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Title: Bacterial diversity and antibiotic resistance in water habitats: searching the links with the human microbiome

One-sentence summary: In this review antibiotic resistance dissemination is discussed based on bacterial diversity and ecology in water habitats and in the human body.

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Abstract

Water is one of the most important bacterial habitats on Earth. As such, water represents also a major way of dissemination of bacteria between different environmental compartments. Human activities led to the creation of the so-called urban water cycle, comprising different sectors (waste-, surface, drinking water), among which bacteria can hypothetically be exchanged. Therefore, bacteria can be mobilized between unclean water habitats (e.g. wastewater) and clean or pristine water environments (e.g. disinfected and spring drinking water) and eventually reach humans. In addition, bacteria can also transfer mobile genetic elements between different water types, other environments (e.g. soil) and humans. These processes may involve antibiotic resistant bacteria and antibiotic resistance genes. In this review, the hypothesis that some bacteria may share different water compartments and be also hosted by humans is discussed based on the comparison of the bacterial diversity in different types of water and with the human-associated microbiome. The role of such bacteria as potential disseminators of antibiotic resistance and the inference that currently only a small fraction of the clinically relevant antibiotic resistome may be known is discussed.

Introduction

The development and spread of antibiotic resistance among bacteria is considered a universal threat to human, animal and environmental health. Numerous studies have demonstrated the importance of the environmental settings (e.g. water or soil) on the cycling of antibiotic resistance in nature, either because antibiotic resistance mechanisms can originate in environmental bacteria or because human and animal commensals and pathogens can contaminate the environment (Riesenfeld *et al.*, 2004; Baquero *et al.*, 2008; Martinez, 2008; Zhang *et al.*, 2009; Allen *et al.*, 2010).

Water is one of the most important bacterial habitats on Earth, is a major way of dissemination of microorganisms in nature and has been recognized as a significant reservoir of antibiotic resistance (Baquero *et al.*, 2008; Zhang *et al.*, 2009; Rizzo *et al.*, 2013). As a microbial habitat, water may represent the origin of resistance genes, be an amplifier and/or reservoir of genes already acquired by human pathogens and released as pollutants in the environment or act as a bioreactor, facilitating the interchange of resistance genes between pathogenic and non-pathogenic bacteria (Poirel *et al.*, 2005; Baquero *et al.*, 2008; Rizzo *et al.*, 2013). However, and in spite of the intense research in this area over the last years, it is not clear under which circumstances water bacteria are important sources of novel mechanisms of antibiotic resistance or when do they act as carriers or helper elements that, somehow, facilitate the spread of antibiotic resistance.

Another question, still unanswered, regards the modes by which antibiotic resistance in water may be relevant for human health. Because antibiotic resistance is harbored and transferred by bacteria, a better understanding of the bacterial diversity and ecology may bring interesting insights into the modes of resistance dissemination from and into humans. This approach is now possible because numerous studies conducted worldwide have explored the bacterial diversity in water habitats over the last decades. In parallel, the human microbiome project has stimulated the thorough characterization of the diversity of bacteria that permanently or transiently can colonize the human body. The combination of both datasets may bring interesting information for the discussion of antibiotic resistance transmission from water to humans and vice-versa.

This work discusses the hypothesis that bacteria sharing different water compartments and also the human body may represent important pieces in the network of antibiotic resistance dissemination. In addition, the cross-comparison of the bacterial diversity in human and water habitats *versus* the currently identified antibiotic resistance genes is used to sustain the hypothesis that an important fraction of the clinically relevant antibiotic resistome may be yet to be unveiled.

The urban water cycle

Over the centuries, humans settled their lives preferentially in sites around water reservoirs, creating high population densities in these areas and also major sources of pollution. The implementation of sanitation processes capable of removing contaminants (chemical pollutants, organic matter, microorganisms) from wastewater before its discharge into the natural environment became a priority. In the same way, the supplying of clean and safe drinking water, often requiring purification and disinfection, is nowadays regarded as a basic human right, essential for an effective policy for health protection (WHO & UNICEF, 2000). Throughout the years, the scientific knowledge and numerous technologic advances contributed to the continuous improvement of processes for the provision of safe water and appropriate disposal and treatment of wastewater. These two stages constitute the man-made or urban water cycle.

Bacterial diversity in water habitats

Freshwater habitats are amongst the natural habitats that harbour the richest bacterial diversity (Tamames *et al.*, 2010). In a comparative study involving 16S rRNA gene sequences from 3502 sampling experiments of natural and artificial bacterial habitats, Tamames *et al.* (2010) concluded that soil and freshwater, represented by aquifers, groundwater, lakes, rivers, drinking water and wastewater, are the natural habitats that harbour the largest number and most diverse group of bacterial lineages. In the current study the bacterial diversity in different freshwater habitats within the urban water cycle was compared (Fig. 1 and Table S1). This comparison was based on studies published after 1995 in journals indexed to the ISI – Web of Knowledge, in which the major objective was the analysis of the water bacterial diversity, supported by 16S rRNA gene sequence analysis.

At high taxonomic ranks of phylum or class, in general, the most predominant bacteria belong to the phyla *Proteobacteria* (mainly of the classes *Alpha*-, *Beta*- and *Gammaproteobacteria*), *Actinobacteria*, *Bacteroidetes* and *Firmicutes*, irrespective of the type of water – surface (lakes, rivers, wetlands), mineral, drinking and wastewater (Fig. 1, Table S1 and Table S1 references). However, different types of water present distinct patterns of bacterial diversity at lower taxonomic ranks, of genus or species. At least this was the conclusion drawn whenever, according to the publications supporting this comparison, the 16S rRNA gene sequence analysis allowed such a discrimination. An apparent specificity for some types of water was observed. For example, members of the class *Betaproteobacteria* and of the phylum *Bacteroidetes* were frequently detected in surface, mineral and drinking water, but not so often in wastewater. In turn, *Firmicutes* were frequently reported in wastewater. Ubiquitous bacteria are those with low specificity, occurring in different environments, including throughout the urban water cycle or in the interface air-water-soil (Tamames *et al.*, 2010) (Fig. 1 and Table S1). At the genus rank, examples of the most ubiquitous bacteria in water habitats, *i.e.* those detected in wastewater, surface- and drinking water, are members of the genera *Acidovorax*, *Curvibacter*, *Sphingomonas*, *Aeromonas*, *Acinetobacter*, *Pseudomonas*, *Legionella*, *Rhodococcus*, *Gordonia*, *Mycobacterium*, *Flavobacterium*, *Bacillus* and *Clostridium* (Fig. 1 and Table S1). Bacteria belonging to these groups, and others still unidentified, are probably capable of circulating between different aquatic habitats, spanning the whole urban water cycle.

The use of culture independent approaches, mainly the high throughput sequencing methods, brought a renewed perspective of the bacterial diversity in water habitats, in which less than 0.1% of bacteria can be cultivated (Amann *et al.*, 1995; Simon & Daniel, 2011; Vaz-Moreira *et al.*, 2013). These approaches revealed that bacteria still unidentified below the phylum or class levels are detected in every type of water (Table S1). This is particularly notorious for some bacterial phyla/classes, which despite the apparent poor culturability are common water inhabitants. Good examples of groups almost or exclusively detected by culture-independent methods are members of *Delta-* and *Epsilonproteobacteria*, *Acidobacteria*, *Verrucomicrobia*, *Cyanobacteria*, *Nitrospirae*, *Planctomycetes*, *Chloroflexi*, *Chlorobi*, *Gemmatimonadetes*, *Spirochaetes*, *Chlamydiae*, *Aquificae*, *Thermotogae*, *Fusobacteria*, *Synergistetes* and *Tenericutes*, some of them including bacteria ubiquitous in water habitats (Fig. 1, Table S1). Nevertheless, culture-independent methods, even high throughput sequencing, may fail on the detection of some bacterial groups, in particular the less abundant organisms (Pinto & Raskin, 2012). Different biases (e.g., DNA extraction, PCR or sequence data analysis) may hamper the detection of certain community members. On the other hand, the 16S rRNA gene sequence analysis, particularly of small gene fragments as those generated with high throughput sequencing methods, may not allow a reliable identification of bacteria (e.g. Clarridge, 2004). These arguments may explain why bacteria of the genera *Escherichia* or *Enterococcus*, used as indicators of faecal contamination, and frequently detected in wastewater habitats at counts as high as 10^4-10^6 colony forming units per mL (Garcia-Armisen & Servais, 2004; Ferreira da Silva *et al.*, 2007; Levantesi *et al.*, 2010) are not detected in studies surveying the bacterial diversity, as those summarized in Fig. 1. The low abundance of these bacteria in water habitats, even in those with faecal contamination, is also suggested by cultivation procedures. Indeed, the cultivation of *Escherichia* or *Enterococcus* usually requires the use of selective culture media, while on general culture media, such as Plate Count Agar, if isolated, they represent a small fraction of the cultivable populations. Although both approaches are truly complementary to explore the bacterial diversity of an ecosystem, the current state of the art suggests a poor synchronization between culture-independent and culture-dependent methods. This represents a serious limitation in a comprehensive analysis of the bacterial diversity, mainly when the assessment of the features such as metabolism, physiology, genetics, virulence and antibiotic resistance of a specific group is under discussion. Expectably, one of the major outcomes of the implementation of culture-independent methods will be the improvement of cultivation methods and the strengthening of studies based on pure cultures (Anonymous, 2012; Lagier *et al.*, 2012; Prakash *et al.*, 2013). These advances will be indispensable to the thorough assessment of possible intersections between distinct microbiomes, for example, environmental and human.

Evidences of the natural antibiotic resistome

Over the last 70 years, clinically-relevant antibiotic resistance, *i.e.* in pathogens and opportunistic bacteria, increased to worrisome levels, mainly in areas with strong human intervention (Baquero *et al.*, 2008; Martinez, 2009; Andersson & Hughes, 2011; Cantón & Morosini, 2011). Nevertheless, antibiotic resistance is a natural property of bacteria, occurring in environments with reduced or null anthropogenic impacts, such as wild life or remote Earth zones (Riesenfeld *et al.*, 2004; D'Costa *et al.*, 2006; Dantas *et al.*, 2008; Allen *et al.*, 2010; D'Costa *et al.*, 2011; Segawa *et al.*, 2013). In part this can be due to the fact that antibiotics production is ancient in nature, with more than 10^6-10^9 years (D'Costa *et al.*, 2011). Functions, as diverse as molecular signaling, transcription activation, enhanced gene transfer, stimulation of bacterial adhesion, increased mutation frequency or virulence suppression, have been attributed to antibiotics produced in nature (Davies *et al.*, 2006; Wright, 2007; Dantas *et al.*, 2008; Sengupta *et al.*, 2013). Eventually these functions will vary among the target bacteria and will depend on the genetic and physiological environment of the cell. Accordingly, natural antibiotic resistance mechanisms are those that make these molecules compatible with the normal cell function (Wright, 2007; Sengupta *et al.*, 2013). Natural antibiotic resistance has been studied in depth in soil bacteria of the phyla *Actinobacteria*, *Proteobacteria*, or *Bacteroidetes*, mainly in those yielding antibiotic production or degradation activity (Riesenfeld *et al.*, 2004; D'Costa *et al.*, 2006; Dantas *et al.*, 2008; D'Costa *et al.*, 2011; Forsberg *et al.*, 2012). However, natural antibiotic resistance is not restricted to soil bacteria, being also reported in other environments, including water.

Mineral and spring waters are good examples of natural water habitats, since these aquifers originate in ground water sources and are protected from human intervention (Rosenberg, 2003; European Comission, 2009). Unlike tap water, mineral and spring water cannot be disinfected by any kind of treatment to remove or destroy microorganisms (European Comission, 2009) and, thus, its microbiota mirrors the natural populations of the aquifer. Because this type of water is known to contain a rich microbiota and it is destined to human consumption, several studies have searched the presence of antibiotic resistant bacteria (Rosenberg & Duquino, 1989; Massa *et al.*, 1995; Mary *et al.*, 2000; Messi *et al.*, 2005; Zeenat *et al.*, 2009; Falcone-Dias *et al.*, 2012). Although in some of these studies the experiments were not designed to survey bacterial diversity and antibiotic resistance, it is possible to infer about the wide diversity of antibiotic resistance patterns and the frequent occurrence of multi-resistance phenotypes. Mineral or spring bottled waters commercialized in Italy, Portugal, France and other world regions contained bacteria resistant to multiple antibiotics, distributed by several genera and species (*Afipia*, *Bosea*, *Brevundimonas*, *Ochrobactrum*, *Curvibacter*, *Ralstonia*, *Variovorax*, *Acinetobacter*, *Klebsiella*, *Moraxella*, *Pseudomonas*, *Flavobacterium*, *Pedobacter*, *Arthrobacter*, *Corynebacterium*, *Microbacterium*, *Micrococcus*, *Bacillus*, *Kurthia*, and *Staphylococcus*) (Massa *et al.*, 1995; Mary *et al.*, 2000; Messi *et al.*, 2005; Zeenat *et al.*, 2009; Falcone-Dias *et al.*, 2012). Bottled spring water bacteria can reach densities as high as 10^2 colony forming units per mL and display resistance to more than 20 antibiotics belonging to eight different classes, including 3^{th} generation cephalosporins, carbapenems and fluoroquinolones (Falcone-Dias *et al.*, 2012). It is remarkable that, in general, studies conducted in different geographic areas and in different occasions demonstrate that the natural microbiota of mineral and spring waters contains a myriad of antibiotic resistant bacteria, as was observed before for pristine soils or ancient permafrost samples (e.g. D'Costa *et al.*, 2006, 2011; Allen *et al.*, 2009). Many of these (multi-)drug resistance phenotypes are probably intrinsic in these bacteria, and resistance transfer to human-related bacteria can be considered highly unlikely. These considerations require a further discussion about the nature of the environmental antibiotic resistome.

Acquired, intrinsic and silent resistance: different assets in the same game

Most of the discussions on antibiotic resistance are centered on acquired resistance, resultant from gene mutation or genetic recombination by horizontal gene transfer (conjugation, transformation or transduction) (Martinez & Baquero, 2000; Livermore, 2003; Tenover, 2006; Zhang *et al.*, 2009; Davies & Davies, 2010). Although these can be random processes, in the presence of selective pressures, such as antimicrobial residues, bacterial lineages with acquired antibiotic resistance will have an improved fitness (i.e. a better capacity to survive and reproduce in comparison with bacteria without acquired resistance), becoming more prevalent in the community (Barbosa & Levy, 2000; Martinez 2009; Andersson & Hughes, 2011).

In contrast, the intrinsic resistome is described as an ensemble of non-acquired genes with influence on the susceptibility to antibiotics (Fajardo *et al.*, 2008; Baquero *et al.*, 2013). This form of resistance comprises diverse mechanisms that can be related with structural, physiological or biochemical properties of bacteria, such as reduced permeability, metabolic functions, efflux systems, among others (Fajardo *et al.*, 2008; Martinez *et al.*, 2008; Wright, 2010; Alvarez-Ortega *et al.*, 2011; Baquero *et al.*, 2013). Intrinsic antibiotic resistance represents a characteristic phenotype of a species or organism, resultant from multiple genes and, hence, is not easily transferable by horizontal gene transfer. In the same way, it is not the direct consequence of adaptation to antibiotics (Alvarez-Ortega *et al.*, 2011).

Since about 3% of the genes in a bacterial genome may be related with intrinsic resistance processes (Fajardo *et al.*, 2008), it is expected that this native resistance form represents an important fraction of the environmental antibiotic resistome. A well characterized intrinsic resistome belongs to the opportunistic pathogen *Pseudomonas aeruginosa*, which displays intrinsic resistance to a wide variety of antibiotics, resultant from a complex network of genes (Fajardo *et al.*, 2008; Alvarez-Ortega *et al.*, 2011; Breidenstein *et al.*, 2011). The low permeability of the external membrane, 12–100 times lower in *P. aeruginosa* than in *E. coli*, and the presence of some proteins involved in the alteration of cell metabolism, leading, for instance, to changes in the cell growth state, are supposed to represent the most important mechanisms of intrinsic resistance in this organism (Hancock, 1998; Alvarez-Ortega *et al.*, 2011; Breidenstein *et al.*, 2011). Although intrinsic resistance may be characteristic of a species, it is not necessarily common to all species members. In *E. coli*, point mutations in different *loci* were observed to promote reduced susceptibility to antibiotics such as ciprofloxacin, rifampin, vancomycin, ampicillin, sulfamethoxazole, gentamicin, or metronidazole (Tamae *et al.*, 2008). The potential of some members of a species to mutate towards significant reduction or increase in antibiotic susceptibility was observed in different species (e.g. *Helicobacter pylori*, *Acinetobacter baylyi*, *P. aeruginosa*), being probably species-specific (Gomez & Neyfakh, 2006; Fajardo *et al.*, 2008; Girgis *et al.*, 2009; Liu *et al.*, 2010). This kind of genome variations in bacterial populations is probably common in nature and may have interesting implications on the ecology of antibiotic resistant bacteria.

The implications of the intrinsic resistome on the evolution of acquired antibiotic resistance are not completely understood. However, the characterization of the intrinsic resistome genes may bring important contributes to predict the stability, emergence and evolution of antibiotic resistance (Martinez *et al.*, 2007; Fajardo *et al.*, 2008). In a community, it is possible that intrinsic resistance will drive bacterial selection, leading to community rearrangements, mainly when selective pressures, as those imposed by antibiotics, are present (Baquero *et al.*, 2013). Hypothetically, if a bacterial population is intrinsically resistant, it will have higher chances to survive in the presence of antimicrobial residues, and to get in contact with potential resistance donors, proliferating more and faster than non-intrinsically resistant organisms. Thus, it can be hypothesized that intrinsic resistance, at least in some highly ubiquitous bacteria, may represent an advantage for resistance acquisition. A good example of how intrinsic resistance may favor resistance acquisition may be represented by *P. aeruginosa*, one of the opportunistic pathogens with highest potential to acquire antibiotic resistance (Breidenstein *et al.*, 2011).

A major question may be whether genes related with intrinsic resistance phenotypes may be transferred horizontally. Although such an event is not supposed to occur, at least at a high frequency, conceivably, it is not impossible. Other resistance determinants not included in the classical antibiotic-resistance genes, may also occur in nature, and bring interesting insights into the ecology of antibiotic resistance. Silent resistance genes are hidden forms of antibiotic resistance that do not confer resistance to its native host, although are capable of conferring resistance when expressed in other hosts (Dantas & Sommer, 2012).

In summary, the natural antibiotic resistome comprises three categories: i) those designated as acquired resistance genes, which correspond to the classical antibiotic-resistance genes, ii) the genes related with intrinsic resistance and iii) the silent resistance genes. Because some of these genes may respond to unspecific stimuli, and not only to antibiotics, they may contribute to the selection of the antibiotic unsusceptible populations (Dantas & Sommer, 2012; Baquero *et al.*, 2013). These arguments reinforce the need to study antibiotic resistance in a global perspective either in the context of the cell genome or the whole bacterial community.

Antibiotic resistance in wastewater

Among the man-made environments, wastewater treatment plants (WWTP) are the most important receptors and suppliers of human derived antibiotic resistance (Manaia *et al.*, 2012; Rizzo *et al.*, 2013). The indicators of faecal contamination, *E. coli* and *Enterococcus* spp., are often used to monitor antibiotic resistance prevalence in urban wastewaters (Ur-WW). In these groups, high resistance prevalence values have been observed for antibiotics with a long history of use, such as aminopenicillins, sulfonamides and tetracyclines for *E. coli* or tetracycline and erythromycin for enterococci (Manaia *et al.*, 2012). Moreover, it is shown that conventional wastewater treatment does not contribute to reduce the fraction of antibiotic resistant bacteria, leading, sometimes, to its increase in the final effluent (Ferreira da Silva *et al.*, 2006, 2007; Łuczkiewicz *et al.*, 2010; Novo *et al.*, 2013). It is impressive that in different world regions and using distinct types of wastewater treatment, WWTP are responsible for the discharge of about one billion of culturable antibiotic resistant coliforms per minute to the environment (exemplified for ciprofloxacin resistance in Fig. 2). Despite the relevance of *E. coli* and *Enterococcus* as indicators of human faecal contamination, apparently these bacteria are not the most prevalent bacterial groups in sewage sludge or in wastewater (Sanapareddy *et al.*, 2009; McLellan *et al.*, 2010; Xia *et al.*, 2010b; Yang *et al.*, 2011; Wang *et al.*, 2012; Ye & Zhang, 2012; Zhang *et al.*, 2012) (Fig. 1). Indeed, *E. coli* and enterococci are probably minor representatives of the water bacterial communities. This conclusion leads us to a new dilemma. If most of the well-known bacteria in terms of antibiotic resistance are minor representatives of wastewater communities, it is reasonable to argue that other community members, mainly the most abundant, may play also important roles as donors, receptors or simply mediators of antibiotic resistance dissemination.

Antibiotic resistance in aquaculture environments

In aquaculture, antimicrobials are routinely used through the direct addition into the water body. However, the negative impacts of this procedure have been demonstrated and include the persistence of antimicrobial residues in water and fish and the selection and spread of resistance genes, with the consequent contamination of the environment and the human food-chain (Sørum, 1998; Cabello *et al.*, 2006; Taylor *et al.*, 2011; Tamminen *et al.*, 2011). The spread of antibiotic resistance among fish pathogens has economic impacts on aquaculture productivity and increases the possibilities of the dissemination of resistance determinants to other bacteria, including human pathogens (Rhodes *et al.*, 2000; Cabello *et al.*, 2006). The long term effects are demonstrated by the fact that, even in the absence of selective pressures, when the antibiotic used was banned from an aquaculture system, genes conferring low susceptibility to that antibiotic will persist (Tamminen *et al.*, 2011). Bacterial diversity studies in aquaculture water bodies are scant, but the presence of some genera, such as *Yersinia*, *Vibrio*, *Photobacterium*, *Pseudomonas* and *Aeromonas,* is consistently reported (Sørum, 1998; Schulze *et al.*, 2006; Ozaktas *et al.*, 2012; Rodríguez-Blanco *et al.*, 2012). These genera comprise also some bacteria with important roles on antibiotic resistance spread, for example *qnr*A, encoding a DNA topoisomerase protector and the extended-spectrum beta-lactamase PER-6 (Poirel *et al.*, 2005; Girlich *et al.*, 2010a). Moreover, the dissemination of antimicrobial resistance in aquaculture environments may be associated with other resistance determinants such as heavy metals or biocides (Akinbowale *et al.*, 2007; Rodríguez-Blanco *et al.*, 2012, Seiler & Berendonk, 2012; Cabello *et al.*, 2013).

Antibiotic resistance in disinfected drinking water

Despite the scarce information regarding antibiotic resistance in disinfected drinking water, it was already demonstrated that it may contain bacteria, such as those of the genera *Sphingobium*, *Sphingomonas*, *Pseudomonas* and *Acinetobacter* or non-faecal *Enterobacteriaceae* capable of resisting different antibiotics (Faria *et al.*, 2009; Xi *et al.*, 2009; Vaz-Moreira *et al.*, 2011b, 2012; Figueira *et al.*, 2012; Narciso-da-Rocha *et al.*, 2013) (Table S2). For instance, *Sphingomonadaceae*, a bacterial group recognizedly ubiquitous, rich in mobile genetic elements, and comprising common inhabitants of environments contaminated with xenobiotcs, can be highly prevalent in disinfected drinking water (Koskinen *et al.*, 2000; Furuhata *et al.*, 2007; Stolz *et al.*, 2009; Aylward *et al.*, 2013). Tap water *Sphingomonadaceae* yield a rich and diversified resistance pattern to penicillins, cephalosporins, carbapenems and aminoglycosides (Vaz-Moreira *et al.*, 2011b), but their relevance on the spread of antibiotic resistance is unknown.

Independent studies have demonstrated that antibiotic resistant bacteria, at least for some classes of antibiotics, may be more prevalent in tap than in the water source (Xi *et al.*, 2009; Gomez-Alvarez *et al.*, 2012; Vaz-Moreira *et al.*, 2012; Narciso-da-Rocha *et al.*, 2013). Such an effect may be due either to the selective effect of the disinfection processes or to the income of antibiotic resistant bacteria downstream the disinfection point (Gomez-Alvarez *et al.*, 2012; Vaz-Moreira *et al.*, 2013). This is a fundamental and difficult to answer question, given the complex rearrangements in the bacterial communities that result from the disinfection processes (Hoefel *et al.*, 2005; Eichler *et* *al.*, 2006; Kormas *et al.*, 2010; Figueira *et al.*, 2011; Vaz-Moreira *et al.*, 2013). However, strain tracking approaches do not support the conclusion that the water source is the most probable origin of the antibiotic resistance detected in tap water (Vaz-Moreira *et al.*, 2011b, 2012; Narciso-da-Rocha *et al.*, 2013). Regarding the origin of the antibiotic resistance found in drinking water, it has been observed that the majority of the resistance phenotypes in bacteria of groups such as *Sphingomonadaceae*, *Pseudomonas* or *Acinetobacter* is species dependent. This observation suggests a pattern of vertical inheritance of resistance and, thus, it can be hypothesized that antibiotic resistance in these organisms is probably intrinsic (Shehabi *et al.*, 2005; Vaz-Moreira *et al.*, 2011b, 2012; Narciso-da-Rocha *et al.*, 2013). Either being acquired or intrinsic, the impacts that antibiotic resistant bacteria present in drinking water may have on human-health are still unknown.

Antibiotic resistance genes throughout the urban water cycle

The tracking of antibiotic resistance genes in different environmental compartments is an important tool to assess the ecology and epidemiology of antibiotic resistance. Antibiotic resistance genes, encoding every known type of mechanism (target protection, target modification, drug modification, reduced permeability or efflux), are found throughout the urban water cycle (Table S2). These genes have been detected either in bacterial isolates or in total genomic DNA samples, using, most of the times, primers or probes targeting antibiotic resistance genes that are already known. Most of such primers and probes were designed based on genome sequences of bacterial isolates yielding a given resistance phenotype. Therefore, the vast majority of surveys of antibiotic resistance genes rely, directly or indirectly, on cultivable bacteria recognized as opportunists or pathogens. Examples of the most common hosts of the well-known antibiotic resistance genes are members of the family *Enterobacteriaceae* (e.g. genera *Klebsiella*, *Citrobacter*, *Enterobacter*, *Raoultella*) or the genera *Acinetobacter*, *Aeromonas*, *Burkholderia*, *Pseudomonas*, *Enterococcus*, *Staphylococcus* and some other that in total represent a humble fraction of the bacterial groups thriving in water habitats.

Wastewater, in particular raw, is the richest water habitat in known antibiotic resistance genes. There, can be found a typical signature of genes encoding resistance to “old” antibiotics such as tetracyclines, sulfonamides, aminoglycosides and beta-lactams (e.g. *tet*, *aac*, *dfr*, *sul*, class A beta-lactamases) (Table S2). Most of these genes are located in plasmids and some are part of the variable gene cassettes of integrons and, probably, can easily be mobilized amongst bacteria (Garcillán-Barcia *et al.*, 2011; Partridge, 2011). Recently, Zhang *et al.* (2011) demonstrated that plasmids, mainly harbored by *Proteobacteria* of the classes *Alpha*-, *Beta*- and *Gamma*- and members of the genera *Bacillus*, *Mycobacterium* and *Nocardiopsis*, some of which are abundant in wastewater habitats, are relevant vectors of tetracycline, macrolide and multidrug resistance genes in these environmental niches.

Studies reporting the diversity and abundance of antibiotic resistance genes in drinking water are scarce. However, the occurrence of genes also detected in clinical isolates, encoding resistance to beta-lactams, aminoglycosides, macrolides or sulfonamides is described even in disinfected water (Table S2) (Faria *et al.*, 2009; Xi *et al.*, 2009; Figueira *et al.*, 2012). The origin of these resistance genes in drinking water is still unknown, being unclear in which cases it results from environmental contamination. A major limitation to answer this question is related with the fact that most of the drinking water bacteria are of environmental origin and poorly or not at all characterized in terms of antibiotic resistance genes (Fig. 1, Table S1).

Commonly used arguments to explain the evolutionary success of acquired antibiotic resistance

Acquired antibiotic resistance is an emblematic example of biological evolution, driven by two major mechanisms – genetic variability (mutation and recombination) and selection (Thomas & Nielsen, 2005; Andersson & Hughes, 2010; Wiedenbeck & Cohan, 2011). Genetic variability results from gene mutation and horizontal gene transfer, in which the latter has more dramatic implications on the physiology and ecology of bacteria (Hausner & Wuertz, 1999; Arber, 2000; Miyahara *et al.*, 2011). On the other hand, antibiotics, even at sub-inhibitory concentrations, or other micro-pollutants such as heavy metals, contribute for the selection of resistant bacteria (Alonso *et al.*, 2001; Davies *et al.*, 2006; Tello *et al.*, 2012). However, the selection of antibiotic resistant bacteria may not represent the only consequence of the environmental contamination with antibiotics. Actually, the residues of antibiotics at environmental concentrations (often sub-inhibitory) are also correlated with disturbances on the structure and composition of bacterial communities in water habitats (Huerta *et al.*, 2013; Novo *et al.*, 2013). Moreover, in the environment, pollutants occur in complex mixtures, which make it difficult to predict their effects on the microbial communities. Processes of co- or cross-resistance, for instance, due to genetic linkage or to broad enzyme specificity, may lead to the selection of resistance genes in the absence of a selective pressure by antibiotics (Baker-Austin *et al.*, 2006; Harada & Asai, 2010). If the above mentioned arguments could explain antibiotic resistance proliferation, acquired antibiotic resistance would be detected only in habitats such as wastewater or in the animal or human body, mainly in the gut, during antibiotherapy periods. However, this is not the case and antibiotic resistance determinants are found in environments where none of the above mentioned pressures are present (Harada & Asai, 2010). The strongest argument to explain the occurrence of recognized clinically relevant resistance genes in environments with no apparent selective pressure refers to the low fitness costs of antibiotic resistance genes (*i.e.* when antibiotic resistance acquisition do not reduce the survival and proliferation of a bacterium, even in the absence of selective pressures) (Andersson & Hughes, 2010; Gullberg *et al.*, 2011). The influence of compensatory mutations on the reduction of fitness costs imposed by acquired antibiotic resistance has been demonstrated (Björkman et al., 2000; Maisnier-Patin & Andersson, 2004; Handel *et al.*, 2006; Andersson & Hughes, 2010; Schulz zur Wiesch *et al.*, 2010; Tanaka & Valckenborgh, 2011). Since compensatory mutations may alleviate the fitness costs associated with a given acquired resistance, resistant and susceptible bacteria will display a comparable fitness in the environment, although with different levels of tolerance to antibiotics. As a consequence, strains harboring resistance and compensatory mutations may have a selective advantage in the environment, mainly in the presence of antimicrobial residues (Björkman et al., 2000; Handel *et al.*, 2006; Andersson & Hughes, 2010; Schulz zur Wiesch *et al.*, 2010). The importance of the environmental conditions on the selection of resistance and compensatory mutations is suggested by the fact that different fitness-compensating mutations are observed in bacteria thriving in mice or in a laboratory medium (Björkman *et al.*, 2000). These evidences emphasize the complexity of the antibiotic resistance ecology, although it seems reasonable to assume that as long as bacteria and/or genetic elements are able to move across different water habitats, cross-resistance and low fitness costs may explain why acquired antibiotic resistance can reach habitats such as drinking water.

Intersections between the water and the human-associated microbiome

Increasing evidences on the diversity, metabolic and functional capabilities of the microbiota associated with the human body show that microbial consortia play important roles in disease and health conditions, although their roles are not yet completely understood (Turnbaugh *et al.*, 2007; Eloe-Fadrosh & Rasko, 2013). Microorganisms colonizing or infecting humans may derive from different primary habitats, and not only the human body, and play distinct roles in health or disease status. The expression "human-associated microbiome" is herein used to refer to all microorganisms capable of colonizing or infecting a human host independently of which is their primary habitat.

Two types of intersection between the human-associated microbiome and water habitats are expected. One refers to the release of bacteria from humans to wastewater. The other comprises bacteria that being present in drinking water are also reported in the human-associated microbiome. The first type of intersection was comprehensively analysed by McLellan *et al.* (2010) who concluded that, as expected, only a small fraction of bacteria excreted by humans were represented in sewage and even less were found in surface water. Among the bacterial lineages found throughout these compartments, the predominant were *Lachnospiraceae*, *Bacteroidaceae* and *Ruminococcaceae* (McLellan *et al.*, 2010), groups poorly characterized in terms of antibiotic resistance. Other intersections are widely known as those of the indicators *E. coli* and enterococci, which representativeness in water and human-associated microbiomes is not so evident as could be expected (Table S1) (Qin *et al.*, 2010; Arumugam *et al.*, 2011; The Human Microbiome Project Consortium, 2012).

The assessment of the second type of intersection is even more difficult. The occurrence of antibiotic resistant bacteria in drinking water may be important because of the harmful effects that this could have in the human health. In such case, transmission could be directly of water bacteria to humans or, indirectly, via transmission of resistance genes from water bacteria to human related bacteria (Fig. 3). Lee *et al.* (2010) used germ-free mice to demonstrate a correlation between the microbiota of drinking water and its presence in the gastrointestinal tract. However, this approach hardly can be used to infer about the fate of antibiotic resistant bacteria in the human gastrointestinal tract, given the richness and diversity of such habitat. Considering the value of taxonomy and phylogeny in the prediction of the ecology and physiology of bacteria, the currently available information about human and environmental microbiomes may allow interesting inferences. Using this rationale, the occurrence of the same bacterial lineages in drinking water and in the human-associated microbiome may be an indication of the fitness of those bacteria to the human body. In addition, it may suggest its potential to, under favorable conditions, *e.g.* antibiotherapy, suffer positive selection or promote horizontal gene transfer. The search of bacterial groups found in water habitats (Table S1) in the NIH Human Microbiome Project catalog (http://www.hmpdacc-resources.org/hmp_catalog) revealed that 35 groups, distributed by five phyla (*Proteobacteria*, *Actinobacteria*, *Firmicutes*, *Bacteroidetes*, *Cyanobacteria*), found in treated drinking water can also be detected in the human-associated microbiome (e.g. in the gastrointestinal tract, oral cavity or skin, including lesions). Identically, 19 lineages distributed by three phyla (*Proteobacteria*, *Actinobacteria* and *Firmicutes*), found in mineral water can also be found in the human-associated microbiome (Table S1; Fig. 1). Probably, in the future, when more data are made available, more bacterial groups will be observed to be common to water environments and the human body. Nevertheless, it is already worthy of note that bacteria of the genera *Burkholderia*, *Acinetobacter*, *Aeromonas*, *Klebsiella*, *Pseudomonas*, *Stenotrophomonas* or *Clostridium* (Table S1), all of them with high potential to acquire antibiotic resistance genes (Zhang *et al.*, 2009), can be found in drinking water and in the human-associated microbiome. Others such as members of the genera *Sphingomonas* or *Methylobacterium* which exhibit resistance to several antibiotics, but about which almost nothing is known about antibiotic resistance genetics (Furuhata *et al.*, 2006, 2007; Vaz-Moreira *et al.*, 2011b), can also be found in water habitats and in the human-associated microbiome. The meaning of these evidences is still unclear but it may hint a link between water habitats and the human body, giving support to the hypothesis that water habitats may, directly or indirectly, supply antibiotic resistant bacteria for the human-associated microbiome (Fig. 3).

Missing links between natural and contaminant antibiotic resistance

Water and soil are regarded as important potential antibiotic resistance reservoirs, either natural or due to animal (and manure used as fertilizer) and human derived environmental contamination (Bush *et al.*, 2011; Forsberg *et al.*, 2012). However, except in a few well documented cases (e.g. *qnr* and *bla*$_{\mathrm{CTX\text{-}M}}$) (Poirel *et al.*, 2002, 2005), it is difficult to demonstrate the passage of resistance genes from the environment to clinically-relevant bacteria or to clarify the mechanisms that made such a gene transfer possible. Previous studies have demonstrated that the human gut antibiotic resistome comprises an impressive myriad of antibiotic resistance genes not identified before and evolutionarily distant from the currently known resistance genes (Sommer *et al.*, 2009). The increasing number of complete bacterial genome sequences, support this observation (http://www.ncbi.nlm.nih.gov/genome). Putative annotation data, available in public databases, suggests that multidrug resistance as well as other specific resistance mechanisms are widespread in *Bacteria*. However, the annotated function encoded by these genome sequences is not reliable to infer with accuracy the expected phenotypes, mainly because the phenotype encoded by a gene may depend on the genetic and physiological environment (e.g. silent resistance genes, Dantas & Sommer, 2012). Probably, most of the still unknown resistome is composed by resistance genes not yet validly annotated and others which expression is host-dependent. However, the clinical relevance of these genetic determinants as well as their influence on antibiotic resistance emergence is not clear yet. Although it can be hypothesised that the "unkown" human resistant microbiome may represent the missing link between the environment and the human pathogens, evidences that ingested products (food and water) can be the major sources of antibiotic resistance genes are still missing.

Antibiotic therapy imposes profound and long lasting rearrangements in the human-associated microbiome, characterized by the increase of *Proteobacteria* and the simultaneous reduction of other groups such as *Bacteroidetes* or *Firmicutes* (Young & Schmidt, 2004; Antonopoulos *et al.*, 2009; Jakobsson *et al.*, 2010; Jernberg *et al.*, 2010). Eventually, it can be argued that, under specific conditions (e.g. antibiotherapy), minor or silenced parts of the human antibiotic resistome may lead important microbial and genomic rearrangements responsible for resistance development. Apparently, the environmental and pathogenic resistomes are not distinct, with the same genes being detected in both, although with higher prevalence in the pathogenic resistome (D'Costa *et al.*, 2006; Allen *et al.*, 2010; Forsberg *et al.*, 2012) (Fig. 3). Indeed, antibiotic resistance genes and gene mobilization cassettes, many of which without recognized clinical relevance, are widespread in nature, spanning numerous lineages of the bacterial world (Cantón, 2009; Allen *et al.*, 2010). However, apparently only a small fraction of these genetic elements was successfully spread through animals, humans and the environment, representing a public health threat. Which are the genetic characteristics or the external conditions that support the evolutionary success of an antibiotic resistance gene is still a major question.

Concluding remarks

In summary, the previous discussion on the diversity and ecology of water bacteria and antibiotic resistance led to a few conclusions and raised some new hypothesis:

1. Water habitats host an impressive bacterial diversity. However, only a few lineages are known to harbor antibiotic resistance genes of already recognized clinical relevance. The hypothesis that many bacterial lineages, some of them still unculturable, inhabiting water may represent a reservoir of new or emerging antibiotic resistance determinants cannot be discarded;

2. Bacteria belonging to the same bacterial lineages inhabit different types of water, including pristine water, disinfected water and raw wastewater. The hypothesis that these lineages can transfer relevant properties, mainly those that can be acquired by horizontal gene transfer, from unclean water habitats to clean environments, cannot be discarded;

3. Only a few groups of bacteria found in waters were, so far, identified in the human-associated microbiome. Although it is still uncertain in which cases the same species

and strain can live in water and colonize humans, it is arguable that at least some of those lineages can represent a link between the water habitats and humans. In such case, those bacteria may be involved in the direct or indirect transfer of properties, including antibiotic resistance;

4. Well known human commensal (as coliforms or enterococci) and pathogenic bacteria are minor and often undetected representatives of the water microbial communities assessed based on metagenomic analysis. Therefore, metagenomic approaches may be of limited value to detect antibiotic resistance determinants already described in these organisms, unless enrichment or targeted methods are used.

5. Studies designed to survey the phylogeny of the antibiotic resistance genes and tracking the same gene types over different environmental compartments may contribute to shade some light on the relevance of environmental bacteria on the spread and transfer to humans of antibiotic resistance.

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None to declare

References:

Aizenberg-Gershtein Y, Vaizel-Ohayon D & Halpern M (2012) Structure of bacterial communities in diverse freshwater habitats. *Can J Microbiol* **58**: 326-335.

Akinbowale OL, Peng H, Grant P & Barton MD (2007) Antibiotic and heavy metal resistance in motile aeromonads and pseudomonads from rainbow trout (*Oncorhynchus mykiss*) farms in Australia. *Int J Antimicrob Agents* **30**: 177–182.

Allen HK, Moe LA, Rodbumrer J, Gaarder A & Handelsman J (2009) Functional metagenomics reveals diverse beta-lactamases in a remote Alaskan soil. *ISME J* **3(2)**:243-251.

Allen HK, Donato J, Wang HH, Cloud-Hansen KA, Davies J & Handelsman J (2010) Call of the wild: antibiotic resistance genes in natural environments. *Nat Rev Microbiol* **8**: 251-259.

Alonso A, Sanchez P & Martinez JL (2001) Environmental selection of antibiotic resistance genes. *Environ Microbiol* **3**: 1-9.

Alvarez-Ortega C, Wiegand I, Olivares J, Hancock REW &Martínez JL (2011) The intrinsic resistome of *Pseudomonas aeruginosa* to β-lactams. *Virulence* **2(2)**:144-146.

Amann RI, Ludwig W & Schleifer KH (1995) Phylogenetic identification and *in situ* detection of individual microbial cells without cultivation. *Microbiol Rev* **59**: 143-169.

Andersson DI & Hughes D (2010) Antibiotic resistance and its cost: is it possible to reverse resistance? *Nat Rev Microbiol* **8**: 260-271.

Andersson DI & Hughes D (2011) Persistence of antibiotic resistance in bacterial populations. *FEMS Microbiol Rev* **35**: 901-911.

Anonymous (2012) The cultural revolution. *Nature Rev Microbiol* **11**: 1-1. doi:10.1038/nrmicro2948.

Antonopoulos DA, Huse SM, Morrison HG, Schmidt TM, Sogin ML & Young VB (2009) Reproducible community dynamics of the gastrointestinal microbiota following antibiotic perturbation. *Infect Immun* **77**: 2367-2375.

Araújo C, Torres C, Silva N, *et al*. (2010) Vancomycin-resistant enterococci from Portuguese wastewater treatment plants. *J Basic Microbiol* **50**: 605-609.

Arber W (2000) Genetic variation: molecular mechanisms and impact on microbial evolution. *FEMS Microbiol Rev* **24**: 1-7.

Arumugam M, Raes J, Pelletier E, *et al.* (2011) Enterotypes of the human gut microbiome. *Nature* **473**: 174-180.

Auerbach EA, Seyfried EE & McMahon KD (2007) Tetracycline resistance genes in activated sludge wastewater treatment plants. *Water Res* **41**: 1143-1151.

Aylward FO, McDonald BR, Adams SM, Valenzuela A, Schmidt RA, Goodwin LA, Woyke T, Currie CR, Suen G & Poulsen M (2013) Comparison of 26 Sphingomonad genomes reveals diverse environmental adaptations and biodegradative capabilities. *Appl Environ Microbiol* **79(12)**: 3724-2733.

Baik KS, Park SC, Kim EM, Bae KS, Ahn JH, Ka JO, Chun J & Seong CN (2008) Diversity of bacterial community in freshwater of Woopo wetland. *J Microbiol* **46**: 647-655.

Baker-Austin C, Wright MS, Stepanauskas R & McArthur JV (2006) Co-selection of antibiotic and metal resistance. *Trends Microbiol* **14**: 176-182.

Baquero F, Martínez JL & Cantón R (2008) Antibiotics and antibiotic resistance in water environments. *Curr Opin Biotechnol* **19**: 260-265.

Baquero F, Tedim AP & Coque TM (2013) Antibiotic resistance shaping multi-level population biology of bacteria. *Front Microbiol* **4(15)**, doi:10.3389/fmicb.2013.00015.

Barbosa TM & Levy SB (2000) The impact of antibiotic use on resistance development and persistence. *Drug Resist Updat* **3**: 303–311.

Barker-Reid F, Fox EM & Faggian R (2010) Occurrence of antibiotic resistance genes in reclaimed water and river water in the Werribee Basin, Australia. *J Water Health* **8**: 521-531.

Björkman J, Nagaev I, Berg OG, Hughes D &AnderssonDI (2000) Effects of environment on compensatory mutations to ameliorate costs of antibiotic resistance. *Science* **287**: 1479-1482.

Böckelmann U, Dörries HH, Ayuso-Gabella MN, *et al*. (2009) Quantitative PCR monitoring of antibiotic resistance genes and bacterial pathogens in three European artificial groundwater recharge systems. *Appl Environ Microbiol* **75**: 154-163.

Borjesson S, Mattsson A & Lindgren PE (2010) Genes encoding tetracycline resistance in a full-scale municipal wastewater treatment plant investigated during one year. *J Water Health* **8**: 247-256.

Borjesson S, Melin S, Matussek A & Lindgren PE (2009) A seasonal study of the *mec*A gene and *Staphylococcus aureus* including methicillin-resistant *S. aureus* in a municipal wastewater treatment plant. *Water Res* **43**: 925-932.

Breidenstein EBM, de la Fuente-Núñez C & Hancock REW (2011) *Pseudomonas aeruginosa*: all roads lead to resistance. *Trends Microbiol* **19(8)**: 419-426.

Bush K, Courvalin P, Dantas G, *et al.* (2011) Tackling antibiotic resistance. *Nat Rev Microbiol* **9**: 894-896.

Cabello FC (2006) Heavy use of prophylactic antibiotics in aquaculture: a growing problem for human and animal health and for the environment. *Environ Microbiol* **8(7)**: 1137-1144.

Cabello FC, Godfrey HP, Tomova A, Ivanova L, Dölz H, Millanao A & Buschmann AH (2013) Antimicrobial use in aquaculture re-examined: its relevance to antimicrobial resistance and to animal and human health. *Environ Microbiol* **15(7)**: 1917–1942.

Cantón R (2009) Antibiotic resistance genes from the environment: a perspective through newly identified antibiotic resistance mechanisms in the clinical setting. *Clin Microbiol Infect* **15 Suppl 1**: 20-25.

Cantón R & Morosini MI (2011) Emergence and spread of antibiotic resistance following exposure to antibiotics. *FEMS Microbiol Rev* **35**: 977-991.

Casanovas-Massana A & Blanch AR (2012) Diversity of the heterotrophic microbial populations for distinguishing natural mineral waters. *Int J Food Microbiol* **153**: 38-44.

Cattoir V, Poirel L, Aubert C, Soussy CJ & Nordmann P (2008) Unexpected occurrence of plasmid-mediated quinolone resistance determinants in environmental *Aeromonas* spp. *Emerg Infect Dis* **14**: 231-237.

Chen H, Shu W, Chang X, Chen JA, Guo Y & Tan Y (2010) The profile of antibiotics resistance and integrons of extended-spectrum beta-lactamase producing thermotolerant coliforms isolated from the Yangtze River basin in Chongqing. *Environ Pollut* **158**: 2459-2464.

Chen Z, Zhou Z, Peng X, Xiang H, Xiang S & Jiang Z (2013) Effects of wet and dry seasons on the aquatic bacterial community structure of the Three Gorges Reservoir. *World J Microbiol Biotechnol* **29**: 841-853.

Chouchani C, Marrakchi R, Henriques I & Correia A (2013) Occurrence of IMP-8, IMP-10, and IMP-13 metallo-beta-lactamases located on class 1 integrons and other extended-spectrum beta-lactamases in bacterial isolates from Tunisian rivers. *Scand J Infect Dis* **45**: 95-103.

Clarridge JE, 3rd (2004) Impact of 16S rRNA gene sequence analysis for identification of bacteria on clinical microbiology and infectious diseases. *Clin Microbiol Rev* **17**: 840-862, table of contents.

Colomer-Lluch M, Jofre J & Muniesa M (2011) Antibiotic resistance genes in the bacteriophage DNA fraction of environmental samples. *PLoS One* **6**: e17549.

Cottrell MT, Waidner LA, Yu L & Kirchman DL (2005) Bacterial diversity of metagenomic and PCR libraries from the Delaware River. *Environ Microbiol* **7**: 1883-1895.

Czekalski N, Berthold T, Caucci S, Egli A & Burgmann H (2012) Increased levels of multiresistant bacteria and resistance genes after wastewater treatment and their dissemination into lake geneva, Switzerland. *Front Microbiol* **3**: 106, doi: 10.3389/fmicb.2012.00106

D'Costa VM, McGrann KM, Hughes DW & Wright GD (2006) Sampling the Antibiotic Resistome. *Science* **311**: 374-377.

D'Costa VM, King CE, Kalan L, *et al.* (2011) Antibiotic resistance is ancient. *Nature* **477**: 457-461.

Dantas G & Sommer MO (2012) Context matters - the complex interplay between resistome genotypes and resistance phenotypes. *Curr Opin Microbiol* **15**: 577-582.

Dantas G, Sommer MO, Oluwasegun RD & Church GM (2008) Bacteria subsisting on antibiotics. *Science* **320**: 100-103.

Davies J & Davies D (2010) Origins and evolution of antibiotic resistance. *Microbiol Mol Biol Rev* **74(3)**: 417–433.

Davies J, Spiegelman GB & Yim G (2006) The world of subinhibitory antibiotic concentrations. *Curr Opin Microbiol* **9**: 445-453.

De Boeck H, Miwanda B, Lunguya-Metila O, Muyembe-Tamfum JJ, Stobberingh E, Glupczynski Y & Jacobs J (2012) ESBL-positive Enterobacteria isolates in drinking water. *Emerg Infect Dis* **18**: 1019-1020.

de Figueiredo DR, Pereira MJ, Moura A, Silva L, Bárrios S, Fonseca F, Henriques I & Correia A (2007) Bacterial community composition over a dry winter in meso- and eutrophic Portuguese water bodies. *FEMS Microbiol Ecol* **59**: 638-650.

Dong X & Reddy GB (2010) Nutrient removal and bacterial communities in swine wastewater lagoon and constructed wetlands. *J Environ Sci Health A Tox Hazard Subst Environ Eng* **45**: 1526-1535.

Drudge CN, Elliott AV, Plach JM, Ejim LJ, Wright GD, Droppo IG & Warren LA (2012) Diversity of integron- and culture-associated antibiotic resistance genes in freshwater floc. *Appl Environ Microbiol* **78**: 4367-4372.

Eichler S, Christen R, Höltje C, Westphal P, Bötel J, Brettar I, Mehling A & Höfle MG (2006) Composition and dynamics of bacterial communities of a drinking water supply system as assessed by RNA- and DNA-based 16S rRNA gene fingerprinting. *Appl Environ Microbiol* **72**: 1858-1872.

Eloe-Fadrosh EA & Rasko DA (2013) The Human Microbiome: from symbiosis to pathogenesis. *Annu Rev Med* **64**:145-163.

European Comission (2009) DIRECTIVE 2009/54/EC - on the exploitation and marketing of natural mineral waters.

Fajardo A, Martínez-Martín N, Mercadillo M, *et al.* (2008). The neglected intrinsic resistome of bacterial pathogens. *PLoS One* **3(2)**:e1619. doi: 10.1371/journal.pone.0001619

Falcone-Dias M, Vaz-Moreira I & Manaia CM (2012) Bottled mineral water as a potential source of antibiotic resistant bacteria. *Water Res* **46**: 3612-3622.

Faria C, Vaz-Moreira I, Serapicos E, Nunes OC & Manaia CM (2009) Antibiotic resistance in coagulase negative staphylococci isolated from wastewater and drinking water. *Sci Total Environ* **407**: 3876-3882.

Ferreira da Silva M, Vaz-Moreira I, Gonzalez-Pajuelo M, Nunes OC & Manaia CM (2007) Antimicrobial resistance patterns in *Enterobacteriaceae* isolated from an urban wastewater treatment plant. *FEMS Microbiol Ecol* **60**: 166-176.

Ferreira da Silva M, Tiago I, Verissimo A, Boaventura RA, Nunes OC & Manaia CM (2006) Antibiotic resistance of enterococci and related bacteria in an urban wastewater treatment plant. *FEMS Microbiol Ecol* **55**: 322-329.

Figueira V, Vaz-Moreira I, Silva M & Manaia CM (2011) Diversity and antibiotic resistance of *Aeromonas* spp. in drinking and waste water treatment plants. *Water Res* **45**: 5599-5611.

Figueira V, Serra EA, Vaz-Moreira I, Brandão TRS & Manaia CM (2012) Comparison of ubiquitous antibiotic-resistant *Enterobacteriaceae* populations isolated from wastewaters, surface waters and drinking waters. *J Water Health* **10.1**.

Forsberg KJ, Reyes A, Wang B, Selleck EM, Sommer MO & Dantas G (2012) The shared antibiotic resistome of soil bacteria and human pathogens. *Science* **337**: 1107-1111.

Furuhata K, Kato Y, Goto K, Hara M, Yoshida S & Fukuyama M (2006) Isolation and identification of *Methylobacterium* species from the tap water in hospitals in Japan and their antibiotic susceptibility. *Microbiol Immunol* **50**: 11-17.

Furuhata K, Kato Y, Goto K, Saitou K, Sugiyama J, Hara M & Fukuyama M (2007) Identification of yellow-pigmented bacteria isolated from hospital tap water in Japan and their chlorine resistance. *Biocontrol Sci* **12**: 39-46.

Gajan EB, Abashov R, Aghazadeh M, Eslami H, Oskouei SG & Mohammadnejad D (2008) Vancomycin-resistant *Enterococcus faecalis* from a wastewater treatment plant in Tabriz, Iran. *Pak J Biol Sci* **11**: 2443-2446.

Galvin S, Boyle F, Hickey P, Vellinga A, Morris D & Cormican M (2010) Enumeration and characterization of antimicrobial-resistant *Escherichia coli* bacteria in effluent from municipal, hospital, and secondary treatment facility sources. *Appl Environ Microbiol* **76(14)**: 4772–4779.

Garcia-Armisen T & Servais P (2004) Enumeration of viable *E. coli* in rivers and wastewaters by fluorescent in situ hybridization. *J Microbiol Methods* **58**: 269-279.

Garcillán-Barcia MP, Alvarado A & de la Cruz F (2011) Identification of bacterial plasmids based on mobility and plasmid population biology. *FEMS Microbiol Rev* **35**: 936-956.

Gich F, Schubert K, Bruns A, Hoffelner H & Overmann J (2005) Specific detection, isolation, and characterization of selected, previously uncultured members of the freshwater bacterioplankton community. *Appl Environ Microbiol* **71**: 5908-5919.

Girgis HS, Hottes AK & Tavazoie S (2009) Genetic architecture of intrinsic antibiotic susceptibility. *PLoS One* **4(5)**: e5629, doi: 10.1371/journal.pone.0005629.

Girlich D, Poirel L & Nordmann P (2010a) PER-6, an extended-spectrum beta-lactamase from *Aeromonas allosaccharophila*. *Antimicrob Agents Chemother* **54**: 1619-1622.

Girlich D, Poirel L & Nordmann P (2010b) Novel ambler class A carbapenem-hydrolyzing beta-lactamase from a *Pseudomonas fluorescens* isolate from the Seine River, Paris, France. *Antimicrob Agents Chemother* **54**: 328-332.

Girlich D, Poirel L & Nordmann P (2010c) First isolation of the *bla*OXA-23 carbapenemase gene from an environmental *Acinetobacter baumannii* isolate. *Antimicrob Agents Chemother* **54**: 578-579.

Girlich D, Poirel L & Nordmann P (2011) Diversity of clavulanic acid-inhibited extended-spectrum beta-lactamases in *Aeromonas* spp. from the Seine River, Paris, France. *Antimicrob Agents Chemother* **55**: 1256-1261.

Graham DW, Olivares-Rieumont S, Knapp CW, Lima L, Werner D & Bowen E (2011) Antibiotic resistance gene abundances associated with waste discharges to the Almendares River near Havana, Cuba. *Environ Sci Technol* **45**: 418-424.

Gomez-Alvarez V, Revetta RP & Santo Domingo JW (2012) Metagenomic analyses of drinking water receiving different disinfection treatments. *Appl Environ Microbiol* **78**: 6095-6102.

Gomez MJ & Neyfakh AA (2006) Genes involved in intrinsic antibiotic resistance of *Acinetobacter baylyi*. *Antimicrob Agents Chemother* **50:** 3562–3567.

Gullberg E, Cao S, Berg OG, Ilback C, Sandegren L, Hughes D & Andersson DI (2011) Selection of resistant bacteria at very low antibiotic concentrations. *PLoS Pathog* **7**: e1002158.

Hamelin K, Bruant G, El-Shaarawi A, Hill S, Edge TA, Fairbrother J, Harel J, Maynard C, Masson L & Brousseau R (2007) Occurrence of virulence and antimicrobial resistance genes in *Escherichia coli* isolates from different aquatic ecosystems within the St. Clair River and Detroit River areas. *Appl Environ Microbiol* **73**: 477-484.

Hancock REW (1998) Resistance mechanisms in *Pseudomonas aeruginosa* and other nonfermentative Gram-negative bacteria. *Clin Infect Dis* **27**(Suppl. 1): S93–S99.

Hancock REW & Brinkman FSL (2002) Function of *Pseudomonas* porins in uptake and efflux. *Ann Rev Microbiol* **56**: 17-38.

Handel A, Regoes RR & Antia R (2006) The role of compensatory mutations in the emergence of drug resistance. *PLoS Comput Biol* **2(10)**: e137.

Harada K & Asai T (2010) Role of antimicrobial selective pressure and secondary factors on antimicrobial resistance prevalence in *Escherichia coli* from food-producing animals in Japan. *J Biomed Biotechnol* **2010**: 180682.

Hausner M & Wuertz S (1999) High rates of conjugation in bacterial biofilms as determined by quantitative in situ analysis. *Appl Environ Microbiol* **65**: 3710-3713.

Henriques I, Moura A, Alves A, Saavedra MJ & Correia A (2006a) Analysing diversity among beta-lactamase encoding genes in aquatic environments. *FEMS Microbiol Ecol* **56**: 418-429.

Henriques IS, Fonseca F, Alves A, Saavedra MJ & Correia A (2006b) Occurrence and diversity of integrons and beta-lactamase genes among ampicillin-resistant isolates from estuarine waters. *Res Microbiol* **157**: 938-947.

Henriques IS, Fonseca F, Alves A, Saavedra MJ & Correia A (2008) Tetracycline-resistance genes in gram-negative isolates from estuarine waters. *Lett Appl Microbiol* **47**: 526-533.

Henriques IS, Araújo S, Azevedo JSN, Alves MS, Chouchani C, Pereira A & Correia A (2012) Prevalence and diversity of carbapenem-resistant bacteria in untreated drinking water in Portugal. *Microb Drug Resist* **18(5)**:531-537.

Hiorns WD, Methe BA, Nierzwicki-Bauer SA & Zehr JP (1997) Bacterial diversity in Adirondack mountain lakes as revealed by 16S rRNA gene sequences. *Appl Environ Microbiol* **63**: 2957-2960.

Hoefel D, Monis PT, Grooby WL, Andrews S & Saint CP (2005) Profiling bacterial survival through a water treatment process and subsequent distribution system. *J Appl Microbiol* **99**: 175-186.

Huerta B, Marti E, Gros M, López P. Pompêo M, Armengol J, Barceló D, Balcázar JL, Rodríguez-Mozaz S & Marcé R (2013) Exploring the links between antibiotic occurrence, antibiotic resistance, and bacterial communities in water supply reservoirs. *Sci Total Environ* **456-457**: 161-170.

Jakobsson HE, Jernberg C, Andersson AF, Sjolund-Karlsson M, Jansson JK & Engstrand L (2010) Short-term antibiotic treatment has differing long-term impacts on the human throat and gut microbiome. *PLoS One* **5**: e9836.

Jernberg C, Lofmark S, Edlund C & Jansson JK (2010) Long-term impacts of antibiotic exposure on the human intestinal microbiota. *Microbiol* **156**: 3216-3223.

Kassem, II, Esseili MA & Sigler V (2008) Occurrence of *mec*A in nonstaphylococcal pathogens in surface waters. *J Clin Microbiol* **46**: 3868-3869.

Kim J, Kang HY & Lee Y (2008) The identification of CTX-M-14, TEM-52, and CMY-1 enzymes in *Escherichia coli* isolated from the Han River in Korea. *J Microbiol* **46**: 478-481.

Kormas KA, Neofitou C, Pachiadaki M & Koufostathi E (2010) Changes of the bacterial assemblages throughout an urban drinking water distribution system. *Environ Monit Assess* **165**: 27-38.

Koskinen R, Ali-Vehmas T, Kämpfer P, Laurikkala M, Tsitko I, Kostyal E, Atroshi F & Salkinoja-Salonen M (2000) Characterization of *Sphingomonas* isolates from Finnish and Swedish drinking water distribution systems. *J Appl Microbiol* **89**: 687-696.

Lachmayr KL, Kerkhof LJ, Dirienzo AG, Cavanaugh CM & Ford TE (2009) Quantifying nonspecific TEM beta-lactamase (*bla*TEM) genes in a wastewater stream. *Appl Environ Microbiol* **75**: 203-211.

Lagier JC, Armougom F, Million M, *et al.* (2012) Microbial culturomics: paradigm shift in the human gut microbiome study. *Clin Microbiol Infect* **18**: 1185-1193.

LaPara TM, Burch TR, McNamara PJ, Tan DT, Yan M & Eichmiller JJ (2011) Tertiary-treated municipal wastewater is a significant point source of antibiotic resistance genes into Duluth-Superior Harbor. *Environ Sci Technol* **45**: 9543-9549.

Lee J, Lee CS, Hugunin KM, Maute CJ & Dysko RC (2010) Bacteria from drinking water supply and their fate in gastrointestinal tracts of germ-free mice: a phylogenetic comparison study. *Water Res* **44**: 5050-5058.

Letunic I & Bork P (2007) Interactive Tree Of Life (iTOL): an online tool for phylogenetic tree display and annotation. *Bioinformatics* **23(1)**:127-128.

Letunic I & Bork P (2011) Interactive Tree Of Life v2: online annotation and display of phylogenetic trees made easy. *Nucleic Acids Res* **39(2)**: W475-W478.

Levantesi C, La Mantia R, Masciopinto C, Böckelmann U, Ayuso-Gabella MN, Salgot M, Tandoi V, Van Houtte E, Wintgens T & Grohmann E (2010) Quantification of pathogenic microorganisms and microbial indicators in three wastewater reclamation and managed aquifer recharge facilities in Europe. *Sci Total Environ* **408**: 4923-4930.

Liu A, Tran L, Becket E, Lee K, Chinn L, Park E, Tran K & Miller JH (2010) Antibiotic sensitivity profiles determined with an *Escherichia coli* gene knockout collection: generating an antibiotic bar code. *Antimicrob Agents Chemother* **54(4)**: 1393–1403**.**

Liu B & Pop M (2009) ARDB-Antibiotic Resistance Genes Database. *Nucleic Acids Res* **37**: D443-447.

Livermore DM (2003) Bacterial resistance: origins, epidemiology, and impact. *Clin Infect Dis* **36**(Suppl 1): S11-S23.

Loy A, Beisker W & Meier H (2005) Diversity of bacteria growing in natural mineral water after bottling. *Appl Environ Microbiol* **71**: 3624-3632.

Lu P, Chen C, Wang Q, Wang Z, Zhang X & Xie S (2013) Phylogenetic diversity of microbial communities in real drinking water distribution systems. *Biotechnol Bioproc* **E 18**: 119-124.

Łuczkiewicz A, Jankowska K, Fudala-Ksiazek S & Olanczuk-Neyman K (2010) Antimicrobial resistance of fecal indicators in municipal wastewater treatment plant. *Water Res* **44**: 5089-5097.

Luo Y, Mao D, Rysz M, Zhou Q, Zhang H, Xu L & P JJA (2010) Trends in antibiotic resistance genes occurrence in the Haihe River, China. *Environ Sci Technol* **44**: 7220-7225.

Lymperopoulou DS, Kormas KA & Karagouni AD (2012) Variability of Prokaryotic Community Structure in a Drinking Water Reservoir (Marathonas, Greece). *Microbes Environ* **27**: 1-8.

Macedo AS, Freitas AR, Abreu C, Machado E, Peixe L, Sousa JC & Novais C (2011) Characterization of antibiotic resistant enterococci isolated from untreated waters for human consumption in Portugal. *Int J Food Microbiol* **145**: 315-319.

Maisnier-Patin S & Andersson DI (2004) Adaptation to the deleterious effects of antimicrobial drug resistance mutations by compensatory evolution. *Res Microbiol* **155**: 360–369.

Manaia CM, Novo A, Coelho B & Nunes OC (2010) Ciprofloxacin resistance in domestic wastewater treatment plants. *Water Air Soil Pollut* **208**: 335‑343.

Manaia CM, Vaz-Moreira I & Nunes OC (2012) Antibiotic resistance in waste water and surface water and human health implications. Chapter 6 In *The Handbook of Environmental Chemistry*,(Barceló D, ed.), vol 20: 173-212. Springer-Verlag, Berlin Heidelberg. DOI: 10.1007/698_2011_118.

Martinez JL (2008) Antibiotics and antibiotic resistance genes in natural environments. *Science* **321**: 365-367.

Martinez JL (2009) Environmental pollution by antibiotics and by antibiotic resistance determinants. *Environ Pollut* **157**: 2893-2902.

Martinez JL & Baquero F (2000) Mutation frequencies and antibiotic resitance. *Antimicrob Agents Chemother* **44(7)**: 1771-1777.

Martinez JL, Baquero F & Andersson DI (2007) Predicting antibiotic resistance. *Nat Rev Microbiol* **5**: 958–965.

Mary P, Defives C & Hornez JP (2000) Occurrence and multiple antibiotic resistance profiles of non-fermentative Gram-negative microflora in five brands of non-carbonated french bottled spring water. *Microb Ecol* **39**: 322-329.

Massa S, Petruccioli M, Fanelli M & Gori L (1995) Drug resistant bacteria in non carbonated mineral waters. *Microbiol Res* **150**: 403-408.

McGarvey JA, Miller WG, Sanchez S & Stanker L (2004) Identification of bacterial populations in dairy wastewaters by use of 16S rRNA gene sequences and other genetic markers. *Appl Environ Microbiol* **70**: 4267-4275.

McGarvey JA, Miller WG, Sanchez S, Silva CJ & Whitehand LC (2005) Comparison of bacterial populations and chemical composition of dairy wastewater held in circulated and stagnant lagoons. *J Appl Microbiol* **99**: 867-877.

McLellan SL, Huse SM, Mueller-Spitz SR, Andreishcheva EN & Sogin ML (2010) Diversity and population structure of sewage-derived microorganisms in wastewater treatment plant influent. *Environ Microbiol* **12**: 378-392.

Messi P, Guerrieri E & Bondi M (2005) Antibiotic resistance and antibacterial activity in heterotrophic bacteria of mineral water origin. *Sci Total Environ* **346**: 213-219.

Miyahara E, Nishie M, Takumi S, *et al.* (2011) Environmental mutagens may be implicated in the emergence of drug-resistant microorganisms. *FEMS Microbiol Lett* **317**: 109-116.

Moura A, Jove T, Ploy MC, Henriques I & Correia A (2012) Diversity of gene cassette promoters in class 1 integrons from wastewater environments. *Appl Environ Microbiol* **78**: 5413-5416.

Moura A, Tacão M, Henriques I, Dias J, Ferreira P & Correia A (2009) Characterization of bacterial diversity in two aerated lagoons of a wastewater treatment plant using PCR-DGGE analysis. *Microbiol Res* **164**: 560-569.

Narciso-da-Rocha C, Vaz-Moreira I, Svensson-Stadler L, Moore ERB & Manaia CM (2013) Diversity and antibiotic resistance of *Acinetobacter* spp. in water from the source to the tap. *Appl Microbiol Biotechnol* **97**: 329-340.

Navarro-Noya YE, Suarez-Arriaga MC, Rojas-Valdes A, Montoya-Ciriaco NM, Gomez-Acata S, Fernandez-Luqueno F & Dendooven L (2013) Pyrosequencing analysis of the bacterial community in drinking water wells. *Microb Ecol*. DOI 10.1007/s00248-013-0222-3.

Novo A, Andre S, Viana P, Nunes OC & Manaia CM (2013) Antibiotic resistance, antimicrobial residues and bacterial community composition in urban wastewater. *Water Res* **47**: 1875-1887.

Novo A & Manaia CM (2010) Factors influencing antibiotic resistance burden in municipal wastewater treatment plants. *Appl Microbiol Biotechnol* **87**: 1157–1166.

Okoh AI & Igbinosa EO (2010) Antibiotic susceptibility profiles of some *Vibrio* strains isolated from wastewater final effluents in a rural community of the Eastern Cape Province of South Africa. *BMC Microbiol* **10**: 143.

Otterholt E & Charnock C (2011) Microbial quality and nutritional aspects of Norwegian brand waters. *Int J Food Microbiol* **144**: 455-463.

Ozaktas T, Taskin B & Gozen AG (2012) High level multiple antibiotic resistance among fish surface associated bacterial populations in non-aquaculture freshwater environment. *Water Res* **46**: 6382-6390.

Ozgumus OB, Sandalli C, Sevim A, Celik-Sevim E & Sivri N (2009) Class 1 and class 2 integrons and plasmid-mediated antibiotic resistance in coliforms isolated from ten rivers in northern Turkey. *J Microbiol* **47**: 19-27.

Partridge SR (2011) Analysis of antibiotic resistance regions in Gram-negative bacteria. *FEMS Microbiol Rev* **35**: 820-855.

Picão RC, Poirel L, Demarta A, Silva CS, Corvaglia AR, Petrini O & Nordmann P (2008) Plasmid-mediated quinolone resistance in *Aeromonas allosaccharophila* recovered from a Swiss lake. *J Antimicrob Chemother* **62**: 948-950.

Pinto AJ & Raskin L (2012) PCR biases distort bacterial and archaeal community structure in pyrosequencing datasets. *PLoS One* **7**: e43093.

Pinto AJ, Xi C & Raskin L (2012) Bacterial community structure in the drinking water microbiome is governed by filtration processes. *Environ Sci Technol* **46**: 8851-8859.

Poirel L, Kampfer P & Nordmann P (2002) Chromosome-encoded Ambler class A beta-lactamase of *Kluyvera georgiana*, a probable progenitor of a subgroup of CTX-M extended-spectrum beta-lactamases. *Antimicrob Agents Chemother* **46**: 4038-4040.

Poirel L, Rodriguez-Martinez JM, Mammeri H, Liard A & Nordmann P (2005) Origin of plasmid-mediated quinolone resistance determinant QnrA. *Antimicrob Agents Chemother* **49**: 3523-3525.

Poitelon JB, Joyeux M, Welte B, Duguet JP, Prestel E, Lespinet O & DuBow MS (2009) Assessment of phylogenetic diversity of bacterial microflora in drinking water using serial analysis of ribosomal sequence tags. *Water Res* **43**: 4197-4206.

Prakash O, Shouche Y, Jangid K & Kostka JE (2013) Microbial cultivation and the role of microbial resource centers in the omics era. *Appl Microbiol Biotechnol* **97**:51-62.

Qin J, Li R, Raes J *et al.* (2010) A human gut microbial gene catalogue established by metagenomic sequencing. *Nature* **464**: 59-67.

Revetta RP, Pemberton A, Lamendella R, Iker B & Santo Domingo JW (2010) Identification of bacterial populations in drinking water using 16S rRNA-based sequence analyses. *Water Res* **44**: 1353-1360.

Rhodes G., Huys G, Swings J, McGann P, Hiney M, Smith P & Pickup RW (2000) Distribution of oxytetracycline resistance plasmids between aeromonads in hospital and aquaculture environments: implication of Tn*1721* in dissemination of the tetracycline resistance determinant Tet A. *Appl. Environ Microbiol* **66**: 3883-3890.

Riesenfeld CS, Goodman RM & Handelsman J (2004) Uncultured soil bacteria are a reservoir of new antibiotic resistance genes. *Environ Microbiol* **6**: 981-989.

Rizzo L, Manaia C, Merlin C, Schwartz T, Dagot C, Ploy MC, Michael I & Fatta-Kassinos D (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.

Rodríguez-Blanco A, Lemos ML & Osorio CR (2012) Integrating conjugative elements as vectors of antibiotic, mercury, and quaternary ammonium compound resistance in marine aquaculture environments. *Antimicrob. Agents Chemother* **56(5)**: 2619-2626.

Rosenberg FA (2003) The microbiology of bottled water. *Clin Microbiol Newslett* **26**: 41-44.

Rosenberg FA & Duquino HH (1989) Antibiotic-resistance of *Pseudomonas* from german mineral waters. *Toxic Assess* **4**: 281-294.

Rudi K, Tannaes T & Vatn M (2009) Temporal and spatial diversity of the tap water microbiota in a Norwegian hospital. *Appl Environ Microbiol* **75**: 7855-7857.

Samra ZQ, Naseem M, Khan SJ, Dar N & Athar MA (2009) PCR targeting of antibiotic resistant bacteria in public drinking water of Lahore metropolitan, Pakistan. *Biomed Environ Sci* **22**: 458-463.

Sanapareddy N, Hamp TJ, Gonzalez LC, Hilger HA, Fodor AA & Clinton SM (2009) Molecular diversity of a North Carolina wastewater treatment plant as revealed by pyrosequencing. *Appl Environ Microbiol* **75**: 1688-1696.

Schulze AD, Alabi AO, Tattersall-Sheldrake AR & Miller KM (2006) Bacterial diversity in a marine hatchery: Balance between pathogenic and potentially probiotic bacterial strains. *Aquaculture* **256**: 50–73.

Schulz zur Wiesch P, Engelstädter J & Bonhoeffer S (2010) Compensation of fitness costs and reversibility of antibiotic resistance mutations. A*ntimicrob Agents Chemother* **54(5)**: 2085–2095.

Segawa T, Takeuchi N, Rivera A, Yamada A, Yoshimura Y, Barcaza G, Shinbori K, Motoyama H, Kohshima S & Ushida K (2013) Distribution of antibiotic resistance genes in glacier environments. *Env Microbiol Rep* **5**: 127-134.

Seiler C & Berendonk TU (2012) Heavy metal driven co-selection of antibiotic resistance in soil and water bodies impacted by agriculture and aquaculture. *Front Microbiol* **3**, 399. doi: 10.3389/fmicb.2012.00399

Sengupta S, Chattopadhyay MK & Grossart HP (2013) The multifaceted roles of antibiotics and antibiotic resistance in nature. *Front Microbiol* **4**: 47.

Shehabi AA, Masoud H & Maslamani FA (2005) Common antimicrobial resistance patterns, biotypes and serotypes found among *Pseudomonas aeruginosa* isolates from patient's stools and drinking water sources in Jordan. *J Chemother* **17**: 179-183.

Shi P, Jia S, Zhang XX, Zhang T, Cheng S & Li A (2013) Metagenomic insights into chlorination effects on microbial antibiotic resistance in drinking water. *Water Res* **47**: 111-120.

Simon C & Daniel R (2011) Metagenomic analyses: past and future trends. *Appl Environ Microbiol* **77(4)**: 1153-1161.

Smith P (2008) Antimicrobial resistance in aquaculture. *Rev Sci Tech Off int Epiz* **27(1)**: 243-264.

Sommer MO, Dantas G & Church GM (2009) Functional characterization of the antibiotic resistance reservoir in the human microflora. *Science* **325**: 1128-1131.

Sørum H (1998) Mobile drug resistance genes among fish bacteria. *APMIS* **84(106)**: 74-76.

Stoll C, Sidhu JP, Tiehm A & Toze S (2012) Prevalence of clinically relevant antibiotic resistance genes in surface water samples collected from Germany and Australia. *Environ Sci Technol* **46**: 9716-9726.

Stolz A (2009) Molecular characteristics of xenobiotic-degrading sphingomonads. *Appl Microbiol Biotechnol* **81**: 793-811.

Storteboom H, Arabi M, Davis JG, Crimi B & Pruden A (2010) Identification of antibiotic-resistance-gene molecular signatures suitable as tracers of pristine river, urban, and agricultural sources. *Environ Sci Technol* **44**: 1947-1953.

Su HC, Ying GG, Tao R, Zhang RQ, Zhao JL & Liu YS (2012) Class 1 and 2 integrons, sul resistance genes and antibiotic resistance in *Escherichia coli* isolated from Dongjiang River, South China. *Environ Pollut* **169**: 42-49.

Szczepanowski R, Krahn I, Bohn N, Puhler A & Schluter A (2007) Novel macrolide resistance module carried by the IncP-1beta resistance plasmid pRSB111, isolated from a wastewater treatment plant. *Antimicrob Agents Chemother* **51**: 673-678.

Szczepanowski R, Linke B, Krahn I, Gartemann KH, Gützkow T, Eichler W, Pühler A & Schlüter A (2009) Detection of 140 clinically relevant antibiotic-resistance genes in the plasmid metagenome of wastewater treatment plant bacteria showing reduced susceptibility to selected antibiotics. *Microbiol* **155**: 2306-2319.

Tacão M, Correia A & Henriques I (2012) Resistance to broad-spectrum antibiotics in aquatic systems: anthropogenic activities modulate the dissemination of *bla*(CTX-M)-like genes. *Appl Environ Microbiol* **78**: 4134-4140.

Tamames J, Abellan JJ, Pignatelli M, Camacho A & Moya A (2010) Environmental distribution of prokaryotic taxa. *BMC Microbiol* **10**: 85.

Tamae C, Liu A, Kim K, *et al.* (2008) Determination of antibiotic hypersensitivity among 4,000 single-gene-knockout mutants of *Escherichia coli*. *J Bacteriol* **190(17)**: 5981–5988.

Tamminen M, Karkman A, Lõhmus A, Muziasari WI, Takasu H, Wada S, Suzuki S & Virta M (2011) Tetracycline resistance genes persist at aquaculture farms in the absence of selection pressure. *Environ Sci Technol* **45**: 386–391.

Tanaka MM & Valckenborgh F (2011) Escaping an evolutionary lobster trap: drug resistance and compensatory mutation in a fluctuating environment. *Evolution* **65(5)**: 1376–1387.

Tao R, Ying GG, Su HC, Zhou HW & Sidhu JP (2010) Detection of antibiotic resistance and tetracycline resistance genes in *Enterobacteriaceae* isolated from the Pearl rivers in South China. *Environ Pollut* **158**: 2101-2109.

Taylor NGH, Verner-Jeffreys DW & Baker-Austin C (2011) Aquatic systems: maintaining, mixing and mobilising antimicrobial resistance? *Trends Ecol Evol* **26(6)**: 278-284.

Tello A, Austin B & Telfer TC (2012) Selective pressure of antibiotic pollution on bacteria of importance to public health. *Environ Health Perspect* **120**: 1100-1106.

Tenover FC (2006) Mechanisms of antimicrobial resistance in bacteria. *Am J Med* **119**(6 Suppl 1): S3–S10, discussion S62–S70.

The Human Microbiome Project Consortium (2012) Structure, function and diversity of the healthy human microbiome. *Nature* **486**: 207-214.

Thomas CM & Nielsen KM (2005) Mechanisms of, and barriers to, horizontal gene transfer between bacteria. *Nat Rev Microbiol* **3**: 711-721.

Tokajian ST, Hashwa FA, Hancock IC & Zalloua PA (2005) Phylogenetic assessment of heterotrophic bacteria from a water distribution system using 16S rDNA sequencing. *Can J Microbiol* **51**: 325-335.

Turnbaugh PJ, Ley RE, Hamady M, Fraser-Liggett CM, Knight R & Gordon JI (2007) Feature The Human Microbiome Project. *Nature* **449**: 804-810.

Vaz-Moreira I, Egas C, Nunes OC & Manaia CM (2011a) Culture-dependent and culture-independent diversity surveys target different bacteria: a case study in a freshwater sample. *Antonie Van Leeuwenhoek* **100**: 245-257.

Vaz-Moreira I, Nunes OC & Manaia CM (2011b) Diversity and antibiotic resistance patterns of *Sphingomonadaceae* isolated from drinking water. *Appl Environ Microbiol* **77**: 5697-5706.

Vaz-Moreira I, Nunes OC & Manaia CM (2012) Diversity and antibiotic resistance in *Pseudomonas* spp. from drinking water. *Sci Tot Environ* **426**: 366-374.

Vaz-Moreira I, Egas C, Nunes OC & Manaia CM (2013) Bacterial diversity from the source to the tap: a comparative study based on 16S rRNA gene-DGGE and culture-dependent methods. *FEMS Microbiol Ecol* **83**: 361-374.

Walsh TR, Weeks J, Livermore DM & Toleman MA (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.

Wang X, Hu M, Xia Y, Wen X & Ding K (2012) Pyrosequencing analysis of bacterial diversity in 14 wastewater treatment systems in China. *Appl Environ Microbiol* **78**: 7042-7047.

WHO & UNICEF (2000) Global water supply and sanitation assessment 2000 Report.

Wiedenbeck J & Cohan FM (2011) Origins of bacterial diversity through horizontal genetic transfer and adaptation to new ecological niches. *FEMS Microbiol Rev* **35**: 957-976.

Wright GD (2007) The antibiotic resistome: the nexus of chemical and genetic diversity. *Nat Rev Microbiol* **5**: 175-186.

Wright GD (2010) The antibiotic resistome. *Expert Opin Drug Discov* **5(8)**: 779-788.

Xi C, Zhang Y, Marrs CF, Ye W, Simon C, Foxman B & Nriagu J (2009) Prevalence of antibiotic resistance in drinking water treatment and distribution systems. *Appl Environ Microbiol* **75**: 5714-5718.

Xia R, Guo X, Zhang Y & Xu H (2010a) *qnr*VC-like gene located in a novel complex class 1 integron harboring the ISCR1 element in an *Aeromonas punctata* strain from an aquatic environment in Shandong Province, China. *Antimicrob Agents Chemother* **54**: 3471-3474.

Xia S, Duan L, Song Y, Li J, Piceno YM, Andersen GL, Alvarez-Cohen L, Moreno-Andrade I, Huang CL & Hermanowicz SW (2010b) Bacterial community structure in geographically distributed biological wastewater treatment reactors. *Environ Sci Technol* **44**: 7391-7396.

Xu H, Broersma K, Miao V & Davies J (2011a) Class 1 and class 2 integrons in multidrug-resistant gram-negative bacteria isolated from the Salmon River, British Columbia. Can J Microbiol 57: 460-467.

Xu H, Miao V, Kwong W, Xia R & Davies J (2011b) Identification of a novel fosfomycin resistance gene (*fos*A2) in *Enterobacter cloacae* from the Salmon River, Canada. *Lett Appl Microbiol* **52**: 427-429.

Yang C, Zhang W, Liu R, Li Q, Li B, Wang S, Song C, Qiao C & Mulchandani A (2011) Phylogenetic diversity and metabolic potential of activated sludge microbial communities in full-scale wastewater treatment plants. *Environ Sci Technol* **45**: 7408-7415.

Ye L & Zhang T (2012) Bacterial communities in different sections of a municipal wastewater treatment plant revealed by 16S rDNA 454 pyrosequencing. *Appl Microbiol Biotechnol* **97**: 2681-2690.

Young VB & Schmidt TM (2004) Antibiotic-associated diarrhea accompanied by large-scale alterations in the composition of the fecal microbiota. *J Clin Microbiol* **42**: 1203-1206.

Zeenat A, Hatha AA, Viola L & Vipra K (2009) Bacteriological quality and risk assessment of the imported and domestic bottled mineral water sold in Fiji. *J Water Health* **7**: 642-649.

Zeng DN, Fan ZY, Chi L, Wang X, Qu WD & Quan ZX (2013) Analysis of the bacterial communities associated with different drinking water treatment processes. *World J Microbiol Biotechnol*. DOI 10.1007/s11274-013-1321-5

Zhang T, Zhang XX & Ye L (2011) Plasmid metagenome reveals high levels of antibiotic resistance genes and mobile genetic elements in activated sludge. *PLoS One* **6**: e26041.

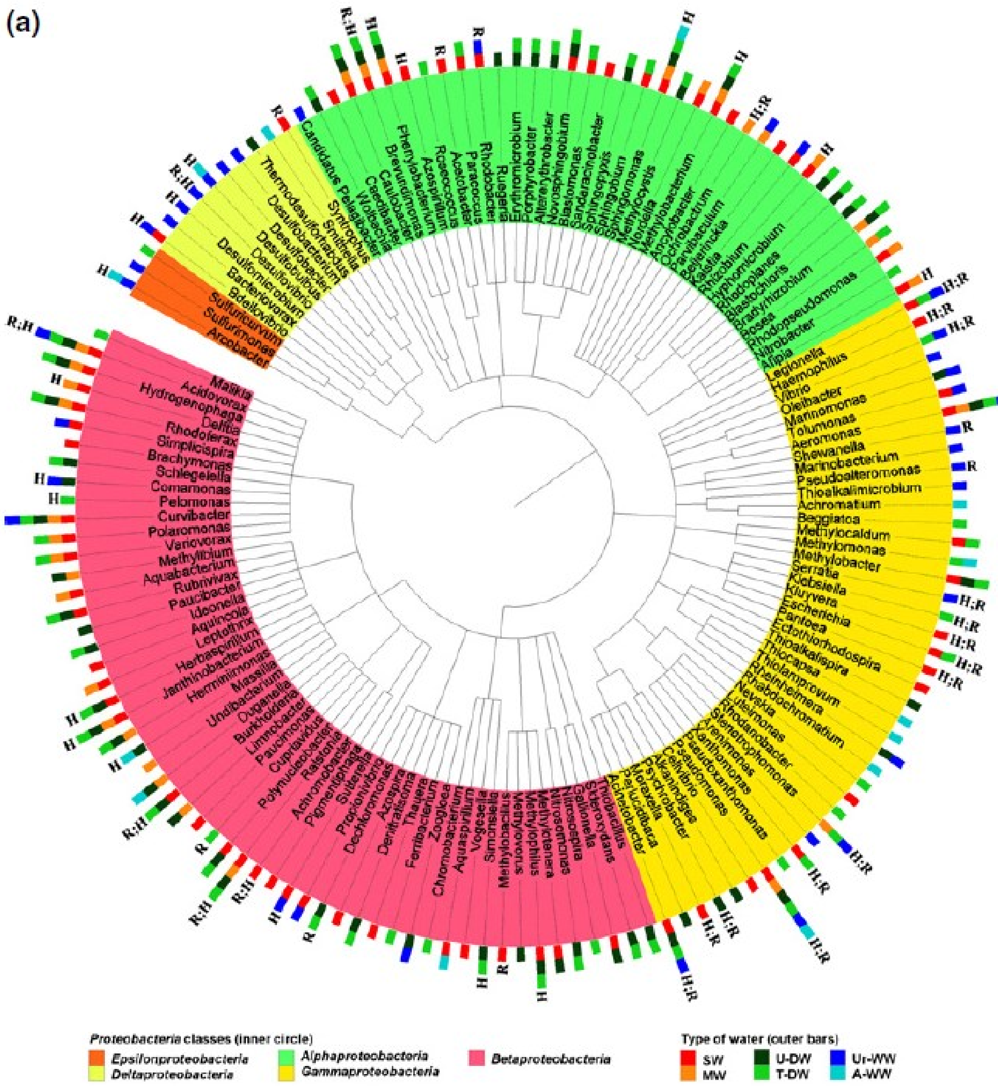
Zhang T, Shao MF & Ye L (2012) 454 pyrosequencing reveals bacterial diversity of activated sludge from 14 sewage treatment plants. *ISME J* **6**: 1137-1147.

Zhang XX, Zhang T & Fang HH (2009) Antibiotic resistance genes in water environment. *Appl Microbiol Biotechnol* **82**: 397-414.

Zwart G, Crump BC, van Agterveld MP, Hagen F & Han S-K (2002) Typical freshwater bacteria: an analysis of available 16S rRNA gene sequences from plankton of lakes and rivers. *Aquat Microb Ecol* **28**: 141–155.

Table S1. Bacterial diversity observed in different types of water, presence in the human-associated microbiome and occurrence of antibiotic resistance genes already characterized

Table S2. Examples of antibiotic resistance genes detected in surface water, drinking water and wastewater


(a)
Arcobacter
Sulfurimonas
Sulfuricurvum
Bdellovibrio
Bacteriovorax
Desulfomicrobium
Desulfovibrio
Desulfobulbus
Desulfobacter
Desulfobacterium
Thermodesulforhabdus
Smithella
Syntrophus
Candidatus Pelagibacter
Wolbachia
Caedibacter
Caulobacter
Brevundimonas
Phenylobacterium
Azospirillum
Roseococcus
Acetobacter
Paracoccus
Rhodobacter
Ruegeria
Erythromicrobium
Porphyrobacter
Altererythrobacter
Novosphingobium
Blastomonas
Sandaracinobacter
Sphingopyxis
Sphingobium
Sphingomonas
Methylocystis
Nordella
Methylobacterium
Ancylobacter
Ochrobactrum
Parvibaculum
Beijerinckia
Kaistia
Rhizobium
Hyphomicrobium
Rhodoplanes
Blastochloris
Bradyrhizobium
Rosea
Rhodopseudomonas
Nitrobacter
Afipia
Legionella
Haemophilus
Vibrio
Oleibacter
Marinomonas
Tolumonas
Aeromonas
Shewanella
Marinobacterium
Pseudoalteromonas
Thioalkalimicrobium
Achromatium
Beggiatoa
Methylocaldum
Methylomonas
Methylobacter
Serratia
Klebsiella
Kluyvera
Escherichia
Pantoea
Ectothiorhodospira
Thioalkalispira
Thiocapsa
Thiolamprovum
Rheinheimera
Rhabdochromatium
Nevskia
Luteimonas
Rhodanobacter
Stenotrophomonas
Arenimonas
Xanthomonas
Pseudoxanthomonas
Pseudomonas
Alkanindiges
Cellvibrio
Psychrobacter
Moraxella
Perlucidibaca
Acinetobacter
Thiobacillus
Sideroxydans
Gallionella
Nitrosospira
Nitrosomonas
Methylotenera
Methylophilus
Methylovorus
Methylobacillus
Simonsiella
Vogesella
Aquaspirillum
Chromobacterium
Zoogloea
Ferribacterium
Thauera
Denitratisoma
Azospira
Dechloromonas
Propionivibrio
Sutterella
Pigmentiphaga
Achromobacter
Ralstonia
Polynucleobacter
Cupriavidus
Paucimonas
Limnobacter
Burkholderia
Duganella
Undibacterium
Massilia
Herminiimonas
Janthinobacterium
Herbaspirillum
Leptothrix
Aquincola
Ideonella
Paucibacter
Rubrivivax
Aquabacterium
Methylibium
Variovorax
Polaromonas
Curvibacter
Pelomonas
Comamonas
Schlegelella
Brachymonas
Simplicispira
Rhodoferax
Delftia
Hydrogenophaga
Acidovorax
Malikia
Proteobacteria classes (inner circle)
Epsilonproteobacteria
Deltaproteobacteria
Alphaproteobacteria
Gammaproteobacteria
Betaproteobacteria
Type of water (outer bars)
SW
MW
U-DW
T-DW
Ur-WW
A-WW

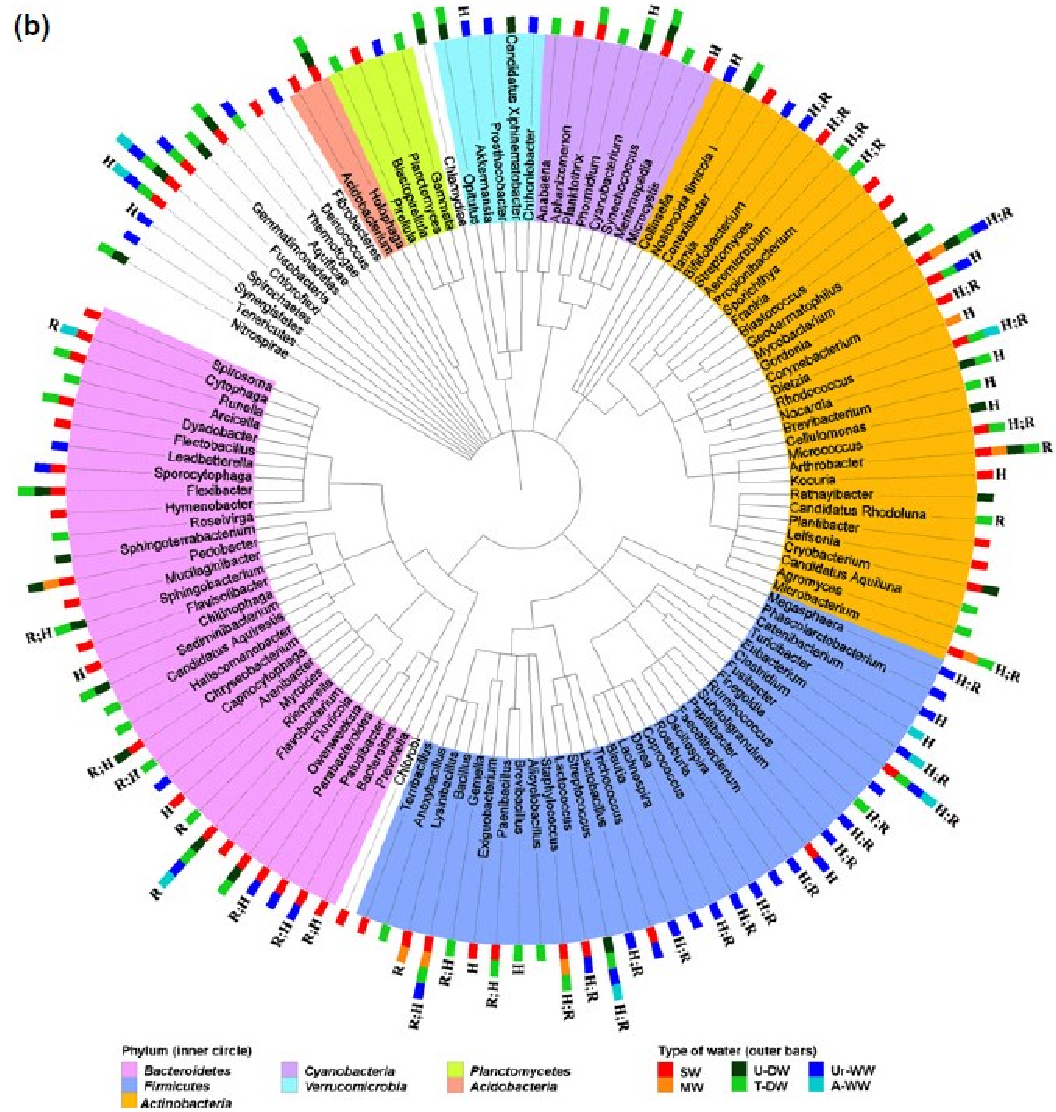


Fig. 1. Dendrogram representations of the bacterial diversity (a. *Proteobacteria* classes and b. other phyla) observed in different types of water, occurrence in the human-associated microbiome (H) and previous description of antibiotic resistance genes (R). The dendrograms were constructed with the iTOL – interactive tree of life (Letunic & Bork, 2007, 2011), based on the taxon ID codes, corresponding to the identifications provided in each of the publications cited (see Table S1).

Different phyla or *Proteobacteria* classes (inner circle) are represented by different colours (when are represented by two or more bacterial genera), and the presence in different types of water are represented by the outer bars. Types of water: SW, surface water that includes W (wetlands), R (rivers), L (lakes); MW, mineral drinking water that also includes spring water; U-DW, untreated drinking water; T-DW, treated drinking water; Ur-WW, urban domestic wastewater that may also include industrial wastewaters; A-WW, animal wastewater.

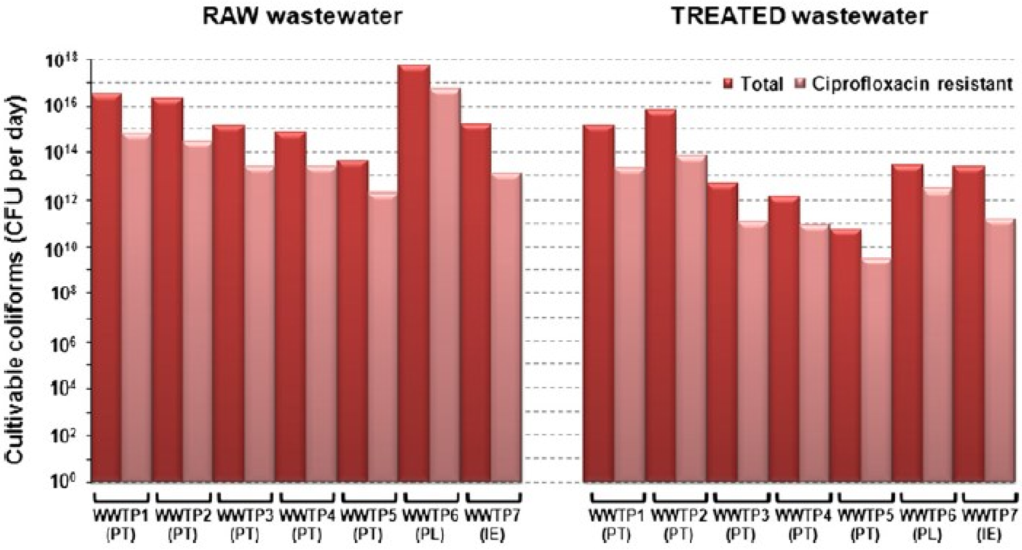


Fig. 2. A domestic wastewater treatment plant (WWTP) discharges about one billion (10^9) ciprofloxacin resistant coliforms per minute.

Total and ciprofloxacin resistant coliforms (CFU per day) discharged by WWTP in different countries [WWTP1-WWTP5, Portugal (PT); WWTP6, Poland (PL); WWTP7, Ireland (IE)], with different sizes (average day flow of 20 000, 32 500, 900, 890, 200, 96 000 and 49 000 m^3, respectively) and treatment processes [activated sludge (WWTP1 and WWTP6), trickling filter (WWTP2), submerged aerated filter (WWTP3), aeration lagoon (WWTP4), anaerobic lagoon (WWTP5), unknown secondary treatment (WWTP7), with bacterial removal rates above of 1.5-4 log(CFU) (Galvin *et al.*, 2010; Łuczkiewicz *et al.*, 2010; Manaia *et al.*, 2010; Novo & Manaia, 2010).

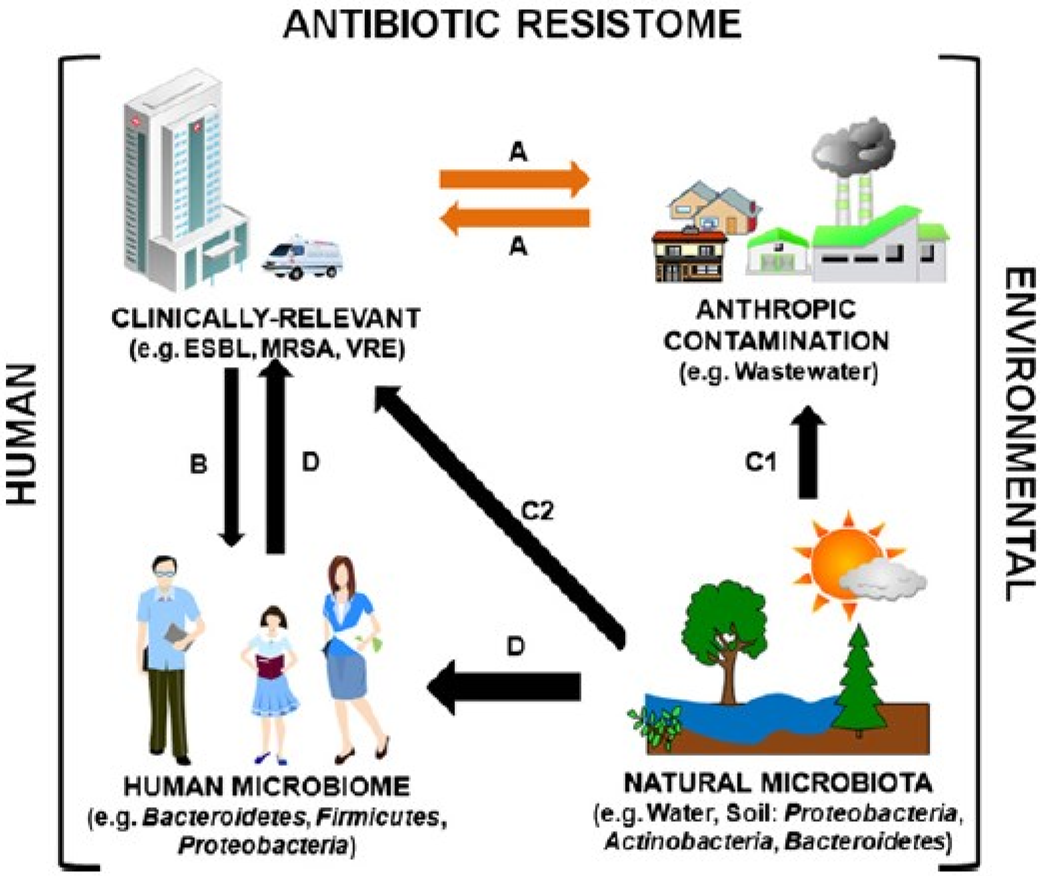


Fig. 3. Hypothesis about the relationship between environmental and human antibiotic resistome. A) cycle of known clinically-relevant antibiotic resistance determinants; B) transfer of antibiotic resistance genetic determinants from clinically-relevant bacteria to commensal human microbiota; C) transfer of antibiotic resistance genetic determinants from the natural resistome to clinically-relevant bacteria either thriving in the environment (C1) or hosted by humans (C2); D) indirect transfer of antibiotic resistance determinants from the natural resistome to clinically-relevant bacteria via human microbiome.