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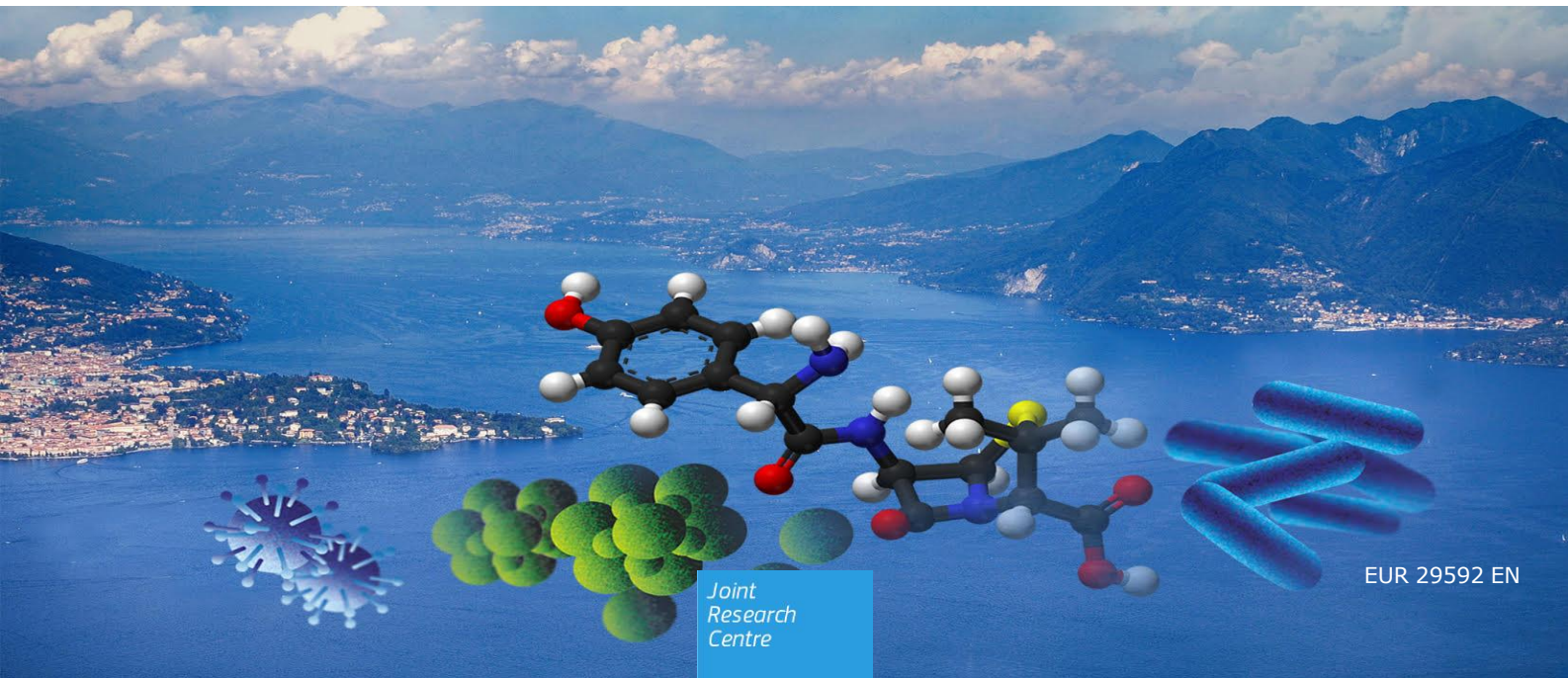
State of the Art on the Contribution of Water to Antimicrobial Resistance

Isabella Sanseverino, Anna Navarro Cuenca, Robert Loos, Dimitar Marinov and Teresa Lettieri

2018



EUR 29592 EN

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JRC 114775

EUR 29592 EN

PDF	ISBN 978-92-79-98478-5	ISSN 1831-9424	doi:10.2760/771124
Print	ISBN 978-92-79-98479-2	ISSN 1018-5593	doi:10.2760/82376

Luxembourg: Publications Office of the European Union, 2018

How to cite this report: Isabella Sanseverino, Anna Navarro Cuenca, Robert Loos, Dimitar Marinov and Teresa Lettieri, *State of the Art on the Contribution of Water to Antimicrobial Resistance,* EUR 29592 EN, Publications Office of the European Union, Luxembourg, ISBN 978-92-79-98478-5, doi:10.2760/771124, JRC114775

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Acknowledgements

We would like to acknowledge Ramón Pérez de Lara for producing all of the figures and Úna Cullinan for making available all required literature. We also thank Magdalena Niegowska for the bibliographic support and for critically reading the report.

Abstract

Antimicrobial resistance (AMR) is defined as the ability of microorganisms to withstand the effects of antibiotics. It is considered to be a universal threat to humans, animals and the environment. The resistance mechanisms developed by bacteria originate from the overuse of antibiotics in medical care and animal farming, or from the spread of resistance genes among microorganisms. Worldwide, 700 000 people die annually from resistant infections. Therefore, unless action is taken, the estimated annual deaths attributable to AMR will be 10 million by 2050.

The aim of this report is to discuss the mechanisms of antibiotic action and antibiotic resistance, focusing on potential effects in water. Waterbodies have been recognised as a significant reservoir of antibiotics and antibiotic resistance genes (ARG). They facilitate the interchange of resistance genes between pathogenic and non-pathogenic bacteria and may contribute to the maintenance of antimicrobial resistance in the environment.

In this report, a review of the global scientific literature was conducted to show the levels of antibiotics in wastewater treatment plants (WWTP), surface waters, agricultural runoff and drinking waters. The most frequently monitored antibiotics in WWTP were found to be sulfamethoxazole, ciprofloxacin and trimethoprim, while the most important substances in surface waters were erythromycin, sulfamethoxazole and trimethoprim.

The antibiotics monitored in inland surface waters are identified, and the co-occurrence of heavy metals and antibiotic resistance in bacteria is discussed. The chemical environmental pollution caused by heavy metals such as silver (Ag), copper (Cu) and zinc (Zn) can co-select for antibiotic resistance. Antibiotics have been frequently detected in different aquatic environments within urban water cycles (in waste, surface and drinking water). Even though the detected levels of such antibiotics are low (in the range of ng/L to µg/L), they could promote antimicrobial resistance through gene transfer between bacteria.

General safety measures to improve the effectiveness of wastewater treatment processes and to control the use of antibiotics in animal husbandry and in human medical practices could help constrain the spread of AMR. New research should also be conducted to understand the relationship between antibiotics' concentration and the selection of resistance determinants in order to define the minimal concentration of antibiotics (separately and combined) that induces resistance in bacteria. This should also be considered in the evaluation of the risk assessment of antibiotics in water in order to define their environmental impact.

1. Introduction

Antibiotics are chemical agents that kill or inhibit the growth of microorganisms and are widely used in the treatment of bacterial diseases. Most of the antibiotics were discovered during the "antibiotic golden age", a period that began in 1941 with the production of Penicillin, the first antibacterial agent extracted in 1928 from the fungus *Penicillium notatum* by Alexander Fleming. Starting from 1941, many other antibiotics have been discovered and currently they are mainly obtained semi-synthetically or synthetically by chemical variations of pre-existing natural antibiotics in order to improve their effectiveness.

The discovery of antibiotics is considered one of the most important events in the history of medicine. Their use in human health care and in animal health management has indeed assured the treatment of many bacterial infections for years. However, they are now becoming less efficient due to the apparent overuse in medical and veterinary applications and high concern has been expressed worldwide due to the increasing development and spread of antimicrobial resistance (AMR), which occurs when bacteria resist the effects of antimicrobial treatments. In Europe, about 25000 people die of resistant infections every year. Unfortunately, resistance has been reported for almost all the available antibiotics but, despite the increase of AMR, the development of new antimicrobial agents is declining. The decreasing interest in the discovery of new antibiotics has principally economic and regulatory reasons. Most pharmaceutical companies are not interested in developing a product which requires a huge investment to be commercialised and then placed on the market at a low price[1,2]. In addition, antibiotics are used for a short period of time, differently from the drugs prescribed to treat chronic diseases that guarantee a high return on investment. The result is that the number of new antibiotics developed and approved has reduced progressively over the past 30 years, increasing the problem to treat resistant bacteria[1].

In 2014, a review published in the United Kingdom (UK) recommended actions to address the growing global problem of drug-resistant infections. Amongst these recommendations, the UK report proposed new alternative approaches for treating bacterial infections in order to cut the unnecessary use of antibiotics and improve a global surveillance of drug resistance and antimicrobial consumption in humans and animals[3]. The European Commission (EC) recognised early the importance in addressing the AMR issue in humans and animals with the publication of the "Action Plan" in 2011[4]. The "One Health" approach published in 2017 reinforced the previous Plan since it encompasses also the environmental contribution to the spread of AMR[5,6].

The European attention towards the environmental problems posed by antibiotics in water was also shown by the inclusion of three antibiotics (azithromycin, clarithromycin and erythromycin) in the first surface water Watch List (WL) of the European Water Framework Directive (WFD) (in 2015) (EU, 2015/495)[7], a list of substances potentially harmful for the aquatic environment but for which monitoring data were not sufficient to establish their environmental risk. The WL mechanism should provide high-quality monitoring data on the concentrations of the substances in the aquatic environment and other two antibiotics, ciprofloxacin and amoxicillin have been added in the next WL exercise (in 2018) (EU, 2018/840)[8].

So far, many scientific publications reported the antibiotic concentrations in waterbodies and more recently, several papers aimed to focus on genes involved in AMR, however still missing the mechanism leading to the selection of resistance determinants in bacteria.

Nowadays, the greatest concern about the antibiotics in waterbodies is their potential role in disseminating and maintaining AMR in the environment and their contribution to the spreading of the resistance from environmental microbes to human or animal pathogens. Antibiotics can enter the aquatic environment as a result of inadequate wastewater treatment, the disposal of unused medicines or through agricultural runoff[9]. Once in the environment, antibiotics can be easily degraded or can persist and therefore accumulate.

Antibiotics are only partially removed during the conventional water treatments and although their concentrations in many wastewater treatment plant (WWTP) effluents and surface waters are low (usually at levels of ng/L to µg/L), such concentrations could promote the acquisition of new resistances.

Considering the relevance of the AMR issue at global level and the key role played by water in the AMR spread and persistence, this report aimes at collecting the environmental concentration levels of antibiotics reported in the scientific literature for WWTP, surface waters, agricultural runoff, aquacultures and drinking waters. Due to the extensive scientific data available, the collected information is not exhaustive but is merely meant to give an overview on this topic. A database including the antibiotics' measurements at European level was also consulted to extract the number of countries and sites where antibiotics were measured by competent authorities. The co-occurrence of heavy metals and antibiotic resistance was then discussed to assess the potential role of metals as a selective force in propagating the antibiotic resistance genes.

2. Mode of action of antibiotics

Antibiotics are commonly classified as bactericidal when they kill the infecting bacteria or as bacteriostatic when they inhibit the growth without killing bacteria[10]. They can be grouped in different classes such as aminoglycosides, β-lactams, tetracyclines and quinolones according to their chemical structure and mode of action (Figure 1 and Table 1). Antibiotics can have different bacterial targets or act on the same target. They can disrupt the bacterial cell membrane, inhibit the cell wall synthesis, the nucleic acids, the protein synthesis or the metabolic pathways (Figure 1)[11]. All these mechanisms impair the multiplication and growth of bacteria. For example, the antibiotics of the sulphonamides class can inhibit the folate metabolism required for purine and pyrimidine biosynthesis and hence nucleic acid synthesis which is essential for survival and replication of bacteria. Indeed, as shown in Figure 1, sulfonamides act as competitive antagonists of *para*-aminobenzoic acid (PABA), a molecule needed to form dihydrofolic acid (DHF) and tetrahydrofolic acid (THF), two precursors of folic acid. Examples of antibiotics involved in each mechanism are shown in Figure 1.

Antibiotics are extensively applied in human and veterinary medicine and, as shown in Figure 2, there are no antibiotics in Europe which, among those considered, are specific for veterinary medicine applications only, meaning that they are also used for treatments in humans. The broad use of antibiotics has contributed to spread these compounds in the environment and the different routes of antibiotic exposure to the natural ecosystem, including waterbodies, are described below in the following paragraph.

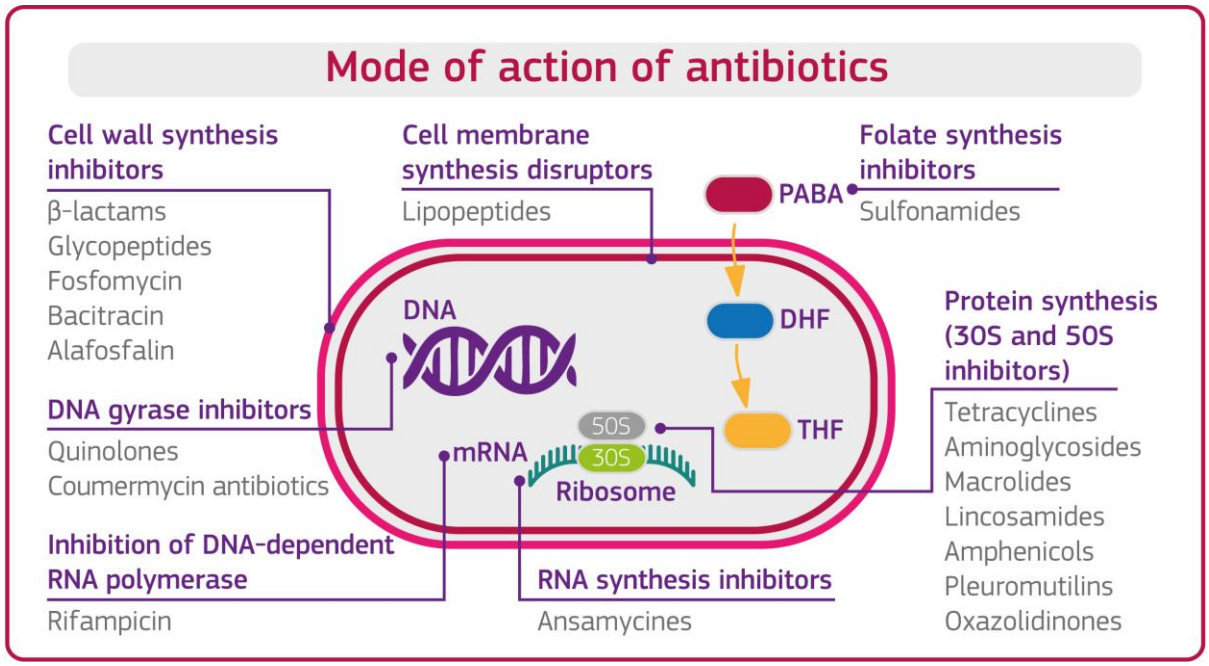

Figure 1. Mode of action of antibiotics. Antibiotics can inhibit the growth of bacteria by targeting the bacterial cell wall or the cell membrane. Other targets are the nucleic acid synthesis and the protein synthesis. The latter is a process performed by ribosomes, nucleoprotein complexes which consist of a small and large subunit (30S and 50S in bacteria, as shown in the figure). Antibiotics can also act as antimetabolites by inhibiting the folate metabolism (and consequently the DNA synthesis) in a pathway involving *para*-aminobenzoic acid (PABA) and two precursors of folic acid, dihydrofolic acid (DHF) and tetrahydrofolic acid (THF). Antibiotics can inhibit DNA gyrase, an enzyme which modifies the DNA conformation, playing a role in replication and transcription. Class of antibiotics involved in each mechanism are shown in grey.

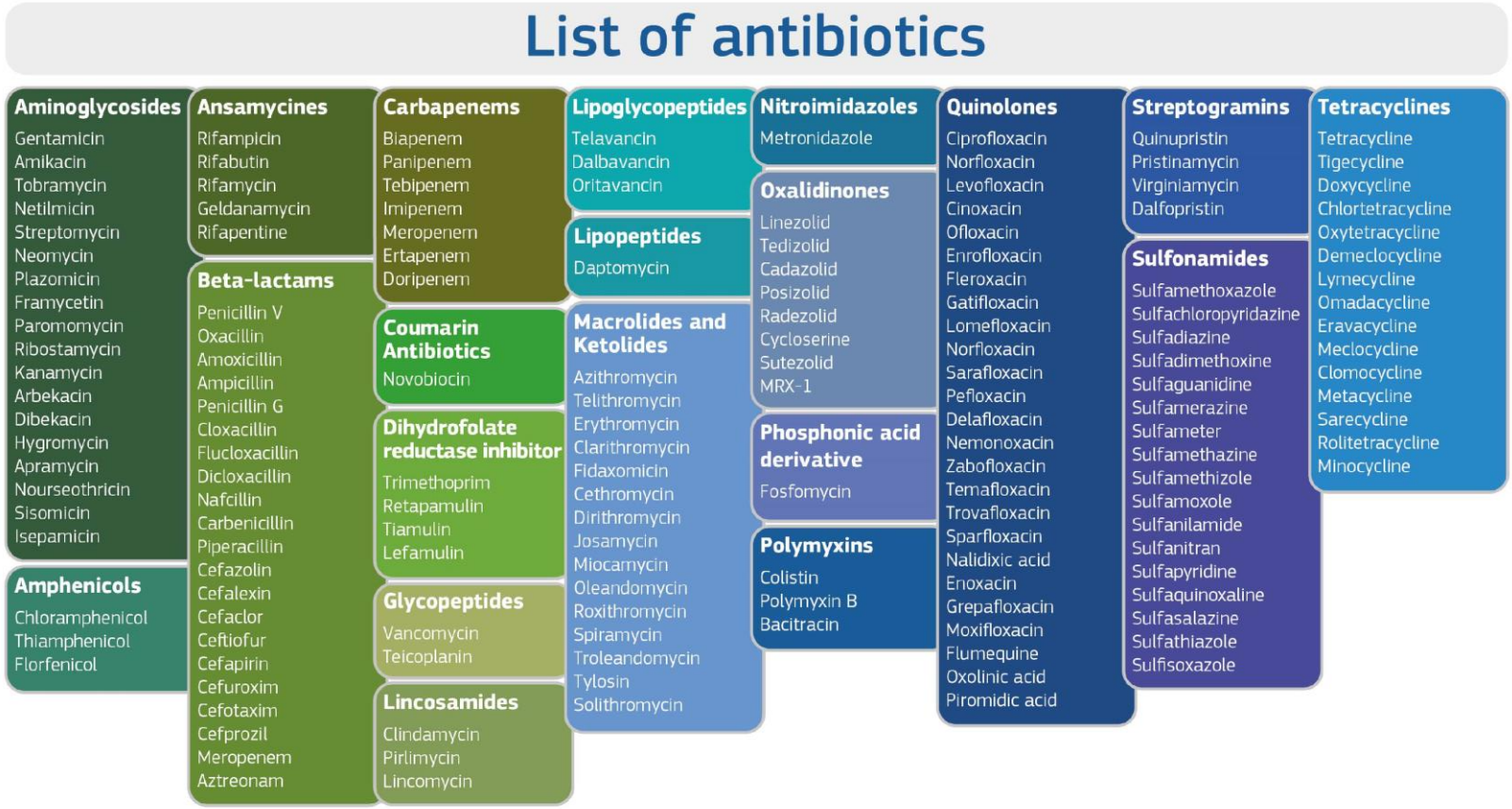

Table 1. List of Antibiotics. List of the most common antibiotics. Each box includes a list of antibiotics belonging to different classes (in bold). Carbapenems are a subclass of β-lactams antibiotics.

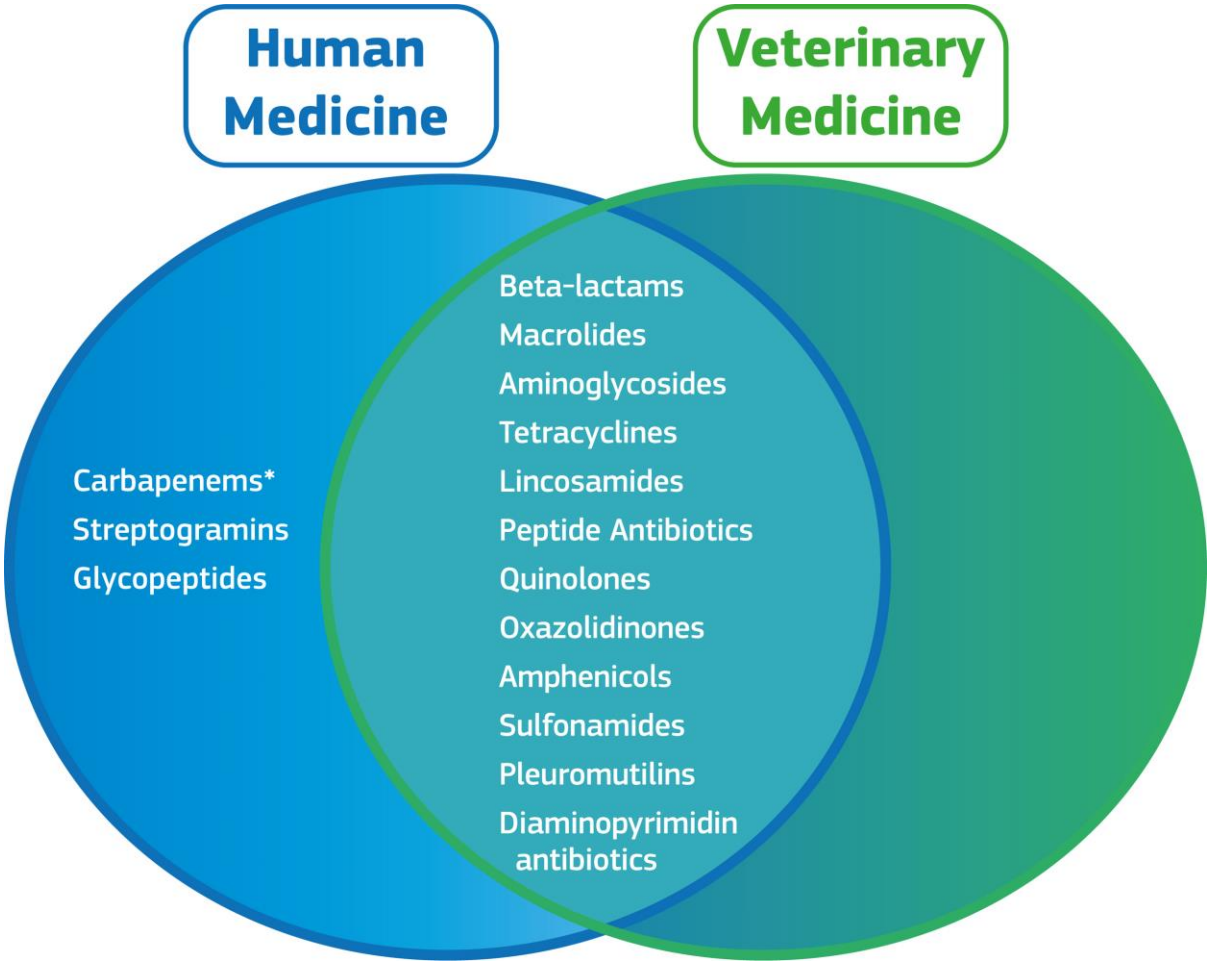

Figure 2. Classes of antibiotics used in human and veterinary medicine in Europe. The Venn diagram shows representative classes of antibiotics and their uses in human or veterinary medicine in Europe. Among the classes listed in the figure, none of them is specific for veterinary medicine while there are classes of antibitics (carbapenem, streptpgramins and glycopeptides) that are only used for treatments in humans.
(*) Carbapenems are a sub-class of β-lactam antibiotics.
Sources: "ECDC/EFSA/EMA second joint report on the integrated analysis of the consumption of antimicrobial agents and occurrence of antimicrobial resistance in bacteria from humans and food-producing animals. European Centre for Disease Prevention and Control (ECDC), European Food Safety Authority (EFSA) and European Medicines Agency (EMA). 28 June 2017;
http://www.agenziafarmaco.gov.it/; http://www.ema.europa.eu/ema/

3. Routes of exposure to aquatic ecosystem

Antibiotics can enter into the environment by different routes[12] including urban and industrial waste or agricultural runoff, as shown in Figure 3.

Antibiotics used in human medicine are mainly discharged into the environment from wastewater treatment plants (WWTP). Indeed, after ingestion, a large amount of antibiotics is only partially metabolised and the metabolites might retain their antibiotic activity[13,14]. For some antibiotics such as β-lactams, quinolones, tetracyclines, phenicols and trimethoprim, the excretion generally exceeds the 50% of the administrated dose, while only around 19% of ciprofloxacin dose is excreted as active metabolites (sulfociprofloxacin, oxociprofloxacin, desethylene ciprofloxacin and formyl-ciprofloxacin)[15]. Therefore, a mixture of antibiotics and their metabolites travel through the sewage system to the WWTP where their complete elimination is not possible so that the antibiotics can reach the natural aquatic systems (surface waters and soils and the sewage sludge)[16].

The impact of veterinary antibiotics discharged in water depends on the farm practices and it mainly occurs *via* excretion. As in humans, after the administration in livestock, both metabolised and unmetabolised antibiotics end up in the manure or slurry. The direct entry of antibiotics in the environment takes place when cattle are out door or manure is used as fertilizer. Once in the soil, antibiotics may enter aquatic systems indirectly *via* surface runoff to surface water and/or by leaching to groundwater[12]. Antibiotics from veterinary use can be also introduced directly into aquatic systems due to their use in aquaculture[12,17].

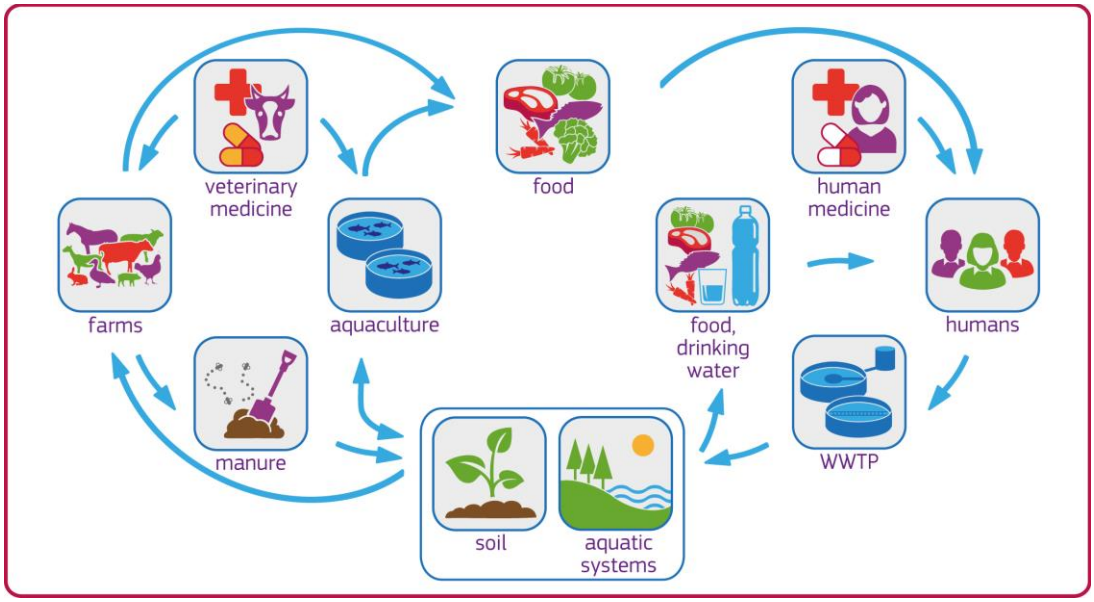

Figure 3. Schematic representation of the environmental routes for antibiotics from human and veterinary uses. This figure shows the environmental compartments where antibiotics from veterinary and human uses are mobilised and transported. The arrows show the connection among the compartments and the aquatic ecosystems. The presence of veterinary antibiotics in soil and in the aquatic system is mainly due to their use in aquaculture or in farms. Administered antibiotics are excreted from animals and the application of animal manure for soil fertilisation purposes represents a route to spread antibiotics in the environment. Antibiotics used in human medicine can enter the natural ecosystem mainly due to the insufficient removal of these compounds in the wastewater treatment plants (WWTP). Humans and animals are exposed to antibiotics also through the food chain. Besides the excretion, other possible routes that should be considered are *via* accidental spills and discharges during their manufacture, as well as the disposal of the unused or expired antibiotics that are not recycled.

As pharmaceuticals are constantly released into the environment, organisms could be exposed to many of these compounds for long time periods. Antibiotics have highly differentiated structures and their behaviour, fate, transport and persistence in the environment may depend on their partial transformation, bioaccumulation and deposition in sediment, soil, surface water and groundwater[18]. Depending on their mobility and persistence in the environment, antibiotics and their metabolites can reach surface and groundwater, and potentially drinking water[12]. It has been reported that quinolones, sulphonamides and trimethoprim are the most detected antibiotics in the environment because of their high use in human and veterinary medicine and their persistence in aquatic systems[12]. Compounds that have high sorption coefficients tend to interact with solid particles and accumulate in sediments and/or sludge, while the compounds with low sorption coefficients tend to remain in aqueous phase favouring their mobility. A study reported that tetracycline binds to particulate matter due to its high sorption coefficient and therefore will be primarily found on suspended particles and sediments/sludge[19].

3.1 Concentration of antibiotics in wastewater treatment plants

Antibiotics are widely used in human and animal healthcare but, once in the body, these drugs are not completely metabolised or eliminated and a percentage ranging from 30% to 90% is excreted unchanged into the wastewater system. The traditional water treatment systems partly degrade or leave the antibiotics unchanged. The derived degradation products can sometimes be as toxic as their parents or having even a higher ecotoxicity[20].

Antibiotics can be detected in surface and wastewaters at concentrations from ng/L to µg/L[21,22]. The occurrence of antibiotics in the water cycle is well documented and the wastewater treatment plants (WWTP) represent one of the most important sources of pharmaceuticals in waterbodies[23]. The traditional WWTP follow specific processes consisting of physico-chemical and biological water treatments to eliminate contaminants like organic matter, solids and nutrients. Pharmaceuticals, like antibiotics, are only partially removed in WWTP and these compounds can therefore be consistently present in waterbodies. One of the stages in water treatment process is disinfection, which is applied to remove microbial population in order to protect humans from exposure to pathogenic microorganisms, like for example *Escherichia coli*, whose recommended concentration in Italy needs to be below 5000 colony-forming unit (CFU)/100 ml according to the limits established by the local authorities for wastewater. A study comparing two disinfection processes, chlorination and ultraviolet (UV) light, found that concentrations of antibiotics were significantly lower in the chlorination effluent than in the UV disinfection effluent[24]. However, additional data are necessary to confirm this evidence.

The most frequently detected pharmaceuticals in wastewaters and surface waters are usually antibiotics, anti-inflammatory, analgesics/antipyretics, lipid regulators, beta blockers (cardiovascular drugs), radiocontrast agents, hormones, psychotropic drugs (antidepressants) and anticonvulsants[25,26]. A research performed in a Portuguese WWTP identified the antibiotics sulfamethoxazole, ciprofloxacin, erythromycin and sulfapyridine in wastewater influents. Their average concentrations were in the range of 0.28-0.69 µg/L and the removal efficiency following the traditional water treatments was lower or equal to 50%. The seasonality did not impact this trend but the removal efficiency observed at the end of all the treatment steps was higher during spring than in autumn and winter[25].

Even when the removal of ciprofloxacin in WWTP is high (90%) due to the sorption of the antibiotic to sewage sludge, the poor biological degradation of this compound results in its accumulation. If the sludge is used as fertilizer, antibiotic residues can be transferred to crops and soil where ciprofloxacin can persist for more than 90 days with only minimal transformations[13,27].

In the United States (USA) the presence of ciprofloxacin, ofloxacin, sulfamethoxazole and trimethoprim was reported in wastewaters at respective concentrations of 0.130, 0.255, 0.485 and 0.373 µg/L[24]. Another confirmation of the removal inefficiency of antibiotics in WWTP is given by the concentrations of sulfamethoxazole, trimethoprim, ciprofloxacin, tetracycline, and clindamycin detected in the receiving water at levels ranging from 0.090 to 6.0 µg/L[28]. It has been reported that antibiotics like ciprofloxacin and ofloxacin were detected at higher concentrations in hospital effluents discharging into the Ter River, in Spain[29]. Similar results were found in Portugal where ciprofloxacin was frequently detected during the analysis of influents and effluents of 15 different WWTP[30]. In Italy, eight antibiotics were detected at concentrations between 0.008 and 1.1 µg/L in samples collected from effluents of different treatment plants[31]. In Brisbane, Australia, the application of two different wastewater treatment processes (conventional activated sludge treatment and advanced microfiltration/reverse osmosis) showed that both plants were efficient in reducing the antibiotic concentrations even if the effluents still contained these compounds at low to mid ng/L level[32]. The importance of checking the occurrence and removal of antibiotics in samples collected at different points within the WWTP and during different times of the year is crucial to evaluate the efficiency of the current treatments and to start thinking about new strategies to minimise the impact of antibiotics in the water environment. The improvement of treatment plant methods will enhance water quality and can significantly reduce the diffusion of antibiotics in the environment[18].

3.1.1 Data analysis of global antibiotics' concentrations in wastewater treatment plants effluents

The collected literature data for antibiotics measured worldwide in effluents of wastewater treatment plants (WWTP) include 267 samples. The considered publications are listed in Table A in the Annex I, and a general statistical overview of the collected data is presented in Figure 4. It shows the number of total samples collected for each antibiotic and indicates the number of countries where antibiotics were measured (numbers at the bottom of each box). The most frequently observed antibiotics in WWTP effluents are sulfamethoxazole (31 samples in 13 countries), ciprofloxacin (26 samples in 11 countries), and trimethoprim (26 samples in 10 countries) followed by clarithromycin (17 samples in 7 countries), erythromycin (15 samples in 7 countries), clindamycin (13 samples in 6 countires) and azithromycin (13 samples in 6 countries).

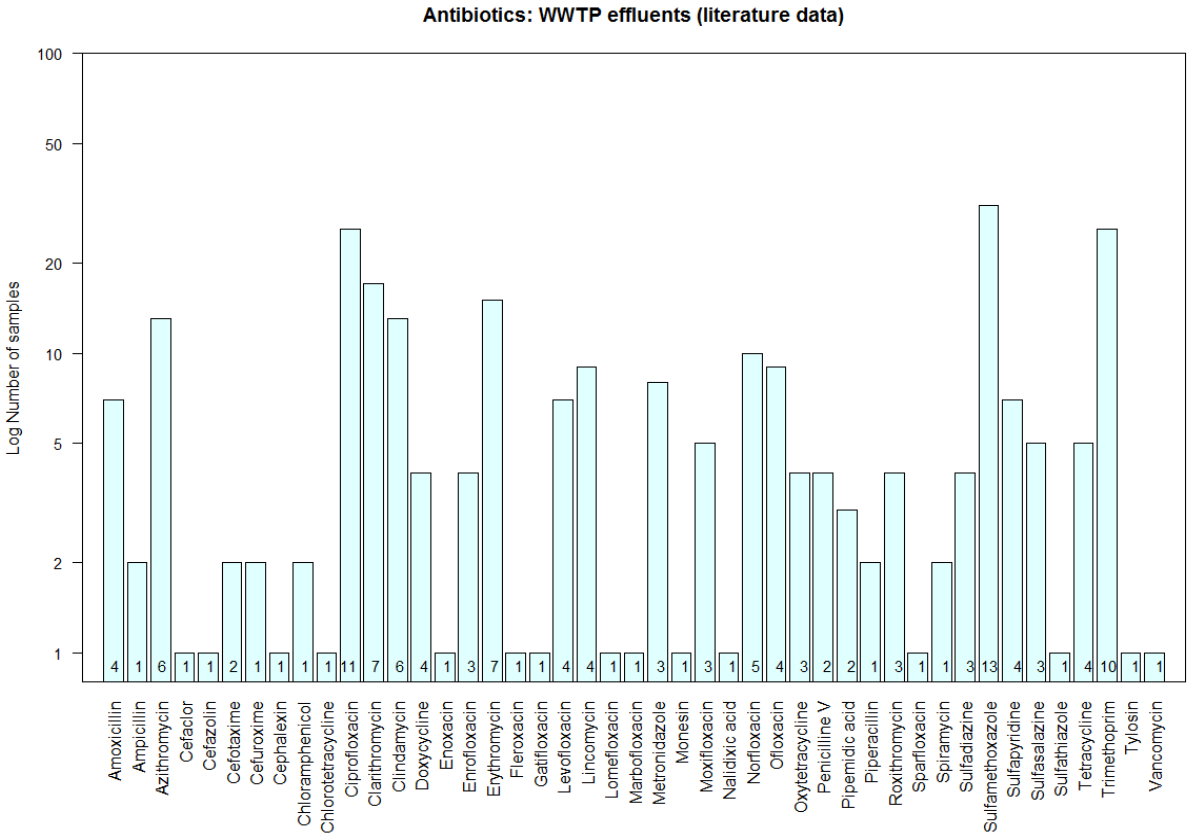

Figure 4. Number of collected samples for antibiotics in wastewater treatment plants (WWTP) effluents all over the world (literature data). Numbers of collected samples are reported in logarithmic scale. The reported monitoring data are for 45 antibiotics and the numbers at the bottom of each box indicate in how many countries each antibiotic was measured. Sulfamethoxazole, ciprofloxacin and trimethoprim are the most frequently monitored antibiotics.

The range of antibiotics' concentrations measured globally in WWTP effluents according to the scientific literature review (see Table A in the Annex I) is shown as boxplot in Figure 5. The boxes were built by using minimal and maximal concentrations and the figure also shows the average (mean) concentrations (red bars in the boxes) for each compound. For several antibiotics, the minimal, mean and maximal concentrations overlap since only a single measurement was available for them. According to the collected data, the maximal concentrations in WWTP effluents for the majority of antibiotics (18 out of 45) are between 0.1 µg/L and 1 µg/L. Thirteen antibiotics have maximal concentrations between 1 and 10 µg/L, however, 3 amongst them (ciprofloxacin, ofloxacin, and sulfamethoxazole) have max concentrations approaching or equal to 10 µg/L (for the others the max is below 4 µg/L). The remaining 14 antibiotics showed max concentrations below 0.1 µg/L.

Regarding the average concentrations, 10 out of 45 antibiotics showed values higher than 0.5 µg/L while all the other substances (35 out of 45) have mean concentrations below 0.5 µg/L.

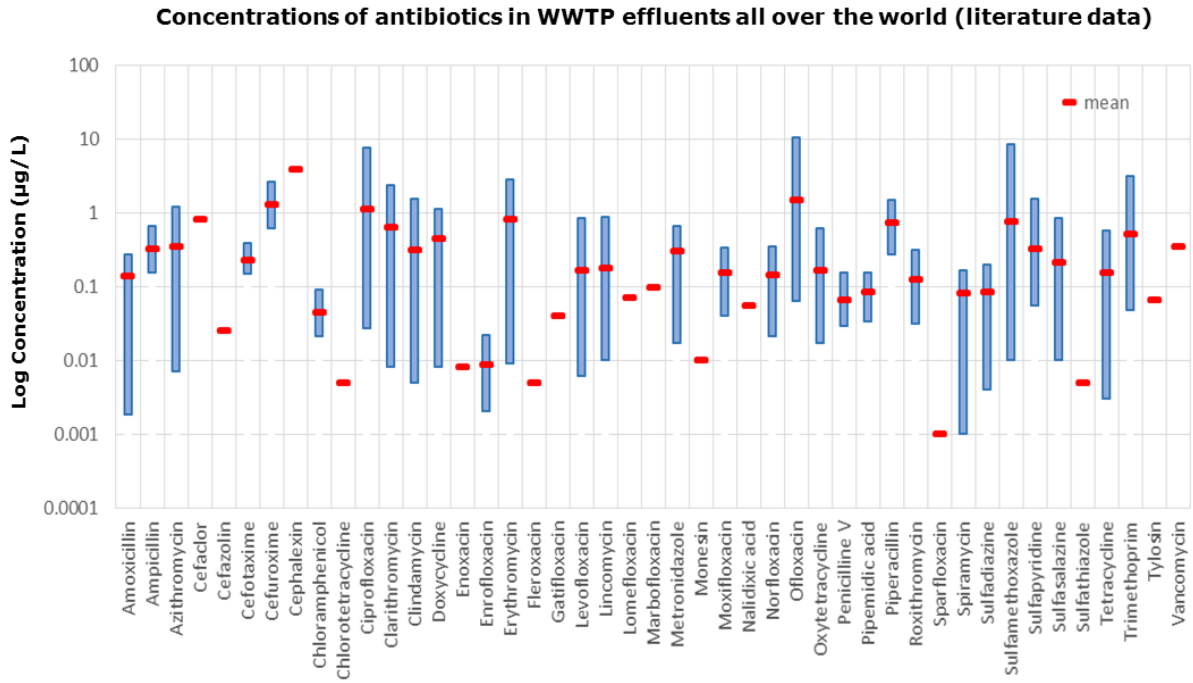

Figure 5. Antibiotics' concentrations in wastewater treatment plants (WWTP) effluents all over the world (literature data). The antibiotics' concentrations are reported in logarithmic scale. Boxes were built by using minimal and maximal values and mean concentrations are indicated by red bars in each box. The figure shows that the maximal concentrations for the majority of antibiotics in WWTP effluents are in the range of 0.1-1 µg/L. For thirteen antibiotics, the maximal concentration is between 1 and 10 µg/L, and only for three antibiotics (ciprofloxacin, ofloxacin, and sulfamethoxazole) it is around or equal to 10 µg/L. The remaining 14 substances showed max concentrations below 0.1 µg/L (for 5 of them the max is lower than 0.01 µg/L).

About 57% of the data reported in Table A (see Annex I) and represented in Figure 4 and Figure 5 refer to the maximal concentrations of the antibiotics detected in WWTP effluents; 36% are median or mean value concentrations, while the remaining 8% represent minimal concentrations. The reported monitoring data are for 45 antibiotics from 13 countries all over the world but 79% of effluent samples come from European countries while the remaining data are from Australia, China, and United States (USA). The quality of reported data is difficult to check since the publications sometimes lack information about the analytical methods and limits of quantification for the measurements.

3.2 Antibiotics in surface water

Inland surface waters refer to waterbodies like rivers or lakes and represent an important source for community water needs, such as urban water supply and irrigation. These waterbodies can also be used for drinking water (DW) production but only when treatments based on filtration and disinfection are correctly applied to assure good DW quality. Antibiotics detected in surface waters can derive from industrial sources, from households or from hospitals. Indeed, as described in section 3.1, the wastewater treatment plants (WWTP) may not completely remove antibiotics leading to their release into the freshwater environment[33]. In addition, antibiotics are also washed away and may accumulate in biosolids, nutrient-rich organic materials generated by WWTP that can be later used on farms as fertilizers. This practice can cause the release of pharmaceuticals

or antibiotics in surface waters by direct leaching into the river bed, biosolid runoff or from sewer overflow[34].

When antibiotics are detected in surface water, their concentrations are usually lower than the levels found in WWTP effluents. Potential factors responsible for this reduction in concentration include the dilution of antibiotics in surface water, the bioaccumulation, biodegradation, photodegradation as well as their ability to be absorbed on solids, colloids or dissolved organic matters.

3.2.1 Data analysis of global antibiotics' concentration in surface water

Antibiotics' concentrations measured worldwide in inland surface waters (including Europe) were gathered from literature sources; the considered publications are listed in Table B in the Annex II.

These collected surface water data contain 728 samples for 43 antibiotics from 24 countries. However, about 66% of these records refers only to 5 countries (Spain, China, Italy, United States (USA) and Germany).

A general overview of the collected literature data is given in Figure 6, showing the number of samples and the number of countries (given at the bottom of each box) where antibiotics were measured. The top three most frequently monitored antibiotics were erythromycin, sulfamethoxazole and trimethoprim, which were measured in more than 10 countries.

The range of antibiotics' concentrations measured in inland surface water is presented in Figure 7. The boxes were built by using minimal and maximal concentrations and the figure also shows the average (mean) concentrations (red bars in the boxes) for each compound. The reported maximal concentrations for the majority of antibiotics (19 out of 43) are between 0.1 µg/L and 1 µg/L. Sixteen compounds have max concentrations exceeding 1 µg/L, however, four antibiotics (azithromycin, ciprofloxacin, sulfamethoxazole, and sulfapyridine) have the maximal concentrations slightly higher than 10 µg/L (these records coming from one single country in Europe). The remaining 8 substances showed max concentrations below 0.1 µg/L.

Considering the average concentrations, only 4 out of 43 antibiotics (azithromycin, sulfamethoxypyridazine, sulfapyridine, and sulfamethazine) showed values higher than 0.5 µg/L while all other compounds have mean values under this threshold (sometimes considerably below).

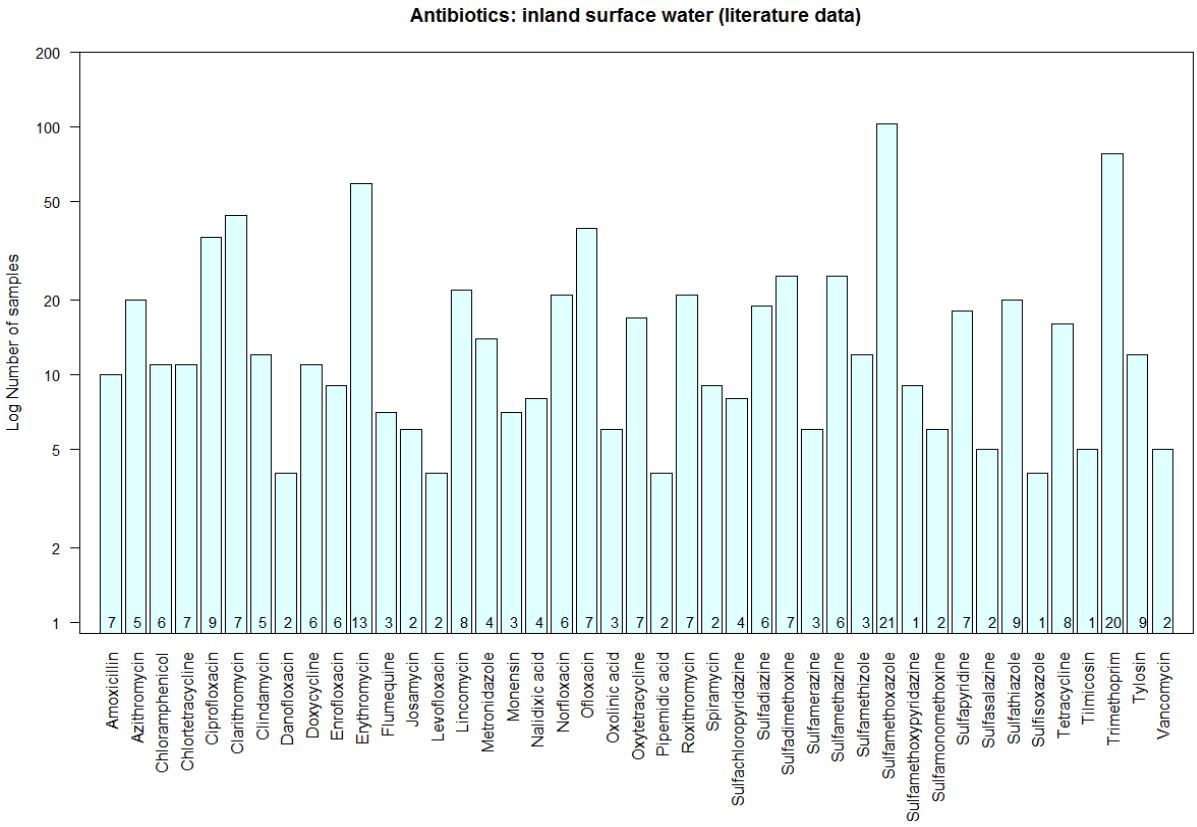

Figure 6. **Number of collected samples for antibiotics in inland surface water all over the world (literature data)**. Numbers of collected samples are reported in logarithmic scale. The reported monitoring data are for 43 antibiotics from 24 countries and the numbers at the bottom of each box indicate in how many countries each antibiotic was measured. As shown in the figure, the most frequently monitored antibiotics in inland surface water are erythromycin, sulfamethoxazole and trimethoprim which were also measured in more than 10 countries.

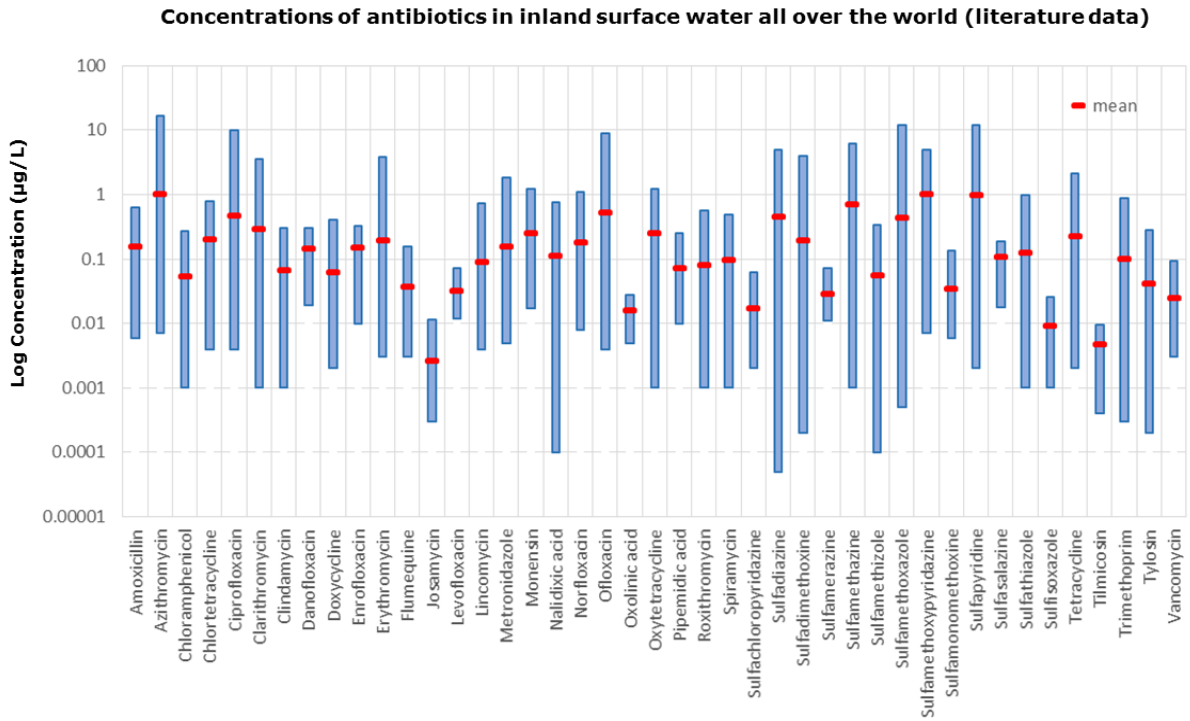

Figure 7. Antibiotics' concentrations in inland surface water all over the world (literature data). Antibiotics' concentrations are reported in logarithmic scale. The maximal concentrations for the majority of antibiotics are in the range of 0.1-1 µg/L. For 16 of them, the highest concentrations exceed 1 µg/L. Azithromycin, ciprofloxacin, sulfamethoxazole, and sulfapyridine have the maximal concentrations slightly higher than 10 µg/L. The remaining 8 substances showed max concentrations below 0.1 µg/L.

3.3 Comparison between wastewater treatment plants and surface water

A comparison of the concentrations of the 267 wastewater treatment plants (WWTP) samples with the 728 surface water samples collected from the literature is difficult because in most cases maximal, median or mean concentrations but no disaggregated data are given in scientific publications. At first glance, the values of both the maximal and the average concentrations in surface waters appear to be similar to those reported for the WWTP effluents. However, the surface water levels in Figure 7 clearly show for most of the substances a higher distribution at lower concentrations below 0.1 or 0.01 µg/L than for WWTP in Figure 5. Examples are sulfamethoxazole or clarithromycin.

The concentrations in surface waters should certainly be lower considering the water dilution factor which assumes a level of dilution of antibiotics in the receiving water. The antibiotic concentration in water is also dependent on the distance from the WWTP (higher is the distance, lower is the concentration) and considering that the place of sampling was not indicated in the articles, it was not clear if the water was collected close to or far away from WWTP effluents, explaining why the values we have reported for WWTP and surface waters are comparable.

3.4 Antibiotics' concentration in European inland surface water

To examine if recent measurements for antibiotics are available from European inland surface waters, a starting list of more than 700 antibiotics taken from the United States (USA) National Library of Medicine (https://chem.nlm.nih.gov/chemidplus/) was matched

against the European monitoring data set collected by the JRC during the last prioritisation exercise of the Water Framework Directive (WFD)[35], coming mainly from the European national competent authorities. This data set contains more than 16.6 million records for 1390 individual substances measured in inland surface waters during 2006-2014 time period.

Thirty-five antibiotics were identified and Figure 8 shows the amount of samples collected in the period 2006-2014, including the number of European countries which performed measurements. However, the EU dataset didn't distinguish between measurements performed close to or away from effluents of wastewater treatment plant (WWTP).

The statistical analysis showed that only two antibiotics are widely monitored in Europe by the national monitoring authorities; sulfamethoxazole was measured in 14 Member States (MS) with 11684 samples, and sulfamethazine in 11 MS with 3798 samples. All other antibiotics were found to be monitored only in a few European countries. For instance, the three antibiotics azithromycin, roxithromycin and trimethoprim were measured in only four countries (660, 2094 and 4613 samples, respectively). Six other antibiotics (ciprofloxacin, clarithromycin, erythromycin, norfloxacin, ofloxacin and spiramycin) were monitored in three countries. The remaining twenty-four antibiotics were monitored occasionally in one or two countries and only few samples are available for them (in most cases between 10 and 100).

Three of the above-mentioned antibiotics (azithromycin, clarithromycin and erythromycin) are included in the Watch List (WL) program (EU, 2015/495)[7], and two more (ciprofloxacin and amoxicillin) have been added in the updated WL (EU, 2018/840)[8]. The substances in the WL are selected from amongst those that may pose a significant risk at Union level but for which monitoring data are insufficient to come to a conclusion on the potential risk they may pose. Thus, the monitoring of antibiotics in the WL should generate high quality data on their concentrations introduced to or *via* the aquatic environment.

Thereby, the statistical analysis of the official European surface water monitoring data from the national competent authorities suggests that except for some antibiotics (e.g. sulfamethoxazole and sulfamethazine), very few measurements are available for the European aquatic environment.

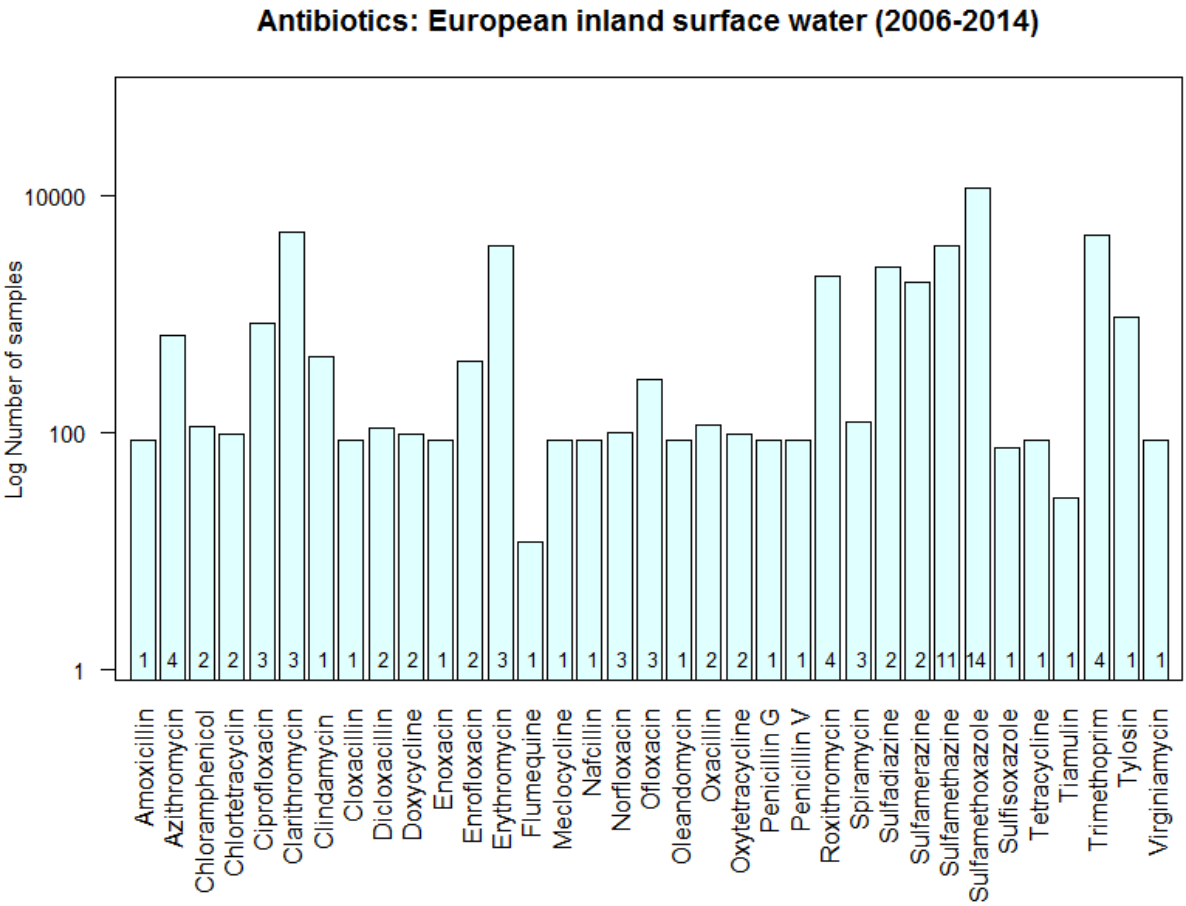

Figure 8. Number of collected samples for antibiotics in inland surface water in Europe. Total number of samples for antibiotics (logarithmic scale) with measurements reported in the official European inland surface water dataset for the period 2006-2014. The reported monitoring data are for 35 antibiotics and for each of them, the number at the bottom of the boxes indicates in how many countries they were measured. As shown in the figure, the most frequently and widely monitored antibiotics in Europe are sulfamethoxazole and sulfamethazine.

Then, Figure 9 shows the range of measured environmental concentrations for the antibiotics found in the European inland surface water (official) dataset (measured close to or away from WWTP effluents) during the period 2006-2014. The boxes were built by using minimal and maximal concentrations. The figure also shows the average (mean) concentrations (red bars in the boxes) in Europe for each single antibiotic. For some antibiotics (amoxicillin, cloxacillin, enoxacin, flumequine, oleandomycin, tetracycline and virginiamycin) the minimal, mean and maximal concentrations are overlapping because identical or repeating measurements were reported (suggesting the reporting of non-quantified samples due to a low sensitivity of the analytical methods used in the monitoring process). Therefore, the available measured environmental concentrations for them could not be considered as reliable.

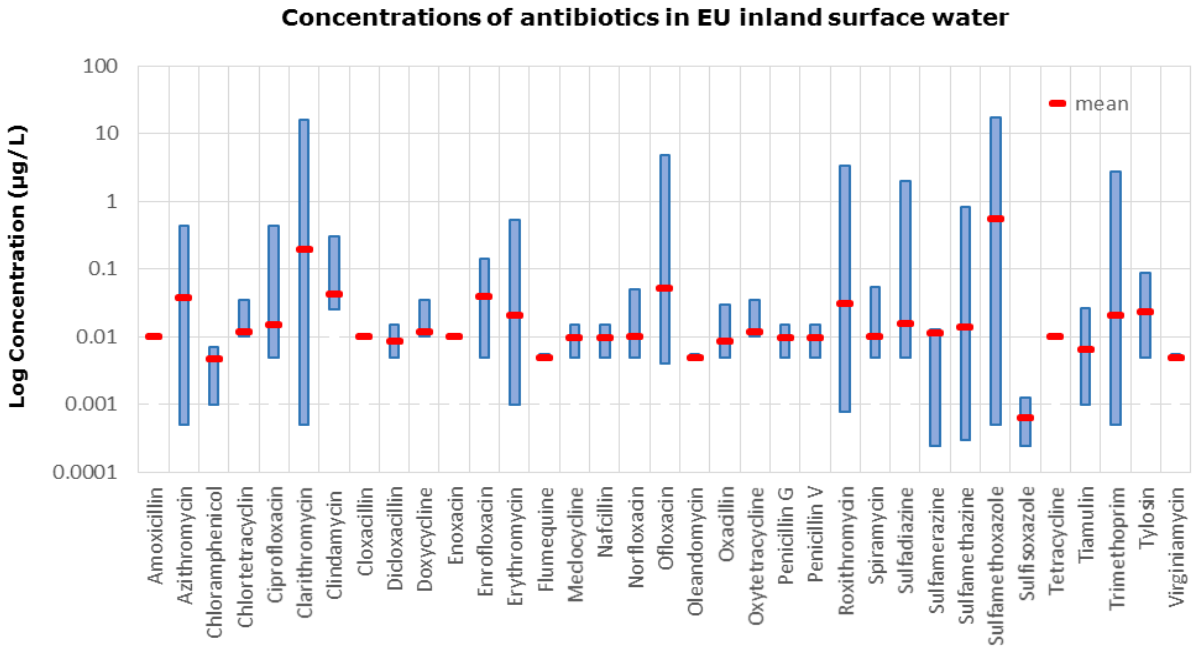

Figure 9. Antibiotics' concentration in European inland surface water. Range of measured environmental concentrations (boxes are built only by using minimal and maximal values) and mean concentrations (red bars in each box) of the antibiotics reported in the European inland surface water dataset (measuremens close to or away from effluents of wastewater treatment plants - WWTP) during the period 2006-2014. For some antibiotics (amoxicillin, cloxacillin, enoxacin, flumequine, oleandomycin, tetracycline and virginiamycin), the minimal, mean and maximal concentrations overlap suggesting a low sensitivity of the analytical methods used in their monitoring process.

In addition, Figure 9 shows that across Europe, the inland surface water concentrations of some antibiotics could span several orders of magnitude. For instance, sulfamethoxazole showed a minimal concentration equal to 0.5 ng/L and a maximal concentration of 17 µg/L while for clarithromycin the range was between 0.5 ng/L and 16 µg/L. Besides, four other antibiotics (ofloxacin, roxithromycin, sulfadiazine and trimethoprim) had maximal concentrations between 2 and 5 µg/L. The remaining 29 antibiotics showed max values below 1 µg/L (amongst them 24 with max under 0.1 µg/L).

The average concentrations of antibiotics in European inland surface waters for the considered thirty-five compounds showed a range of 0.0006–0.548 µg/L. Only two substances have average concentrations higher than 0.1 µg/l; these are clarithromycin (0.193 µg/L) and sulfamethoxazole (0.548 µg/L). Sixteen antibiotics showed a mean concentration ≤ 0.01 µg/L and seventeen antibiotics have average concentrations in the range of 0.01-0.06 µg/L.

3.5 Antibiotics' concentration in drinking water

The antibiotic profile in waterbodies may change in different countries because of the differences in the treatment habits and in the prevalence of diseases. The amount of antibiotics in drinking water (DW) depends on several factors including the traditional water treatment systems used to remove microorganisms and their property to be easily degraded or persistent in water.

Safe DW is needed to assure the public health and well-being. The Drinking Water Directive (DWD) 2015/1787/EC[36] should guarantee good quality of DW consumed in Europe and its basic purpose is "*to protect human health from the adverse effects of any contamination of water intended for human consumption by ensuring that it is wholesome and clean*"[37]. Antibiotics are not included in the list of parameters usually checked for testing the quality of DW in Europe. Indeed, the contamination of antibiotics in tap water is usually low, generally in the low ng/L range, but the presence of low antibiotic concentrations detected in natural environments are highly relevant because of their potential ability to select for new bacterial mutants[38].

The antibiotics amoxicillin, lincomycin, erythromycin and tylosin were detected in Italian DW at concentration in the ng/L range which is unlikely to pose a risk to humans following an acute exposure to the drugs. Instead, possible negative effects due to a chronic low-level exposure to antibiotics over a lifetime could not be excluded[39]. An American study has also shown the presence of macrolides (1-5 ng/L), and quinolones (3-4 ng/L) in drinking waters[40]. In addition, traces of the antibiotic oxytetracycline (1 ng/L) were detected in tap water by a survey performed in Tai Po (Hong Kong), and the veterinary antibiotics florfenicol and thiamphenicol were identified in tap water samples in Shanghai (China) with respective median concentrations of 12 and 11 ng/L[41,42]. A small number and amounts of antibiotics have been also detected in DW in North Carolina (United States of America, USA). The levels have been compared to those reported in source water indicating the incomplete removal of some antibiotics like ciprofloxacin, norfloxacin, lincomycin, doxycycline and tylosin during physico-chemical water treatment processes[40]. A different scenario has been instead observed in south-east Queensland, Australia, where antibiotics have been detected in surface water in the low ng/L to few µg/L concentration range but no antibiotics were observed in any of the DW samples[43]. The non-detection of antibiotics in finished water has also been reported in water samples collected from a drinking-water-treatment facility in the USA, indicating that the concentrations were under the analytical detection limits or that antibiotics were degraded during the conventional treatment process[44].

So far, there is little research available reporting the occurrence of antibiotics in DW (see Table 2) and the potential health consequences related to the long-period exposure through DW are not known. It is therefore important to check the antibiotics' concentrations in DW even if according to a World Health Organisation (WHO) report, the low levels of pharmaceuticals in DW are unlikely to be considered as a potential risk to human health[45].

Table 2. Antibiotics in drinking water (DW) or sources of DW

Substance	Country	Source of monitoring data	MEC (µg/L)	Reference
Azithromycin	USA	Ground- (n=25) and surface-water (n=49) sources of DW	0.029 (max)	Focazio et al., 2008[46]
	Spain	DW in Barcelona	0.017 (max)	López-Serna et al., 2010[47]
Chlortetracycline	Japan	Source water of DW purification plants (n=6)	0.012 (max)	Simazaki et al., 2015[48]
Ciprofloxacin	Finland	Raw drinking water	0.036 (max)	Vieno et al., 2007[49]
	Switzerland	Raw DW from Lake Geneva	0.032 (median)	Morasch et al., 2010[50]
	China	Tap water in Macao (n=12)	0.002-0.008	Yiruhan et al., 2010[51]
	China	Tap water in Guangzhou (n=10)	0.006-0.680	Yiruhan et al., 2010[51]

	Spain	Llobregat River Aquifer (GW)	0.014-0.324	Cabeza et al., 2012[52]
	Spain	DW in Barcelona	0.013 (max)	López-Serna et al., 2010[47]
	Switzerland	Raw DW from Lake Geneva	0.014 (median)	Morasch et al., 2010[50]
	Spain	DW in Barcelona	0.004 (max)	López-Serna et al., 2010[47]
Clarithromycin	Switzerland	GW in Switzerland	0.004 (max)	Huntscha et al., 2012[53]
Clindamycin	Switzerland	Raw DW from Lake Geneva	0.004 (median)	Morasch et al., 2010[50]
Enoxacin	Spain	DW in Barcelona	0.016 (max)	López-Serna et al., 2010[47]
	USA	Ground- (n=25) and surface-water (n=49) sources of DW	0.040 (max)	Focazio et al., 2008[46]
	China	Tap water in Macao (n=12)	0.003-0.005	Yiruhan et al., 2010[51]
	China	Tap water in Guangzhou (n=10)	0.008 (max)	Yiruhan et al., 2010[51]
Enrofloxacin	Spain	DW in Barcelona	0.019 (max)	López-Serna et al., 2010[47]
	USA	Finished DW in North Carolina	0.005 (max)	Ye et al., 2007[40]
	USA	Ground- (n=25) and surface-water (n=49) sources of DW	0.040 (max)	Focazio et al., 2008[46]
	Portugal	GW, Lisbon	0.004 (max)	de Jesus Gaffney et al., 2015[54]
	Portugal	DW, Lisbon	0.005 (max)	de Jesus Gaffney et al., 2015[54]
Erythromycin	Spain	Llobregat River Aquifer (GW)	0.154 (max)	Cabeza et al., 2012[52]
Florfenicol	China	Tap water in Shanghai	0.00082-0.024	Wang et al., 2016[42]
Flumequine	USA	Finished DW in North Carolina	0.003 (max)	Ye et al., 2007[40]
Josamycin	Spain	DW in Barcelona	0.001 (max)	López-Serna et al., 2010[47]
Lincomycin	Netherlands	DW produced from Rhine, Meuse or Polder River	0.001 (max)	Houtman et al., 2014[55]
	China	Tap water in Macao (n=12)	0.009-0.037	Yiruhan et al., 2010[51]
Lomefloxacin	China	Tap water in Guangzhou (n=10)	0.179 (max)	Yiruhan et al., 2010[51]
	China	Tap water in Macao (n=12)	0.007-0.017	Yiruhan et al., 2010[51]
	China	Tap water in Guangzhou (n=10)	0.083 (max)	Yiruhan et al., 2010[51]
Norfloxacin	Spain	DW in Barcelona	0.033 (max)	López-Serna et al., 2010[47]
	Switzerland	Raw DW from Lake Geneva	0.006 (median)	Morasch et al., 2010[50]
Ofloxacin	Spain	Llobregat River Aquifer (GW)	0.006 (max)	Cabeza et al., 2012[52]

	Spain	DW in Barcelona	0.015 (max)	López-Serna et al., 2010[47]
Oxolinic acid	USA	Finished DW in North Carolina	0.004 (max)	Ye et al., 2007[40]
Oxytetracycline	China	Tap water in Hong Kong	0.001	Li et al., 2017[41]
Roxithromycin	USA	Finished DW in North Carolina	0.001 (max)	Ye et al., 2007[40]
Sarafloxacin	USA	Ground- (n=25) and surface-water (n=49) sources of DW	0.020 (max)	Focazio et al., 2008[46]
Spiramycin	Spain	DW in Barcelona	0.021 (max)	López-Serna et al., 2010[47]
Sulfabenzamide	Spain	GW in Catalonia	0.002 (max)	García-Galán et al., 2010[56]
	Portugal	GW, Lisbon	0.002 (max)	de Jesus Gaffney et al., 2015[54]
	Portugal	DW, Lisbon	0.001 (max)	de Jesus Gaffney et al., 2015[54]
Sulfadiazine	Spain	GW in Catalonia	0.001 (max)	García-Galán et al., 2010[56]
	Switzerland	Raw DW from Lake Geneva	0.002 (median)	Morasch et al., 2010[50]
Sulfadimethoxine	Spain	GW in Catalonia	0.002 (max)	García-Galán et al., 2010[56]
Sulfadoxine	Spain	GW in Catalonia	0.004 (max)	García-Galán et al., 2010[56]
Sulfamerazine	Spain	GW in Catalonia	0.003 (max)	García-Galán et al., 2010[56]
	Portugal	GW, Lisbon	0.001 (max)	de Jesus Gaffney et al., 2015[54]
	Portugal	DW, Lisbon	0.001 (max)	de Jesus Gaffney et al., 2015[54]
	Spain	Llobregat River Aquifer (GW)	0.023-0.084	Cabeza et al., 2012[52]
	Spain	GW in Catalonia	0.004 (max)	García-Galán et al., 2010[56]
	Spain	DW in Barcelona	0.004 (max)	López-Serna et al., 2010[47]
Sulfamethazine	Switzerland	GW in Switzerland	0.006 (max)	Huntscha et al., 2012[53]
	USA	Finished drinking water	0.003 (max)	Benotti et al., 2009[57]
	Switzerland	Raw DW from Lake Geneva	0.014 (median)	Morasch et al., 2010[50]
	Europe	164 GW from 23 countries	0.002 (mean)	Loos et al., 2010[58]
	Netherlands	DW produced from Rhine, Meuse or Polder River	0.013 (max)	Houtman et al., 2014[55]
	Japan	Source water of DW purification plants (n=6)	0.019 (max)	Simazaki et al., 2015[48]
	USA	DW samples from 29 DWTPs	0.008 (max)	Glassmeyer et al., 2017[59]
	Portugal	GW, Lisbon	0.002 (max)	de Jesus Gaffney et al., 2015[54]
Sulfamethoxazole	Portugal	DW, Lisbon	0.001 (max)	de Jesus Gaffney et al., 2015[54]

	Spain	Llobregat River Aquifer (GW)	0.009-0.046	Cabeza et al., 2012[52]
	Spain	GW in Catalonia	0.064 (max)	García-Galán et al., 2010[56]
	Switzerland	GW in Switzerland	0.015 (max)	Huntscha et al., 2012[53]
Sulfamethoxypyridazine	Spain	GW in Catalonia	0.001 (max)	García-Galán et al., 2010[56]
	Portugal	GW, Lisbon	0.007 (max)	de Jesus Gaffney et al., 2015[54]
	Portugal	DW, Lisbon	0.002 (max)	de Jesus Gaffney et al., 2015[54]
	Spain	Llobregat River Aquifer (GW)	0.016-0.021	Cabeza et al., 2012[52]
Sulfapyridine	Spain	GW in Catalonia	0.001 (max)	García-Galán et al., 2010[56]
	Netherlands	DW produced from Rhine, Meuse or Polder River	0.026 (max)	Houtman et al., 2014[55]
Sulfaquinoxaline	Spain	GW in Catalonia	0.001 (max)	García-Galán et al., 2010[56]
Sulfathiazole	USA	Finished water in DW purification plant	0.01 (max)	Stackelberg et al., 2007[60]
Sulfisoxazole sodium	Japan	Source water of DW purification plants (n=6)	0.013 (max)	Simazaki et al., 2015[48]
Tiamulin	Netherlands	DW produced from Rhine, Meuse or Polder River	0.055 (max)	Houtman et al., 2014[55]
Thiamphenicol	China	Tap water in Shanghai	0.00084-0.022	Wang et al., 2016[42]
	USA	Ground- (n=25) and surface-water (n=49) sources of DW	0.020 (max)	Focazio et al., 2008[46]
	Switzerland	Raw DW from Lake Geneva	0.009 (median)	Morasch et al., 2010[50]
	Netherlands	DW produced from Rhine, Meuse or Polder River	0.056 (max)	Houtman et al., 2014[55]
Trimethoprim	Spain	DW in Barcelona	0.001 (max)	López-Serna et al., 2010[47]
Tylosin	USA	Finished DW in North Carolina	0.004 (max)	Ye et al., 2007[40]

3.6 Antibiotics' concentration in aquaculture

Aquaculture is the farming of aquatic animals and plants in fresh, marine and brackish water. The first aquaculture farms were small in size with low stock density and minimal additional treatments to promote food production. The rapid population growth during the 20th century and the parallel increased demand for high-quality proteins supplied by finfish and shellfish has determined a subsequent strong diffusion of aquaculture practices. In 2009, almost 50% of the world's consumed seafood was produced by aquaculture and in 2011, the aquaculture's revenue has been estimated at around €3 billion per year in Europe, with greater gains than the catching sector[61,62]. Among the species primarily reared in aquaculture farms, the seabass, the trout, the salmon and mussels are the most representative in Europe, where the five Member States (MS), United Kingdom, Italy, France, Greece and Spain, are the main producers of aquaculture products, accounting for almost 70% of the total European production[62]. The aquaculture industry has contributed to increase the seafood production and to meet their demand on the market since 1970. Due to the growing request for seafood, aquaculture methods have increasingly shifted from extensive systems (where no feeding activity are provided) to semi-intensive (where food is supplemented) or intensive systems (where all nutritional requirements are provided by commercial feeds) in order to boost the production. Increases in industrialisation of aquacultures has been followed by the intensification of fish density, stressful conditions and nutrient pollution, resulting in poor water quality and the growing use of antibiotics to avoid the spread of diseases that would cause serious losses in production and sales. One of the examples of environmental sustainability in aquaculture is given by the reduction in use of antibiotics in aquaculture in favour of vaccines. Currently, the situation in Europe does not seem to be a cause for concern. Indeed a European Report published in 2015 shows that only 0.32% of the total samples (1,546) derived from aquaculture environments and analysed in 28 MS for the presence of antibacterials (which also include antibiotics), were considered non-compliant samples[63].

Regarding vaccines, this practice is one of the factors which has fostered the development of the salmonid aquaculture industry in countries such as Norway. Nowadays, most of the fish vaccines are administered by intra-peritoneal injection, but other methods include the immersion of the fish for a few second in a vaccine solution and the oral vaccination obtained by mixing the antigens into the feed[64,65]. Several licensed vaccines are today available for the Atlantic salmon, one of the most representative salmonid species in global aquaculture, whose worldwide production in 2011 was 1.619.200 tonnes[66]. In Norway, salmons are prevalently vaccinated against diseases like furunculosis, vibriosis, cold-water vibriosis and winter ulcer, contributing to the decrease in antibiotic use without causing negative effects for the industry[65,66].

Although the large industrial scale vaccination was initially developed for salmonid species including Atlantic salmon, coho salmon, rainbow trout and ayu, vaccines are today available for 17 species of fish and target more than 28 diseases caused by viruses or bacteria[66]. Efforts are now focused on creating more oral vaccines due to their simple delivery and low-cost production or using expression systems like yeast to get round the problem of producing vaccines for viruses not easily culturable in the laboratory. The final goal is to develop alternative methods to reduce the cost of vaccination and produce vaccines with a strong and long-lasting protection for most of the fish species.

There are very few studies on antibiotic residues in aquacultures and considering the increasing human consumption of aquaculture products, more investigations should be carried out and data from farmers and institutions should be made public to be aware of antibiotic concentrations in aquacultures.

3.7 Antibiotic use in farms

The ever-increasing demand of food animal production has been the main reason for the intensification of antibiotic use in livestocks not only as a therapy but also as metaphylaxis. The metaphylaxis involves the administration of high doses of antibiotics to the whole flock for a short period of time even if the clinical symptoms are exhibited only in a few animals. The aim of this mass medication is to eliminate or decrease the outbreak of diseases that could harm the livestock, preventing health problems and economic losses at the subproductive level.

Subtherapeutic levels of antibiotics have been extensively used as growth promoters in animal farming. The growth promotion refers to the administration of antibiotics in healthy animals to increase the growth rates and food efficiency. The exact mechanism of antimicrobial growth promoters is still unclear but it seems that it is related to interactions between antibiotics with intestinal microbial population[67].

These above-mentioned livestock antibiotic use practices have promoted a selective pressure for bacteria that are resistant to antibiotics, resulting in an increasing awareness of the effects of antimicrobial drug use in animal's commensal flora. In Sweden, the use of antibiotics for growth promotion in animal farming was banned in 1986, while in Denmark, the addition of the two antibiotics avoparcin and virginiamycin to animal feeds was outlawed in 1995[68]. In the European Union (EU), avoparcin was banned as growth promoter in 1997 and two years later the use of bacitracin, spiramycin, tylosin and virginiamycin was also prohibited[68]. The general use of antimicrobial drugs for growth promotion was definitively banned in Europe since January 2006 and their use was phased out in the United States (USA) in 2017[69]. As of today, antibiotics can be used in the USA and in Europe to treat, control or prevent infections in livestocks. In 2011, the European Commission has published a report stating the importance of the "*Introduction of the new Animal Health Law, which will focus on prevention of diseases, reducing the use of antibiotics and replacing current Animal Health provisions based on disease control*"[70]. A recent joint opinion has been published by the European Medicines Agency (EMA) and the European Food Safety Authority (EFSA) on measures to reduce the antimicrobial use in animal husbandry and the impacts on food safety in Europe[71]. In this document is underlined the need to phase out the preventive use of antimicrobials at national level and to reduce metaphylaxis by adopting recognised alternative measures[71]. Outlawing the use of antibiotics for prevention or growth promotion purposes represents an important step to assure a responsible use of antimicrobial drugs. This approach will surely contribute to reduce the phenomena of resistance as well as the spreading of the resistant strains in the environment.

The ongoing broad use of antibiotics by livestock industry results in their constant introduction into the environment besides being a risk to public health due to human consumption of meat and animal derivatives. However, in Italy, only 53 of 159.543 bovine milk samples analysed during routine quality control in 2001 were found positive for some antibiotics like penicillin G, amoxicillin and cephalosporin. Penicillin G was detected in 26 samples at concentrations ranging from 3.7±0.4 µg/l to 6240±550 µg/l; amoxicillin was found in 3 samples at concentrations ranging from 8.5±0.1 µg/l to 53.7±2.3 µg/l and cephapirin was identified in 2 samples at the concentration of 5.7±0.1 µg/l and 6.4±0.3 µg/l[72]. The antibiotic concentrations were high respect to the Maximum Residue Limit (MRL) set by the European Union (EU) Regulation 2377/90[73] but the total number of milk samples found positive at the microbial test was very low and not worrying in terms of human health. In 2015, a European report summarised the monitoring data on the detection of veterinary medical product residues and other substances in live animals and animal products in the EU. The total number of samples analysed by 28 Member States (MS) for antibacterial detection was 114.485. For the antibacterial category, which also includes antibiotics, a percentage of 0.20% of the samples analysed under the Directive 96/23/European Commission (EC) and including bovines, pigs, sheep/goats, horses, poultry, aquaculture, milk, eggs, rabbits and honey, were non-compliant samples. The

highest percentage of non-compliant samples was reported for honey (0.95%) and in general, the percentage of non-compliant samples (0.20%) was comparable to the previous eight years (0.18-0.29%)[74].

It should be mentioned that when livestocks are treated with antibiotics, the application of manure as organic fertilizer in agriculture can mediate the contamination of soil and surface waters. A tetracycline concentration equivalent to 23 mg/kg has been detected in pig manure samples from Austria, while in China, the antibiotics norfloxacin and enrofloxacin were found in chicken manure at concentrations of 225 and 1420 mg/kg, respectively[75,76]. In addition, accumulation of antibiotics was observed in crops under manure fertilisation[77,78]. This means that antibiotics may be transferred into the environment through the cyclic application of manure, thus causing potential ecological risks from exposure to these contaminants.

Agricultural runoff is also considered a source of antibiotics. It refers to the water leaving farms that runs over agricultural land and then flows in surface water rather than being absorbed into groundwater or evaporating. Agricultural runoff can be caused by meteorological factors (e.g. type of precipitations, rainfall intensity) or can be influenced by agricultural activities which are not well-managed (e.g overgrazing). Concentrations of antibiotics detected in agricultural runoff are listed in the Table 3.

Table 3. Antibiotics in agricultural runoff

Substance	Country	Source of monitoring data	MEC (µg/L)	Reference
	France	Cojeul River (agricultural livestock impact)	0.001	Tlili et al., 2016[79]
Ampicillin	France	Fresnoy Lagoon (agricultural livestock impact)	0.006	Tlili et al., 2016[79]
	France	Cojeul River (agricultural livestock impact)	0.004	Tlili et al., 2016[79]
Chlortetracycline	France	Fresnoy Lagoon (agricultural livestock impact)	0.017	Tlili et al., 2016[79]
	France	Cojeul River (agricultural livestock impact)	0.007	Tlili et al., 2016[79]
Ciprofloxacin	France	Fresnoy Lagoon (agricultural livestock impact)	0.006	Tlili et al., 2016[79]
Clarithromycin	USA	Agricultural runoff at the coastline of Maumee Bay (Lake Erie)	0.072 (max)	Wu et al., 2009[80]
Clindamycin	USA	Agricultural runoff at the coastline of Maumee Bay (Lake Erie)	0.011 (max)	Wu et al., 2009[80]
	France	Cojeul River (agricultural livestock impact)	0.085	Tlili et al., 2016[79]
Danofloxacin	France	Fresnoy Lagoon (agricultural livestock impact)	0.05	Tlili et al., 2016[79]
	France	Cojeul River (agricultural livestock impact)	0.036	Tlili et al., 2016[79]
Difloxacin	France	Fresnoy Lagoon (agricultural livestock impact)	0.026	Tlili et al., 2016[79]
	Poland	Water from water supply systems of 25 food-producing animal farms	1650 (max)	Gbylik-Sikorska et al., 2015[81]
	France	Cojeul River (agricultural livestock impact)	0.005	Tlili et al., 2016[79]
Doxycycline	France	Fresnoy Lagoon (agricultural livestock impact)	0.012	Tlili et al., 2016[79]
	Poland	Water from water supply systems of 25 food-producing animal farms	1670 (max)	Gbylik-Sikorska et al., 2015[81]
	France	Cojeul River (agricultural livestock impact)	0.04	Tlili et al., 2016[79]
Enrofloxacin	France	Fresnoy Lagoon (agricultural livestock impact)	0.025	Tlili et al., 2016[79]
Erythromycin	USA	Agricultural runoff at the coastline of Maumee Bay (Lake Erie)	0.438 (max)	Wu et al., 2009[80]
Flumequine	Poland	Water from water supply systems of 25 food-producing animal farms	3.48 (max)	Gbylik-Sikorska et al., 2015[81]
	USA	Agricultural runoff at the coastline of Maumee Bay (Lake Erie)	0.005 (max)	Wu et al., 2009[80]
Lincomycin	Poland	Water from water supply systems of 25 food-producing animal farms	304 (max)	Gbylik-Sikorska et al., 2015[81]
	France	Cojeul River (agricultural livestock impact)	0.018	Tlili et al., 2016[79]
Monensin	France	Fresnoy Lagoon (agricultural livestock impact)	0.017	Tlili et al., 2016[79]
Neomycin	Poland	Water from water supply systems of 25 food-producing animal farms	32 (max)	Gbylik-Sikorska et al., 2015[81]
	France	Cojeul River (agricultural livestock impact)	0.008	Tlili et al., 2016[79]
Norfloxacin	France	Fresnoy Lagoon (agricultural livestock impact)	0.008	Tlili et al., 2016[79]
	France	Cojeul River (agricultural livestock impact)	0.008	Tlili et al., 2016[79]
Ofloxacin	France	Fresnoy Lagoon (agricultural livestock impact)	0.007	Tlili et al., 2016[79]
Orbifloxacin	France	Cojeul River (agricultural livestock impact)	0.033	Tlili et al., 2016[79]

	France	Fresnoy Lagoon (agricultural livestock impact)	0.028	Tlili et al., 2016[79]
	France	Cojeul River (agricultural livestock impact)	0.001	Tlili et al., 2016[79]
Oxytetracycline	France	Fresnoy Lagoon (agricultural livestock impact)	0.001	Tlili et al., 2016[79]
	France	Cojeul River (agricultural livestock impact)	0.018	Tlili et al., 2016[79]
Sulfadiazine	France	Fresnoy Lagoon (agricultural livestock impact)	0.02	Tlili et al., 2016[79]
	France	Cojeul River (agricultural livestock impact)	0.021	Tlili et al., 2016[79]
Sulfadimethoxine	France	Fresnoy Lagoon (agricultural livestock impact)	0.023	Tlili et al., 2016[79]
	France	Cojeul River (agricultural livestock impact)	0.011	Tlili et al., 2016[79]
Sulfamerazine	France	Fresnoy Lagoon (agricultural livestock impact)	0.02	Tlili et al., 2016[79]
Sulfamethazine	USA	Agricultural runoff at the coastline of Maumee Bay (Lake Erie)	0.010 (max)	Wu et al., 2009[80]
	USA	Agricultural runoff at the coastline of Maumee Bay (Lake Erie)	0.112 (max)	Wu et al., 2009[80]
	Poland	Water from water supply systems of 25 food-producing animal farms	58.7 (max)	Gbylik-Sikorska et al., 2015[81]
	France	Cojeul River (agricultural livestock impact)	0.014	Tlili et al., 2016[79]
Sulfamethoxazole	France	Fresnoy Lagoon (agricultural livestock impact)	0.013	Tlili et al., 2016[79]
	France	Cojeul River (agricultural livestock impact)	0.008	Tlili et al., 2016[79]
Sulfathiazole	France	Fresnoy Lagoon (agricultural livestock impact)	0.013	Tlili et al., 2016[79]
	France	Cojeul River (agricultural livestock impact)	0.011	Tlili et al., 2016[79]
Tetracycline	France	Fresnoy Lagoon (agricultural livestock impact)	0.01	Tlili et al., 2016[79]
Tiamulin	Poland	Water from water supply systems of 25 food-producing animal farms	66.8 (max)	Gbylik-Sikorska et al., 2015[81]
Tilmicosin	Poland	Water from water supply systems of 25 food-producing animal farms	1.73 (max)	Gbylik-Sikorska et al., 2015[81]
	USA	Agricultural runoff at the coastline of Maumee Bay (Lake Erie)	0.252 (max)	Wu et al., 2009[80]
	Poland	Water from water supply systems of 25 food-producing animal farms	17.8 (max)	Gbylik-Sikorska et al., 2015[81]
	France	Cojeul River (agricultural livestock impact)	0.026	Tlili et al., 2016[79]
Trimethoprim	France	Fresnoy Lagoon (agricultural livestock impact)	0.028	Tlili et al., 2016[79]

4. Antibiotic Resistance

The antimicrobial resistance (AMR) is defined as the ability of microorganisms to resist the effects of antimicrobial treatments, especially antibiotics. However, since the advent of the antimicrobial medicine, the increasing use and misuse of antibiotics have contributed to the spread of resistant bacteria. The antibiotic resistance (ABR) results in the ineffectiveness of medical treatment for bacterial diseases thus increasing the morbidity/mortality rates of affected patients.

ABR is a natural process in bacteria. The intrinsic or natural resistance is a mechanism attributed to an innate inability of responding to certain antibiotic agents in order to guarantee the normal cell functions. The Gram-negative bacteria are for example intrinsically resistant to the antibiotic vancomycin, the large molecular size of which does not allow the penetration through the outer bacterial membrane[82]. Another example is *Pseudomonas aeruginosa*, whose innate resistance to many antibiotics is likely to be due to its low membrane permeability[83]. Bacteria can also acquire resistance to antibiotics. The acquired or active resistance implies genetic modifications in microorganisms so that a particular antibiotic agent that was once effective against the organism, becomes ineffective. The acquired resistance is the major mechanism of antimicrobial resistance.

Resistance mutations may confer a significant fitness cost for bacteria. Bacterial fitness is defined as the ability to replicate in a given environment and when bacteria become resistant to an antibiotic, their growth rate decreases hence compromising their virulence and transmissibility. However, the fitness cost may be reduced or eliminated as a result of additional genetic modifications that increase fitness without compromising resistance. This is of particular concern because it may cause the stabilisation of the resistance in a bacterial population[84].

A recent European report published in 2017 underlines the relationship between the consumption of antibiotics and the occurrence of antimicrobial resistance in bacteria from humans and food-producing animals[85]. The analysis was made by antimicrobial classes including fluoroquinolones, cephalosponins, polymixins and macrolides and suggests the prudent use of antibiotics for both humans and veterinary purposes.

4.1 Mechanisms of Antibiotic Resistance

Bacteria are characterised by a genetic plasticity that allows them to adapt to different environmental threats including the presence of antibiotic molecules that may compromise their survival. Antibiotic resistance (ABR), developed as a strategy to respond to the antibiotic occurrence, can be genetically mediated through either the acquisition of resistance genes from other bacteria or through the occurrence of spontaneous resistance mechanisms which favour the survival of microorganisms[86]. While some bacterial strains display intrinsic resistance, a bacterial population can gain resistance to antibiotics by the recombination of foreign DNA into the chromosome or *via* the mutation in key genes during replication. This mutation can then be passed to the subsequent generations leading to a population of resistant bacteria (vertical transmission), as shown in Figure 10A. More commonly, resistance genes can be acquired from other strains and species (horizontal transmission) through different mechanisms (Figure 10B): transformation (uptake of the free DNA from the environment), transduction (transfer of DNA from a virus to bacteria) and conjugation (transfer of DNA between bacteria by direct cell-to-cell contact) (Figure 10B)[87].

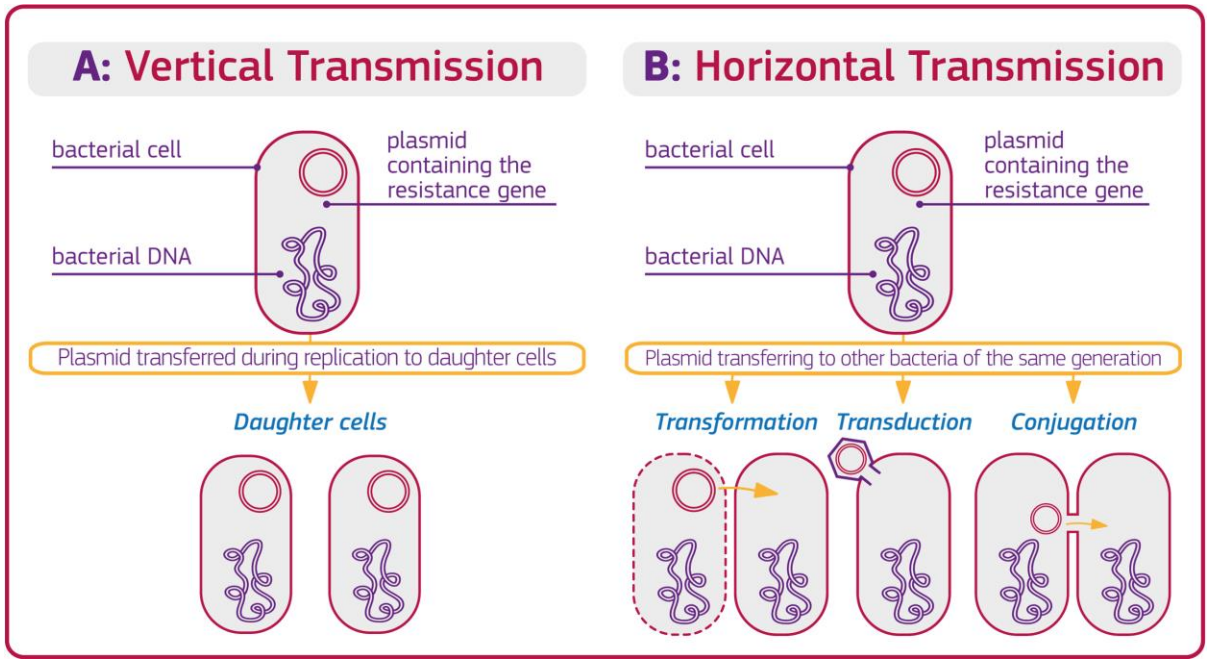

Figure 10. Mechanism of vertical and horizontal transmission in bacteria. A. During replication, the bacteria can transfer a resistance gene contained in a plasmid from a parent cell to the next generation (vertical transmission). **B.** The horizontal transmission in bacteria can be mediated by three principal mechanisms: transformation (uptake of the free DNA), transduction (virus-mediated gene transfer) and conjugation (transfer of DNA through a close contact between donor and recipient bacteria).

The reported increasing prevalence of ABR may be in large part caused by the misuse of antibiotics and by other factors including the use of antibiotics in agriculture, animal husbandry and household chores or by the prolonged hospitalisation and the ineffective infection-control practices in ill patients. The spread of ABR in humans is therefore influenced by the development of resistant organisms as a result of selective pressure of antimicrobial use and by their transmission from person to person. In this scenario, when resistant bacteria emerge following mutational events, the antibiotic acts on the susceptible bacteria and leaves the resistant population unchanged, making the treatment ineffective. Conjugation is the main strategy through which the resistance spreads. As shown in Figure 10, conjugation involves transfer of genetic material by cell-to-cell contact and the main mobile genetic elements (MGE) taking part in this mechanism are plasmids, transposons and integrons which ensure a genetic interchange in bacteria and play a crucial role in the dissemination of antimicrobial resistance[87]. The role of the environment and in particular of waterbodies like lakes, rivers or wastewater effluents in the spread of antimicrobial resistance is a matter of growing relevance. Indeed, waterbodies receive bacteria from different sources (e.g. hospitals, industries, farms) where specific strains have been probably selected by intensive antibiotic usage, and could promote a genetic exchange among environmental strains and allochthonous bacteria, leading to acquisition of new antibiotic resistances. The horizontal gene transfer favours the spread of antibiotic resistance in waterbodies because the resistance genes in bacteria can be localised on the bacterial chromosome as well as on the extrachromosomal elements like transposons and plasmids[88]. Bacteriophages, the viruses that infect bacteria, may also represent an efficient vector for the acquisition and dissemination of antibiotic resistance genes (ARG) and they could be an important source of ARG for their high survival capacity and their abundance in waterbodies[89-91]. Discharge of antibiotics into waterbodies could also have an impact on the introduction of new resistance genes in the environmental bacteria, which in turn can transfer their intrinsic resistance genes to humans, and therefore linking ABR in the environment with the resistance observed in clinic. In order to survive in the

presence of antibiotics, bacteria have evolved different resistance mechanisms. The most relevant are described in the following paragraphs (4.1.1 to 4.1.3).

4.1.1 Inactivation of antibiotics

One of the main mechanisms of antibiotic resistance is the bacterial ability to produce enzymes capable to inactivate the drug by hydrolysis or chemical modifications (Figure 11). The biochemical reactions catalysed by the enzymes include: acetylation, phosphorylation and adenylation.

Aminoglycoside modifying enzymes (AME) are an example giving resistance through biochemical modification of the aminoglycosides, antibiotics that inhibit protein synthesis. The AME covalently modify the OH and NH_2 groups of the substrate, and three cases can be distinguished: i) the aminoglycoside acetyltransferases which modify the target antibiotics by transferring the acetyl group from acetyl-CoA; ii) the aminoglycoside phosphatases which transfer the phosphoryl group from adenosine triphosphate (ATP) to the substrate; and finally iii) the aminoglycoside adenylyltransferases which catalyse the transfer of the adenosine monophosphate (AMP) group from ATP[92]. All these enzymatic modifications have the final effect of lowering the affinity of the drug for the target so that antibiotics are not able anymore to exert their antibacterial properties.

The chloramphenicol acetyltransferases (CAT) represent another class of enzymes capable to modify chemically the substrate. They transfer the acetyl group from acetyl-CoA to chloramphenicol and as for the AME, the enzymatic inactivation of the drug catalysed by CAT results in the reduced binding between the antibiotic and the target[83].

One of the best examples of resistance *via* hydrolysis is represented by the well-characterised enzymes β-lactamases. The β-lactam antibiotics including penicillin and cephalosporin contain a chemical structure named β-lactam ring. This structure is capable of binding to the enzymes Penicillin Binding Proteins (PBP) that help build the peptidoglycan layer. The β-lactams interfere with the crosslinking of the peptidoglycan by binding the PBP and thus preventing the bacterial cell wall synthesis. By inhibiting the cell wall synthesis, the bacterial cell is damaged due to osmotic instability or autolysis. The enzymes β-lactamases hydrolyse the β-lactam ring and prevent the binding between antibiotics and PBP, thus rendering the antimicrobial ineffective[86].

To date, many different types of β-lactamases have been described and classified according to their biochemical functions (Bush-Jacob classification) or their structural characteristics (Ambler classification) (Table 4). As shown in Table 4, the Ambler classification suggests four different molecular classes: A, B, C and D[93]. Class A enzymes include penicillinases, carbapenemases, cephalosporinases and extended-spectrum β-lactamases (ESBL). These enzymes are inhibited by sulbactam, tazobactam and clavulanic acid including monobactams but not cephamycins. Cefotaximase-M (CTX-M) type enzymes belong to the ESBL and they were probably acquired from *Kluyvera*, a bacterial genus found in the soil. The genes coding for CTX-M enzymes have been found associated to transposons or insertion sequences and they have been isolated especially in cephalosporin-resistant *Escherichia coli* and *Klebsiella pneumoniae*. Class B enzymes include metallo-β-lactamases, so called because they utilise zinc^{2+} (Zn^{2+}) as a cofactor for the hydrolysis of the β-lactam ring (Table 4). One example of metallo-β-lactamases is represented by the New Delhi metallo-beta-lactamase-1 (NMD-1). The *blaNMD* gene has been found located on plasmids and on the host chromosome and it is characterised by a high mobility in a short span of time. This enzyme is particularly found in Gram-negative bacteria including *Escherichia coli* and *Klebsiella pneumoniae* worldwide. In addition, its discovery in soil and drinking water (DW) suggests a potential threat to human health.

Finally, Class C and Class D β-lactamases are enzymes like AmpC and oxacillin hydrolysing enzymes (OXA), respectively (Table 4). The AmpC β-lactamase is a cephalosporinase and the gene *blaAmpC* is located both on the chromosome and on plasmids. The OXA enzymes

have the ability to hydrolyse oxacillin and are often associated to mobile gene elements (MGE). They are now widely spread in *Acinetobacter baumannii* but also in other bacteria like *Escherichia coli* and *Klebsiella pneumoniae.*

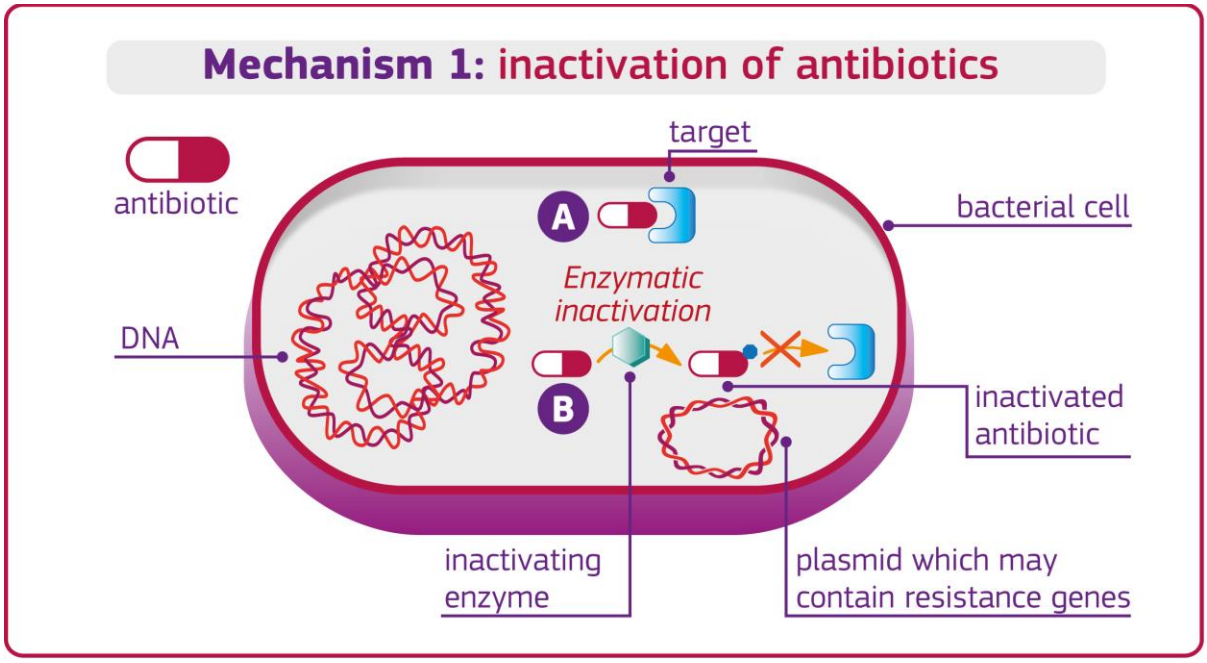

Figure 11. Mechanisms of antimicrobial resistance: inactivation of antibiotics. A. When the antibiotics enter the bacteria, they exert their activity by binding to a specific target. **B.** Bacteria can acquire or develop resistance to antibiotics through the activity of enzymes which hydrolyse or chemically modify the antibiotics preventing their binding to the target (e.g. β-lactamases, aminoglycosides, glycopeptides, tetracyclines).

Table 4. Ambler classification of β-lactamases

Ambler Class	Representative enzyme types	Examples of enzymes
Class A	Penicillinases Carbapenemases Cephalosporinases Extended-Spectrum β-Lactamases Broad-spectrum-β-Lactamases	CTX-M, TEM, SHV, KPC
Class B	Metallo-β-lactamases	IMP, VIM, NDM-1
Class C	Penicillinases Cephalosporinases	AmpC
Class D	Oxacillin hydrolyzing enzymes	OXA

CTX-M: cefotaximase-M, **SHV**: sulfhydryl variable enzymes, **KPC**: *Klebsiella pneumoniae* carbapenemases, **IMP**: imipenemase metallo-β-lactamases, **VIM**: Verona integron encoded metallo-β-lactamases, **NDM-1:** New Delhi metallo-β-lactamase-1, **OXA**: oxacillin hydrolyzing enzymes.

4.1.2 Decrease of antibiotic penetration and pumping of antibiotics out of cells

The susceptibility of bacteria to a specific antibiotic is determined by their ability to reduce the cellular internalisation of the drug, or on the contrary, to favour antibiotic extrusion mechanisms as shown in Figure 12 and 13. Bacteria have therefore developed strategies to reduce the quantity of antibiotics able to cross the cell membrane to survive in their presence. Mechanisms used by bacteria to reduce the intracellular antibiotic accumulation include either the downregulation of protein channels (porins) localised on the outer membrane to reduce the drug influx into the cells (Figure 12 and Figure 14), or the expression of efflux pumps to remove the antibiotics already present within the cells (Figure 13 and Figure 14)[94]. The amount of porins is particularly relevant for Gram-negative bacteria whose outer membrane is an important barrier that provides protection against toxic compounds and that must be overcome to allow antibiotics to penetrate the bacteria cell envelope and reach their intracellular targets. Bacteria can regulate outer membrane permeability by modulating the expression of porins (Figure 14). The downregulation of these proteins or their replacement with selective channels, implies a limited access of antibiotics into the cells. This intrinsic mechanism of antibiotic resistance can be achieved by two main processes: a) a modulation of porins expression; and b) an impairment of porins functions. All these mechanisms result in a decreased antibiotic penetration in bacteria and they mainly affect molecules such as β-lactams and tetracyclines which often use porins to pass through the cell membrane. As observed in clinical isolates, the low susceptibility of *Pseudomonas aeruginosa* to imipenem is due to the downregulation of the porin protein OprD and the reduced number of specific porins is responsible for resistance of *Pseudomonas* spp. and *Acinetobacter baumanii* to β-lactams[95,96]. An important contribution to the intrinsic antibiotic resistance is also given by the overexpression of efflux pumps, membrane proteins capable of extruding antibiotics from both the periplasm and cytoplasm of bacteria (Figure 14). Efflux pumps are able to export a wide range of substances so that the antibacterial concentration into the cells is so low that the drug cannot exert its function. Most efflux pumps have a broad substrate specificity but they can also be substrate-specific like the tetracycline (Tet) efflux pump, a membrane protein involved in the extrusion of tetracycline[97]. The ability of some efflux

pumps to interact with a wide range of antibiotics is explained by the formation of hydrophobic and electrostatic interactions between substrates and the central cavity of the membrane proteins. Up to date, there are five different families of efflux pumps, grouped according to their mechanisms and structural conformation, as shown in Figure 14: a) the major facilitator superfamily (MFS); b) the resistance-nodulation-cell division family (RND); c) the small multidrug resistance family (SMR); d) the multidrug and toxic compounds extrusion family (MATE); and e) the ATP-binding cassette superfamily (ABC)[87].

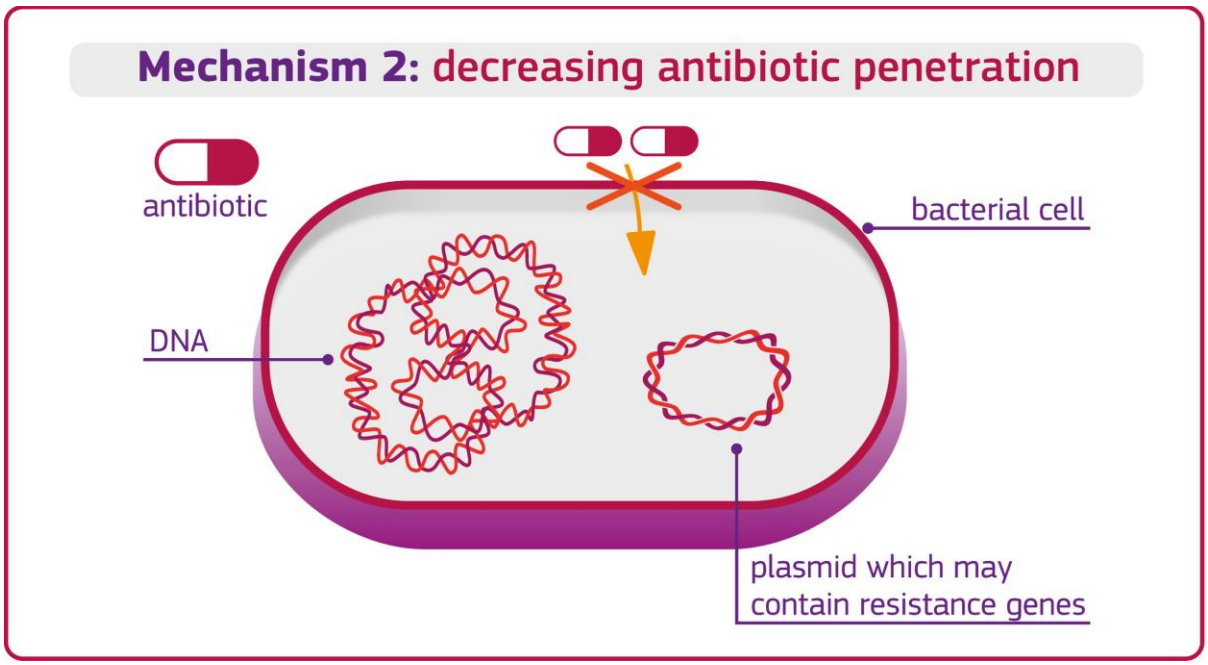

Figure 12. Mechanisms of antimicrobial resistance: decreasing antibiotic penetration. Bacteria can acquire or develop resistance to antibiotics by reducing the antibiotic intracellular concentration as a result of their low penetration into the bacteria (e.g. β-lactams, aminoglycosides, quinolones).

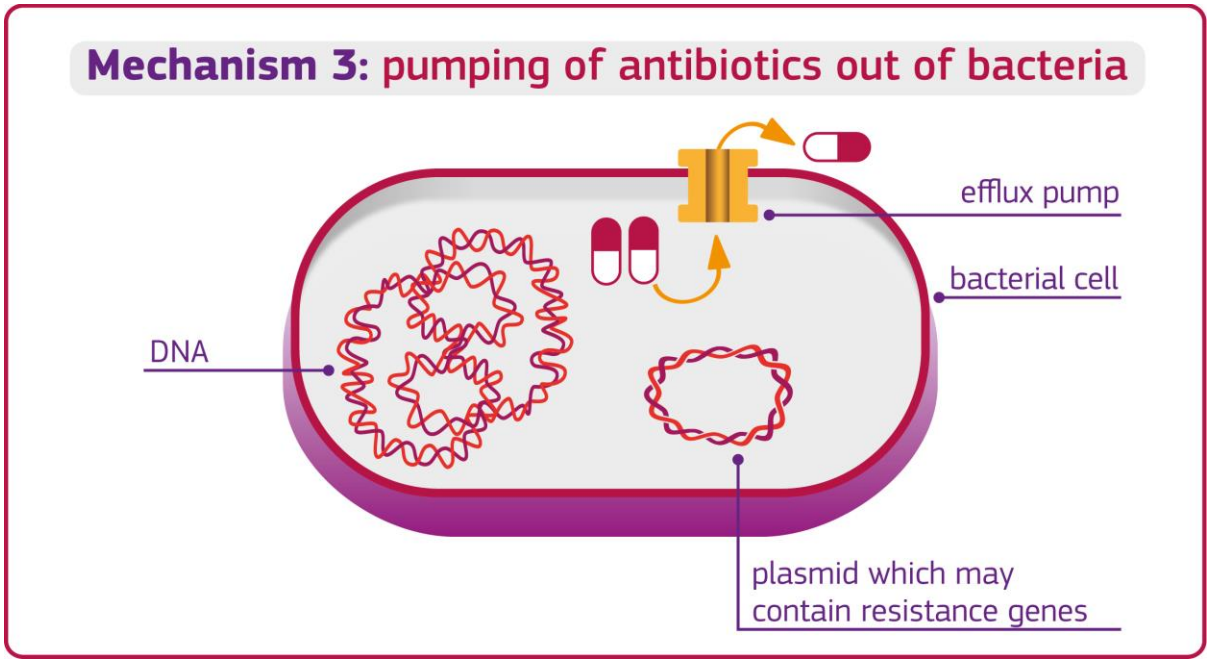

Figure 13. Mechanisms of antimicrobial resistance: pumping of antibiotics out of bacteria. Bacteria can acquire or develop resistance to antibiotics by reducing the antibiotic intracellular concentration as a result of their extrusion by efflux pumps (e.g. β-lactams, aminoglycosides, macrolides, quinolones).

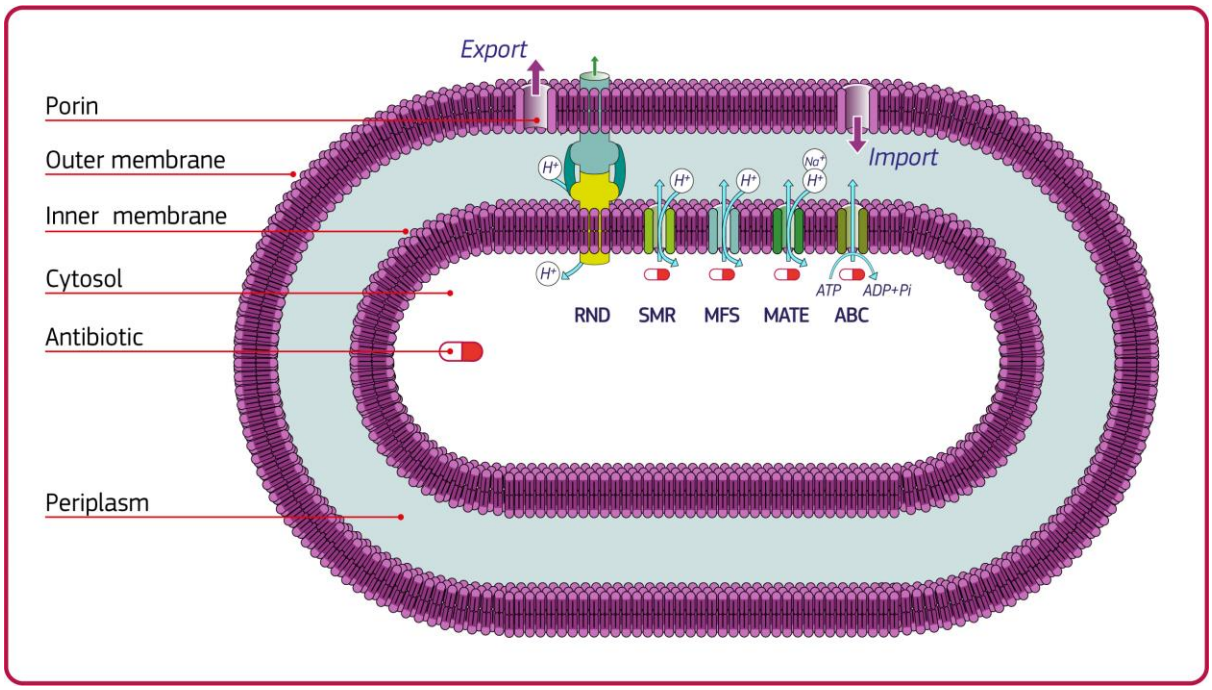

Figure 14. Porins and efflux pumps. Schematic representation of porins and efflux pumps in bacteria. Porins are membrane proteins allowing the uptake and extrusion of molecules while efflux pumps are transport proteins involved in mechanisms of drug extrusion. The main families of efflux transporters are: 1) resistance-nodulation-cell division family (RND), 2) small multidrug resistance family (SMR), 3) major facilitator superfamily (MFS), 4) multidrug and toxic compounds extrusion family (MATE), and 5) ATP-binding cassette superfamily (ABC). As shown in the figure, the drugs are pumped out of the cell by efflux pumps while H^+ or Na^+ are pumped into the cell. In the ABC family, the pumps are powered by adenosine triphosphate (ATP) which is hydrolysed to adenosine diphosphate (ADP) and inorganic phosphate.

Efflux pumps are encoded by genes located in mobile genetic elements (MGE) or on the chromosome. Efflux pumps included in the first four groups (Figure 14) use proton exchange as source of energy while the ABC family necessitates the energy generated by adenosine triphosphate (ATP) hydrolysis. Among these groups, the efflux pumps belonging to the RND superfamily are associated with resistance to a wide range of antibiotics (e.g. tetracycline, some β-lactams, fluoroquinolones) and other toxic compounds like dyes, bile salts and disinfectants[98]. In particular, the efflux pumps MexAB-OprM and MexCD-OprJ play a role in resistance to carbapenems, fluoroquinolones and aminoglycosides, thus contributing to the multidrug resistance (MDR) in bacteria[99]. Moreover, MexCD-OprJ has been found in many clinical isolates of *Pseudomonas aeruginosa*[100]. Sometimes, the conjunct modulated expression of porins and efflux pumps can result in the resistance to different antibiotics as in the case of imipenem and carbapenems in *Pseudomonas aeruginosa* clinical strains[101]. The expression of efflux proteins is controlled by specific transcription factors and by mutations localised in genes encoding these proteins or their molecular regulators. A deeper comprehension of molecular basis of the expression of efflux pumps could be helpful in preventing antibiotic efflux mechanisms and in designing novel therapeutics to prevent the overexpression of these proteins.

4.1.3 Change in target site

The target alteration is one of the mechanisms used by bacteria to induce antibiotic resistance. Indeed, most antibiotics exert their activities by binding to a specific target, preventing its function and consequently killing the bacteria or inhibiting their growth. As shown in Figure 15, the target modification comprises: i) the mutation in gene encoding the target; ii) the enzymatic modification of the target; and iii) the substitution of the classical target.

During a bacterial infection, a point mutation could compromise the functionality of an antibiotic target (see Figure 15A) so generating a strain with a resistance to the antibiotic which gives it a proliferative advantage over the strains without the mutation. The bacterial ribosome represents a major antibiotic target and the linezolid resistance is an example of target alteration. Briefly, the 23S ribosomal ribonucleic acid (rRNA), a linezolid target, may undergo a series of mutational events which decrease its affinity binding to the linezolid and induce the resistance reducing the efficiency of the antibiotic. However, as the genes encoding for the ribosomal target exist in multiple copies, the accumulation of mutations is necessary to observe a functional effect[102]. Another example of mutational alteration is the rifampicin (RIF) resistance due to a single-step point mutation event of the DNA-dependent RNA polymerase enzyme which inhibits the ability of RIF to block the bacterial transcription permitting this process to continue[103].

The modification of an antimicrobial target may involve not only mutational changes but can also be mediated by a chemical alteration of the target (see Figure 15A). The erythromycin ribosomal methylation (*erm*) gene encodes for an enzyme which catalyses the methylation of the 23S rRNA and results in the resistance to macrolide, lincosamine and streptogramin B[87]. The methylation of the target can be also mediated by the chloramphenicol-florfenicol resistance methyltrasferase. This enzyme specifically methylates the 23S rRNA and prevents its binding to phenicols, lincosamides, pleuromutilins and streptogramin A[104].

Another route to inhibit the antimicrobial activity is the replacement of natural targets with new molecules having a low affinity for the drug (see Figure 15B). This is the case of the Penicillin Binding Protein 2a (PBP2a) which acts as a substitute of the original Penicillin Binding Protein (PBP). As shown in Figure 15B, the protein PBP2a is expressed in methicillin-resistant *Staphylococcus aureus* (MRSA) by the acquired foreign gene *mecA* whose induction confers the resistant phenotype. The *mecA* gene is located in a gene cassette and scientific evidences suggest the high mobility rate of the allele. The methicillin binds weakly to the PBP2a thus enabling the bacteria to survive despite high levels of antibiotics[105].

The vancomycin resistance is another example of target alteration. Vancomycin blocks the bacterial cell wall synthesis by binding the terminal residues D-Alanine-D-Alanine (D-Ala-D-Ala) of peptidoglycan precursors. In vancomycin resistant strains, the D-Ala-D-Ala moiety of the growing peptidoglycan is substituted by the D-Lactate-D-Lactate or D-Serine-D-Serine groups and the affinity of vancomycin to its target is significantly reduced. Another mechanism is the destruction of the terminal group D-Ala-D-Ala preventing the binding between the antibiotic and its target[106].

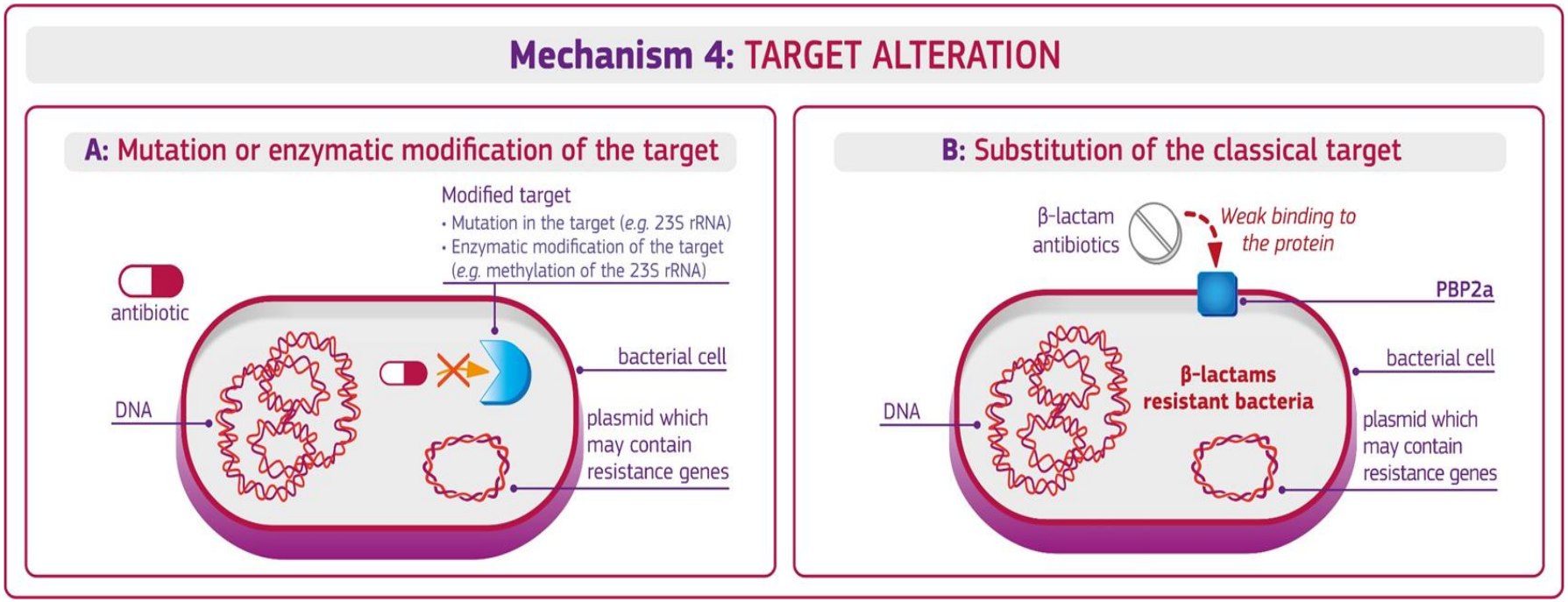

Figure 15. Mechanisms of antimicrobial resistance. A. A target alteration can be mediated by mutations or enzymatic modifications generating a protein (modified target) with reduced or null affinity for the antibiotics (e.g. β-lactamases, aminoglycosides, glycopeptides, macrolides, tetracyclines, sulfonamides). **B.** Substitution of the classical target can occur in β-lactams resistant bacteria (e.g. methicillin-resistant *Staphylococcus aureus* (MRSA)) where a resistance gene codifies for an altered form of Penicillin Binding Protein (PBP), called Penicillin Binding Protein 2a (PBP2a), which has a reduced affinity for β-lactams antibiotics. When PBP2a is expressed, it binds weakly to the antibiotics enabling bacteria to survive.

4.2 Antibiotic resistance in aquatic systems

Aquatic ecosystems contribute to the ecological productivity and provide many services to the society. These services include water for drinking, irrigation, and recreational activities. The quality and access to safe water is a matter of great importance. It is indeed known that pathogen contamination of water resources has a potential health risk and the recent spread of antibiotic resistance bacteria make this risk even more severe[107]. Moreover, antibiotic resistance has developed over time from a single antibiotic resistance to a multidrug resistance[108]. Multidrug resistant bacteria are insensitive to the administrated microbial medicines (which have different structures and molecular targets) and facilitate the spread of antibiotic resistance because of the failure of the microbial responses to standard treatments which can lead to a protracted illness[109].

Water is not only a way to disseminate antibiotic resistance genes (ARG) among human and animal populations but also a route by which ARG are introduced in natural ecosystems[110]. The resistant bacteria in the environment can act as an unlimited source of resistance genes not yet encountered in human pathogens and these genes can be then introduced in clinic, and vice versa[9,88]. An example of a direct exchange between the environmental and clinical resistome (defined as the complete set of resistance genes in bacteria) is given by the identification of the quinolone resistance gene *qnr*[110-112]. This gene was first detected in clinical isolates of *Klebsiella pneumoniae* in United States (USA) and it was more recently found in *Shewanella algae* (a Gram-negative species widely distributed in marine and freshwater) and in *Aeromonas* spp. in the Seine River (France)[113-115].

Resistant bacteria can be induced in the environment under selection pressures of the antibiotics and when this pressure disappears, ARG are not readily lost[116], meaning that ARG can be also detected in waterbodies without antibiotic contamination[117,118].

Several studies have investigated the presence of ARG in wastewater treatment plant (WWTP) effluents, where the maximal antibiotic concentrations, according to the data collected from the literature (see Table A in the Annex I), are usually between 0.1 µg/L and 1 µg/L[119]. One hundred and twenty-three different plasmid-encoded resistance-gene-specific amplicons have been detected in bacteria isolated from the effluent of a WWTP in Germany. Some of the genes detected are known to confer resistance to β-lactam, chloramphenicol, fluoroquinolone, tretracycline and sulfonamide antibiotics[120]. Another study reported the expression of ARG for quinolones, tetracyclines and sulfonamides in two WWTP in China and showed that the gene abundance decreased in the WWTP effluents respect to the influents[121].

From WWTP, ARG can be spread to different aquatic compartments such as lakes and rivers. A study showed that ARG for sulfonamide (*sul*), tetracycline (*tet*) and quinolone (*qnr*) antibiotics were widely distributed in selected urban lakes in China[122]. The abundance and diversity of 258 ARG were investigated in a highly polluted urban river in eastern China, and a Rijksinstituut voor Volksgezondheid en Milieu (RIVM) report showed high-levels of ampicillin-, aminoglycoside- and quinolone-resistant bacteria in the rivers Meuse, Rhine and New Meuse[123,124].

When bacteria adhere to surfaces in aqueous environments, they can form a complex matrix known as biofilm. These biofilms may be composed of a single species or, more frequently, by a complex community of microorganisms[125]. Biofilms represent a reservoir of ARG but their role in the acquisition and spread of antibiotic resistance has not been fully investigated in aquatic systems[125]. In biofilms, the antibiotic resistance is probably due to a combination of factors including a poor antibiotic penetration, the presence of slow-growing or stationary phase-cells and an altered microenvironment in which the oxygen availability and pH gradients may impact the antibiotic efficacy[126]. A study reported the expression of ARG for β-lactams, tetracyclines and sulfonamides in biofilms samples collected in WWTP in Spain and showed a significant increase in the relative abundances

of ARG when the samples were collected downstream of the WWTP discharge[127]. In New Zealand, ARG including one which conferred resistance to vancomycin were detected in freshwater biofilms collected in Taieri River[128]. The discovery that ARG are also expressed in biofilms in water contributes to a better understanding of the spread and persistence of antibiotic resistance in the environment.

4.3 Co-selection of antibiotic resistance

Non-antibiotic compounds like biocides and heavy metals may promote the antibiotic resistance through a phenomenon called co-selection[129-134]. As shown in Figure 16, co-selection occurs when a biochemical mechanism induces resistance to different compounds (e.g. biocides/metals and/or antibiotics) (cross-resistance), when genes conferring resistant phenotyphes are located on the same genetic element (e.g. resistance genes for antibiotics and/or metals/biocides) (co-resistance), or when a resistance gene for different substances (e.g. biocides/metals and/or antibiotics) is regulated by a single regulatory gene (co-regulation).

Biocides have a broad spectrum of antimicrobial activity and are commonly used as disinfectants in hospitals and farms as well as preservatives in pharmaceutical products, cosmetics and food[135]. Heavy metals are also used as antimicrobial agents in hospitals, industries and agricultures[136]. For example, urinary catheter coated with silver (Ag) showed a significant reduction of urinary tract infections in hospitalised patients[137].

The exposure of bacteria to environmental pollutants could result in a selection of resistance genes to biocides/metals and antibiotics[132].

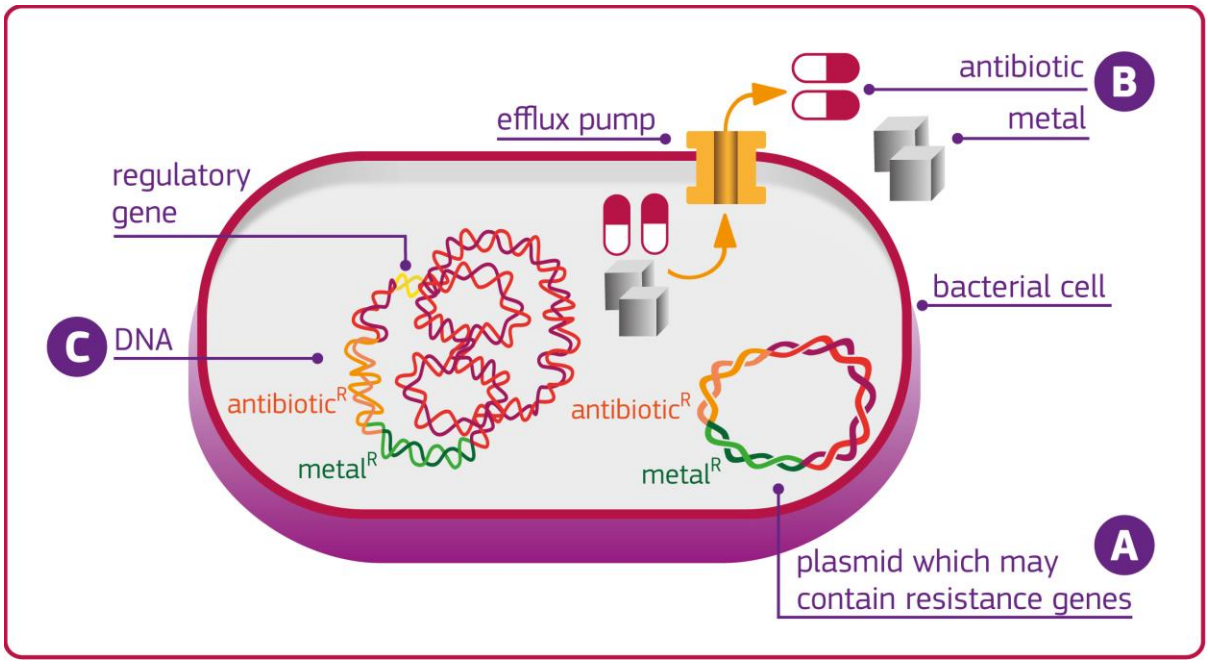

Figure 16. Co-selection mechanisms of antibiotic resistance. Antibiotic resistance can be co-selected through different mechanisms such as: **A.** Co-resistance that occurs when two or more genes are located on the same genetic element, **B.** Cross-resistance that takes place when one resistance mechanism provides resistance for different substances (e.g. antibiotics and/or metals) and **C.** Co-regulation that occurs when resistance genes for different substances (e.g. antibiotics and/or metals) are regulated by a single regulatory gene. **Antibiotic^R** stands for antibiotic resistance; **Metal^R** stands for metal resistance.

The co-selection process is mainly caused by co-resistance and cross-resistance (Figure 16). The co-resistance occurs when two or more genes that codify resistance to different agents are located on the same genetic element such as a plasmid (Figure 16A)[132]. These genetic elements can be transferred from one bacterium to another by horizontal gene transfer, hence causing the spread of resistance (see section 4.1)[138,139]. Resistance genes can also be carried in integrons, mobile genetic elements that play an important role in the worldwide dissemination of antibiotic resistance[132,140]. Class I integrons are assumed to catalyse co-selection because they often contain gene cassets that mediate resistance to antibiotics. Although they were originally associated with transposons and/or plasmids, they have also been detected on chromosomes[141]. A study in freshwater biofilms has demonstrated a dynamic exchange of gene cassets between different integron classes found in environmental, commensal and pathogenic bacteria allowing them to rapidly adapt to new environmental conditions[142].

Cross-resistance refers to the presence of a single mechanism that provides resistance to more than one substance (Figure 16B)[143]. It can occur when a single efflux pump can provide resistance to different classes of antibiotics and other substances such as metals[100,144]. Cross-resistance can also be mediated by mutations such as those observed in the cell membrane of *Pseudomonas aeruginosa* which confer resistance to different antibiotics as a consequence of a lower cell wall permeability and the activation of efflux systems[145,146].

The co-regulation mechanism happens when multiple resistance genes that provide resistance to different compounds are regulated by a single regulatory gene (Figure 16C)[132]. For example, the overexpression of efflux pumps and the simultaneous downregulation of porin pathways seem to be an effective mechanism to prevent intracellular accumulation of different substances[147].

Bacteria can be found in large proportions in aquatic systems, and their exposure to different chemical pollutants has the potential to allow them to develop resistance to different compounds, even to ones that they have never been exposed to, so increasing the risk of selecting organisms adapted to antibiotic agents[131]. Considering the levels of metal pollution in the environment, of particular concern is the role that these substances can play in the maintenance and spread of resistance to antibiotics.

4.3.1 Heavy metals and resistance mechanisms

Heavy metals are metals with a density above 5 g/cm^3 and their distribution in the environment is governed by natural and human activities. Potential sources of heavy metals include soil erosion, mining, industrial wastes, urban and agricultural runoff, insecticides applied to crops, and many others. Heavy metals are persistent in the environment where they tend to accumulate causing damages to plants and animals[148,149]. Some heavy metals such as zinc (Zn), nickel (Ni) and copper (Cu) are physiologically essential for biological systems while others like mercury (Hg) and cadmium (Cd), have no known biological functions[133,150].

Most of the heavy metals are non-toxic for humans at low concentrations, however they can become toxic at higher concentrations[151,152]. They are often used as antimicrobial agents and some metals and products containing metals are also used in medicine for treatment of various diseases. For example, arsenic (As) has been administered to patients with acute promyelocytic leukemia, and bismuth (Bi) has been used for treating infections caused by *Helicobacter pylori* or against gastric lymphoma[153]. Other metals such as Cu, Zn, Cd and As are used in agricultural activities as growth promoters, fungicides and herbicides, as well as antifouling in fish farms[133,154-157].

In bacteria, heavy metal toxicity also depends on their concentration even if some metals like silver (Ag) and Hg are poisonous at very low concentrations[136]. In order to avoid cellular damage, bacteria have evolved regulatory mechanisms of resistance to metals. Metal-resistant bacteria were first detected by Moore and his team in 1960, when Hg resistant (Hg^R) bacteria (*Staphylococcus aureus*) were isolated from wounds[143]. Some

studies have then showed evidences that heavy metals can induce co-selection of antimicrobial resistance in bacteria[132,133]. In 2016, Lloyd and her team found that bacteria with resistance to three or more antibiotics were more common in Hg^R isolates than in Hg-sensitive (Hg^S) isolates[158]. Figure 17 shows the three main metal resistance mechanisms are known in bacteria[133,136]: A) extracellular sequestration of metals, which minimises the concentration of free metal ions in the cell; B) reduction of metal uptake and increased elimination of toxic metals by efflux systems; C) inactivation of metals through reduction of intracellular ions by enzymes like the Hg reductase (MerA) which reduces Hg ions (Hg^{2+}) to the less toxic form, the elemental Hg (Hg^0); and D) repair mediated by cellular chaperones, enzymes or antioxidants of molecules that are vulnerable to oxidation by metals.
It was also observed that metal resistance shares the common mode of actions which confer resistance to antibiotics. These mechanisms are listed in Table 5.

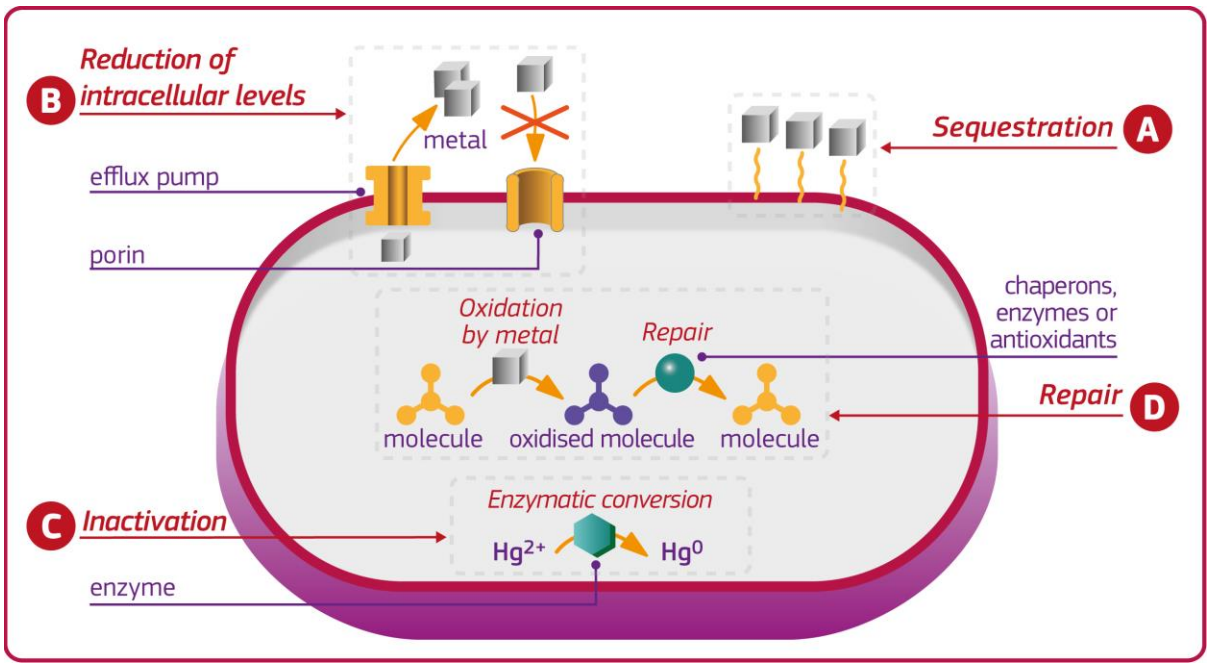

Figure 17. Metal resistance mechanisms in bacteria. A. Sequestration of metals; **B.** Reduction of metal uptake and increase of efflux outside the cell; **C.** Inactivation of metals in a less toxic form (e.g. from Hg^{2+} to Hg^0); **D.** Repair of molecules vulnerable to oxidation by metals which is mediated by cellular chaperones, enzymes or antioxidants.

Table 5. List of common resistance mechanisms between heavy metals and antibiotics

Mechanism of resistance	Metals	Antibiotics	Section
Inactivation of antibiotics and metals	As and Hg	β-lactams and chloramphenicol	4.1.1
Decrease of antibiotic penetration and increase of efflux out of the cell	As, Cu, Co, Zn, Mn, Ag, Cd, Ni	β-lactams, tetracycline and ciprofloxacin	4.1.2
Change in target site	Hg, Zn and Cu	β-lactams and rifampicin	4.1.3

As: arsenic; **Ag:** silver; **Cd**: cadmium; **Co**: cobalt; **Cu**: copper; **Hg**: mercury; **Mn**: manganese; **Ni**: nickel; **Zn**: zinc.

Antibiotic and metal resistance can be associated with co-selection mechanisms in which genes encoding resistance to both metals and antibiotics may reside in the same genetic element (co-resistance) or when there is a coregulation of resistance genes expression (co-regulation) or again, when a single mechanism is responsible for the induced resistances (cross-resistance) (see Figure 16)[159].

Multidrug efflux pumps can extrude a variety of compounds including antibiotics and heavy metals mainly through cross-resistance mechanisms[100]. An example is the multidrug efflux pump in *Listeria monocytogenes* which can export metals and antibiotics[160].

In 2016, Fang and co-authors described that in *Escherichia coli* strains isolated from diseased food-producing animals, genes encoding efflux systems to detoxify Cu, Ag and As co-existed with antimicrobial and heavy metal resistance determinants on the same plasmids, giving an example of co-resistance[138]. Co-resistance of Hg-resistant bacteria (Hg^R) to antibiotics has been observed in a study where the Hg exposure in fish increased the expression of the Hg reductase gene (*merA)*, providing resistance to Hg and showing a higher probability for these bacteria to be resistant to multiple antibiotics compared to the Hg-sensitive bacteria[158]. As an example of co-regulation, a study performed in *Pseudomonas aeruginosa* showed that the expression of an efflux system conferring resistance to Zn, Cd and cobalt (Co) was regulated by mechanisms also responsible for the resistance to carbapenems[147].

A study in China found a significant positive correlation between antibiotic resistance genes (ARG) and metals like Cu, Zn, and Hg in agricultural soil and manure showing the potential role of heavy metals in the co-selection of antibiotic resistance. In the same study, the correlation between ARG and the corresponding antibiotic concentration was instead much weaker[161]. Similar results were found in Western Australia, where even low concentrations of metals could select antibiotic resistance in residential soil underlying the possible contribution of metals in the spread of ARG[162].

4.3.2 Heavy metals and co-selection in water

As heavy metals are persistent in the environment, metal contamination may act as a long-term selective pressure for antibiotic resistance. Indeed, while most antibiotics are readily degraded in water, metals are not and they can accumulate in natural ecosystems, including water. A positive correlation between antibiotics and heavy metals concentrations has been indeed observed in water samples collected in the final effluents of two wastewater treatment plants (WWTP) in China. A significant correlation was in

particular found between antibiotic resistance genes (ARG) and the concentration of arsenic (As), zinc (Zn), lead (Pb) and mercury (Hg), suggesting that their combined presence in WWTP may favour the propagation of ARG[121]. In another study in China, a cluster analyses was used to assess a positive correlation between the expression of ARG and the concentration of anthropogenic pollutants (antibiotics and metals) in three artificial city park lakes[122].

To address the molecular mechanisms involved in the association between metal exposure and the spread of ARG, water samples collected from three different WWTP in Italy were analysed for the abundance of ARG and heavy metals. A strict correlation between the expression of the class I Integron gene (*int1*), the ARG for sulfonamides (*sulII*), and the two genes *czcA* and *arsB*, the first encoding resistance for cadmium (Cd), cobalt (Co) and Zn, and the second for As was also identified suggesting a mechanism of co-selection between ARG and metals in water[163]. In Colorado, candidates for co-selection were identified using the high-throughput DNA sequencing in order to obtain a metagenomic profile of ARG and metal resistance genes (MRG) in river waters[164]. The same metagenomic approach was performed in samples derived from influents and effluents of Korean WWTP. Also in this case, both ARG and MRG were detected in the microbial community[165].

Other studies tried to investigate the relationship between the antibiotic and heavy metal resistance but they did not focus on the molecular mechanisms involved in this association. A bacterial strain highly resistant to Cd (the Minimal Inhibitory Concentration – MIC, intended as the minimal concentration that inhibits the growth of a microorganism, was 250 mg/L), penicillin and ampicillin was isolated from electronic industry effluents during a study aimed at finding novel bacterial strains to be used in bioremediation techniques[166]. A concomitant antibiotic and heavy metal resistance was observed in Hg-resistant bacteria isolated from different sampling sites in an Indian river contaminated with heavy metals including chromium (Cr), Pb and Hg. Specifically, Hg-resistant bacteria were found to be resistant to different metals and in particular to Pb and copper (Cu), suggesting that the resistance to both metals and antibiotics may be genetically linked by co-selection. Hg-resistant bacterial strains checked for the antibiotic resistance pattern showed that the isolates were also sensitive to antibiotics like teicoplanin, azithromycin and vancomycin. The contamination of water with Hg may therefore act as a driving force for the carriage of ARG in water[167]. Again, in Turkey, samples were collected from seawater and sediment in a polluted Bay. Gram-negative bacterial strains were isolated from water samples and tested for their susceptibility to different antibiotics. The MIC for metals (Cd, Cu, Pb, Cr and manganese (Mg)) was also derived. More specifically, it was found that metal resistant-bacteria isolated from seawater also showed the resistance to streptomycin, ampicillin and trimethoprim-sulphamethoxazole[168]. A similar study was performed by the same authors and in the same marine environment (in Turkey) but this time it was focused only on *Aeromonas* spp. and *Pseudomonas* spp. and the authors found strains that were resistant to both metals and antibiotics[169]. The bacterial tolerance to metals and antibiotics was also checked in an American study conducted in two streams where the incubation of bacteria with different antibiotics and metals showed a positive correlation between the antibiotic and metal tolerance values. This evidence supported the hypothesis that metal contamination may result in an increased frequency of antibiotic resistance in bacteria[170]. In a microcosm study, freshwater bacteria have been exposed to individual metals and antibiotics and it was observed that each pollutant selected for multiresistant microorganisms. The antibiotic concentrations used in this study were higher than the levels detected in waterbodies, while the metal concentrations applied were in a more environmentally realistic ranges, underlying that metals, rather than antibiotics, may select for ARG in water[171]. Additionally, the occurrence of antibiotic and metal resistance was investigated in the River Indus, the major river in Pakistan, where the discovery of bacteria resistant to both antibiotics and metals suggested a possible concomitant gene regulation by these pollutants through co-selection.

As described above, most of the studies performed in water did not analyse the relationship between metal and antibiotic resistance at molecular level but they determined the levels of resistance after exposure of bacteria to these pollutants. More studies are therefore needed to better understand the molecular mechanisms involved in the association between antibiotics and metals in water and also to assess the presence of plasmid-encoded resistance genes.

5. Conclusions

Antibiotic resistance represents a European and global problem which has already reached high levels of concern in many parts of the world. Aquatic environments are considered a reservoir of antibiotic resistance determinants and the identification of the abundance and distribution of antibiotics and antibiotic resistance genes (ABR) in waterbodies can aid in establishing how antrophogenic inputs affect their spread and which strategies could be developed to combat this worldwide issue. In this technical report, a review of the global scientific literature was conducted to analyse the levels of antibiotics in water (e.g. wastewater treatment plants (WWTP), surface waters, drinking water). For WWTP, the monitoring data collected were related to 45 antibiotics from 13 countries all over the world. Most of the data came from Europe (79.2%) and for the antibiotics detected, the concentrations were in the range of 0,1-1 µg/L. The antibiotics sulfamethoxazole, trimethoprim and ciprofloxacin were the most frequently observed in WWTP effluents. Similar concentrations of antibiotics were also reported in surface waters, although a reduction in their levels due to the dilutions of these substances from effluents into receiving water should have been expected. Complementary to the literature data, measured concentrations of antibiotics were also gathered from a European database containing more than 16.6 million records for 1390 individual substances monitored for the period from 2006 to 2014. Data show that among 35 antibiotics, sulfamethoxazole and sulfamethazine were the most frequently monitored in Europe.

Concerning drinking water, antibiotic residues are unlikely to be considered as a potential risk to humans. Indeed, the detected antibiotic concentrations in drinking water (DW) are usually low and in the range of ng/L. A particular attention should be instead posed to the aquaculture. So far, there is little research available reporting the occurrence of antibiotics in this sector. Unless in Europe the use of antibiotics in aquacultures is carefully managed and aquaculture products must not contain pharmacologically active substances above an established Maximum Residue Limit (MRL) (Commission Regulation (EU) No 37/2010[172]), there is anyway a need to have public data available to be aware of the antibiotic residues in aquaculture products. Aquaculture is indeed considered the fastest growing animal food-producing sector and it is estimated to account for approximately half of the total food-fish supply. It is therefore necessary to prevent the bacterial diseases in aquaculture products and the use of vaccines could limit the use of chemicals and antibiotics in this sector.

There are also evidences suggesting a link between the environmental resistome (defined as a collection of naturally-occurring antibiotic resistance genes in water and soil) and clinically relevant resistance genes; moreover, a deeper knowledge about how the genes are transferred from the environment to the clinic will be useful for the discovery and the management of antibiotics and for controlling the dissemination of antibiotic resistance.

Metals, unlike antibiotics which are usually rapidly degraded in water, are more persistent in the environment and bacteria have evolved different mechanisms of resistance to tolerate their actions. Literature data show a co-selection between antibiotic resistance genes and genes conferring resistance to metals suggesting a role of heavy metals in the spreading of antibiotic resistance. However, for the majority of the studies, the antibiotic concentrations used to induce resistance were higher than the levels found in the environment and until now the potential molecular mechanisms to assess the role of metals as a selective force in the spread of the antibiotic resistance genes have been investigated in very few studies. Therefore, a clear and detailed understanding of the relationships between metals, antibiotics and the dissemination of antibiotic resistance need additional investigations, as well as new research should be carried out to define the minimal concentration of antibiotics which would induce the resistance. An environmental risk assessment for these substances needs to be defined taking into consideration the relative effects of the main determinants of antibiotic resistance and to estimate the risk of emergence and spread of this event.

Annex I

Table A: Antibiotics in wastewater treatment plant (WWTP) effluents (*)

Substance	Country	WWTP effluents / description	MEC (µg/L)	Reference
Amoxicillin	Italy	Different WWTP effluents	0.0018 – 0.120	Andreozzi et al., 2004[173]
	Italy	WWTP effluents	0.015 – 0.120	Castiglioni et al., 2005[31]
	Australia	WWTP effluent in Brisbane	0.270 (max)	Watkinson et al., 2007[32]
	Spain	Hospital wastewater, and urban WWTP effluent in Girona	0.258 (max)	Gros et al., 2013[174]
	Europe	90 WWTP effluents from 18 countries	< 0.025	Loos et al., 2013[175]
	Germany	WWTP effluent in Dresden	0.187 (max)	Rossmann et al., 2014[176]
Ampicillin	Greece	WWTP effluent in Volos	0.151 (mean)	Papageorgiou et al., 2016[177]
	Greece	WWTP effluent in Volos	0.498 (max)	Papageorgiou et al., 2016[177]
Azithromycin	Spain	Hospital wastewater, and urban WWTP effluent in Girona	0.085-0.592	Gros et al., 2013[174]
	Czech Republic	WWTP effluent in Ceské Budejovice	0.050 (median)	Golovko et al., 2014[178]
	Czech Republic	WWTP effluent in Ceské Budejovice	0.22 (max)	Golovko et al., 2014[178]
	Germany	WWTP effluent in Dresden	0.277 (median)	Rossmann et al., 2014[176]
	Germany	WWTP effluent in Dresden	0.956 (max)	Rossmann et al., 2014[176]
	Slovakia	WWTP effluents (n=2)	0.504 (mean)	Birošová et al., 2014[179]
	Slovakia	WWTP effluents (n=2)	1.2 (max)	Birošová et al., 2014[179]
	Spain	WWTP effluents in Girona	0.135 (max)	Rodriguez-Mozaz et al., 2015[29]
	Portugal	WWTP effluents (n=15)	0.007 (mean)	Pereira et al., 2015[30]
	Portugal	WWTP effluents (n=15)	0.2 (max)	Pereira et al., 2015[30]
	England (south)	WWTP effluents (n=4)	0.035 – 0.264	Johnson et al.; 2017[180]
Cefaclor	Australia	WWTP effluent in Brisbane	0.800 (max)	Watkinson et al., 2007[32]
Cefazolin	Spain	WWTP effluents in Girona	0.025 (max)	Rodriguez-Mozaz et al., 2015[29]
Cefotaxime	Spain	WWTP effluents in Girona	0.229 (max)	Rodriguez-Mozaz et al., 2015[29]

	Germany	WWTP effluent in Dresden	0.217 (max)	Rossmann et al., 2014[176]
Cefuroxime	Germany	WWTP effluent in Dresden	0.599 (median)	Rossmann et al., 2014[176]
	Germany	WWTP effluent in Dresden	2.0 (max)	Rossmann et al., 2014[176]
Cephalexin	Australia	WWTP effluent in Brisbane	3.9 (max)	Watkinson et al., 2007[32]
Chloramphenicol	UK	WWTP effluent Coslech (Wales)	0.021 (mean)	Kasprzyk-Hordern et al., 2009[181]
	UK	WWTP effluent Coslech (Wales)	0.069 (max)	Kasprzyk-Hordern et al., 2009[181]
Chlorotetracycline	Australia	WWTP effluent in Brisbane	0.005 (max)	Watkinson et al., 2007[32]
Ciprofloxacin	USA	WWTP effluents (n=2)	0.130 (median)	Renew et al., 2004[24]
	USA	WWTP effluents (n=2)	0.370 (max)	Renew et al., 2004[24]
	Italy	WWTP effluents	0.027 – 0.514	Castiglioni et al., 2005[31]
	USA	WWTP effluents (n=3)	5.6 (max)	Batt et al., 2006[28]
	Australia	WWTP effluent in Brisbane	0.640 (median)	Watkinson et al., 2007[32]
	Australia	WWTP effluent in Brisbane	6.9 (max)	Watkinson et al., 2007[32]
	China	WWTP effluent	0.037 (mean)	Jia et al., 2012[182]
	Spain	WWTP effluents (n=14) in Castellon province (Valencia)	0.70 (median)	Gracia-Lor et al., 2012[183]
	Spain	WWTP effluents (n=14) in Castellon province (Valencia)	1.1 (max)	Gracia-Lor et al., 2012[183]
	Spain	Hospital wastewater, and urban WWTP effluent in Girona	7.4 (max)	Gros et al., 2013[174]
	Europe	90 WWTP effluents from 18 countries	0.096 (mean); 0.264 (max)	Loos et al., 2013[175]
	Czech Republic	WWTP effluent in Ceské Budejovice	0.065 (median)	Golovko et al., 2014[178]
	Czech Republic	WWTP effluent in Ceské Budejovice	0.19 (max)	Golovko et al., 2014[178]
	Germany	WWTP effluent in Dresden	0.146 (median)	Rossmann et al., 2014[176]
	Germany	WWTP effluent in Dresden	0.920 (max)	Rossmann et al., 2014[176]
	Slovakia	WWTP effluents (n=2)	0.211 (mean)	Birošová et al., 2014[179]
	Slovakia	WWTP effluents (n=2)	0.338 (max)	Birošová et al., 2014[179]
	Spain	WWTP effluents in Girona	0.175 (max)	Rodriguez-Mozaz et al., 2015[29]

	Portugal	WWTP effluents (n=15)	0.137 (mean)	Pereira et al., 2015[30]
	Portugal	WWTP effluents (n=15)	0.836 (max)	Pereira et al., 2015[30]
	Greece	WWTP effluent in Volos	0.199 (mean)	Papageorgiou et al., 2016[177]
	Greece	WWTP effluent in Volos	0.591 (max)	Papageorgiou et al., 2016[177]
	Portugal	WWTP effluent in Beirolas, Lisbon	0.35 (median)	de Jesus Gaffney et al., 2017[25]
	Portugal	WWTP effluent in Beirolas, Lisbon	1.4 (max)	de Jesus Gaffney et al., 2017[25]
Clarithromycin	Switzerland	WWTP effluents (n=3)	0.057-0.328	McArdell et al., 2003[184]
	Italy	WWTP effluents	0.008 – 0.059	Castiglioni et al., 2005[31]
	Spain	WWTP effluents (n=14) in Castellon province (Valencia)	0.02 (median)	Gracia-Lor et al., 2012[183]
	Spain	WWTP effluents (n=14) in Castellon province (Valencia)	0.06 (max)	Gracia-Lor et al., 2012[183]
	Spain	Hospital wastewater, and urban WWTP effluent in Girona	0.113-0.973	Gros et al., 2013[174]
	Czech Republic	WWTP effluent in Ceské Budejovice	0.93 (median)	Golovko et al., 2014[178]
	Czech Republic	WWTP effluent in Ceské Budejovice	2.31 (max)	Golovko et al., 2014[178]
	Germany	WWTP effluent in Dresden	0.366 (median)	Rossmann et al., 2014[176]
	Germany	WWTP effluent in Dresden	1.8 (max)	Rossmann et al., 2014[176]
	Slovakia	WWTP effluents (n=2)	1.2 (mean)	Birošová et al., 2014[179]
	Slovakia	WWTP effluents (n=2)	1.8 (max)	Birošová et al., 2014[179]
	Spain	WWTP effluents in Girona	0.129 (max)	Rodriguez-Mozaz et al., 2015[29]
	England (south)	WWTP effluents (n=4)	0.024 – 0.377	Johnson et al.; 2017[180]
Clindamycin	USA	WWTP effluents (n=3)	1.0 (max)	Batt et al., 2006[28]
	Australia	WWTP effluent in Brisbane	0.005 (median)	Watkinson et al., 2007[32]
	Australia	WWTP effluent in Brisbane	0.005 (max)	Watkinson et al., 2007[32]
	Spain	WWTP effluents (n=14) in Castellon province (Valencia)	0.02 (median)	Gracia-Lor et al., 2012[183]
	Spain	WWTP effluents (n=14) in Castellon province (Valencia)	0.02 (max)	Gracia-Lor et al., 2012[183]

	Spain	Hospital wastewater, and urban WWTP effluent in Girona	0.018-1.5	Gros et al., 2013[174]
	Europe	90 WWTP effluents from 18 countries	0.070 (mean); 0.277 (max)	Loos et al., 2013[175]
	Germany	WWTP effluent in Dresden	0.151 (median)	Rossmann et al., 2014[176]
	Germany	WWTP effluent in Dresden	0.882 (max)	Rossmann et al., 2014[176]
	Slovakia	WWTP effluents (n=2)	0.056 (mean)	Birošová et al., 2014[179]
	Slovakia	WWTP effluents (n=2)	0.069 (max)	Birošová et al., 2014[179]
Clindamycin sulfoxide	Germany	WWTP effluent in Dresden	0.423 (median)	Rossmann et al., 2014[176]
	Germany	WWTP effluent in Dresden	1.3 (max)	Rossmann et al., 2014[176]
Doxycycline	Australia	WWTP effluent in Brisbane	0.040 (max)	Watkinson et al., 2007[32]
	China	WWTP effluent	0.632 (mean)	Gao et al., 2012[185]
	Germany	WWTP effluent in Dresden	1.1 (max)	Rossmann et al., 2014[176]
	Slovakia	WWTP effluents (n=2)	0.008 (max)	Birošová et al., 2014[179]
Enoxacin	Slovakia	WWTP effluents (n=2)	0.008 (max)	Birošová et al., 2014[179]
Enrofloxacin	Australia	WWTP effluent in Brisbane	0.010 (median)	Watkinson et al., 2007[32]
	Australia	WWTP effluent in Brisbane	0.020 (max)	Watkinson et al., 2007[32]
	China	WWTP effluent	0.002 (mean)	Jia et al., 2012[182]
	Slovakia	WWTP effluents (n=2)	0.003 (max)	Birošová et al., 2014[179]
Erythromycin	Switzerland	WWTP effluents (n=3)	0.199 (max)	McArdell et al., 2003[184]
	Italy	WWTP effluents	0.009 – 0.353	Castiglioni et al., 2005[31]
	UK	WWTP effluent in Cilfynydd (Wales)	1.4 (mean)	Kasprzyk-Hordern et al., 2009[181]
	UK	WWTP effluent in Cilfynydd (Wales)	2.8 (max)	Kasprzyk-Hordern et al., 2009[181]
	UK	WWTP effluent Coslech (Wales)	0.696 (mean)	Kasprzyk-Hordern et al., 2009[181]
	UK	WWTP effluent Coslech (Wales)	2.8 (max)	Kasprzyk-Hordern et al., 2009[181]
	Spain	WWTP effluents (n=14) in Castellon province (Valencia)	0.08 (median)	Gracia-Lor et al., 2012[183]

	Spain	WWTP effluents (n=14) in Castellon province (Valencia)	0.12 (max)	Gracia-Lor et al., 2012[183]
	Czech Republic	WWTP effluent in Ceské Budejovice	0.11 (median)	Golovko et al., 2014[178]
	Czech Republic	WWTP effluent in Ceské Budejovice	0.35 (max)	Golovko et al., 2014[178]
	Slovakia	WWTP effluents (n=2)	0.015 (mean)	Birošová et al., 2014[179]
	Slovakia	WWTP effluents (n=2)	0.020 (max)	Birošová et al., 2014[179]
	Portugal	WWTP effluent in Beirolas, Lisbon	0.51 (median)	de Jesus Gaffney et al., 2017[25]
	Portugal	WWTP effluent in Beirolas, Lisbon	2.8 (max)	de Jesus Gaffney et al., 2017[25]
Fleroxacin	China	WWTP effluent	0.005 (mean)	Jia et al., 2012[182]
Gatifloxacin	China	WWTP effluent	0.04 (mean)	Jia et al., 2012[182]
Levofloxacin	Czech Republic	WWTP effluent in Ceské Budejovice	0.006 (median)	Golovko et al., 2014[178]
	Czech Republic	WWTP effluent in Ceské Budejovice	0.018 (max)	Golovko et al., 2014[178]
	Germany	WWTP effluent in Dresden	0.150 (median)	Rossmann et al., 2014[176]
	Germany	WWTP effluent in Dresden	0.836 (max)	Rossmann et al., 2014[176]
	Slovakia	WWTP effluents (n=2)	0.042 (mean)	Birošová et al., 2014[179]
	Slovakia	WWTP effluents (n=2)	0.058 (max)	Birošová et al., 2014[179]
	England (south)	WWTP effluents (n=4)	0.047 (max)	Johnson et al.; 2017[180]
Lincomycin	Italy	WWTP effluents	0.011 – 0.846	Castiglioni et al., 2005[31]
	Australia	WWTP effluent in Brisbane	0.050 (median)	Watkinson et al., 2007[32]
	Australia	WWTP effluent in Brisbane	0.070 (max)	Watkinson et al., 2007[32]
	Spain	WWTP effluents (n=14) in Castellon province (Valencia)	0.01 (median)	Gracia-Lor et al., 2012[183]
	Spain	WWTP effluents (n=14) in Castellon province (Valencia)	0.16 (max)	Gracia-Lor et al., 2012[183]
	Spain	Hospital wastewater, and urban WWTP effluent in Girona	0.119 (max)	Gros et al., 2013[174]
	Europe	90 WWTP effluents from 18 countries	0.031 (mean); 0.317 (max)	Loos et al., 2013[175]
Lomefloxacin	China	WWTP effluent	0.071 (mean)	Jia et al., 2012[182]
Marbofloxacin	Spain	Hospital wastewater, and urban WWTP effluent in Girona	0.096 (max)	Gros et al., 2013[174]

Metronidazole	UK	WWTP effluent in Cilfynydd (Wales)	0.265 (mean)	Kasprzyk-Hordern et al., 2009[181]
	UK	WWTP effluent in Cilfynydd (Wales)	0.421 (max)	Kasprzyk-Hordern et al., 2009[181]
	UK	WWTP effluent Coslech (Wales)	0.353 (mean)	Kasprzyk-Hordern et al., 2009[181]
	UK	WWTP effluent Coslech (Wales)	0.561 (max)	Kasprzyk-Hordern et al., 2009[181]
	Spain	Hospital wastewater, and urban WWTP effluent in Girona	0.017-0.643	Gros et al., 2013[174]
	Spain	WWTP effluents in Girona	0.144 (max)	Rodriguez-Mozaz et al., 2015[29]
	Greece	WWTP effluent in Volos	0.035 (max)	Papageorgiou et al., 2016[177]
Monesin	Australia	WWTP effluent in Brisbane	0.010 (max)	Watkinson et al., 2007[32]
Moxifloxacin	China	WWTP effluent	0.04 (mean)	Jia et al., 2012[182]
	Spain	WWTP effluents (n=14) in Castellon province (Valencia)	0.16 (median)	Gracia-Lor et al., 2012[183]
	Spain	WWTP effluents (n=14) in Castellon province (Valencia)	0.18 (max)	Gracia-Lor et al., 2012[183]
	Greece	WWTP effluent in Volos	0.085 (mean)	Papageorgiou et al., 2016[177]
	Greece	WWTP effluent in Volos	0.298 (max)	Papageorgiou et al., 2016[177]
Nalidixic acid	Australia	WWTP effluent in Brisbane	0.055 (max)	Watkinson et al., 2007[32]
Norfloxacin	Australia	WWTP effluent in Brisbane	0.025 (median)	Watkinson et al., 2007[32]
	Australia	WWTP effluent in Brisbane	0.145 (max)	Watkinson et al., 2007[32]
	China	WWTP effluent	0.256 (mean)	Jia et al., 2012[182]
	Spain	WWTP effluents (n=14) in Castellon province (Valencia)	0.13 (median)	Gracia-Lor et al., 2012[183]
	Spain	WWTP effluents (n=14) in Castellon province (Valencia)	0.15 (max)	Gracia-Lor et al., 2012[183]
	Spain	Hospital wastewater, and urban WWTP effluent in Girona	0.327 (max)	Gros et al., 2013[174]
	Czech Republic	WWTP effluent in Ceské Budejovice	0.083 (median)	Golovko et al., 2014[178]
	Czech Republic	WWTP effluent in Ceské Budejovice	0.25 (max)	Golovko et al., 2014[178]
	Slovakia	WWTP effluents (n=2)	0.021 (mean)	Birošová et al., 2014[179]

	Slovakia	WWTP effluents (n=2)	0.033 (max)	Birošová et al., 2014[179]
Ofloxacin	USA	WWTP effluents (n=2)	0.255 (median)	Renew et al., 2004[24]
	USA	WWTP effluents (n=2)	0.350 (max)	Renew et al., 2004[24]
	Italy	WWTP effluents	0.150 – 1.1	Castiglioni et al., 2005[31]
	China	WWTP effluent	0.528 (mean)	Jia et al., 2012[182]
	Spain	WWTP effluents (n=14) in Castellon province (Valencia)	0.44 (median)	Gracia-Lor et al., 2012[183]
	Spain	Hospital wastewater, and urban WWTP effluent in Girona	0.063-10.3	Gros et al., 2013[174]
	Spain	WWTP effluents in Girona	0.172 (max)	Rodriguez-Mozaz et al., 2015[29]
Oxytetracycline	Australia	WWTP effluent in Brisbane	0.020 (max)	Watkinson et al., 2007[32]
	China	WWTP effluent	0.021 (mean)	Gao et al., 2012[185]
	England (south)	WWTP effluents (n=4)	0.017 – 0.602	Johnson et al.; 2017[180]
Penicilline V	Australia	WWTP effluent in Brisbane	0.030 (median)	Watkinson et al., 2007[32]
	Australia	WWTP effluent in Brisbane	0.080 (max)	Watkinson et al., 2007[32]
	Europe	90 WWTP effluents from 18 countries	0.029 (mean); 0.122 (max)	Loos et al., 2013[175]
Pipemidic acid	China	WWTP effluent	0.033 (mean)	Jia et al., 2012[182]
	Spain	WWTP effluents (n=14) in Castellon province (Valencia)	0.10 (median)	Gracia-Lor et al., 2012[183]
	Spain	WWTP effluents (n=14) in Castellon province (Valencia)	0.12 (max)	Gracia-Lor et al., 2012[183]
Piperacillin	Germany	WWTP effluent in Dresden	0.274 (median)	Rossmann et al., 2014[176]
	Germany	WWTP effluent in Dresden	1.2 (max)	Rossmann et al., 2014[176]
Roxithromycin	Switzerland	WWTP effluents (n=3)	0.031 (max)	McArdell et al., 2003[184]
	Australia	WWTP effluent in Brisbane	0.100 (max)	Watkinson et al., 2007[32]
	Germany	WWTP effluent in Dresden	0.084 (median)	Rossmann et al., 2014[176]
	Germany	WWTP effluent in Dresden	0.281 (max)	Rossmann et al., 2014[176]
Sparfloxacin	China	WWTP effluent	0.001 (mean)	Jia et al., 2012[182]
Spiramycin	Italy	WWTP effluents	0.001 – 0.161	Castiglioni et al., 2005[31]

	China	WWTP effluent	0.027 (mean)	Gao et al., 2012[185]
	Europe	90 WWTP effluents from 18 countries	0.004 (mean); 0.105 (max)	Loos et al., 2013[175]
	Greece	WWTP effluent in Volos	0.194 (max)	Papageorgiou et al., 2016[177]
Sulfamethoxazole	USA	WWTP effluents (n=2)	0.485 (median)	Renew et al., 2004[24]
	USA	WWTP effluents (n=2)	1.6 (max)	Renew et al., 2004[24]
	Italy	WWTP effluents	0.046 – 0.317	Castiglioni et al., 2005[31]
	USA	WWTP effluents (n=3)	6.0 (max)	Batt et al., 2006[28]
	Australia	WWTP effluent in Brisbane	0.270 (median)	Watkinson et al., 2007[32]
	Australia	WWTP effluent in Brisbane	0.570 (max)	Watkinson et al., 2007[32]
	UK	WWTP effluent in Cilfynydd (Wales)	0.010 (mean)	Kasprzyk-Hordern et al., 2009[181]
	UK	WWTP effluent in Cilfynydd (Wales)	0.023 (max)	Kasprzyk-Hordern et al., 2009[181]
	UK	WWTP effluent Coslech (Wales)	0.019 (mean)	Kasprzyk-Hordern et al., 2009[181]
	UK	WWTP effluent Coslech (Wales)	0.044 (max)	Kasprzyk-Hordern et al., 2009[181]
	China	WWTP effluent	0.192 (mean)	Gao et al., 2012[185]
	Spain	WWTP effluents (n=14) in Castellon province (Valencia)	0.05 (median)	Gracia-Lor et al., 2012[183]
	Spain	WWTP effluents (n=14) in Castellon province (Valencia)	0.06 (max)	Gracia-Lor et al., 2012[183]
	Spain	Hospital wastewater, and urban WWTP effluent in Girona	0.019-0.198	Gros et al., 2013[174]
	Europe	90 WWTP effluents from 18 countries	0.142 (mean); 1.1 (max)	Loos et al., 2013[175]
	Czech Republic	WWTP effluent in Ceské Budejovice	0.090 (median)	Golovko et al., 2014[178]
	Czech Republic	WWTP effluent in Ceské Budejovice	0.26 (max)	Golovko et al., 2014[178]
	Greece	WWTP effluents (n=8)	0.481 (max)	Kosma et al., 2014[186]
	Germany	WWTP effluent in Dresden	0.199 (median)	Rossmann et al., 2014[176]
	Germany	WWTP effluent in Dresden	8.3 (max)	Rossmann et al., 2014[176]
	Slovakia	WWTP effluents (n=2)	0.049 (mean)	Birošová et al., 2014[179]

	Slovakia	WWTP effluents (n=2)	0.108 (max)	Birošová et al., 2014[179]
	Spain	WWTP effluents in Girona	0.073 (max)	Rodriguez-Mozaz et al., 2015[29]
	Greece	WWTP effluent in Volos	0.020 (mean)	Papageorgiou et al., 2016[177]
	Greece	WWTP effluent in Volos	0.080 (max)	Papageorgiou et al., 2016[177]
	England (south)	WWTP effluents (n=4)	0.227 (max)	Johnson et al.; 2017[180]
	Portugal	WWTP effluent in Beirolas, Lisbon	0.69 (median)	de Jesus Gaffney et al., 2017[25]
	Portugal	WWTP effluent in Beirolas, Lisbon	2.0 (max)	de Jesus Gaffney et al., 2017[25]
Sulfapyridine	Spain	Hospital wastewater, and urban WWTP effluent in Girona	0.059 (max)	Gros et al., 2013[174]
	Czech Republic	WWTP effluent in Ceské Budejovice	0.055 (median)	Golovko et al., 2014[178]
	Czech Republic	WWTP effluent in Ceské Budejovice	0.20 (max)	Golovko et al., 2014[178]
	Slovakia	WWTP effluents (n=2)	0.078 (mean)	Birošová et al., 2014[179]
	Slovakia	WWTP effluents (n=2)	0.120 (max)	Birošová et al., 2014[179]
	Portugal	WWTP effluent in Beirolas, Lisbon	0.28 (median)	de Jesus Gaffney et al., 2017[25]
	Portugal	WWTP effluent in Beirolas, Lisbon	1.5 (max)	de Jesus Gaffney et al., 2017[25]
Sulfasalazine	Czech Republic	WWTP effluent in Ceské Budejovice	0.050 (median)	Golovko et al., 2014[178]
	Czech Republic	WWTP effluent in Ceské Budejovice	0.83 (max)	Golovko et al., 2014[178]
	Slovakia	WWTP effluents (n=2)	0.055 (mean)	Birošová et al., 2014[179]
	Slovakia	WWTP effluents (n=2)	0.124 (max)	Birošová et al., 2014[179]
	Australia	WWTP effluent in Brisbane	0.010 (max)	Watkinson et al., 2007[32]
Sulfathiazole	Australia	WWTP effluent in Brisbane	0.005 (max)	Watkinson et al., 2007[32]
Tetracycline	USA	WWTP effluents (n=3)	0.56 (max)	Batt et al., 2006[28]
	Australia	WWTP effluent in Brisbane	0.030 (max)	Watkinson et al., 2007[32]
	Slovakia	WWTP effluents (n=2)	0.003 (max)	Birošová et al., 2014[179]
	England (south)	WWTP effluents (n=4)	0.045 – 0.133	Johnson et al.; 2017[180]

Trimethoprim	USA	WWTP effluents (n=2)	0.373 (median)	Renew et al., 2004[24]
	USA	WWTP effluents (n=2)	1.2 (max)	Renew et al., 2004[24]
	USA	WWTP effluents (n=3)	0.53 (max)	Batt et al., 2006[28]
	Australia	WWTP effluent in Brisbane	0.050 (median)	Watkinson et al., 2007[32]
	Australia	WWTP effluent in Brisbane	0.480 (max)	Watkinson et al., 2007[32]
	UK	WWTP effluent in Cilfynydd (Wales)	1.2 (mean)	Kasprzyk-Hordern et al., 2009[181]
	UK	WWTP effluent in Cilfynydd (Wales)	3.1 (max)	Kasprzyk-Hordern et al., 2009[181]
	UK	WWTP effluent Coslech (Wales)	0.876 (mean)	Kasprzyk-Hordern et al., 2009[181]
	UK	WWTP effluent Coslech (Wales)	1.2 (max)	Kasprzyk-Hordern et al., 2009[181]
	Spain	WWTP effluents (n=14) in Castellon province (Valencia)	0.09 (median)	Gracia-Lor et al., 2012[183]
	Spain	WWTP effluents (n=14) in Castellon province (Valencia)	0.1 (max)	Gracia-Lor et al., 2012[183]
	Spain	Hospital wastewater, and urban WWTP effluent in Girona	0.216 (max)	Gros et al., 2013[174]
	Europe	90 WWTP effluents from 18 countries	0.229 (mean); 0.800 (max)	Loos et al., 2013[175]
	Czech Republic	WWTP effluent in Ceské Budejovice	0.25 (median)	Golovko et al., 2014[178]
	Czech Republic	WWTP effluent in Ceské Budejovice	0.44 (max)	Golovko et al., 2014[178]
	Greece	WWTP effluents (n=8)	0.533 (max)	Kosma et al., 2014[186]
	Germany	WWTP effluent in Dresden	0.208 (median)	Rossmann et al., 2014[176]
	Germany	WWTP effluent in Dresden	0.554 (max)	Rossmann et al., 2014[176]
	Slovakia	WWTP effluents (n=2)	0.087 (mean)	Birošová et al., 2014[179]
	Slovakia	WWTP effluents (n=2)	0.088 (max)	Birošová et al., 2014[179]
	Spain	WWTP effluents in Girona	0.125 (max)	Rodriguez-Mozaz et al., 2015[29]
	Greece	WWTP effluent in Volos	0.047 (mean)	Papageorgiou et al., 2016[177]
	Greece	WWTP effluent in Volos	0.096 (max)	Papageorgiou et al., 2016[177]

	England (south)	WWTP effluents (n=4)	0.087 – 0.455	Johnson et al.; 2017[180]
Tylosin	Australia	WWTP effluent in Brisbane	0.065 (max)	Watkinson et al., 2007[32]
Vancomycin	Germany	WWTP effluent in Dresden	0.348 (max)	Rossmann et al., 2014[176]

(*) The name "Europe" indicated in the column "Country" for eight antibiotics (amoxicillin, ciprofloxacin, clindamycin, lincomycin, penicilline V, sulfadiazine, sulfamethoxazole and trimethoprim) was used for reporting the mean concentration calculated from 90 measurements from samples collected in wastewater treatment plants (WWTP) effluents located in 18 European countries. For sulfamethoxazole, we included in the dataset concentrations measured not only in single countries but also mean concentrations calculated on: i) totally 6633 samples collected all over the world; ii) measurements performed in 122 European river samples; iii) data collected from Danube river; and iv) totally 5536 surface water samples across Europe and in tributaries of Danube Rivers. For trimethoprim, we incorporated to the dataset both the concentrations in different countries and the mean concentration calculated on a total of 1899 surface water samples in Europe.

Annex II

Table B: Antibiotics in surface water

Substance	Country	River / Lake	MEC (µg/L)	Reference
Amoxicillin	UK (Wales)	River Taff	0.058 (median); 0.245 (max)	Kasprzyk-Hordern et al., 2007[187]
	UK	River Taff and Ely (Wales)	0.117 (median); 0.622 (max)	Kasprzyk-Hordern et al., 2008[188]
	Australia	River water	0.200 (max)	Watkinson et al., 2009[43]
	Italy	Surface water, River Arno	0.006 (mean); 0.010 (max)	Zuccato et al., 2010[189]
	Canada	Wascana Creek, Qu'Appelle River	0.080 (max)	Waiser et al., 2010[190]
	France	Seine River	0.068	Tuc Dinh et al., 2011[191]
	Greece	Saronikos Gulf and Elefsis Bay in central Aegean Sea (WWTP impact from Athens)	0.128 (max)	Alygizakis et al., 2016[192]
Ampicillin	China	Sindian, Dahan and Gaoping Rivers	0.100 (max)	Lin et al., 2009[193]
	France	Canche River (urban impact)	0.001	Tlili et al., 2016[79]
Azithromycin	Spain	Ebro River (n=7; downstream WWTPs)	0.017 (median); 0.068 (max)	Gros et al., 2007[194]
	Japan	Tone River basin	0.012 (median); 0.070 (max)	Nakada et al., 2007[195]
	USA	Loup River, Big Blue River, Wood River, Salt Creek, Missouri River (downstream WWTPs)	0.670 (median); 1.5 (max)	Bartelt-Hunt et al., 2009[196]
	Serbia	Danube, Sava and Tamis Rivers	0.055 (median); 0.081 (max)	Grujić et al., 2009[197]
	Spain	Llobregat River	0.072 (max)	López-Serna et al., 2010[47]
	Spain	Ebro River	0.037 (max)	López-Serna et al., 2011[198]
	Spain	Jarama, Manzanares, Guadarrama, Henares, and Tagus Rivers	0.569 (max)	Valcárcel et al., 2011[199]
	Italy	Receiving water in the Po Valley	0.090 (max)	Al Aukidy et al., 2012[200]
	Spain	Llobregat River	0.037 (max)	Osorio et al., 2012[201]
	Spain	Ebro River and tributaries	0.041 (max)	López-Serna et al., 2012[202]
	Spain	Llobregat River	0.018 (max)	Boleda et al., 2013[203]
	Italy	Receiving water of a large WWTP in the Po Valley	0.007 (mean)	Verlicchi et al., 2014[204]
	Spain	El Albujón River	16.6 (max)	Moreno-González et al., 2014[205]

	Spain	Mar Menor lagoon (SE Spain, Murcia)	0.164 (max)	Moreno-González et al., 2015[206]
	Spain	Llobregat, Ebro, Júcar, and Guadalquivir Rivers	0.154 (max)	Osorio et al., 2016[207]
	Spain	Ter River downstream WWTP in Girona	0.115 (max)	Rodriguez-Mozaz et al., 2015[29]
Cefaclor	Australia	River water	0.200 (max)	Watkinson et al., 2009[43]
Cefalexin	Australia	River water	0.100 (max)	Watkinson et al., 2009[43]
	Spain	Llobregat, Ebro, Júcar, and Guadalquivir Rivers	0.001 (max)	Osorio et al., 2016[207]
Cefazolin	Spain	Ter River downstream WWTP in Girona	0.008 (max)	Rodriguez-Mozaz et al., 2015[29]
Cefotaxime	Spain	Ter River downstream WWTP in Girona	0.165 (max)	Rodriguez-Mozaz et al., 2015[29]
Chloramphenicol	Germany	Rhine, Urselbach, Nidda and Main Rivers	0.060 (max)	Hirsch et al., 1999[208]
	China	Pearl River at Guangzhou	0.084 (median); 0.266 (max)	Xu et al., 2007b[209]
	South Korea	Han River, North and South Han River, Kyung-Ahn Stream	0.031 (median); 0.054 (max)	Choi et al., 2008a[210]
	UK	River Taff and Ely (Wales)	0.002 (median); 0.040 (max)	Kasprzyk-Hordern et al., 2008[188]
	UK	River Ely (Wales)	0.005 (mean)	Kasprzyk-Hordern et al., 2009[181]
	China	Huangpu River	0.028 (max)	Jiang et al., 2011[211]
	Spain	Llobregat River	0.001 (max)	Osorio et al., 2012[201]
	Romania	Danube, Olt, Siret, and Argeş Rivers	0.013 (max)	Chitescu et al., 2015[212]
Chlortetracycline	USA	Streams and rivers (n=139)	0.69 (max)	Kolpin et al., 2002[213]
	Canada	Grand River watershed	0.192 (max)	Lissemore et al., 2006[214]
	USA	Cache La Poudre River	0.080 (median); 0.210 (max)	Sung-Chul et al., 2007[215]
	Australia	River water	0.060 (max)	Watkinson et al., 2009[43]
	China	Sindian, Dahan and Gaoping Rivers	0.090 (max)	Lin et al., 2009[193]
	China	Huangpu River	0.017 (max)	Jiang et al., 2011[211]
	Spain	Ebro River	0.059 (max)	López-Serna et al., 2011[198]
	Spain	Llobregat River	0.011 (max)	Osorio et al., 2012[201]
	France	Canche River (urban impact)	0.004	Tlili et al., 2016[79]
	South Korea	River Han	0.793 (max)	Kim et al., 2016[216]
Ciprofloxacin	USA	Streams and rivers (n=139)	0.030 (max)	Kolpin et al., 2002[213]

	Italy	Po and Lambro River	0.020 (median); 0.026 (max)	Calamari et al., 2003[217]
	Italy	Po and Lambro Rivers	0.020 (median); 0.026 (max)	Zuccato et al., 2005[218]
	USA	Streams downstream WWTPs	0.170 (median); 0.360 (max)	Batt et al., 2006[28]
	Finland	Vantaa River	0.025 (max)	Vieno et al., 2006[219]
	USA	Upper Tennessee River	0.007 (median); 0.054 (max)	Conley et al., 2008[220]
	China	Pearl River	0.459 (max)	Peng et al., 2008[221]
	China	Tonghui River	0.010 (median); 0.020 (max)	Xiao et al., 2008[222]
	Australia	River water	1.3 (max)	Watkinson et al., 2009[43]
	France	Arc River (WWTP impact)	10 (max)	Feitosa et al., 2009[223]
	Italy	Surface water, River Po	0.009 (mean); 0.016 (max)	Zuccato et al., 2010[189]
	Italy	Surface water, River Arno	0.019 (mean); 0.038 (max)	Zuccato et al., 2010[189]
	Spain	Llobregat River	0.028 (max)	López-Serna et al., 2010[47]
	France	Seine River	0.017	Tuc Dinh et al., 2011[191]
	France	Charmoise River, upstream WWTP	0.004	Tuc Dinh et al., 2011[191]
	France	Charmoise River, downstream WWTP	0.135	Tuc Dinh et al., 2011[191]
	Spain	Ebro River	0.115 (max)	López-Serna et al., 2011[198]
	Spain	Surface water in Castellon and Valencia provinces	0.740 (max)	Gracia-Lor et al., 2011[224]
	Spain	Jarama, Manzanares, Guadarrama, Henares, and Tagus Rivers	0.224 (max)	Valcárcel et al., 2011[199]
	Italy	Receiving water in the Po Valley	0.100 (max)	Al Aukidy et al., 2012[200]
	Spain	Llobregat River	0.271 (max)	Osorio et al., 2012[201]
	Italy	Receiving water of a large WWTP in the Po Valley	0.025 (mean)	Verlicchi et al., 2014[204]
	Poland	Gościcina and Reda Rivers	2.7 (max)	Wagil et al., 2014[225]
	China	Wenyu River	0.066 (max)	Zhang et al., 2014[226]
	Romania	Danube, Olt, Siret, and Argeş Rivers	0.006 (max)	Chitescu et al., 2015[212]
	USA	River in Maryland, upstream WWTP	0.01	He et al., 2015[227]
	USA	River in Maryland, downstream WWTP	0.031 (max)	He et al., 2015[227]

	Spain	Ter River downstream WWTP in Girona	0.072 (max)	Rodriguez-Mozaz et al., 2015[29]
	France	Canche River (urban impact)	0.007	Tlili et al., 2016[79]
Clarithromycin	Germany	Rhine, Urselbach, Nidda and Main Rivers	0.260 (max)	Hirsch et al., 1999[208]
	Italy	Po and Lambro River	0.002 (median); 0.020 (max)	Calamari et al., 2003[217]
	Germany	River Elbe	0.034 (median); 0.040 (max)	Weigel et al., 2004[228]
	Italy	Po and Lambro Rivers	0.008 (median); 0.020 (max)	Zuccato et al., 2005[218]
	Japan	Tone River basin	0.026 (median); 0.060 (max)	Nakada et al., 2007[195]
	Germany	River Havel	0.009 (median); 0.043 (max)	Heberer et al., 2008[229]
	USA	A stream in Ohio	0.005 (median)	Spongberg & Witter, 2008[230]
	France	Arc River (WWTP impact)	0.700 (median); 2.3 (max)	Feitosa et al., 2009[223]
	Italy	Surface water, River Po	0.002 (mean); 0.002 (max)	Zuccato et al., 2010[189]
	Italy	Surface water, River Arno	0.025 (mean); 0.045 (max)	Zuccato et al., 2010[189]
	Germany	River Leine and Baltic Sea	0.077 (max)	Nödler et al., 2010[231]
	USA	Surface water in Colorado	0.005 (max)	Ferrer et al., 2010[232]
	Germany	Rhine River	0.013 (median); 0.030 (max)	Ter Laak et al., 2010[233]
	Spain	Llobregat River	0.089 (max)	López-Serna et al., 2010[47]
	Spain	Ebro River	0.037 (max)	López-Serna et al., 2011[198]
	Spain	Surface water in Castellon and Valencia provinces	0.091 (max)	Gracia-Lor et al., 2011[224]
	Spain	Ebro River	0.037 (max)	Silva et al., 2011[234]
	Spain	Jarama, Manzanares, Guadarrama, Henares, and Tagus Rivers	1.7 (max)	Valcárcel et al., 2011[199]
	Spain	Ebro River and tributaries	0.141 (max)	López-Serna et al., 2012[202]
	Spain	Llobregat River	0.232 (max)	Osorio et al., 2012[201]
	Spain	River water of Pego-Oliva Marshlands	0.035 (max)	Vazquez-Roig et al., 2012[235]
	Italy	Receiving water in the Po Valley	0.100 (max)	Al Aukidy et al., 2012[200]
	Spain	Llobregat River	0.054 (max)	Boleda et al., 2013[203]
	Spain	El Albujón River	2.4 (max)	Moreno-González et al., 2014[205]

	Italy	Receiving water of a large WWTP in the Po Valley	0.006 (mean)	Verlicchi et al., 2014[204]
	Spain	Ter River downstream WWTP in Girona	0.096 (max)	Rodriguez-Mozaz et al., 2015[29]
	Spain	Surface waters in Castellón	0.034 (max)	Boix et al., 2015[236]
	Spain	Mar Menor lagoon (SE Spain, Murcia)	0.010 (max)	Moreno-González et al., 2015[206]
	Spain	Llobregat, Ebro, Júcar, and Guadalquivir Rivers	0.066 (max)	Osorio et al., 2016[207]
	Germany	Large Rivers	0.070 (max)	Baumann et al., 2015[237]
	Germany	Small Rivers	3.6 (max)	Baumann et al., 2015[237]
	Germany	7 Rivers in Bavaria	0.030 (median); 0.100 (max)	Baumann et al., 2015[237]
	China	Yangtze, Huai, Yellow, Hai, Liao River	0.001 (median); 0.012 (max)	Sun et al., 2015[238]
Clindamycin	USA	Streams downstream WWTPs	0.066 (median); 0.140 (max)	Batt et al., 2006[28]
	Germany	River Havel	0.031 (median); 0.048 (max)	Heberer et al., 2008[229]
	USA	A stream in Ohio	0.001 (median)	Spongberg & Witter, 2008[230]
	Australia	River water	0.010 (max)	Watkinson et al., 2009[43]
	Canada	Wascana Creek, Qu'Appelle River	0.300 (max)	Waiser et al., 2010[190]
	Germany	Rhine River	0.016 (median); 0.090 (max)	Ter Laak et al., 2010[233]
	Netherlands	Rhine and Meuse River	0.005 (mean); 0.016 (max)	de Jongh et al., 2012[239]
Danofloxacin	France	Seine, Marne, Oise rivers	0.019 (max)	Tamtam et al., 2008[240]
	Spain	Llobregat River	0.280 (max)	Osorio et al., 2012[201]
	Spain	Ebro River	0.207 (max)	López-Serna et al., 2011[198]
	France	Canche River (urban impact)	0.068	Tlili et al., 2016[79]
Demecolcycline	USA	Cache La Poudre River	0.030 (median); 0.050 (max)	Sung-Chul et al., 2007[215]
Difloxacin	France	Canche River (urban impact)	0.032	Tlili et al., 2016[79]
Dimetridazole	Spain	El Albujón River	0.028 (max)	Moreno-González et al., 2014[205]
	Spain	Llobregat, Ebro, Júcar, and Guadalquivir Rivers	0.047 (max)	Osorio et al., 2016[207]
Doxycycline	Canada	Grand River watershed	0.008 (median); 0.073 (max)	Lissemore et al., 2006[214]
	USA	Cache La Poudre River	0.020 (median); 0.050 (max)	Sung-Chul et al., 2007[215]
	Australia	River water	0.400 (max)	Watkinson et al., 2009[43]

	Spain	Ebro River	0.048 (max)	López-Serna et al., 2011[198]
	China	Huangpu River	0.047 (max)	Jiang et al., 2011[211]
	Spain	Llobregat River	0.018 (max)	Osorio et al., 2012[201]
	China	Wenyu River	0.008 (max)	Zhang et al., 2014[226]
	France	Allier River	0.002 (max)	Celle-Jeanton et al., 2014[241]
	France	Canche River (urban impact)	0.004	Tlili et al., 2016[79]
Enoxacin	Spain	Llobregat River	0.005 (max)	López-Serna et al., 2010[47]
	Spain	Ebro River	0.140 (max)	López-Serna et al., 2011[198]
	Spain	Llobregat River	0.279 (max)	Osorio et al., 2012[201]
Enrofloxacin	USA	Streams in Iowa	0.010 (max)	Kolpin et al., 2004[242]
	Australia	River water	0.300 (max)	Watkinson et al., 2009[43]
	Spain	Llobregat River	0.040 (max)	López-Serna et al., 2010[47]
	Spain	Ebro River	0.178 (max)	López-Serna et al., 2011[198]
	Spain	Surface water in Castellon and Valencia provinces	0.070 (max)	Gracia-Lor et al., 2011[224]
	Spain	Llobregat River	0.313 (max)	Osorio et al., 2012[201]
	Poland	Gościcina and Reda Rivers	0.249 (max)	Wagil et al., 2014[225]
	France	Canche River (urban impact)	0.038	Tlili et al., 2016[79]
	South Korea	River Han	0.133 (max)	Kim et al., 2016[216]
Epitetracycline	China	Wenyu River	0.026 (max)	Zhang et al., 2014[226]
Erythromycin	USA	Streams and rivers (n=139)	1.7 (max)	Kolpin et al., 2002[213]
	Italy	Po and Lambro River	0.004 (median); 0.016 (max)	Calamari et al., 2003[217]
	UK	Rivers	1.0 (max)	Ashton et al., 2004[243]
	USA	Streams in Iowa	0.220 (max)	Kolpin et al., 2004[242]
	Germany	River Elbe	0.040 (median); 0.070 (max)	Weigel et al., 2004[228]
	Canada	Grand River watershed	0.006 (median); 0.051 (max)	Lissemore et al., 2006[214]
	Canada	Grand River watershed	0.007 (median); 0.007 (max)	Hao et al., 2006[244]
	UK	Tyne River	0.070 (max)	Roberts and Thomas, 2006[245]

	USA	Cache La Poudre River	0.120 (median); 0.450 (max)	Sung-Chul et al., 2007[215]
	South Korea	Surface waters	0.003 (median); 0.005 (max)	Kim et al., 2007[246]
	UK (Wales)	River Taff	0.022 (max)	Kasprzyk-Hordern et al., 2007[187]
	Spain	Ebro River (n=7; downstream WWTPs)	0.071 (max)	Gros et al., 2007[194]
	China	Pearl River Delta	0.489	Xu et al., 2007a[247]
	China	Pearl River at Guangzhou	0.245 (median); 0.636 (max)	Xu et al., 2007b[209]
	Canada	Little River and Upper Detroir River	0.178 (max)	Hao et al., 2008[248]
	UK	River Taff (Wales)	0.015 (median); 0.121 (max)	Kasprzyk-Hordern et al., 2009[181]
	UK	River Ely (Wales)	0.015 (mean)	Kasprzyk-Hordern et al., 2009[181]
	China	Sindian, Dahan and Gaoping Rivers	0.078 (median)	Lin et al., 2009[193]
	Spain	Middle and lower Llobregat and Anoia Rivers	0.030 (median); 0.070 (max)	Muñoz et al., 2009[249]
	Spain	Llobregat, Cardener and Anoia rivers	0.033 (median); 0.112 (max)	López-Roldán et al., 2010[250]
	Italy	Surface water, River Po	0.009 (mean); 0.010 (max)	Zuccato et al., 2010[189]
	Italy	Surface water, River Arno	0.023 (mean); 0.038 (max)	Zuccato et al., 2010[189]
	Germany	River Leine and Baltic Sea	0.022 (max)	Nödler et al., 2010[231]
	USA	Surface water in Colorado	0.052 (max)	Ferrer et al., 2010[232]
	South Korea	5 rivers in Busan	0.072 (median)	Sim et al., 2010[251]
	Germany	Rhine River	0.021 (median); 0.110 (max)	Ter Laak et al., 2010[233]
	Spain	Llobregat River	0.119 (max)	López-Roldán et al., 2010[250]
	Spain	Llobregat River	0.175 (max)	López-Serna et al., 2010[47]
	France	Seine River	0.004	Tuc Dinh et al., 2011[191]
	France	Prédecelle River, downstream WWTP	0.004	Tuc Dinh et al., 2011[191]
	France	Charmoise River, downstream WWTP	0.131	Tuc Dinh et al., 2011[191]
	Canada	Wascana Creek, Qu'Appelle River	0.300 (max)	Waiser et al., 2010[190]
	Spain	Ebro River	0.052 (max)	López-Serna et al., 2011[198]
	Spain	Surface water in Castellon and Valencia provinces	0.078 (max)	Gracia-Lor et al., 2011[224]
	Spain	Ebro River	0.042 (max)	Silva et al., 2011[234]

	Spain	Jarama, Manzanares, Guadarrama, Henares, and Tagus Rivers	3.8 (max)	Valcárcel et al., 2011[199]
	Netherlands	Rhine and Meuse River	0.010 (mean); 0.035 (max)	de Jongh et al., 2012[239]
	Spain	Llobregat River	0.362 (max)	Osorio et al., 2012[201]
	Spain	Llobregat River	0.040 (max)	Boleda et al., 2013[203]
	Spain	El Albujón River	0.065 (max)	Moreno-González et al., 2014[205]
	Romania	Prahova, Timis, Danube, Siret, Prut, and Jijia Rivers	0.025 (max)	Chițescu and Nicolau, 2014[252]
	Portugal	River Tagus and Zezere (Lisbon)	0.031 (max)	de Jesus Gaffney et al., 2015[54]
	Spain	Surface waters in Castellón	0.010 (max)	Boix et al., 2015[236]
	Spain	Mar Menor lagoon (SE Spain, Murcia)	0.078 (max)	Moreno-González et al., 2015[206]
	Spain	Llobregat, Ebro, Júcar, and Guadalquivir Rivers	0.019 (max)	Osorio et al., 2016[207]
Florfenicol	China	Huangpu River	0.010 (median)	Jiang et al., 2011[211]
	South Korea	River Han	0.340 (max)	Kim et al., 2016[216]
Flumequine	France	Seine, Marne, Oise rivers	0.012 (median); 0.032 (max)	Tamtam et al., 2008[240]
	France	Seine River	0.005	Tuc Dinh et al., 2011[191]
	Spain	Surface water in Castellon and Valencia provinces	0.020 (max)	Gracia-Lor et al., 2011[224]
	Spain	Ebro River	0.030 (max)	López-Serna et al., 2011[198]
	China	Wenyu River	0.154 (max)	Zhang et al., 2014[226]
	Spain	Surface waters in Castellón	0.003 (max)	Boix et al., 2015[236]
Gatifloxacin	China	Tonghui River	0.030 (median); 0.042 (max)	Xiao et al., 2008[222]
	China	Wenyu River	0.116 (max)	Zhang et al., 2014[226]
Josamycin	Japan	Tone River basin	0.0003 (median); 0.0004 (max)	Nakada et al., 2007[195]
	Spain	Llobregat River	0.002 (max)	López-Serna et al., 2010[47]
	Spain	Ebro River	0.001 (max)	López-Serna et al., 2011[198]
	Spain	Ebro River	0.001 (max)	Silva et al., 2011[234]
	Spain	Llobregat River	0.011 (max)	Osorio et al., 2012[201]
Levofloxacin	Japan	Tone River basin	0.023 (median); 0.032 (max)	Nakada et al., 2007[195]
	USA	Upper Tennessee River	0.012 (median); 0.059 (max)	Conley et al., 2008[220]
Lincomycin	USA	Streams and rivers (n=139)	0.73 (max)	Kolpin et al., 2002[213]

	Italy	Po and Lambro River	0.028 (median); 0.249 (max)	Calamari et al., 2003[217]
	USA	Streams in Iowa	0.010 (max)	Kolpin et al., 2004[242]
	Italy	Po and Lambro Rivers	0.033 (median); 0.249 (max)	Zuccato et al., 2005[218]
	Canada	Grand River watershed	0.012 (median); 0.355 (max)	Lissemore et al., 2006[214]
	Canada	Grand River watershed	0.026 (median); 0.046 (max)	Hao et al., 2006[244]
	Canada	Little River and Upper Detroir River	0.010 (max)	Hao et al., 2008[248]
	Australia	River water	0.050 (max)	Watkinson et al., 2009[43]
	Italy	Surface water, River Po	0.006 (mean); 0.007 (max)	Zuccato et al., 2010[189]
	Italy	Surface water, River Arno	0.008 (mean); 0.011 (max)	Zuccato et al., 2010[189]
	South Korea	5 rivers in Busan	0.034 (median)	Sim et al., 2010[251]
	Spain	Surface water in Castellon and Valencia provinces	0.047 (max)	Gracia-Lor et al., 2011[224]
	Netherlands	Meuse River	0.004 (max)	Houtman et al., 2013[253]
	China	Yangtze, Huai, Yellow, Hai, Liao River	0.008 (median); 0.012 (max)	Sun et al., 2015[238]
	Spain	Surface waters in Castellón	0.012 (max)	Boix et al., 2015[236]
Lomefloxacin	China	Tonghui River	0.003 (median); 0.005 (max)	Xiao et al., 2008[222]
	China	Wenyu River	0.038 (max)	Zhang et al., 2014[226]
Marbofloxacin	Spain	Surface water in Castellon and Valencia provinces	0.205 (max)	Gracia-Lor et al., 2011[224]
Metronidazole	UK	River Taff and Ely (Wales)	0.005 (median); 0.024 (max)	Kasprzyk-Hordern et al., 2008[188]
	UK	River Taff (Wales)	0.012 (median); 0.024 (max)	Kasprzyk-Hordern et al., 2009[181]
	UK	River Ely (Wales)	0.012 (mean)	Kasprzyk-Hordern et al., 2009[181]
	Spain	Llobregat River	0.045 (max)	López-Serna et al., 2010[47]
	Spain	Ebro River	0.030 (max)	López-Serna et al., 2011[198]
	Spain	Ebro River	0.030 (max)	Silva et al., 2011[234]
	Spain	Jarama, Manzanares, Guadarrama, Henares, and Tagus Rivers	1.8 (max)	Valcárcel et al., 2011[199]
	Italy	Receiving water in the Po Valley	0.011 (max)	Al Aukidy et al., 2012[200]
	Spain	Llobregat River	0.049 (max)	Osorio et al., 2012[201]
	Spain	Ter River downstream WWTP in Girona	0.028 (max)	Rodriguez-Mozaz et al., 2015[29]

	Spain	Llobregat, Ebro, Júcar, and Guadalquivir Rivers	0.066 (max)	Osorio et al., 2016[207]
	Greece	Saronikos Gulf and Elefsis Bay in central Aegean Sea (WWTP impact from Athens)	0.008 (max)	Alygizakis et al., 2016[192]
Minocycline	China	Wenyu River	0.006 (max)	Zhang et al., 2014[226]
Monensin	Canada	Grand River watershed	0.044 (median); 1.2 (max)	Lissemore et al., 2006[214]
	Canada	Grand River watershed	0.092 (median); 0.220 (max)	Hao et al., 2006[244]
	Canada	Little River and Upper Detroir River	0.022 (max)	Hao et al., 2008[248]
	Australia	River water	0.150 (max)	Watkinson et al., 2009[43]
	France	Canche River (urban impact)	0.017	Tlili et al., 2016[79]
Moxifloxacin	China	Tonghui River	0.009 (median); 0.014 (max)	Xiao et al., 2008[222]
	China	Wenyu River	0.015 (max)	Zhang et al., 2014[226]
Nalidixic acid	Japan	Tone River basin	0.004 (median); 0.009 (max)	Nakada et al., 2007[195]
	Australia	River water	0.750 (max)	Watkinson et al., 2009[43]
	Spain	Surface water in Castellon and Valencia provinces	0.014 (max)	Gracia-Lor et al., 2011[224]
	China	Wenyu River	0.113 (max)	Zhang et al., 2014[226]
	China	Yangtze, Huai, Yellow, Hai, Liao River	0.0001 (median); 0.001 (max)	Sun et al., 2015[238]
	Spain	Surface waters in Castellón	0.004 (max)	Boix et al., 2015[236]
Norfloxacin	USA	Streams and rivers (n=139)	0.12 (max)	Kolpin et al., 2002[213]
	USA	Streams in Iowa	0.030 (max)	Kolpin et al., 2004[242]
	China	Pearl River Delta	0.166	Xu et al., 2007a[247]
	China	Pearl River at Guangzhou	0.081 (median); 0.251 (max)	Xu et al., 2007b[209]
	China	Tonghui River	0.030 (median); 0.066 (max)	Xiao et al., 2008[222]
	France	Seine, Marne, Oise rivers	0.022 (median); 0.163 (max)	Tamtam et al., 2008[240]
	Australia	River water	1.1 (max)	Watkinson et al., 2009[43]
	Spain	Llobregat River	0.016 (max)	López-Serna et al., 2010[47]
	Spain	Ebro River	0.090 (max)	López-Serna et al., 2011[198]
	Spain	Surface water in Castellon and Valencia provinces	0.054 (max)	Gracia-Lor et al., 2011[224]

	France	Seine River	0.037	Tuc Dinh et al., 2011[191]
	France	Prédecelle River, downstream WWTP	0.075	Tuc Dinh et al., 2011[191]
	France	Charmoise River, downstream WWTP	0.017	Tuc Dinh et al., 2011[191]
	Spain	Llobregat River	0.405 (max)	Osorio et al., 2012[201]
	Spain	River water of Pego-Oliva Marshlands	0.037 (max)	Vazquez-Roig et al., 2012[235]
	China	Wenyu River	0.512 (max)	Zhang et al., 2014[226]
	Poland	Gościcina and Reda Rivers	0.443 (max)	Wagil et al., 2014[225]
	France	Canche River (urban impact)	0.008	Tlili et al., 2016[79]
Ofloxacin	Italy	Po and Lambro Rivers	0.037 (median); 0.306 (max)	Zuccato et al., 2005[218]
	Finland	Vantaa River	0.005 (max)	Vieno et al., 2006[219]
	Spain	Ebro River (n=7; downstream WWTPs)	0.146 (max)	Gros et al., 2007[194]
	China	Pearl River Delta	0.074	Xu et al., 2007a[247]
	China	Pearl River at Guangzhou	0.044 (median); 0.108 (max)	Xu et al., 2007b[209]
	China	Pearl River	0.439 (max)	Peng et al., 2008[221]
	China	Tonghui River	0.300 (median); 0.535 (max)	Xiao et al., 2008[222]
	France	Seine, Marne, Oise rivers	0.030 (median); 0.055 (max)	Tamtam et al., 2008[240]
	Spain	Middle and lower Llobregat and Anoia Rivers	2.1 (median); 8.8 (max)	Muñoz et al., 2009[249]
	Spain	Llobregat, Cardener and Anoia rivers	0.285 (median); 1.9 (max)	López-Roldán et al., 2010[250]
	Italy	Surface water, River Po	0.011 (mean); 0.018 (max)	Zuccato et al., 2010[189]
	Italy	Surface water, River Arno	0.005 (mean); 0.011 (max)	Zuccato et al., 2010[189]
	Spain	Llobregat River	1.9 (max)	López-Roldán et al., 2010[250]
	Spain	Llobregat River	0.075 (max)	López-Serna et al., 2010[47]
	Canada	Wascana Creek, Qu'Appelle River	0.020 (max)	Waiser et al., 2010[190]
	France	Seine River	0.018 (max)	Tuc Dinh et al., 2011[191]
	France	Prédecelle River, upstream WWTP	0.004	Tuc Dinh et al., 2011[191]
	France	Prédecelle River, downstream WWTP	0.065	Tuc Dinh et al., 2011[191]
	France	Charmoise River, upstream WWTP	0.004	Tuc Dinh et al., 2011[191]

	France	Charmoise River, downstream WWTP	0.231	Tuc Dinh et al., 2011[191]
	Spain	Ebro River	0.105 (max)	López-Serna et al., 2011[198]
	Spain	Surface water in Castellon and Valencia provinces	0.400 (max)	Gracia-Lor et al., 2011[224]
	Spain	Jarama, Manzanares, Guadarrama, Henares, and Tagus Rivers	0.552 (max)	Valcárcel et al., 2011[199]
	Spain	Llobregat River	0.488 (max)	Osorio et al., 2012[201]
	Spain	River water of Pego-Oliva Marshlands	0.050 (max)	Vazquez-Roig et al., 2012[235]
	Spain	Ebro River and tributaries	0.080 (max)	López-Serna et al., 2012[202]
	China	Wenyu River	1.1 (max)	Zhang et al., 2014[226]
	USA	River in Maryland, upstream WWTP	0.009	He et al., 2015[227]
	Spain	Ter River downstream WWTP in Girona	0.137 (max)	Rodriguez-Mozaz et al., 2015[29]
	USA	River in Maryland, downstream WWTP	0.039 (max)	He et al., 2015[227]
	France	Canche River (urban impact)	0.008	Tlili et al., 2016[79]
Oleandomycin	Italy	Po and Lambro River	0.003 (max)	Calamari et al., 2003[217]
	Australia	River water	0.020 (max)	Watkinson et al., 2009[43]
Orbifloxacin	France	Canche River (urban impact)	0.03	Tlili et al., 2016[79]
Ornidazole	France	Seine, Marne, Oise rivers	0.022 (median); 0.058 (max)	Tamtam et al., 2008[240]
Oxolinic acid	France	Seine, Marne, Oise rivers	0.013 (median); 0.019 (max)	Tamtam et al., 2008[240]
	France	Seine River	0.023	Tuc Dinh et al., 2011[191]
	Spain	Surface water in Castellon and Valencia provinces	0.023 (max)	Gracia-Lor et al., 2011[224]
	China	Wenyu River	0.013 (max)	Zhang et al., 2014[226]
	Spain	Surface waters in Castellón	0.005 (max)	Boix et al., 2015[236]
Oxytetracycline	USA	Streams and rivers (n=139)	0.34 (max)	Kolpin et al., 2002[213]
	Italy	Po and Lambro River	0.009 (median); 0.019 (max)	Calamari et al., 2003[217]
	USA	Cache La Poudre River	0.180 (median); 1.2 (max)	Sung-Chul et al., 2007[215]
	China	Tonghui River	0.002 (median)	Jia et al., 2009[254]
	France	Arc River (WWTP impact)	0.200 (median); 0.68 (max)	Feitosa et al., 2009[223]
	Luxembourg	Alzette River	0.007 (max)	Pailler et al., 2009[255]

	Italy	Surface water, River Po	0.001 (mean); 0.002 (max)	Zuccato et al., 2010[189]
	Spain	Ebro River	0.037 (max)	López-Serna et al., 2011[198]
	China	Huangpu River	0.021 (median)	Jiang et al., 2011[211]
	Spain	Llobregat River	0.081 (max)	Osorio et al., 2012[201]
	China	Wenyu River	0.214 (max)	Zhang et al., 2014[226]
	South Korea	River Han	1.2 (max)	Kim et al., 2016[216]
	France	Canche River (urban impact)	0.001	Tlili et al., 2016[79]
Pefloxacin	Spain	Surface water in Castellon and Valencia provinces	0.064 (max)	Gracia-Lor et al., 2011[224]
	China	Wenyu River	0.022 (max)	Zhang et al., 2014[226]
Penicillin G	Australia	River water	0.250 (max)	Watkinson et al., 2009[43]
Penicillin V	Australia	River water	0.010 (max)	Watkinson et al., 2009[43]
Pipemidic acid	China	Tonghui River	0.010 (median); 0.013 (max)	Xiao et al., 2008[222]
	Spain	Surface water in Castellon and Valencia provinces	0.245 (max)	Gracia-Lor et al., 2011[224]
	China	Wenyu River	0.020 (max)	Zhang et al., 2014[226]
Piromidic acid	China	Wenyu River	0.129 (max)	Zhang et al., 2014[226]
Ronidazole	Spain	Llobregat, Ebro, Júcar, and Guadalquivir Rivers	0.008 (max)	Osorio et al., 2016[207]
Roxithromycin	Germany	Rhine, Urselbach, Nidda and Main Rivers	0.560 (max)	Hirsch et al., 1999[208]
	USA	Streams and rivers (n=139)	0.18 (max)	Kolpin et al., 2002[213]
	Germany	River Elbe	0.033 (median); 0.040 (max)	Weigel et al., 2004[228]
	Canada	Grand River watershed	0.002 (max)	Lissemore et al., 2006[214]
	China	Pearl River Delta	0.07	Xu et al., 2007a[247]
	China	Pearl River at Guangzhou	0.041 (median); 0.169 (max)	Xu et al., 2007b[209]
	South Korea	Han River, North and South Han River, Kyung-Ahn Stream	0.031 (median); 0.054 (max)	Choi et al., 2008a[210]
	Germany	River Havel	0.011 (median); 0.069 (max)	Heberer et al., 2008[229]
	Australia	River water	0.350 (max)	Watkinson et al., 2009[43]
	Germany	River Leine and Baltic Sea	0.016 (max)	Nödler et al., 2010[231]
	Germany	Rhine River	0.014 (median); 0.018 (max)	Ter Laak et al., 2010[233]

	China	Huangpu River	0.002 (median)	Jiang et al., 2011[211]
	Spain	Surface water in Castellon and Valencia provinces	0.012 (max)	Gracia-Lor et al., 2011[224]
	Spain	Llobregat River	0.008 (max)	Osorio et al., 2012[201]
	China	Yangtze, Huai, Yellow, Hai, Liao River	0.001 (median); 0.008 (max)	Sun et al., 2015[238]
Salinomycin	Australia	River water	0.150 (max)	Watkinson et al., 2009[43]
Sarafloxacin	Spain	Surface water in Castellon and Valencia provinces	0.055 (max)	Gracia-Lor et al., 2011[224]
Spiramycin	Italy	Po and Lambro River	0.044 (median); 0.074 (max)	Calamari et al., 2003[217]
	Italy	Po and Lambro Rivers	0.044 (median); 0.074 (max)	Zuccato et al., 2005[218]
	Italy	Surface water, River Po	0.001 (mean); 0.002 (max)	Zuccato et al., 2010[189]
	Italy	Surface water, River Arno	0.008 (mean); 0.018 (max)	Zuccato et al., 2010[189]
	Spain	Llobregat River	0.068 (max)	López-Serna et al., 2010[47]
	Spain	Ebro River	0.488 (max)	López-Serna et al., 2011[198]
	Spain	Llobregat River	0.152 (max)	Osorio et al., 2012[201]
Sulfabenzamide	Spain	Ebro River	0.002 (max)	García-Galán et al., 2010[56]
	Spain	Ebro River	0.008 (median)	García-Galán et al., 2011[256]
	Spain	Ebro River	0.015 (max)	García-Galán et al., 2011[256]
Sulfachloro-pyridazine	Canada	Grand River watershed	0.004 (median); 0.007 (max)	Lissemore et al., 2006[214]
	Canada	Grand River watershed	0.020 (max)	Hao et al., 2006[244]
	USA	Cache La Poudre River	0.030 (max)	Sung-Chul et al., 2007[215]
	USA	Loup River, Big Blue River, Wood River, Salt Creek, Missouri River (downstream WWTPs)	0.002 (max)	Bartelt-Hunt et al., 2009[196]
	China	Huangpu River	0.006 (median)	Jiang et al., 2011[211]
	China	Wenyu River	0.010 (max)	Zhang et al., 2014[226]
	South Korea	River Han	0.060 (max)	Kim et al., 2016[216]
Sulfadiazine	China	Pearl River at Guangzhou	0.124 (median); 0.336 (max)	Xu et al., 2007b[209]
	Japan	Koyama River	0.00005 (max)	Chang et al., 2008[257]
	Spain	Llobregat, Segre and Anoia River	0.119 (median); 2.3 (max)	Diaz-Cruz et al., 2008[258]
	Spain	Llobregat, Cardener and Anoia Rivers	0.008 (median); 0.013 (max)	García-Galán et al., 2010[56]

	Spain	Llobregat River	0.013 (max)	López-Serna et al., 2010[47]
	Spain	Llobregat and Anoia Rivers	5.0 (max)	García-Galán, et al., 2010[56]
	China	Huangpu River	0.013 (median)	Jiang et al., 2011[211]
	Spain	Ebro River	0.002 (median)	García-Galán et al., 2011[256]
	Spain	Ebro River	0.023 (max)	López-Serna et al., 2011[198]
	Spain	Ebro River and tributaries	0.136 (max)	López-Serna et al., 2012[202]
	Spain	Ebro River	0.006 (max)	García-Galán et al., 2011[256]
	Spain	Llobregat River	0.107 (max)	Osorio et al., 2012[201]
	China	Wenyu River	0.321 (max)	Zhang et al., 2014[226]
	Portugal	River Tagus and Zezere (Lisbon)	0.026 (max)	de Jesus Gaffney et al., 2015[54]
	Greece	Saronikos Gulf and Elefsis Bay in central Aegean Sea (WWTP impact from Athens)	0.002 (max)	Alygizakis et al., 2016[192]
	France	Canche River (urban impact)	0.022	Tlili et al., 2016[79]
Sulfadimethoxine	USA	Streams and rivers (n=139)	0.060 (max)	Kolpin et al., 2002[213]
	Canada	Grand River watershed	0.001 (median); 0.056 (max)	Lissemore et al., 2006[214]
	USA	Cache La Poudre River	0.020 (median); 0.040 (max)	Sung-Chul et al., 2007[215]
	Japan	Tone River basin	0.002 (median); 0.003 (max)	Nakada et al., 2007[195]
	Japan	Koyama River	0.0002 (max)	Chang et al., 2008[257]
	South Korea	Han River	0.011 (median); 0.013 (max)	Choi et al., 2008b[259]
	Spain	Llobregat, Segre and Anoia River	0.012 (median); 0.182 (max)	Diaz-Cruz et al., 2008[258]
	USA	Loup River, Big Blue River, Wood River, Salt Creek, Missouri River (downstream WWTPs)	0.003 (median); 4.0 (max)	Bartelt-Hunt et al., 2009[196]
	China	Sindian, Dahan and Gaoping Rivers	0.020 (max)	Lin et al., 2009[193]
	Spain	Llobregat, Cardener and Anoia Rivers	0.005 (median); 0.136 (max)	García-Galán et al., 2010[56]
	Spain	Henares-Jarama-Tajo river system (Madrid)	0.001 (median)	Fernandez et al., 2010[260]
	Spain	Ebro River	0.018 (max)	García-Galán et al., 2010[56]
	Spain	Llobregat and Anoia Rivers	0.136 (max)	García-Galán et al., 2010[56]

	Spain	Ebro River	0.002 (median)	García-Galán et al., 2011[256]
	Spain	Ebro River	0.023 (max)	García-Galán et al., 2011[256]
	Spain	Llobregat River	0.043 (max)	Boleda et al., 2013[203]
	South Korea	River Han	0.080 (max)	Kim et al., 2016[216]
	France	Canche River (urban impact)	0.022	Tlili et al., 2016[79]
Sulfadimidine	China	Pearl River at Guangzhou	0.126 (median); 0.323 (max)	Xu et al., 2007b[209]
	Japan	Koyama River	0.0001 (max)	Chang et al., 2008[257]
Sulfadoxine	Spain	Ebro River	0.020 (max)	García-Galán et al., 2010[56]
	Spain	Ebro River	0.013 (median)	García-Galán et al., 2011[256]
	Spain	Ebro River	0.043 (max)	García-Galán et al., 2011[256]
Sulfaguanidine	China	Wenyu River	0.003 (max)	Zhang et al., 2014[226]
Sulfamerazine	USA	Cache La Poudre River	0.020 (median); 0.060 (max)	Sung-Chul et al., 2007[215]
	Spain	Ebro River	0.016 (max)	García-Galán et al., 2010[56]
	Spain	Ebro River	0.021 (median)	García-Galán et al., 2011[256]
	Spain	Ebro River	0.042 (max)	García-Galán et al., 2011[256]
	France	Canche River (urban impact)	0.011	Tlili et al., 2016[79]
Sulfameter	China	Wenyu River	0.010 (max)	Zhang et al., 2014[226]
Sulfamethazine	USA	Streams and rivers (n=139)	0.22 (max)	Kolpin et al., 2002[213]
	Canada	Grand River watershed	0.003 (median); 0.408 (max)	Lissemore et al., 2006[214]
	Canada	Grand River watershed	0.002 (median); 0.038 (max)	Hao et al., 2006[244]
	USA	Cache La Poudre River	0.020 (max)	Sung-Chul et al., 2007[215]
	Spain	Llobregat, Segre and Anoia River	0.674 (median); 6.2 (max)	Diaz-Cruz et al., 2008[258]
	USA	Loup River, Big Blue River, Wood River, Salt Creek, Missouri River (downstream WWTPs)	0.005 (median); 0.427 (max)	Bartelt-Hunt et al., 2009[196]
	China	Sindian, Dahan and Gaoping Rivers	0.020 (max)	Lin et al., 2009[193]
	Spain	Llobregat, Cardener and Anoia Rivers	0.023 (median); 2.5 (max)	García-Galán et al., 2010[56]
	Spain	Llobregat River	0.112 (max)	López-Serna et al., 2010[47]

	Spain	Ebro River	0.020 (max)	García-Galán et al., 2010[56]
	Spain	Llobregat and Anoia Rivers	5.0 (max)	García-Galán et al., 2010[56]
	China	Huangpu River	0.159 (median)	Jiang et al., 2011[211]
	Spain	Ebro River	0.010 (median)	García-Galán et al., 2011[256]
	Spain	Ebro River	0.065 (max)	García-Galán et al., 2011[256]
	Spain	Llobregat River	0.281 (max)	Osorio et al., 2012[201]
	Spain	Ebro River and tributaries	0.641 (max)	López-Serna et al., 2012[202]
	Spain	Llobregat River	0.113 (max)	Boleda et al., 2013[203]
	China	Wenyu River	0.267 (max)	Zhang et al., 2014[226]
	Portugal	River Tagus and Zezere (Lisbon)	0.001 (max)	de Jesus Gaffney et al., 2015[54]
	South Korea	River Han	0.067 (max)	Kim et al., 2016[216]
Sulfamethizole	USA	Streams and rivers (n=139)	0.13 (max)	Kolpin et al., 2002[213]
	Japan	Koyama River	0.0001 (max)	Chang et al., 2008[257]
	Spain	Llobregat, Segre and Anoia River	0.004 (median); 0.007 (max)	Diaz-Cruz et al., 2008[258]
	USA	Loup River, Big Blue River, Wood River, Salt Creek, Missouri River (downstream WWTPs)	0.141 (median); 0.343 (max)	Bartelt-Hunt et al., 2009[196]
	Spain	Llobregat, Cardener and Anoia Rivers	0.002 (median); 0.010 (max)	García-Galán et al., 2010[56]
	Spain	Ebro River	0.003 (max)	García-Galán et al., 2010[56]
	Spain	Llobregat and Anoia Rivers	0.010 (max)	García-Galán et al., 2010[56]
	Spain	Ebro River	0.002 (median)	García-Galán et al., 2011[256]
	Spain	Ebro River	0.005 (max)	García-Galán et al., 2011[256]
Sulfamethoxazole	Germany	Rhine, Urselbach, Nidda and Main Rivers	0.030 (median); 0.480 (max)	Hirsch et al., 1999[208]
	USA	Streams and rivers (n=139)	1.9 (max)	Kolpin et al., 2002[213]
	Germany	River Elbe	0.047 (median); 0.070 (max)	Weigel et al., 2004[228]
	Sweden	Hoje River	0.015 (median); 0.050 (max)	Bendz et al., 2005[261]
	USA	Rio Grande River	0.300 (max)	Brown et al., 2006[262]
	Canada	Grand River watershed	0.003 (median); 0.009 (max)	Lissemore et al., 2006[214]

	USA	Colorado River	0.672 (max)	Vanderford & Snyder, 2006[263]
	USA	Cache La Poudre River	0.110 (median); 0.320 (max)	Sung-Chul et al., 2007[215]
	South Korea	Surface waters	0.020 (median); 0.036 (max)	Kim et al., 2007[246]
	Poland	River Warta	0.060 (max)	Kasprzyk-Hordern et al., 2007[187]
	Spain	Ebro River (n=7; downstream WWTPs)	0.169 (max)	Gros et al., 2007[194]
	Japan	Tone River basin	0.039 (median); 0.160 (max)	Nakada et al., 2007[195]
	China	Pearl River Delta	0.143	Xu et al., 2007a[247]
	China	Pearl River at Guangzhou	0.086 (median); 0.193 (max)	Xu et al., 2007b[209]
	Japan	Koyama River	0.0005 (max)	Chang et al., 2008[257]
	South Korea	Han River	0.026 (mean)	Choi et al., 2008b[259]
	USA	Upper Tennessee River	0.008 (median); 0.010 (max)	Conley et al., 2008[220]
	Spain	Llobregat, Segre and Anoia River	0.145 (median); 1.5 (max)	Diaz-Cruz et al., 2008[258]
	China	Pearl River	0.510 (max)	Peng et al., 2008[221]
	Germany	River Havel	0.151 (median); 0.326 (max)	Heberer et al., 2008[229]
	France	Seine, Marne, Oise rivers	0.044 (median); 0.544 (max)	Tamtam et al., 2008[240]
	Canada	Little River and Upper Detroir River	0.381 (max)	Hao et al., 2008[248]
	UK	River Taff (Wales)	0.002 (mean)	Kasprzyk-Hordern et al., 2009[181]
	UK	River Ely (Wales)	0.001 (mean)	Kasprzyk-Hordern et al., 2009[181]
	Europe	122 river water samples	0.015 (median); 4.1 (max)	Loos et al., 2009[264]
	Australia	River water	2.0 (max)	Watkinson et al., 2009[43]
	China	Sindian, Dahan and Gaoping Rivers	0.054 (median); 0.300 (max)	Lin et al., 2009[193]
	Spain	Middle and lower Llobregat and Anoia Rivers	1.1 (median); 11.9 (max)	Muñoz et al., 2009[249]
	Europe	European rivers	0.015 (median); 4.1 (max)	Loos et al., 2009[264]
	Luxembourg	Alzette River	0.005 (max)	Pailler et al., 2009[255]
	UK	River Ouse	0.010 (max)	Zhou et al., 2009[265]
	Spain	Guadiamar River	0.010 (max)	Camacho-Munoz et al., 2010[266]
	Spain	Llobregat, Cardener and Anoia Rivers	0.008 (median); 4.3 (max)	García-Galán et al., 2010[56]

	Europe	Danube River	0.016 (median); 0.028 (max)	Loos et al., 2010a[267]
	Europe (East)	Danube Tributary Rivers	0.021 (median); 0.204 (max)	Loos et al., 2010a[267]
	Spain	Llobregat, Cardener and Anoia rivers	0.024 (median); 0.119 (max)	López-Roldán et al., 2010[250]
	Italy	Surface water, River Po	0.002 (mean); 0.002 (max)	Zuccato et al., 2010[189]
	Italy	Surface water, River Arno	0.005 (mean); 0.011 (max)	Zuccato et al., 2010[189]
	Spain	Henares-Jarama-Tajo river system (Madrid)	0.007 (median)	Fernandez et al., 2010[260]
	Germany	River Leine and Baltic Sea	0.093 (max)	Nödler et al., 2010[231]
	Portugal	Douro River estuary	0.053 (max)	Madureira et al., 2010[268]
	USA	Surface water in Colorado	0.210 (max)	Ferrer et al., 2010[232]
	South Korea	Han River	0.061 (median); 0.190 (max)	Yoon et al., 2010[269]
	Spain	Ebro River	0.032 (max)	García-Galán et al., 2010[56]
	Spain	Llobregat and Anoia Rivers	0.653 (max)	García-Galán, et al., 2010[56]
	Spain	Llobregat River	0.119 (max)	López-Roldán et al., 2010[250]
	Spain	Llobregat River	0.078 (max)	López-Serna et al., 2010[47]
	Germany	Rhine River	0.030 (median); 0.110 (max)	Ter Laak et al., 2010[233]
	Spain	Ebro River	0.060 (median)	García-Galán et al., 2011[256]
	China	Huangpu River	0.018 (median)	Jiang et al., 2011[211]
	France	Seine River	0.018 (max)	Tuc Dinh et al., 2011[191]
	France	Prédecelle River, downstream WWTP	0.025	Tuc Dinh et al., 2011[191]
	France	Charmoise River, upstream WWTP	0.006	Tuc Dinh et al., 2011[191]
	France	Charmoise River, downstream WWTP	1.4	Tuc Dinh et al., 2011[191]
	Spain	Ebro River	0.036 (max)	García-Galán et al., 2011[256]
	Spain	Ebro River	0.055 (max)	López-Serna et al., 2011[198]
	Spain	Surface water in Castellon and Valencia provinces	0.033 (max)	Gracia-Lor et al., 2011[224]
	Spain	Jarama, Manzanares, Guadarrama, Henares, and Tagus Rivers	0.952 (max)	Valcárcel et al., 2011[199]
	Spain	Llobregat River	1.5 (max)	Osorio et al., 2012[201]

	Spain	River water of Pego-Oliva Marshlands	0.016 (max)	Vazquez-Roig et al., 2012[235]
	Spain	Ebro River and tributaries	0.017 (max)	López-Serna et al., 2012[202]
	Italy	Receiving water in the Po Valley	0.005 (max)	Al Aukidy et al., 2012[200]
	Spain	Llobregat River	0.149 (max)	Boleda et al., 2013[203]
	Netherlands	Meuse River	0.033 (max)	Houtman et al., 2013[253]
	Spain	Miño River, Galicia	0.064 (max)	Iglesias et al., 2013[270]
	Romania	Prahova, Timis, Danube, Siret, Prut, and Jijia Rivers	0.030 (max)	Chițescu and Nicolau, 2014[252]
	Spain	El Albujón River	0.065 (max)	Moreno-González et al., 2014[205]
	China	Wenyu River	0.443 (max)	Zhang et al., 2014[226]
	Spain	Surface waters in Castellón	0.025 (max)	Boix et al., 2015[236]
	Spain	Mar Menor lagoon (SE Spain, Murcia)	0.094 (max)	Moreno-González et al., 2015[206]
	Portugal	River Tagus and Zezere (Lisbon)	0.022 (max)	de Jesus Gaffney et al., 2015[54]
	Romania	Danube, Olt, Siret, and Argeș Rivers	0.030 (max)	Chitescu et al., 2015[212]
	Spain	Llobregat, Ebro, Júcar, and Guadalquivir Rivers	0.042 (max)	Osorio et al., 2016[207]
	Spain	Ter River downstream WWTP in Girona	0.072 (max)	Rodriguez-Mozaz et al., 2015[29]
	South Korea	River Han	0.270 (max)	Kim et al., 2016[216]
	Europe	5536 surface water samples	0.052 (median)	Straub, 2015[271]
	World	6633 surface water samples	0.049 (median)	Straub, 2015[271]
	France	Canche River (urban impact)	0.014	Tlili et al., 2016[79]
	Greece	Saronikos Gulf and Elefsis Bay in central Aegean Sea (WWTP impact from Athens)	0.006 (max)	Alygizakis et al., 2016[192]
Sulfamethoxy-pyridazine	Spain	Llobregat, Segre and Anoia River	0.032 (median); 3.7 (max)	Diaz-Cruz et al., 2008[258]
	Spain	Llobregat, Cardener and Anoia Rivers	0.096 (median); 0.165 (max)	García-Galán et al., 2010[56]
	Spain	Ebro River	0.015 (max)	García-Galán et al., 2010[56]
	Spain	Llobregat and Anoia Rivers	5.0 (max)	García-Galán, et al., 2010[56]
	Spain	Ebro River	0.007 (median)	García-Galán et al., 2011[256]
	Spain	Ebro River	0.018 (max)	García-Galán et al., 2011[256]

	Spain	Miño River, Galicia	0.011 (max)	Iglesias et al., 2013[270]
Sulfamono-methoxine	Japan	Tone River basin	0.040 (median); 0.130 (max)	Nakada et al., 2007[195]
	China	Sindian, Dahan and Gaoping Rivers	0.006 (max)	Lin et al., 2009[193]
	China	Wenyu River	0.012 (max)	Zhang et al., 2014[226]
	China	Yangtze, Huai, Yellow, Hai, Liao River	0.007 (median); 0.013 (max)	Sun et al., 2015[238]
Sulfanilamide	China	Wenyu River	0.003 (max)	Zhang et al., 2014[226]
Sulfanitran	Spain	Ebro River	0.004 (median)	García-Galán et al., 2011[256]
	Spain	Ebro River	0.127 (max)	García-Galán et al., 2011[256]
Sulfapyridine	UK (Wales)	River Taff	0.010 (max)	Kasprzyk-Hordern et al., 2007[187]
	Poland	River Warta	0.039 (max)	Kasprzyk-Hordern et al., 2007[187]
	Japan	Koyama River	0.003 (max)	Chang et al., 2008[257]
	Spain	Llobregat, Segre and Anoia River	0.012 (median); 12.0 (max)	Diaz-Cruz et al., 2008[258]
	UK	River Taff and Ely (Wales)	0.015 (median); 0.142 (max)	Kasprzyk-Hordern et al., 2008[188]
	UK	River Ely (Wales)	0.019 (median); 0.060 (max)	Kasprzyk-Hordern et al., 2009[181]
	Spain	Ebro River	0.011 (max)	García-Galán et al., 2010[56]
	Spain	Llobregat and Anoia Rivers	5.0 (max)	García-Galán, et al., 2010[56]
	Spain	Llobregat, Cardener and Anoia Rivers	0.010 (median); 0.092 (max)	García-Galán et al., 2010[56]
	China	Huangpu River	0.010 (median)	Jiang et al., 2011[211]
	Spain	Ebro River	0.004 (median)	García-Galán et al., 2011[256]
	Spain	Ebro River	0.043 (max)	García-Galán et al., 2011[256]
	China	Wenyu River	0.091 (max)	Zhang et al., 2014[226]
	Portugal	River Tagus and Zezere (Lisbon)	0.002 (max)	de Jesus Gaffney et al., 2015[54]
Sulfaquinoxaline	Japan	Koyama River	0.009 (max)	Chang et al., 2008[257]
	Spain	Ebro River	0.021 (max)	García-Galán et al., 2010[56]
	Spain	Ebro River	0.043 (median)	García-Galán et al., 2011[256]
	Spain	Ebro River	0.040 (max)	García-Galán et al., 2011[256]
	China	Wenyu River	0.002 (max)	Zhang et al., 2014[226]

Sulfasalazine	UK	River Taff and Ely (Wales)	0.018 (median); 0.168 (max)	Kasprzyk-Hordern et al., 2008[188]
	UK	River Ely (Wales)	0.030 (median); 0.168 (max)	Kasprzyk-Hordern et al., 2009[181]
	Australia	River water	0.150 (max)	Watkinson et al., 2009[43]
Sulfathiazole	Canada	Grand River watershed	0.001 (median); 0.016 (max)	Lissemore et al., 2006[214]
	USA	Cache La Poudre River	0.010 (median); 0.030 (max)	Sung-Chul et al., 2007[215]
	Japan	Koyama River	0.007 (max)	Chang et al., 2008[257]
	Spain	Llobregat, Segre and Anoia River	0.016 (median); 0.332 (max)	Diaz-Cruz et al., 2008[258]
	USA	Loup River, Big Blue River, Wood River, Salt Creek, Missouri River (downstream WWTPs)	0.005 (median); 0.007 (max)	Bartelt-Hunt et al., 2009[196]
	Australia	River water	0.040 (max)	Watkinson et al., 2009[43]
	Luxembourg	Alzette River	0.002 (max)	Pailler et al., 2009[255]
	Spain	Ebro River	0.014 (max)	García-Galán et al., 2010[56]
	Spain	Llobregat and Anoia Rivers	0.960 (max)	García-Galán et al., 2010[56]
	Spain	Llobregat, Cardener and Anoia Rivers	0.002 (median); 0.960 (max)	García-Galán et al., 2010[56]
	Spain	Ebro River	0.007 (median)	García-Galán et al., 2011[256]
	Spain	Ebro River	0.010 (max)	García-Galán et al., 2011[256]
	China	Wenyu River	0.002 (max)	Zhang et al., 2014[226]
	South Korea	River Han	0.056 (max)	Kim et al., 2016[216]
	France	Canche River (urban impact)	0.009	Tlili et al., 2016[79]
Sulfisomidine	Japan	Koyama River	0.0005 (max)	Chang et al., 2008[257]
	Spain	Ebro River	0.006 (median)	García-Galán et al., 2011[256]
	Spain	Ebro River	0.040 (max)	García-Galán et al., 2011[256]
Sulfisoxazole	Spain	Llobregat, Segre and Anoia River	0.001 (median); 0.003 (max)	Diaz-Cruz et al., 2008[258]
	Spain	Ebro River	0.013 (max)	García-Galán et al., 2010[56]
	Spain	Llobregat and Anoia Rivers	0.025 (max)	García-Galán et al., 2010[56]
	Spain	Llobregat, Cardener and Anoia Rivers	0.025 (max)	García-Galán et al., 2010[56]
	Spain	Ebro River	0.008 (median)	García-Galán et al., 2011[256]

	Spain	Ebro River	0.013 (max)	García-Galán et al., 2011[256]
Tetracycline	USA	Streams and rivers (n=139)	0.11 (max)	Kolpin et al., 2002[213]
	USA	Cache La Poudre River	0.020 (median); 0.030 (max)	Sung-Chul et al., 2007[215]
	Canada	Little River and Upper Detroir River	0.073 (max)	Hao et al., 2008[248]
	Australia	River water	0.080 (max)	Watkinson et al., 2009[43]
	China	Sindian, Dahan and Gaoping Rivers	0.022 (max)	Lin et al., 2009[193]
	China	Tonghui River	0.002 (median)	Jia et al., 2009[254]
	Luxembourg	Alzette River	0.007 (max)	Pailler et al., 2009[255]
	China	Huangpu River	0.114 (max)	Jiang et al., 2011[211]
	Spain	Ebro River	0.228 (max)	López-Serna et al., 2011[198]
	France	Prédecelle River, downstream WWTP	0.007	Tuc Dinh et al., 2011[191]
	Spain	Llobregat River	0.712 (max)	Osorio et al., 2012[201]
	China	Wenyu River	0.091 (max)	Zhang et al., 2014[226]
	Spain	Llobregat, Ebro, Júcar, and Guadalquivir Rivers	0.027 (max)	Osorio et al., 2016[207]
	South Korea	River Han	2.1 (max)	Kim et al., 2016[216]
	France	Canche River (urban impact)	0.013	Tlili et al., 2016[79]
Thiamphenicol	China	Huangpu River	0.014 (median)	Jiang et al., 2011[211]
Tilmicosin	Italy	Po and Lambro River	0.0004 (median)	Calamari et al., 2003[217]
	Italy	Surface water, River Po	0.003 (mean); 0.009 (max)	Zuccato et al., 2010[189]
	Italy	Surface water, River Arno	0.002 (mean); 0.009 (max)	Zuccato et al., 2010[189]
	Spain	Ebro River	0.227 (max)	López-Serna et al., 2011[198]
	Spain	Llobregat River	0.096 (max)	Osorio et al., 2012[201]
Trimethoprim	Germany	Rhine, Urselbach, Nidda and Main Rivers	0.200 (max)	Hirsch et al., 1999[208]
	USA	Streams and rivers (n=139)	0.71 (max)	Kolpin et al., 2002[213]
	UK	Rivers	0.036 (max)	Ashton et al., 2004[243]
	Germany	River Elbe	0.035 (median); 0.040 (max)	Weigel et al., 2004[228]
	Sweden	Hoje River	0.030 (median); 0.040 (max)	Bendz et al., 2005[261]
	Canada	Grand River watershed	0.003 (median); 0.015 (max)	Lissemore et al., 2006[214]

	USA	Colorado River	0.080 (max)	Vanderford & Snyder, 2006[263]
	Canada	Grand River watershed	0.001 (median); 0.002 (max)	Hao et al., 2006[244]
	UK	Tyne River	0.019 (max)	Roberts and Thomas, 2006[245]
	South Korea	Surface waters	0.004 (median); 0.005 (max)	Kim et al., 2007[246]
	Poland	River Warta	0.027 (max)	Kasprzyk-Hordern et al., 2007[187]
	Spain	Ebro River (n=7; downstream WWTPs)	0.069 (max)	Gros et al., 2007[194]
	Japan	Koyama River	0.0003 (max)	Chang et al., 2008[257]
	South Korea	Han River, North and South Han River, Kyung-Ahn Stream	0.031 (median); 0.054 (max)	Choi et al., 2008a[210]
	South Korea	Han River	0.011 (mean)	Choi et al., 2008b[259]
	USA	Upper Tennessee River	0.003(median); 0.007 (max)	Conley et al., 2008[220]
	UK	River Taff and Ely (Wales)	0.044 (median); 0.183 (max)	Kasprzyk-Hordern et al., 2008[188]
	Germany	River Havel	0.012 (median); 0.049 (max)	Heberer et al., 2008[229]
	France	Seine, Marne, Oise rivers	0.018 (median); 0.045 (max)	Tamtam et al., 2008[240]
	Canada	Little River and Upper Detroir River	0.346 (max)	Hao et al., 2008[248]
	UK	River Taff (Wales)	0.040 (median); 0.089 (max)	Kasprzyk-Hordern et al., 2009[181]
	UK	River Ely (Wales)	0.062 (mean)	Kasprzyk-Hordern et al., 2009[181]
	Serbia	Danube, Sava and Tamis Rivers	0.025 (median); 0.174 (max)	Grujić et al., 2009[197]
	Australia	River water	0.150 (max)	Watkinson et al., 2009[43]
	Spain	Middle and lower Llobregat and Anoia Rivers	0.140 (median); 0.470 (max)	Muñoz et al., 2009[249]
	Spain	Guadiamar River	0.075 (max)	Camacho-Munoz et al., 2010[266]
	Spain	Llobregat, Cardener and Anoia rivers	0.038 (median); 0.252 (max)	López-Roldán et al., 2010[250]
	Spain	Henares-Jarama-Tajo river system (Madrid)	0.012 (median)	Fernandez et al., 2010[260]
	Germany	River Leine and Baltic Sea	0.095 (max)	Nödler et al., 2010[231]
	Portugal	Douro River estuary	0.016 (max)	Madureira et al., 2010[268]
	Spain	Llobregat River	0.252 (max)	López-Roldán et al., 2010[250]
	USA	Surface water in Colorado	0.105 (max)	Ferrer et al., 2010[232]

	South Korea	Han River	0.041 (median); 0.080 (max)	Yoon et al., 2010[269]
	Spain	Llobregat River	0.034 (max)	López-Serna et al., 2010[47]
	Germany	Rhine River	0.007 (median); 0.020 (max)	Ter Laak et al., 2010[233]
	Spain	Surface water in Castellon and Valencia provinces	0.151 (max)	Gracia-Lor et al., 2011[224]
	Spain	Ebro River	0.030 (max)	Silva et al., 2011[234]
	Spain	Jarama, Manzanares, Guadarrama, Henares, and Tagus Rivers	0.690 (max)	Valcárcel et al., 2011[199]
	Spain	Ebro River	0.030 (max)	López-Serna et al., 2011[198]
	France	Prédecelle River, downstream WWTP	0.008	Tuc Dinh et al., 2011[191]
	France	Charmoise River, downstream WWTP	0.254	Tuc Dinh et al., 2011[191]
	Spain	Llobregat River	0.036 (max)	Osorio et al., 2012[201]
	Spain	River water of Pego-Oliva Marshlands	0.003 (max)	Vazquez-Roig et al., 2012[235]
	Italy	Receiving water in the Po Valley	0.015 (max)	Al Aukidy et al., 2012[200]
	Spain	Ebro River and tributaries	0.060 (max)	López-Serna et al., 2012[202]
	Netherlands	Meuse River	0.013 (max)	Houtman et al., 2013[253]
	Europe	1899 surface water samples	0.012 (median)	Straub, 2013[272]
	Spain	Llobregat River	0.081 (max)	Boleda et al., 2013[203]
	Spain	Miño River, Galicia	0.085 (max)	Iglesias et al., 2013[270]
	China	Wenyu River	0.165 (max)	Zhang et al., 2014[226]
	Ireland	Marine surface waters	0.870 (max)	McEneff et al., 2014[273]
	Romania	Prahova, Timis, Danube, Siret, Prut, and Jijia Rivers	0.020 (max)	Chițescu and Nicolau, 2014[252]
	Spain	El Albujón River	0.025 (max)	Moreno-González et al., 2014[205]
	Italy	Receiving water of a large WWTP in the Po Valley	0.002 (mean)	Verlicchi et al., 2014[204]
	Romania	Danube, Olt, Siret, and Argeş Rivers	0.012 (max)	Chitescu et al., 2015[212]
	Spain	Ter River downstream WWTP in Girona	0.093 (max)	Rodriguez-Mozaz et al., 2015[29]
	Spain	Surface waters in Castellón	0.005 (max)	Boix et al., 2015[236]
	Spain	Mar Menor lagoon (SE Spain, Murcia)	0.002 (max)	Moreno-González et al., 2015[206]
	Spain	Llobregat, Ebro, Júcar, and Guadalquivir Rivers	0.150 (max)	Osorio et al., 2016[207]

	South Korea	River Han	0.587 (max)	Kim et al., 2016[216]
	France	Canche River (urban impact)	0.027	Tlili et al., 2016[79]
	Greece	Saronikos Gulf and Elefsis Bay in central Aegean Sea (WWTP impact from Athens)	0.003 (max)	Alygizakis et al., 2016[192]
Tylosin	USA	Streams and rivers (n=139)	0.28 (max)	Kolpin et al., 2002[213]
	Italy	Po and Lambro River	0.003 (max)	Calamari et al., 2003[217]
	USA	Cache La Poudre River	0.050 (max)	Sung-Chul et al., 2007[215]
	Canada	Little River and Upper Detroir River	0.024 (max)	Hao et al., 2008[248]
	Australia	River water	0.060 (max)	Watkinson et al., 2009[43]
	China	Sindian, Dahan and Gaoping Rivers	0.010 (max)	Lin et al., 2009[193]
	Spain	Ebro River	0.001 (max)	López-Serna et al., 2011[198]
	China	Huangpu River	0.0002 (median)	Jiang et al., 2011[211]
	France	Seine River	0.003	Tuc Dinh et al., 2011[191]
	Spain	Llobregat River	0.030 (max)	Osorio et al., 2012[201]
	Romania	Danube, Olt, Siret, and Argeş Rivers	0.039 (max)	Chitescu et al., 2015[212]
	Greece	Saronikos Gulf and Elefsis Bay in central Aegean Sea (WWTP impact from Athens)	0.002 (max)	Alygizakis et al., 2016[192]
Vancomycin	Italy	Surface water, River Po	0.005 (mean); 0.012 (max)	Zuccato et al., 2010[189]
	Italy	Surface water, River Arno	0.003 (mean); 0.012 (max)	Zuccato et al., 2010[189]
	France	Charmoise River, downstream WWTP	0.09	Tuc Dinh et al., 2011[191]
Virginiamycin	South Korea	River Han	0.187 (max)	Kim et al., 2016[216]

List of abbreviations and definitions

ABC ATP-binding Cassette Superfamily

ABR Antibiotic Resistance

ADP Adenosine Diphosphate

Ag Silver

Ag^R Silver resistance

Ag^+ Silver Ion

AME Aminogycoside Modifying Enzymes

AMP Adenosine Monophosphate

AMR Antimicrobial Resistance

$Antibiotic^R$ Antibiotic Resistance

ARG Antibiotic Resistance Gene

As Arsenic

ATP Adenosine Triphosphate

Bi Bismuth

CAT Chloramphenicol Acetyltransferase

Cd Cadmium

cfr Chloramphenicol-Florfenicol Resistance

CFU Colony Forming Unit

Co Cobalt

CoA Coenzyme A

Cr Chromium

CTX-M Cefotaximase M

Cu Copper

Cu^R Copper resistance

D-Ala-D-Ala D-Alanine-D-Alanine

DHF Dihydrofolic Acid

DNA Deoxyribonucleic Acid

DW Drinking Water

DWD Dirking Water Directive

DWTP Drinking Water Treatment Plants

EC European Commission

ECDC European Centre for Disease Prevention and Control

EFSA European Food Safety Authority

EMA European Medicines Agency

erm Erythromycin Ribosomal Methylation

ESBL Extended Spectrum β-Lactamases

EU European Union

GW Ground Water

Hg Mercury

Hg^R Mercury Resistant

Hg^S Mercury Sensitive

Hg^0 Elemental Mercury

Hg^{2+} Mercuric ions

IMP Imipenemase Metallo β-Lactamase

KPC *Klebsiella pneumoniae* Carbapenemase

MATE multidrug and Toxic Compounds Extrusion Family

MDR Multidrug Resistance

$Metal^R$ Metal Resistance

MFS Major Facilitator Superfamily

MGE Mobile Genetic Element

MIC Minimal Inhibitory Concentration

Mg Manganese

merA Mercury Reductase

MRG Metal Resistance Genes

MRL Maximum Residue Limit

MRSA methicillin-resistant *Staphylococcus aureus*

MS Member States

NDM-1 New Delhi Metallo-β-Lactamase 1

NH_2 Amino Radical

Ni Nickel

OH Hydroxide

OXA Oxacillin Hydrolysing Enzymes

PABA Para-aminobenzoic Acid

Pb Lead

PBP Penicillin Binding Proteins

PBP2a Penicillin Binding Protein 2a

qnr Quinolone resistance gene

RIF Rifampicin

RIVM Rijksinstituut voor Volksgezondheid en Milieu

RNA Ribonucleic Acid

RND Resistance-Nodulation-Cell Division Family

rRNA Ribosomal Ribonucleic Acid

SHV Sulfhydryl Variable Enzymes

SMR Small Multidrug Resistance Family

Sul Sulfonamide

Tet Tetracycline

THF Tetrahydrofolic Acid

UK United Kingdom

USA United States of America

UV Ultraviolet light

VIM Verona Integron Metallo β-Lactamase

WFD Water Framework Directive

WHO World Health Organization

WL Watch List

WWTP Wastewater Treatment Plant

Zn Zinc

Zn^{2+} Zinc cation

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KJ-NA-29592-EN-N

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Publications Office

doi:10.2760/771124

ISBN 978-92-79-98478-5