Institute for Water Research (ICRA), Scientific and Technological Park of the University of Girona, Emili Grahit, 101, 17003 Girona, Spain

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

Corresponding author: Balcazar, J.L. (jlbalcazar@icra.cat).
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

Box 1. Further research on antibiotic resistance in the aquatic environment

The high efficiency of MGEs transferring ARGs among phylogenetically distant bacteria from different environments makes it difficult to distinguish between naturally occurring resistance and the resistance promoted by antibiotics released from anthropogenic sources. However, the European Council concluded in 1998 that there was a relationship between the consumption of antimicrobial compounds and the prevalence of antibiotic-resistant bacteria. Since then, some government agencies have launched several programs for antibiotic resistance surveillance to assess the actual risk for public health associated with the consumption of antimicrobial compounds. Nevertheless, recent advances in metagenomics have shown that very little is known about the antibiotic resistome of the vast majority of environmental bacteria. Antibiotic resistance is by far better understood at the small scale (i.e., within an individual bacterium) than at the large scale (the natural environment). Given that antibiotics are the main weapon against bacterial pathogens, monitoring and surveillance programs and further research of environmental reservoirs is needed to better understand the mechanisms of antibiotic resistance and counteract this major, growing problem.

that replicate independently from chromosomal DNA, may encode a wide variety of genetic determinants. Insertion sequences (ISs) are the smallest and simplest autonomous MGEs, which possess one or two open reading frames and encode a transposase surrounded by linker regions that frequently end with short terminal inverted repeat sequences. Insertion sequence common region (IS*CR*) elements, which differ from the ISs by lacking terminal inverted repeat sequences, are thought to transpose by a mechanism termed rolling circle transposition. Transposons are gene systems flanked by inverted repeat sequences and encode transposases, which specifically recognize and introduce nicks at the ends of these elements to allow for integration at ISs. Integrons possess a site-specific recombination system for the capture of genes, notably those encoding antibiotic resistance, that are contained within mobile gene cassettes. Genomic islands are large DNA segments present in most bacterial genomes that have been acquired by HGT and often contain blocks of genes offering a selective advantage for host bacteria. Finally, integrating conjugative elements (ICEs) are self-transmissible MGEs that integrate into and replicate along with the host cell chromosome. The role of these MGEs as efficient vehicles for HGT has been previously described in detail [40–44]; however, the contribution of bacteriophages to the dissemination of ARGs has not been extensively explored in environmental settings.

Bacteriophages, also known as phages, are viruses that infect bacteria in order to replicate and assemble themselves using the metabolism and machinery of the host cell. Some phages may act as mediators of HGT, through transduction, whereby DNA is transferred from one bacterial cell to another by a phage. Transduction can be generalized, when fragments of bacterial DNA are packaged at random in the phage particles, or specialized, where certain regions of bacterial DNA are carried along with the viral genome [45].

Phages are considered to be the most abundant known organisms, and have a powerful influence on bacterial community structure and function through predation and gene transfer [46]. The relatively recent introduction of molecular techniques has led to a greater understanding of microbial diversity and its role in nature. A recent study revealed the presence of β-lactamases and putative transposases in the phage metagenome of activated sludge [47]. Interestingly, Colomer-Lluch *et al*. [38] determined the presence of ARGs conferring resistance to β-lactams and methicillin in phage DNA from a WWTP and water samples of the receiving river. The authors also demonstrated that ARGs from phage DNA were transferred to susceptible strains, which became resistant to ampicillin. ARGs have also been observed to confer resistance to β-lactams and quinolones in phage DNA from different hospital and WWTP effluents [48]. Altogether, these results undoubtedly demonstrate the contribution of phages in the dissemination of ARGs into the environment.

Aquatic environments as reservoirs of ARGs

It is now clearer than ever that antibiotics act as promoters of antibiotic resistance, and thus may contribute to the

Table 1. Summary of MGEs found in prokaryotes

Genetic element	Characteristics	Examples of resistance determinants	Refs
Plasmid	Variable size (1 to >100 kb), self-transmissible or mobilizable	Plasmid pP2G1 contains ARGs, which confer resistance to different antibiotics	[32]
Insertion sequence	Small (<2.5 kb), contains terminal inverted repeats, and encodes a transposase	IS18 mediates overexpression of the $bla_{OXA-257}$ gene	[33]
IS*CR* elements	Transpose adjacent DNA sequences by rolling circle transposition	IS*CR1* mediates the mobilization of the $bla_{CTX-M-1}$ gene	[34]
Transposon	Large (<12 kb), flanked by IS or inverted repeats; encodes a transposase and other functional genes such as ARGs	Tn*1* and Tn*3* confer resistance to β-lactams	[35]
Integron	Mediates the capture and expression of gene cassettes; encodes for an integrase, attachments sites, and transcriptional elements	Class 1 contains different cassettes conferring multidrug resistance	[32]
Genomic island	Large mobile regions of DNA that encode complex biological functions	SGI1 confers resistance to streptomycin, spectinomycin, β-lactams, and sulfonamides	[36]
Integrating conjugative elements	MGEs that are integrated in the chromosome and transfer via conjugation	ICE*Vch*Hai1 contains different ARGs	[37]
Bacteriophages	Viruses that infect prokaryotic cells; their replication allows the DNA transfer from one bacterium to another, known as transduction	β-Lactamase genes have been isolated from environmental phages	[38]

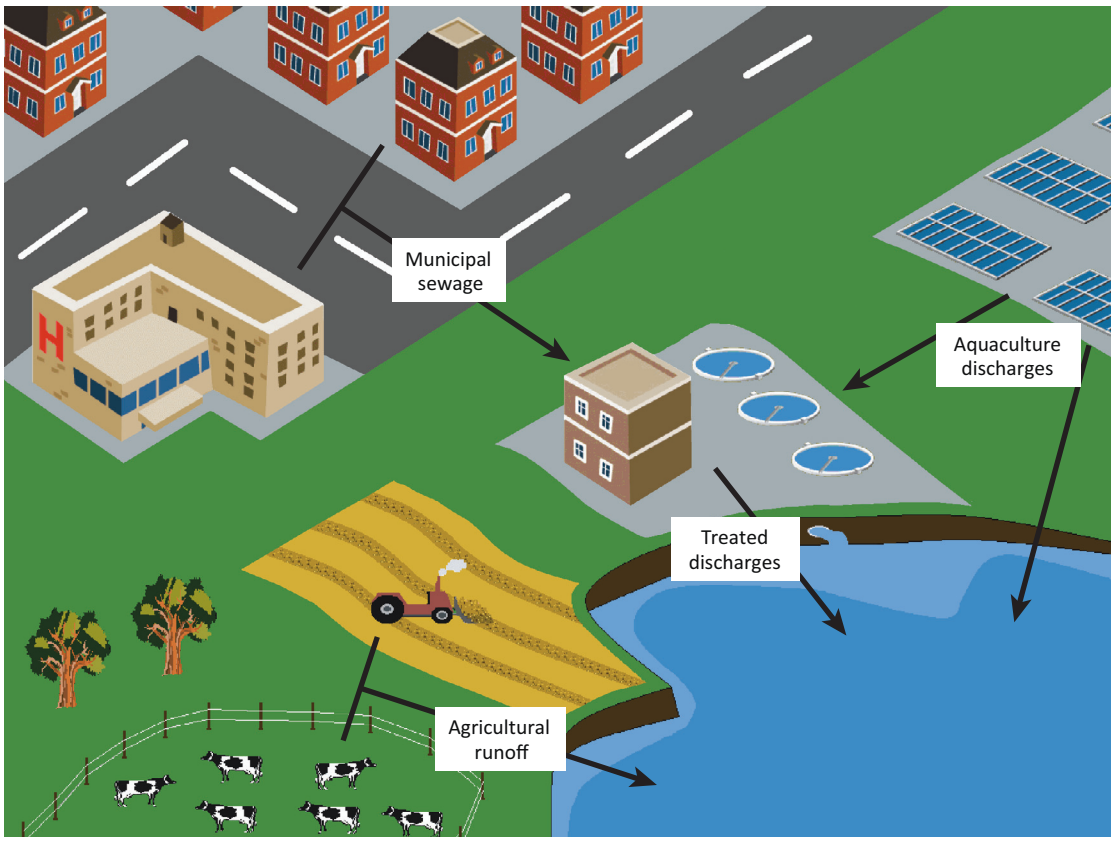

Figure 1. Different anthropogenic activities that result in the dissemination of antibiotic resistance genes (ARGs) in aquatic environments.

acquisition of MGEs [49]. As mentioned above, a diverse mixture of antibiotics and other pollutants, their metabolites and resistant bacteria, reaches the aquatic environment through treated and untreated sewage, hospital waste, aquaculture discharges, and agricultural runoff (Figure 1). These aquatic compartments, such as water and sediment, may therefore have a significant role in driving ARG transfer, ecology, and evolution [5].

WWTPs represent the main sites through which antibiotics are released into the environment. Some studies have suggested that common technologies that are being applied in WWTPs, such as biological treatment, provide an ideal environment (i.e., high bacterial densities, high oxygen, and high nutrient concentrations) for HGT, because bacteria are in continuous direct contact with antibiotics and resistant bacteria [50]. A metagenomic study recently found 140 different ARGs in bacteria from activated sludge and 123 ARGs in the final effluent of a WWTP [51]. Moreover, LaPara *et al*. [52] investigated the impact of tertiary treated municipal wastewater on the quantity of several ARGs in Duluth–Superior Harbor and determined that the treated water was a significant point source of three tetracycline resistance determinants as well as the *intI1* gene into the receiving harbor. We also investigated the effect of WWTP effluents on the prevalence of several ARGs in the Ter River and observed a significant increase in the relative abundance of ARGs in biofilm samples collected downstream of the WWTP discharge [53]. These studies suggest that WWTP discharges could promote the dissemination of ARGs into the environment.

The majority of bacteria in natural aquatic ecosystems are organized in biofilms, which facilitate their survival and dispersal. Biofilms are aggregations of bacteria that live in a highly structured and organized community. Recent studies have suggested that biofilms may contribute to antibiotic resistance, and this may be due to high cell density, close proximity, and accumulation of MGEs. Schwartz *et al*. [54] demonstrated that the *vanA* gene, which confers high-level resistance to vancomycin, was detected not only in wastewater biofilms but also in drinking water biofilms in the absence of enterococci, suggesting possible gene transfer to autochthonous drinking water bacteria. Engemann *et al*. [55] found evidence that the abundance of tetracycline resistance genes was reduced at different rates in the water column, and some genes readily migrated into biofilms, which suggest that biofilms serve as long-term reservoirs for ARGs.

Aquatic sediments also represent an important environmental matrix within which genetic transfer and recombination occur. Antibiotics used in aquaculture and other anthropogenic activities may be retained in these sediments, which can act as an interface for a complex and dynamic community of microorganisms, facilitating the transfer, maintenance, and dissemination of MGEs. Kristiansson *et al*. [30] demonstrated that MGEs, such as class 1 integrons and IS*CR* elements, were highly overrepresented in river sediments exposed to antibiotics. Moreover,

Cummings *et al*. [56] detected five different plasmid-mediated quinolone resistance (PMQR) determinants (including *qnrA*, *qnrB*, *qnrS*, *qepA*, and *aac(6′)-Ib-cr*) in surface sediments from a sewage-impacted coastal wetland along the US–Mexico border, whereas sediments of a nearby urban wetland that was largely unaffected by sewage contained only three different PMQR determinants (*qnrB*, *qnrS*, and *qepA*). Likewise, Chen *et al*. [57] studied the accumulation of ARGs in surface and bottom waters as well as sediments from the Pearl River estuary. The authors found that concentrations of tetracycline resistance genes in the sediments were at least 100 times higher than those in the water, and their concentrations depended on the degree of anthropogenic impacts from the river to the coast. Yang *et al*. [58] investigated the resistome from marine sediments using high-throughput sequencing and metagenomic analysis. They observed that several contigs shared high identity with transposons or plasmids from human pathogens. Together with biofilms and sediments, aquatic organisms may also be important intermediates in the development and dissemination of ARGs [5]. Meibom *et al*. [59] suggested that chitin, an important structural component of many crustaceans and mollusks, not only serves as a surface for biofilm formation in aquatic environments but it also induces competence for natural transformation. The authors demonstrated that competence was experimentally mediated by the acquisition of ARGs during growth of *Vibrio cholerae* on crab shell fragment immersed in seawater. Jiang *et al*. [60] recently demonstrated high levels of ARGs, including ESBLs and PMQR determinants, in bacterial strains isolated from fish intestinal samples in China. Interestingly, Oguri *et al*. [61] observed that ciliates promote the transfer of genes encoding ESBLs among *E. coli* strains, suggesting that the presence of these organisms, which are widely distributed in nature, may provide gene exchange among bacterial populations from different environments. Additionally, several environmental factors, such as temperature, may affect HGT and thereby delineate exchange community boundaries [62]. Walsh *et al*. [63] isolated some $bla_{\text{NDM-1}}$-positive strains from water samples and observed that the highest frequency of conjugation between cells in suspension occurred at 30 °C compared with 25 °C and 37 °C, which suggests that gene transfer may be more frequent in geographic areas with higher temperatures.

Concluding remarks

With the increasing use and accessibility to culture-independent techniques that allow the functional and structural characterization of microbial communities, the exploration of ARGs may extend to a wide range of organisms, including uncultured species. These advances will increase our understanding of the evolutionary pathways through which the MGEs encoding ARGs spread within a community. Taking into consideration that ARGs are 'easy to get, hard to lose' pollutants and that they may be detected in antibiotic-free environments or even when the selective pressure has disappeared, the scientific community and public authorities should decisively collaborate for the implementation of strategies, policies, and programs that will limit the use of antibiotics, and thus the development and dissemination of ARGs in clinical and environmental settings.

Acknowledgments

This study was supported by the Fundación Eugenio Rodríguez Pascual, Spain. E.V. acknowledges the Leonardo da Vinci fellowship (2012-1-GR1 LEO02-09765) from the European Union. J.L.B. acknowledges the Ramon y Cajal research fellowship (RYC-2011-08154) from the Spanish Ministry of Economy and Competitiveness.

References

1 Cosgrove, S.E. (2006) The relationship between antimicrobial resistance and patient outcomes: mortality, length of hospital stay, and health care costs. *Clin. Infect. Dis.* 42, S82–S89
2 Walsh, F. (2013) Investigating antibiotic resistance in non-clinical environments. *Front. Microbiol.* 4, 19
3 Zell, B.L. and Goldmann, D.A. (2007) Healthcare-associated infection and antimicrobial resistance: moving beyond description to prevention. *Infect. Control Hosp. Epidemiol.* 28, 261–264
4 Muto, C.A. (2005) Why are antibiotic-resistant nosocomial infections spiraling out of control. *Infect. Control Hosp. Epidemiol.* 26, 10–12
5 Taylor, N.G.H. *et al.* (2011) Aquatic systems: maintaining, mixing and mobilising antimicrobial resistance? *Trends Ecol. Evol.* 26, 278–284
6 Gullberg, E. *et al.* (2011) Selection of resistant bacteria at very low antibiotic concentrations. *PLoS Pathog.* 7, e1002158
7 Thiele-Bruhn, S. (2003) Pharmaceutical antibiotic compounds in soils – a review. *J. Plant Nutr. Soil Sci.* 166, 145–167
8 Kümmerer, K. (2009) Antibiotics in the aquatic environment – a review – part I. *Chemosphere* 75, 417–434
9 Aarestrup, F.M. (ed.) (2005) *Antimicrobial Resistance in Bacteria of Animal Origin*, ASM Press
10 Aarestrup, F.M. (2005) Veterinary drug usage and antimicrobial resistance in bacteria of animal origin. *Basic Clin. Pharmacol. Toxicol.* 96, 271–281
11 Cabello, F.C. (2006) Heavy use of prophylactic antibiotics in aquaculture: a growing problem for human and animal health and for the environment. *Environ. Microbiol.* 8, 1137–1144
12 Zhang, X.X. *et al.* (2009) Antibiotic resistance genes in water environment. *Appl. Microbiol. Biotechnol.* 82, 397–414
13 Olivares, J. *et al.* (2013) The intrinsic resistome of bacterial pathogens. *Front. Microbiol.* 4, 103
14 Tamae, C. *et al.* (2008) Determination of antibiotic hypersensitivity among 4,000 single-gene-knockout mutants of *Escherichia coli*. *J. Bacteriol.* 190, 5981–5988
15 Gaze, W.H. *et al.* (2013) Influence of humans on evolution and mobilization of environmental antibiotic resistome. *Emerg. Infect. Dis.* 19, http://dx.doi.org/10.3201/eid1907.120871
16 Galán, J.C. *et al.* (2013) Antibiotics as selectors and accelerators of diversity in the mechanisms of resistance: from the resistome to genetic plasticity in the β-lactamases world. *Front. Microbiol.* 4, 9
17 Riesenfeld, C.S. *et al.* (2004) Uncultured soil bacteria are a reservoir of new antibiotic resistance genes. *Environ. Microbiol.* 6, 981–989
18 Allen, H.K. *et al.* (2009) Functional metagenomics reveals diverse β-lactamases in a remote Alaskan soil. *ISME J.* 3, 243–251
19 Humeniuk, C. *et al.* (2002) β-Lactamases of *Kluyvera ascorbata*, probable progenitors of some plasmid-encoded CTX-M types. *Antimicrob. Agents Chemother.* 46, 3045–3049
20 Poirel, L. *et al.* (2002) Chromosome-encoded Ambler class A β-lactamase of *Kluyvera georgiana*, a probable progenitor of a subgroup of CTX-M extended-spectrum β-lactamases. *Antimicrob. Agents Chemother.* 46, 4038–4040
21 Rodríguez, M.M. *et al.* (2004) Chromosome-encoded CTX-M-3 from *Kluyvera ascorbata*: a possible origin of plasmid-borne CTX-M-1-derived cefotaximases. *Antimicrob. Agents Chemother.* 48, 4895–4897
22 Poirel, L. *et al.* (2005) Origin of plasmid-mediated quinolone resistance determinant QnrA. *Antimicrob. Agents Chemother.* 49, 3523–3525
23 Poirel, L. *et al.* (2005) Vibrionaceae as a possible source of Qnr-like quinolone resistance determinants. *J. Antimicrob. Chemother.* 56, 1118–1121
24 Forsberg, K.J. *et al.* (2012) The shared antibiotic resistome of soil bacteria and human pathogens. *Science* 337, 1107–1111

25 Baquero, F. *et al.* (2008) Antibiotics and antibiotic resistance in water environments. *Curr. Opin. Biotechnol.* 19, 260–265
26 Wright, M.S. *et al.* (2008) Influence of industrial contamination on mobile genetic elements: class 1 integron abundance and gene cassette structure in aquatic bacterial communities. *ISME J.* 2, 417–428
27 Rosewarne, C.P. *et al.* (2010) Class 1 integrons in benthic bacterial communities: abundance, association with Tn*402*-like transposition modules and evidence for coselection with heavy-metal resistance. *FEMS Microbiol. Ecol.* 72, 35–46
28 Pruden, A. and Arabi, M. (2012) Quantifying anthropogenic impacts on environmental reservoirs of antibiotic resistance. In *Antimicrobial Resistance in the Environment* (Keen, P.L. and Montforts, M.H.M.M., eds), pp. 173–202, Wiley-Blackwell
29 Storteboom, H. *et al.* (2010) Tracking antibiotic resistance genes in the South Platte River basin using molecular signatures of urban, agricultural, and pristine sources. *Environ. Sci. Technol.* 44, 7397–7404
30 Kristiansson, E. *et al.* (2011) Pyrosequencing of antibiotic-contaminated river sediments reveals high levels of resistance and gene transfer elements. *PLoS ONE* 6, e17038
31 Heuer, H. *et al.* (2011) Antibiotic resistance gene spread due to manure application on agricultural fields. *Curr. Opin. Microbiol.* 14, 236–243
32 Marti, E. and Balcázar, J.L. (2012) Multidrug resistance-encoding plasmid from *Aeromonas* sp. strain P2G1. *Clin. Microbiol. Infect.* 18, E366–E368
33 Zander, E. *et al.* (2013) Insertion sequence IS18 mediates overexpression of $bla_{\text{OXA-257}}$ in a carbapenem-resistant *Acinetobacter bereziniae* isolate. *J. Antimicrob. Chemother.* http://dx.doi.org/10.1093/jac/dkt313
34 Su, Z. *et al.* (2008) The $bla_{\text{CTX-M-1}}$ gene located in a novel complex class I integron bearing an IS*CR1* element in *Escherichia coli* isolates from Zhenjiang, China. *J. Antimicrob. Chemother.* 62, 1150–1164
35 Alekshun, M.N. and Levy, S.B. (2007) Molecular mechanisms of antibacterial multidrug resistance. *Cell* 128, 1037–1050
36 Levings, R.S. *et al.* (2005) The genomic island SGI1, containing the multiple antibiotic resistance region of *Salmonella enterica* serovar Typhimurium DT104 or variants of it, is widely distributed in other *S. enterica* serovars. *J. Bacteriol.* 187, 4401–4409
37 Sjölund-Karlsson, M. *et al.* (2011) Drug-resistance mechanisms in *Vibrio cholerae* O1 outbreak strain, Haiti, 2010. *Emerg. Infect. Dis.* 17, 2151–2154
38 Colomer-Lluch, M. *et al.* (2011) Antibiotic resistance genes in the bacteriophage DNA fraction of environmental samples. *PLoS ONE* 6, e17549
39 Wellington, E.M. *et al.* (2013) The role of natural environment in the emergence of antibiotic resistance in Gram-negative bacteria. *Lancet Infect. Dis.* 13, 155–165
40 Stalder, T. *et al.* (2012) Integron involvement in environmental spread of antibiotic resistance. *Front. Microbiol.* 3, 119
41 van Hoek, A.H.A.M. *et al.* (2011) Acquired antibiotic resistance genes: an overview. *Front. Microbiol.* 2, 203
42 El Salabi, A. *et al.* (2013) Extended spectrum β-lactamases, carbapenemases and mobile genetic elements responsible for antibiotics resistance in Gram-negative bacteria. *Crit. Rev. Microbiol.* 39, 113–122
43 Robinson, D.G. *et al.* (2012) OASIS: an automated program for global investigation of bacterial and archaeal insertion sequences. *Nucleic Acids Res.* 40, e174
44 Toleman, M.A. *et al.* (2006) IS*CR* elements: novel gene-capturing systems of the 21st century? *Microbiol. Mol. Biol. Rev.* 70, 296–316
45 Yin, X. and Stotzky, G. (1997) Gene transfer among bacteria in natural environments. In *Advances in Applied Microbiology* (Neidleman, S.L. and Laskin, A.L., eds), pp. 153–212, Academic Press
46 Kitchener, V. (2003) Phage genomics comes of age. *Nat. Rev. Genet.* 4, 406
47 Parsley, L.C. *et al.* (2010) Identification of diverse antimicrobial resistance determinants carried on bacterial, plasmid, or viral metagenomes from an activated sludge microbial assemblage. *Appl. Environ. Microbiol.* 76, 3753–3757
48 Marti, E. *et al.* (2013) Bacteriophages as a reservoir of extended-spectrum β-lactamase and fluoroquinolone resistance genes in the environment. *Clin. Microbiol. Infect.* http://dx.doi.org/10.1111/1469-0691.12446 (in press)
49 Rodríguez-Rojas, A. *et al.* (2013) Antibiotics and antibiotic resistance: a bitter fight against evolution. *Int. J. Med. Microbiol.* 303, 293–297
50 Rizzo, L. *et al.* (2013) Urban wastewater treatment plants as hotspots for antibiotic resistant bacteria and genes spread into the environment: a review. *Sci. Total Environ.* 447, 345–360
51 Szczepanowski, R. *et al.* (2009) Detection of 140 clinically relevant antibiotic-resistance genes in the plasmid metagenome of wastewater treatment plant bacteria showing reduced susceptibility to selected antibiotics. *Microbiology* 155, 2306–2319
52 LaPara, T.M. *et al.* (2011) Tertiary-treated municipal wastewater is a significant point source of antibiotic resistance genes into Duluth–Superior Harbor. *Environ. Sci. Technol.* 45, 9543–9549
53 Marti, E. *et al.* (2013) Prevalence of antibiotic resistance genes and bacterial community composition in a river influenced by a wastewater treatment plant. *PLoS ONE* 8, e78906
54 Schwartz, T. *et al.* (2003) Detection of antibiotic-resistant bacteria and their resistance genes in wastewater, surface water, and drinking water biofilms. *FEMS Microbiol. Ecol.* 43, 325–335
55 Engemann, C.A. *et al.* (2008) Fate of tetracycline resistance genes in aquatic systems: migration from the water column to peripheral biofilms. *Environ. Sci. Technol.* 42, 5131–5136
56 Cummings, D.E. *et al.* (2011) Broad dissemination of plasmid-mediated quinolone resistance genes in sediments of two urban coastal wetlands. *Environ. Sci. Technol.* 45, 447–454
57 Chen, B. *et al.* (2013) Differentiating anthropogenic impacts on ARGs in the Pearl River Estuary by using suitable gene indicators. *Water Res.* 47, 2811–2820
58 Yang, J. *et al.* (2013) Marine sediment bacteria harbor antibiotic resistance genes highly similar to those found in human pathogens. *Microb. Ecol.* 65, 975–981
59 Meibom, K.L. *et al.* (2005) Chitin induces natural competence in *Vibrio cholerae*. *Science* 310, 1824–1827
60 Jiang, H.X. *et al.* (2012) Prevalence and characteristics of β-lactamase and plasmid-mediated quinolone resistance genes in *Escherichia coli* isolated from farmed fish in China. *J. Antimicrob. Chemother.* 67, 2350–2353
61 Oguri, S. *et al.* (2011) Ciliates promote the transfer of the gene encoding the extended-spectrum β-lactamase CTX-M-27 between *Escherichia coli* strains. *J. Antimicrob. Chemother.* 66, 527–530
62 Richaume, A. *et al.* (1989) Influence of soil variables on in situ plasmid transfer from *Escherichia coli* to *Rhizobium fredii*. *Appl. Environ. Microbiol.* 55, 1730–1734
63 Walsh, T.R. *et al.* (2011) Dissemination of NDM-1 positive bacteria in the New Delhi environment and its implications for human health: an environmental point prevalence study. *Lancet Infect. Dis.* 11, 355–362