Microchemical Journal 136 (2018) 25–39

Contents lists available at ScienceDirect

Microchemical Journal

journal homepage: www.elsevier.com/locate/microc

Ecological effects of antibiotics on natural ecosystems: A review

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ARTICLE INFO

Article history:
Received 10 August 2016
Received in revised form 14 January 2017
Accepted 7 February 2017
Available online 10 February 2017

Keywords:
Antibiotic effects
Natural microbial communities
Biodegradation
Antibiotic resistance genes

ABSTRACT

Among the different pharmaceuticals present in soil and water ecosystems as micro-contaminants, considerable attention has been paid to antibiotics, since their increasing use and the consequent development of multi-resistant bacteria pose serious risks to human and veterinary health. Moreover, once they have entered the environment, antibiotics can affect natural microbial communities. The latter play a key role in fundamental ecological processes, most importantly the maintenance of soil and water quality. In fact, they are involved in biogeochemical cycling and organic contaminant degradation thanks to their large reservoir of genetic diversity and metabolic capability. When antibiotics occur in the environment, they can hamper microbial community structure and functioning in different ways and have both direct (short-term) and indirect (long-term) effects on microbial communities. The short-term ones are bactericide and bacteriostatic actions with a consequent disappearance of some microbial populations and their ecological functioning. The indirect impact includes the development of antibiotic resistant bacteria and in some cases bacterial strains able to degrade them by metabolic or co-metabolic processes. Biodegradation makes it possible to completely remove a toxic compound from the environment if it is mineralized.

Several factors can influence the significance of such direct and indirect effects, including the antibiotic's concentration, the exposure time, the receiving ecosystem (e.g. soil or water) and the co-occurrence of other antibiotics and/or other contaminants.

This review describes the current state of knowledge regarding the effects of antibiotics on natural microbial communities in soil and water ecosystems.

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1. Introduction

Pharmaceuticals are essential for the maintenance of public health and life quality. Thousands of different active compounds are currently in use in large quantities to treat or prevent human and animal diseases; however, they have also been found over the last 20 years as micro-contaminants in soil and water ecosystems [1,2] thanks to an increase in the ability to detect them with advanced chemical analyses. Among the various pharmaceuticals, the presence of antibiotics in soil and water ecosystems causes particular concern since their increasing use and the consequent development of multi-resistant bacteria pose serious risks for human and animal health [3,4,5]. Antimicrobials are compounds that can kill or inhibit the growth of microorganisms (bacteria, archaea, viruses, protozoa, microalgae and fungi). Antibiotics (Table 1) are a type of antimicrobial that specifically act on bacteria or fungi in human and animal hosts, which distinguishes them from disinfectants or other antimicrobials [6]. Some antibiotics are used for other therapeutic uses, such as anticancer drugs (e.g. actinomycin D, anthracyclines, bleomycin, mitosanes, anthracenones, enediynes and epothilones) or pesticides (such as oxytetracycline and streptomycin) [7,8].

Antibiotics currently in use are natural, synthetic and semi-synthetic molecules. Natural antibiotics are produced by bacteria and fungi (e.g. benzylpenicillin and gentamicin) to inhibit or kill other competitor microorganisms (with bacteriostatic or bactericidal effect). Semi-synthetic compounds are natural antibiotics chemically altered by inserting an additive within the drug formulation, which improves its effectiveness (more stable and less biodegradable).

Antibiotics are complex molecules that can have different functional groups within their chemical structure and can be divided into different categories (Table 1), based on their action mechanism: the inhibition of cell wall synthesis, alteration of cell membranes, protein synthesis inhibition, synthesis of nucleic acids inhibition and metabolic or anti-competitive antagonism [4], Fig. 1.

Following administration, they are only partially metabolized and, therefore, a large amount is excreted unaltered or as active metabolites via urine and faeces [9,10]; consequently, human antibiotics reach wastewater treatment plants (WWTPs) [11]. Conventional WWTPs are not specifically designed for antibiotic removal, and consequently these molecules are released directly into the receiving environment

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http://dx.doi.org/10.1016/j.microc.2017.02.006
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Table 1
Main classes, active ingredients and their main use of antibiotics. Information and figures are taken from EMA, the European Medicine Agency (www.ema.europa.eu/ema), The FOOTPRINT Pesticide Properties Database (www.eufootprint.org/ppdb.html), PUBCHEM (pubchem.ncbi.nlm.nih.gov), ChemSpider (http://www.chemspider.com). Data of consumption from EMA [8]. In bold the antibiotics with some information regarding the effects on natural microbial communities of soil and water reported in Table 3.

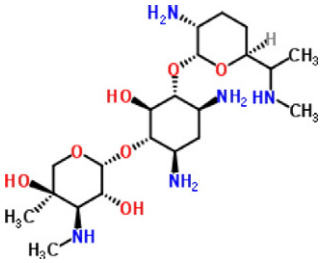
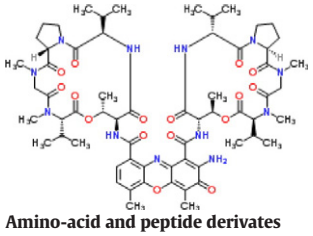
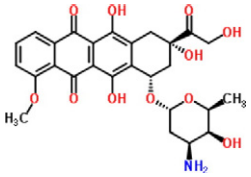
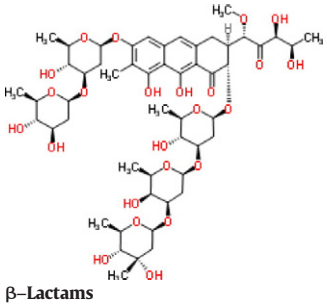
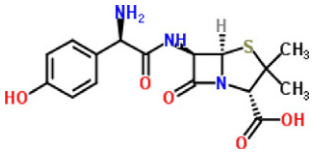
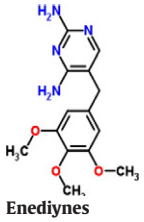
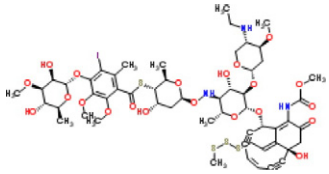
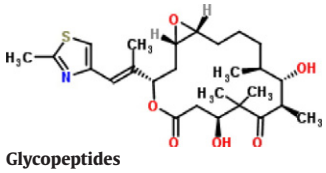
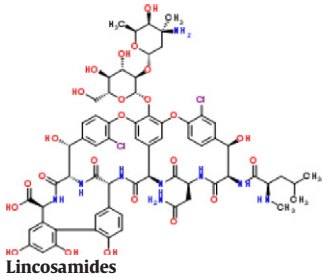
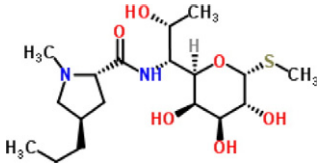
Class and antibiotic function	Active ingredient (some examples)	Main use	Consumption ton active ingredient Human - Vet
Aminoglycosides Inhibition of protein synthesis 	Amikacin Apramycin Dihydrostreptomycin Gentamicin Kanamycin Neomycin Sisomicin Spectinomycin Streptomycin	Veterinary Veterinary Veterinary Human, Veterinary, Plants Veterinary Veterinary Human Veterinary Veterinary, Plants	4.7–290.8
Actinomycines Inhibition of the synthesis of nucleic acids (anticancer drugs) 	Actinomycin D	Human	n.r.
Amino-acid and peptide derivates Inhibition of cell wall synthesis 	β-peptides Magainins D-Cycloserine	Human	n.r.
Anthracyclines Inhibition of DNA and RNA synthesis (anticancer drugs) 	Daunorubicin Doxorubicin Epirubicin Pirirubici Valrubicin	Human Human Human Human Human	n.r.
Anthracenones (anticancer drugs) 	Mithramycin Streptozotocin Pentostatin	Human Human Human	n.r.
β–Lactams Inhibition of cell wall synthesis 	**Amoxicillin** Ampicillin Azlocillin Benzylpenicillin Carbenicillin Cloxacilin Cephalexin Cephalotin Cefazolin Ceftiofur Cefotaxim Cefotiam Cefquinome	Veterinary Veterinary Human Veterinary Human Veterinary (Cattle) Veterinary (Dog) Human Human Veterinary (Cattle, Pigs) Human Human Veterinary (Cattle, Pigs)	Penicillines: 2110.9–1779.8 Cephalosporins 1st-and 2nd-gen.: 178.3–7.3 Monobactams and Carbapenems: 8.5 – not allowed in EU for Vet use

Table 1 (*continued*)

Class and antibiotic function	Active ingredient (some examples)	Main use	Consumption ton active ingredient Human - Vet
	Dicloxacilin	Veterinary (Cattle)	
	Flucloxacillin	Human	
	Methicillin	Human	
	Mezlocillin	Human	
	Nafcillin	Human	
	Oxacillin	Veterinary (Cattle)	
	Nafcillin	Human	
	Penicillin G	Human	
	Piperacillin	Human	
Diaminopyrimidine Inhibition of purine and pyrimidine synthesis	Trimethoprim	Human	n.r.
			
Enediynes	Calicheamycin	Human	n.r.
			
Epothilones Inhibition of cell division (anticancer drugs)	Epothilone A	Human	n.r.
	Epothilone B	Human	
			
Glycopeptides Acting on the wall or membrane cell	Polymyxins (Polymyxin A, Polymyxin E)	Human, Veterinary	n.r.
	Teicoplanin	Human	
	Vancomycin	Veterinary	
	Bleomycin (antitumoral)	Human	
			
Lincosamides Inhibition of protein synthesis by reversibly binding to the 50S ribosomal subunit	Clindamycin	Human	58.8–234.7
	Lincomycin	Veterinary	
			
Macrolides Inhibition of protein synthesis by reversibly binding to the 50S ribosomal subunit	Azithromycin	Human	252.3–638.0
	Clarithromycin	Human	
	Erythromycin	Human, Veterinary (Cattle, Poultry)	
	Natamycin	Food additive	
	Oleandomycin	Veterinary	
	Roxythromycin	Human	
	Spiramycin	Veterinary	
	Tilmicosin	Veterinary	

(*continued on next page*)

Table 1 (*continued*)

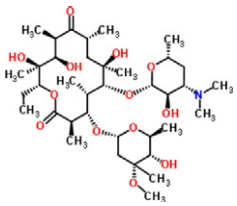
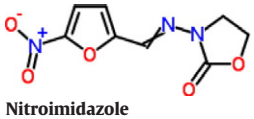
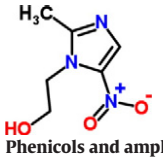
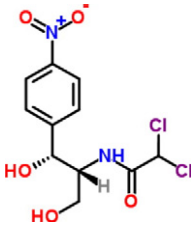
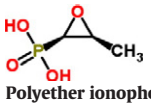
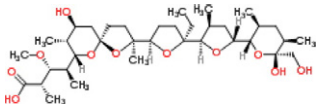
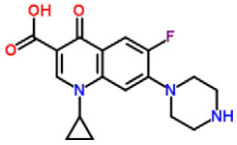
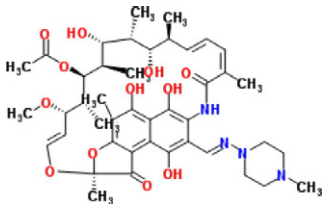
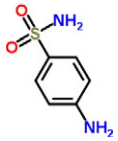
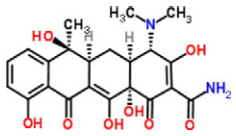
Class and antibiotic function	Active ingredient (some examples)	Main use	Consumption ton active ingredient Human - Vet
	**Tylosin**	Veterinary	
Mitosanes Inhibition of the synthesis of nucleic acids	Mitomycin C	Human (anticancer)	n.r.
			
Nitrofurans Inhibition of the synthesis of nucleic acids	Furaltadone Furazolidone Nitrofurantoin Nitrofurazone	Human, Veterinary[a] Banned from use in the livestock production in European Union (EU) from 1995 due to concerns about the carcinogenicity of their residues in edible tissue	n.r.
			
Nitroimidazole Inibition of nucleic acids synthesis	Metronidazole Tinidazole	Human Human	n.r.
			
Phenicols and amphenicols Inhibition of protein synthesis	**Chloramphenicol** Thiamphenicol	Veterinary (Cat, Dog) Veterinary	2.5–58.0
			
Phosphonates Inhibition of cell wall synthesis	Fosfomycin Phosphonothrixin	Human herbicide	n.r.
			
Polyether ionophores	Laidlomycin Lasalocid acid Maduramycin **Monensin** Narasin Salinomycin Semduramycin	Veterinary Veterinary Veterinary Veterinary Veterinary Veterinary Veterinary	
			
Quinolones and Fluoroquinolones Inhibition of DNA replication	**Ciprofloxacin** Enrofloxacin Flumequine Marbofloxacin Nalidixic acid Ofloxacin **Oxolinic Acid**	Human Veterinary Human Veterinary Human Human Veterinary, Plant	231.5–186.1
			
Rifamycins Inibition of nucleic acids synthesis	Rifampicin Rifapentine	Human Human	n.r.

Table 1 (*continued*)

Class and antibiotic function	Active ingredient (some examples)	Main use	Consumption ton active ingredient Human - Vet
			
Sulfonamides	Mafenide	Veterinary	121.5–826.3
Inhibition of the folic acid synthesis	**Sulfachloropyridazine**	Human, Veterinary	
	Sulfanilamide	Human	
	Sulfadimethoxine	Veterinary (Cattle, Pigs, Poultry)	
	Sulphamethazine	Veterinary (Cattle, Ovine, Poultry)	
	(Sulfadimidine)	Human	
	Sulfamethoxazole	Veterinary (Pigs)	
	Sulfapyridine	Human	
	Sulfathiazole	Human	
	Sulfadiazine	Veterinary	
	Sulfisoxazole		
Tetracyclines	**Chlortetracycline**	Veterinary (Cattle, Pigs)	05.9–2942.6
Inhibition of the protein synthesis	Doxycycline	Veterinary (Cat, Dog), Human	
	**Oxytetracycline**	Human, Veterinary (Cattle, Ovine, Pigs), Plants	
	Tetracycline	Human, Veterinary (Horses, Ovine, Pigs)	

n.r.: Not reported in [8].
[a] Nitrofurantoin, Furazolidone, Furaltadone and Nitrofurazone are nitrofuran derivates.

[12–15]. Although WWTPs are considered the main source of antibiotics and antibiotic-resistance genes for surface waters, the current legislation at a European level does not contain an antibiotic concentration requirement for discharge from WWTPs to receiving water [2]. Much research has been focused on the development of innovative technologies for antibiotic removal in WWTPs (e.g. additional oxidation,

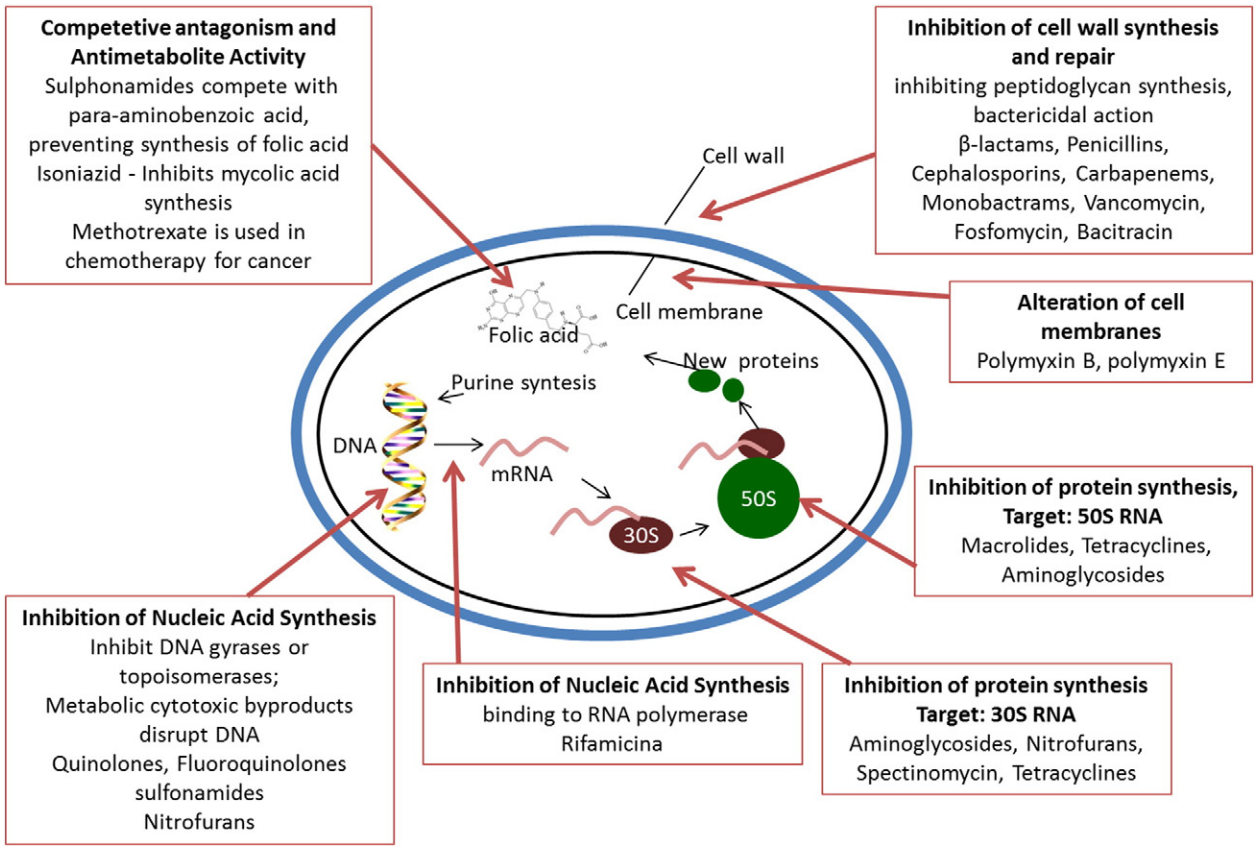


Fig. 1. Main action mechanism of antibiotics.

coagulation, microfiltration on membranes or activated carbon, Clay Micelle Complex). These additional treatments clearly increase management costs and, for this reason, are currently under-utilised [16,17]. Hospital and pharmaceutical industry wastewaters [18–20], uncontrolled and illegal drug disposal [21] and aquaculture [22] can also be a significant source of aquatic contamination [4,13]. The application of manure and sludge to soil as fertilizers, together with irrigation with reclaimed water can contribute to the dissemination of antibiotics and antibiotic resistance genes in the soil [23]. It has also been highlighted that there is the possibility of antibiotic uptake by plants or other organisms from soil fertilized with animal manure or irrigation wastewaters [23–25]. Although the adverse impacts of ingesting antibiotics present in plants are not well known and studied, they might cause allergic or toxic reactions and/or could improve antibiotic resistance in humans [23]. Finally, from surface soil, depending on their specific intrinsic characteristics, antibiotics can be leached to deeper layers and groundwater [26–28].

In this review, we present recent studies of the response by natural microbial communities to antibiotics in terms of detrimental effects, resistance and/or degradative ability.

2. Antibiotics in the environment

Antibiotic concentrations in natural environments such as soil or water range from a few nanograms to hundreds of nanograms per litre or kg soil. The highest quantities are usually found in areas with strong anthropogenic pressures such as hospital effluents [29,30], wastewater influents and effluents [31] and soils treated with manure or soils used for livestock [32]. In wastewaters, antibiotic concentrations are correlated with variations in annual consumption data, being higher in winter. Low concentrations are usually detected in natural environments (see for example Table 2 [15,18,33–41] in which antibiotic concentrations in some Italian rivers are reported). The environmental residual concentrations of antibiotics are due not only to their continuous release into the environment, but also to their intrinsic high persistence. In fact, some antibiotics, such as penicillins, are easily degraded, whereas others, such as fluoroquinolones (e.g. ciprofloxacin), macrolideas (e.g. tylosin) and tetracyclines, are considerably more persistent, resulting in their remaining for longer in the environment, spreading wider and accumulating in higher concentrations [42–44].

A recent review on antibiotics in water found that quinolones, sulfonamides, and trimethoprim are the antibiotics most analyzed and detected, due to their significance for human and veterinary medicine and persistence in the aqueous environment [2].

Table 2
Antibiotic concentration in Italian rivers. Data from [15,18,33–41].

Antibiotic	Class	Concentration (ng L^{-1})		
		Po	Lambro	Tiber
Amoxycillin	B-Lactam	n.d	0–16.7	
Cyprofloxacin	Quinolones	1.3–124	6.7–14.4	8.8–19
Clarithromycin	Macrolide	0.9–128.0	8.3–149.0	
Erythromycin	Macrolide	0.78–15.9	4.5	
Lincomycin	Lincosamide	1.2–248.9	6.8–24.4	
Metronidazole	Nitroimidazole	13–68		
Oleandomycin	Lincosamide	0.1–0.4	0.8–2.8	
Ofloxacin	Fluoroquinolone	33.1	306.1	
Oxytetracycline	Tetracicline	1.2–8.0	14.4	
Sulfamethoxazole	Sulfonamide	1.83–2.39	nd	68
Sulfadiazine	Sulfonamide			236
Sulfadimethoxine	Sulfonamide			28
Sulfapyridine	Sulfonamide			121
Spiramycin	Macrolide	0.66–26.8	8.4–74.2	
Tilmicosin	Macrolide	0.4–8.93	nd	
Tylosin	Macrolide	0.3	2.2–2.8	
Vancomycin	Glycopeptide	0.59–11.69		

Because of their continuous and steady introduction into the environment, aquatic or soil organisms are chronically exposed to these chemicals [45–47]. Moreover, as they are active in very low concentrations, they can have a toxic effect, through both their active ingredients and the additives used in their formulation. The simultaneous presence of several antibiotics with other pharmaceuticals and/or other xenobiotics may result in a synergistic effect, a phenomenon well known in pharmacology [45].

Antibiotic occurrence below the minimal inhibitory concentrations (MICs, the concentration that kills or inhibits growth of at least some bacteria), can select for resistant bacteria. A recent paper reports data on both predicted lowest MICs and no effect concentrations (PNECs) for resistance selection for 122 antibiotics. The PNECs ranged from 0.008 to 64 μg L^{-1}, as compared to the observed/predicted lowest MICs, ranging from 0.69 to 32,000 μg L^{-1}. These values could be used by local authorities to define emissions limits and as guidance in environmental risk assessment [47]. Minimal concentrations in the range of μg L^{-1} can lead to a horizontal transfer of resistance genes in *E. coli*, as found for the broad-spectrum antibiotic tetracycline commonly used to treat both humans and animals [48]. This concentration is in the same range commonly found in soils; serious concern has been consequently raised about the possible role of sub-therapeutic concentrations of tetracycline in promoting antibiotic resistance.

Owing to the important ecological functions played by natural microbial communities, there is a need for more specific protection goals based on the ecosystem service concept. In this regard, antibiotic Ecological Risk Assessments, through the application of a form of toxic action approach, should make more use of ecotoxicological endpoints targeting microorganisms, especially bacteria [6]. In fact, an understanding of the interaction of antibiotic compounds and bacteria in the environment is crucial for a proper risk assessment of these molecules. In general, test methods applied for assessing chemical persistence and toxicity for environmental organisms are performed using OECD and ISO methods; they have been successfully used for decades in the study of the fate of chemicals in the environment. For reasons of efficiency, the risk management has also been used for antibiotics, although it has not yet been fully established whether these tests are valid for antibiotics. Recently, the concept of ecological risk assessment for plant protection products has been improved by considering ecosystem services (supporting services necessary for the production of all other ecosystem services; provisioning services which are the products obtained from ecosystems; and regulating services that impact environmental quality) [49–52]. The same concept has been proposed for antibiotics by Brandt et al. [6], given that natural microbial communities are most probably an antibiotic-sensitive group and play a key role in ecosystem functioning. In this regard, the main ecosystem functions provided by natural bacterial communities are described (e.g. Regulatory, Provisioning and Supporting services) and specific ecological endpoints for natural bacteria are proposed [6].

Antibiotics and their transformation products display a wide range of physico-chemical and biological properties depending on the abiotic properties of the environment. For example, under different pH conditions they can behave as neutral, cationic, anionic or zwitterionic compounds, with both positive and negative charges [4]. Moreover, the octanol-water partition coefficient (log Kow value), absorption, reactivity to light, antibiotic activity and toxicity can change with the pH [4].

According to the European Medicines Agency Guideline of 2006, an environmental risk assessment of human pharmaceuticals is now required to support approval of all new pharmaceutical marketing authorizations [51]. However, many drugs being detected in the environment were approved prior to this guideline and hence there is increasing interest in generating ecological risk data regarding already-in-the-market products. In the European Medicines Agency (EMA) Guidelines, the potential environmental hazard posed by pharmaceuticals is estimated using a tiered approach. If in phase I the predicted environmental concentration in surface water of a drug or its metabolites is > 10 ng L^{-1}

or if the log K_{ow} > 4.5, then a phase II quantitative risk that estimates the environmental fate and effects of the pharmaceutical is required [51–54]. The EMA suggests a concentration limit for surface water of 10 ng L^{-1} below which it is not necessary to carry out further tests for drug toxicity. However, the question is if this limit is sufficiently conservative for antibiotics, given that there can be antibiotic resistance even at lower concentrations (<1 ng L^{-1}), which are often exceeded in current pollution scenarios. While efficient extraction methods combined with high sensitivity analysis by liquid chromatography/mass spectrometry can provide accurate quantification of antibiotics [55] and their transformation products, concentrations measured do not necessarily reflect their bioavailable fractions and effects in the environment. Consequently, biological assays that provide information on bioavailability, biological activity and the effects of mixtures can be essential for an insight into their biological activity.

Improved assessment of the ecological and human health risks associated with antibiotics requires continued advances in analytical accuracy and sensitivity through improvements in sample preparation, instrumentation and screening technologies. The analytical complexity related to the multiplicity of antibiotics and their transformation products, and the simultaneous presence of more than one class of compounds in the environmental compartments (soil, water, sediment, biota) are the main problems in the chemical analyses of these molecules.

In the European contest, the Water Framework Directive (Directive 2000/60/EC), amended in line with advances in scientific knowledge about substances of emerging concern [56,57], establishes the preservation, protection and improvement in the quality and sustainable use of water. The EU Directive 2013/39/EU sets the environmental quality standards (EQS) for a list of 45 substances of priority concern (antibiotics excluded) in surface water and biota across the EU, due to their widespread use and their high concentrations in rivers, lakes, and transitional and coastal waters [56,58]. The recent Decision 2015/495 [53] establishes a watch list of substances that could pose a significant risk for aquatic environments in the EU. For the first time in legislative history macrolide antibiotics (erythromycin, clarithromycin and azithromycin) are included in this list; they will consequently be monitored, with it then being possible that they get to be included in the priority substances list for environmental quality standards [55].

3. Effects of antibiotics on natural microbial communities (environmental side-effects)

Antibiotics are explicitly designed to have an effect on microorganisms and the bacterial responses to them are concentration-dependent [59]. At high concentrations, antibiotics produce antimicrobial actions on susceptible cells, while sub-inhibitory concentrations induce inverse biological responses in bacteria [59].

At high concentrations, antibiotics can act on bacteria with a bactericidal and bacteriostatic effect, although lethal concentrations rarely occur outside of therapeutic applications [60]. Bacteriostatic agents inhibit the growth of bacterial cells but do not kill them, whereas bactericidal agents kill bacteria. The terms bactericidal and bacteriostatic are broad categorizations, and may not apply for a given agent against all organisms, with certain antimicrobials being bactericidal for one bacterial pathogen but bacteriostatic for another. Consequently, these categories are not absolute, since the killing effect of each drug varies with the test method and the species tested [60].

The detrimental effect of antibiotics on natural microbial communities could be the disappearance or inhibition of some microbial groups involved in key ecosystem functions by bactericidal and bacteriostatic effects (direct effect). However, antibiotics can act as a selective force on some microbial populations, which can develop resistance, generating genetic and phenotypic variability and influencing various physiological activities; in some cases bacteria can develop the capability to degrade them (indirect effect) as a homeostatic response to a stress [61].

3.1. Direct effects on microbial community structure

Microbial biodiversity has a functional importance in the maintenance of biological processes in water and soil; in fact, most biogeochemical cycles are exclusively microorganism mediated. Antibiotics can act as an ecological factor in the environment, driving changes in the structure of natural bacterial communities (disappearance or inhibition of some bacterial groups) [62]. The effects can be found even in non-target organisms with important ecological functions [63,64].

Many studies have shown that the presence of antibiotics causes a reduction in microbial biodiversity. Moreover, they can influence the growth and enzyme activities of bacterial communities and ultimately ecological functions such as biomass production and nutrient transformation, leading to loss of functional stability [64–67]. Antibiotics, even broad spectrum ones, have a selective effect on various microbial groups; the group may be large like fungi or bacteria or narrower like a single species. As a result, the antibiotic selective effect alters the relative abundance of microbial species and interferes in interactions between different species. These effects depend on the microbial groups involved [68], on environmental characteristics (e.g. soil or water [64,69]), and on antibiotic concentrations [70]. For example, soil texture, adsorption capacity, pH, water content, temperature and regularity of application, but also climatic natural processes such as freezing, thawing, drying and remoistening can affect the seizure, transformation and release of antibiotics because they alter their physicochemical properties [71].

An updated list of recent studies regarding changes in microbial community structure after the addition of antibiotics in soil and water environments is shown in Table 3 [72–110]. Among the effects of antibiotics on ecological functions can be changes in nitrogen transformation, methanogenesis, sulfate reduction, nutrient cycling and organic matter degradation [111]. For example, sulfonamides have been found to induce a change in microbial diversity by reducing not only microbial biomass, but also the relationship between bacteria and fungi [92]. As regards the nitrogen cycle, it is known that nitrification and denitrification is performed by several prokaryotes and nitrification in particular by ammonium-oxidizing Bacteria and Archaea (AOB and AOA) [79]. Environmentally significant concentrations of fluoroquinolones and sulfonamides could partially inhibit denitrification and the application to soil of swine manure containing the antibiotic tylosine has been shown to change the behavior of nitrogen mediated by these microbial communities [79,111].

Overall, the lack of standardized tests hinders generalizations about the effects of antibiotics on biogeochemical processes.

3.2. Indirect effects: modifications of bacterial ecology, resistance development and pharmaceutical biodegradation

Low concentrations (i.e. nanograms per litre or kg soil) of antibiotics can have long-term indirect effects on microbial species or consortia which are not directly affected by their presence (e.g. via population dynamics). “Low concentrations” means non-lethal and sub-inhibitory ones, which are below the so-called minimal inhibitory concentration (MIC), the lowest drug concentration that, under established in vitro conditions, inhibits visible growth of a target bacterial population [89]. These concentrations can act in three different ways:

- selecting resistance (by enriching for pre-existing resistant bacteria and by selecting for de novo resistance) [13,69,112];
- generating genetic and phenotypic variability (by increasing the rate of adaptive evolution, including resistance development);
- -as signaling molecules (influencing various physiological activities, including virulence, biofilm formation and gene expression) [84].

Table 3
Effects of different antibiotics on soil and water natural microbial communities. Data from [72–110]. The effect is always compared with a control without antibiotic.

Parameter	Effect	Class and antibiotic	Conc.	Experimental condition	Time (days)	Method	Ref.
Bacterial growth/microbial biomass or abundance	Initial decrease (at 8 h)	Sulfonamides (Sulphamethazine)	0.005–50 μM	Marine sediment slurry	2	Cell count by Acridine orange method	[72]
	Decrease	Sulfonamides (Sulphamethazine)	20–100 mg L^{-1}	Soil treated with poultry manure	56	CFU	[73]
	Decrease in soil antibiotic presence and no increase even with an incremental liquid manure addition	Sulfonamides (Sulfadiazine)	0–100 mg kg^{-1} with liquid manure	Soil treated with or without pig liquid manure	32	Biomass by fumigation-extraction method	[74]
Microbial community diversity	Decrease	Quinolones and Fluoroquinolones (Ciprofloxacin)	5 and 50 mg kg^{-1}	Soil microcosms	40	PFLA	[75]
	Increase in fungal and decrease in bacterial PLFAs	Tetracyclines (Tylosin)	>50 mg kg^{-1}	Soil	2	PFLA	[76]
	Change in DGGE bands	β–Lactams (Amoxicillin)	10–100 mg kg^{-1}	Soil treated with manure	18	DGGE	[77]
	Change (abundance in Epsilonproteobacteria in the presence of Penicillin G; abundance in Deltaproteobacteria, Clostridia and Bacilli in the presence of oxytetracycline)	β–Lactams (Penicillin G) and Tetracyclines (Oxytetracycline)	80 μg mL^{-1}	River bacterial community under long term antibiotic stresses by treated wastewater from two antibiotic producing facilities		CFU, PCR	[78]
	Decrease in bacterial community similarities	Glycopeptides (Vancomycin)	1–1000 mg L^{-1}	River sediments sampled 10 m upstream and 10 m downstream from a WWTP	24	PCR, DGGE	[79]
	Alteration (in high-pH and high-clay-content soil diversity was more affected)	Lincosamides (Lincomycin)	0.05–500 mg kg^{-1}	Two forest soils with different pH and clay content	Not mentioned (>10)	T-RFLP	[80]
	Change in the DGGE bands	Macrolides (Natamycin)	50–200 mg L^{-1}	Soil treated with manure	32–61	DGGE profiles *(β-Proteobacteria, Pseudomonas)*	[81]
	The antibiotic favoured sulfate-reducing bacteria and Gram-negative bacteria	Quinolones and Fluoroquinolones (Ciprofloxacin)	0–200 μg mL^{-1}	Salt marsh sediment	30	PFLA richness	[82]
	Decrease in diversity	Quinolones and Fluoroquinolones (Ciprofloxacin)	0.2–2 mg L^{-1}	Marine sediment with ciprofloxacin in the overlying water	49	T-RFLP for Bacteria	[83]
	Difference in diversity of 16S rDNA	Tetracyclines (Tylosin)	2000 mg kg^{-1}	Sandy soil	60	DGGE	[84]
	Alteration in microbial community functioning	Tetracyclines (Chlortetracycline)	0–100 mg kg^{-1}	Soil spiked with DOM extracted from pig manure	45	CLPP using BIOLOG	[85]
	Alteration in microbial community	Tetracyclines (Chlortetracycline)	1, 10, and 100 mg kg^{-1}	Soil microcosms	45	CLPP using BIOLOG	[86]
	Change in microbial community structure	Tetracyclines (Oxytetracycline)	10 mg kg^{-1}	Wheat rhizosphere soil	5–30	CFU count in agar plates for *Bacteria* and *Actinomyces*	[87]
	Shifts in microbial community Structure (Abundance in Gram-positive bacteria, fungi, mycorrhizae, and Protozoa)	Tetracyclines (Oxytetracycline)	5–200 mg kg^{-1}	Grass and agroforestry soils	63	PFLA	[88]
	Change in microbial community diversity	Tetracyclines (Oxytetracycline)	200 ppm	Agricultural soil spiked with swine manure	49	Diversity (Shannon–Wiener and Evenness), BIOLOG	[89]
	Increase in the Ammonia Oxidant Archaea/Ammonia Oxidant Bacteria ratio	Sulfonamides (Sulfadiazine)	10–100 mg kg^{-1}	Soil amended with pig manure	61	qPCR targeting AOA/AOB oxidizing genes	[9]
	Initial decrease and subsequent recovery at 48 days	Sulfonamides (Sulfadiazine)	1–50 mg kg^{-1}	Soil amended with glucose	48	PCR, amplification of rDNA	[91]
	Reduction in total PLFA concentration; variation in total DGGE band intensity	Sulfonamides (Sulfadiazine)	10 and 100 μg g^{-1}	Soil amended with pig manure	32	PFLA, DGGE profiles	[91]
	Alteration in DGGE bands (Additional bands appeared and some bands already visible at the beginning of incubation increased in intensity)	Sulfonamides (Sulfadiazine)	1–50 mg kg^{-1}	Soil amended with glucose	48	DGGE	[92]
	Alteration (predominance of *Burkholderiales*)	Sulfonamides (Sulfamethoxazole)	0.005–50 μM	Enrichment culture with groundwater from	15	PCR, amplification of rDNA	[93]

Table 3 (*continued*)

Parameter	Effect	Class and antibiotic	Conc.	Experimental condition	Time (days)	Method	Ref.
				a pristine zone of a sandy drinking-water aquifer (anaerobic and heterotrophic media)			
	Change in community composition	Sulfonamides (Sulfamethoxazole)	240–520 μg L^{-1}	Microbial communities from an oligotrophic aquifer	30	T-RFLP	[94]
	Alteration (decrease in *Pseudomonas* sequences and increase in those of the genus *Variovorax*)	A mixture of Sulfamethoxazole Erythromycin and Ciprofloxacin	0.33–3.33 μg L^{-1}	Pristine aquifer microbial community	19	ARISA	[91]
	Decrease	Sulfonamides (Sulfadiazine)	10 and 100 mg g^{-1}	Bulk soil	7	PFLA	[92]
	Alteration in the bacterial community composition; increase in Actinobacteria	A mixture of 16 antibiotics (Sulfonamides, Quinolones, Macrolides, macrolides, Tetracycline)	0–1500 ng L^{-1}	Biofilms exposed to river waters from downstream sites	9	CARD-FISH, DGGE	[95]
Bacteria/Fungi ratio	Decrease	Sulfonamides (Sulfamethoxazole)	20–500 mg kg^{-1}	Soil amended with manure from alfalfa or antibiotic-treated pig	7–35	PFLA	[77]
	Decrease	Sulfonamides (Sulfapyridine)	100–1000 mg kg^{-1}	Topsoil	56	PFLA	[96,97]
	Decrease	Tetracyclines (Tetracycline)	5–500 mg kg^{-1}	Soil treated with pig manure	56	PFLA	[98]
	Decrease	Tetracyclines (Oxytetracycline)	100–1000 mg kg^{-1}	Pristine topsoil amended with milled maize straw or glucose	14	PFLA	[23]
	Decrease	Macrolides (Natamycin)	50–200 mg L^{-1}	Bulk soil and rhizosphere soil suspensions on agar medium	32	PFLA	[83]
	Increase (by a factor of 2)	Sulfonamides (Sulfamethoxazole)	20–500 mg kg^{-1}	Soil amended or non-amended with manure from alfalfa or antibiotic-treated pig	7–35	[^3H]leucine incorporation and BIOLOG	[77]
Pollution-Induced Community Tolerance (PICT)	Increase	Sulfonamides (Sulfachloropyridazine)	100 mg kg^{-1}	Soil amended with fresh pig slurry or with alfalfa meal	7	BIOLOG	[99]
	Increase (by a factor of 1.5–5)	Sulfonamides (Sulfadiazine)	1–100 mg kg^{-1}	Soil	35–105	Fluorescence viability staining-flow cytometry PICT protocol; [^3H]leucine incorporation	[99]
	Initial increase; decrease after 25 d and PICT comparable to control at 95 d	Tetracyclines (Tylosin)	50–1500 mg kg^{-1}	Soil with or without alfalfa	95	[^3H]leucine incorporation	[99]
	Decrease	Sulfonamides (Sulfadiazine)	10–100 mg kg^{-1}	Soil amended with manure	32	Microbial activity assays	[100]
Respiration/catabolic activity	Decrease	Quinolones and Fluoroquinolones (Ciprofloxacin)	0.5–2 mg mL^{-1}	Interstitial water samples of wetlands	96 h	BIOLOG	[100]
	Inhibition (75%)	Aminoglycosides (Streptomycin)	400 mg L^{-1}	Activated sludge	2–4 h	Nitrifying activity calculated from nitrite and nitrate production	[101]
Nitrification	Decrease	Sulfonamides (Sulfadiazine)	10–100 mg kg^{-1}	Soil amended with manure	32	ISO 15,685 (2004)	[102]
	Decrease	Sulfonamides (Sulfadimethoxine)	50–200 mg kg^{-1}	Soil	50	Nitrification test	[103]
	Inhibition (≈25%)	Sulfonamides (Sulfadiazine)	100 mg kg^{-1}	Soil	32	ISO 15685 (2004)	[104]
	Inhibition (50%)	Tetracyclines (Oxytetracycline)	12.5–75 mg L^{-1}	Synthetic freshwater plus active cultures of the nitrifying bacteria *Nitrosomonas* and *Nitrobacter*.	7	Measure of changes in the conversion of ammonia to nitrite and nitrate	[105]
	Inhibition	Tetracyclines (Chlortetracycline)	50–200 μg kg^{-1} in poultry manure	Soil	50	Incubation with $NaClO_3$	[106]
	Inhibition	Tetracyclines (Chlortetracycline)	1 mg L^{-1}	Groundwater	5	Nitrate removal and nitrite production	[107]

(*continued on next page*)

Table 3 (*continued*)

Parameter	Effect	Class and antibiotic	Conc.	Experimental condition	Time (days)	Method	Ref.
Denitrification	Decrease (47%)	Sulfonamides (Sulfamethoxazole)	1 μg L^{-1}	Pristine aquifer	19	Denitrification assay	[108]
	Decrease	Sulfonamides (Sulphamethazine)	100 ng L^{-1}	Sediment slurry	50 h	Denitrification genes: nitrite reductase (nirS), nitrous oxide reductase (nosZ)	[72]
	Decrease	Glycopeptides (Vancomycin)	1000 μg L^{-1}	River sediments sampled 10 m upstream and 10 m downstream from a WWTP	24	Resonant FTR approach, PCR, DGGE	[78]
	Decrease (10%)	Tetracyclines (Oxytetracycline)	5.5–7.35 mg kg^{-1}	Pristine topsoil amended with milled maize straw or glucose	7	Iron (III) reduction activity assay	[23]
Iron reduction	Inhibition	Tetracyclines (Chlortetracycline)	100 and 200 μg kg^{-1} in poultry manure	Soil	50	Glucose addition	[104]
	Decrease	Polyether ionophores (Monensin)	0–100 μg kg^{-1} in poultry manure	Soil	50	Glucose addition	[104]
	Decrease	Sulfonamides (Sulfadimethoxine)	0–200 mg kg^{-1}	Soil	50	Iron (III) reduction assay	[108]
	Decrease (10%)	Sulfonamides (Sulfapyridine)		0.003–1.14 mg kg^{-1}	Pristine topsoil amended with milled maize straw or glucose	7	Iron (III) reduction assay [92]
Inhibition	Phenicols and Amphenicols (Chloramphenicol)	50 mg L^{-1}	Sediments collected from a small pond and eutrophic lake	7	Analyses of As(V) and As(III)		[109]
As(III) oxidation	Inhibition (25%)	Quinolones and Fluoroquinolones (Ciprofloxacin)	1 μg kg^{-1}	Soil	5	Analyses of CH_4 production	[110]
Methanogenesis	Stimulation (30%)	Sulfonamides (Sulfamethoxazole)	500 μg kg^{-1}	Soil	5	Analyses of CH_4 production	[110]
	Stimulation (30%)	Sulfonamides (Sulfamethoxazole)	500 μg kg^{-1}	Soil	5	Analyses of CH_4 production	[110]

ARISA = automated ribosomal intergenic spacer analysis; AOA = ammonia-oxidizing archaea; AOB = ammonia-oxidizing bacteria; CFU = colony forming unit; CLPP = community level physiological profiles; DGGE = denaturing gradient gel electrophoresis; FTR = frustrated total reflection; PICT = pollution-induced community tolerance; PLFA = phospholipid fatty acid analysis; qPCR = real time polymerase chain reaction; SIR = substrate induced respiration; T-RFLP = terminal restriction fragment length polymorphism.

It has been well known since the early years of antibiotic use that bacterial resistance has been selected at low antibiotic concentrations [60].

Bacterial resistance is a natural adaptation (homeostatic response) of microorganisms against products trying to prevent their growth. It refers to the ability of a microorganism to survive and multiply, despite the presence of a biocide molecule like an antibiotic [65]. Microorganisms have several mechanisms to avoid the lethal actions of antibiotics (Figure 1): production of enzymes inactivating them (e.g. hydrolysis by β-lactamase); changes in bacterial cell wall permeability (mutations in porins), preventing the entrance of antibiotics; changes in antibiotic target sites within bacterial cells; active transportation systems like efflux pumps in cell walls, which prevent the buildup of antibiotics inside cells; alternative metabolic pathways.

Bacteria can develop resistance towards natural antibiotics promptly because they may have already been pre-exposed to such kind of molecules in nature. Some of the mechanisms can be intrinsic (for example, Gram-negative bacteria are not susceptible to glycopeptides because their membrane is naturally impermeable, in a similar way Gram-positive ones are not inhibited by nalidixic acid or polymyxins), or acquired through resistance gene transmission. The latter can occur by passing genetic material (small molecules of DNA) from one bacterium to another one, even when the latter is phylogenetically distant. This occurs mainly through a plasmid Horizontal Gene Transfer (HGT), via a cytoplasmic bridge. Once introduced into a host cell, the plasmid can remain both integrated in the cell DNA or free in the cytoplasm. The plasmids encode functions not essential to the bacteria's survival, but provide significant advantages in particular conditions of growth or development; the case of R plasmids, responsible for antibiotic resistance, is a good example. Some conjugative plasmids possess a set of genes (transfer genes) which are likely to promote their transfer to different cells (horizontal transmission). Many plasmids accumulate a form of multiple resistance that thwarts the effect of several antibiotics, rendering any antibacterial therapy ineffective [62,113].

In natural ecosystems, the presence of resistance genes can affect the dynamics and physiology of natural microbial populations. For example, resistance to glycopeptides or beta-lactam strongly modifies the structure of peptidoglycan in Gram-positive bacteria [62], while the antibiotic resistance of small colonies of *S. aureus* seems to depend on its bacterial metabolism [113]. The acquisition of resistance can have unforeseen consequences for bacterial metabolism, and afterwards for the evolution of the environmental microbiosphere. However, these aspects have not yet been fully explored at a community level [70,114,115].

The acquisition of antibiotic resistance can produce specific changes in bacterial metabolism that can be useful for bacterial growth in some habitats or in particular environmental conditions [111,116–118]. In fact, some resistance genes lead to a range of metabolic phenotypes, including variations in the ability to use different carbon, nitrogen or phosphate sources for growth [62]. It has been shown that some elements serving in resisting high concentrations of antibiotics also have

other functional roles (e.g. cell homeostasis, signal trafficking and metabolic enzymes) in their original hosts [65]; this phenomenon refers to pleiotropy (a single gene affecting multiple traits). For example, bacteria adapting to increased temperature became resistant to rifampicin antibiotic [119].

At non-lethal concentrations, bacteria can use antibiotics as extracellular chemicals to trigger different cellular responses and they can be considered friendly signals that coordinate and regulate microbial community functioning [85,120]. Consequently, antibiotics have a dual nature: as both weapon and signaling molecules; in the latter case, they can have ecological effects [84,121,122]. In natural settings, microbes are typically in polymicrobial communities, sharing and exchanging a variety of beneficial compounds that serve as cell—cell signals [123]. In this sense, antibiotics are signals that coordinate cooperative social interactions between bacteria and can affect the physiology of some natural microbial populations. For examples, non-lethal levels of antibiotics can alter the expression of genes involved in a variety of bacterial functions like metabolism, regulation, virulence, DNA repair and stress response and modify cellular behaviors in bacteria with the formation of biofilms and persister cells [70]. Consequently, antibiotics induce responses other than those associated with their antimicrobial activities and they are signaling molecules with regulatory functions [124,125].

It has recently been reported that antibiotic resistance may also be developed thanks to the presence of biocides, through co-resistance (selection for clones or mobile elements also carrying antimicrobial resistance) and cross-resistance (selection for genes encoding resistance to both the biocidal substance and one or more therapeutic antibiotic classes) [126]. Moreover, heavy metal pollution may also co-select for bacterial antibiotic resistance in the environment and the resistance genes for metals and antibiotic resistance gens (ARGs) are often located together on the same genetic element [123]. In any case, the co-selection potential of biocides and metals is specific towards certain antibiotics; as an example, the resistance genes to quaternary ammonium compounds (QAC) and class 1 integrons (resistance genes for almost all antibiotic families including beta-lactams, aminoglycosides, trimethoprim, chloramphenicol, fosfomycin, macrolides, lincosamides, rifampicin and quinolones) are more prevalent in bacteria exposed to detergents and biocides [125,126]. Plasmids provide limited opportunities for biocides and metals to promote horizontal transfer of antibiotic resistance through co-selection, whereas ample possibilities exist for indirect selection via chromosomal biocide/metal resistance genes (BMRGs) [127,128].

Moreover, there may be selection for resistant bacteria in addition to effects against other, sensitive bacteria and other microorganisms. For example, the reason for applying antibiotics as growth promoters in sub-therapeutic doses (5–40 ppm) is that they change microbial populations in animals' guts. There would be a problem if the same phenomena occurred in the environment, especially with compounds being enriched in certain samples, e.g. by sorption [127,128]. Information in this field is still scarce.

Regarding the possible degradation of antibiotics, natural microbial communities are key players in several processes controlling the quality of soil and water ecosystems and regulating the fate of pollution released into the environment and, in this sense, they provide the Ecosystem Service termed “Regulation”. Microorganisms are involved in ecosystem self-purification processes since they can degrade contaminants by metabolic and/or co-metabolic pathways. Biodegradation is the most important process for eliminating the majority of xenobiotics, including pharmaceuticals [129–132]. Recovery from contamination is possible only if the toxicity of the molecules does not inhibit microbial activity. Antibiotics are designed to be refractory to biodegradation and many of them (e.g. quinolones, sulphonamides and diaminopyrimidine) are reported to have a high persistence in soil (DT_{50} > 100 d) [26]. Similarly, ciprofloxacin and oxolinic acid are considered quite persistent in water (DT_{50} > 90 d) [133,134].

The main degradation process of an antibiotic depends on its chemical structure. For example, some antibiotics, such as fluoroquinolones, are photosensitive molecules and so photodegradation has been reported as their main transformation pathway. In other cases some bacterial strains or populations able to degrade some antibiotics have been identified as in the case of some quinolones and sulphonamides [135].

The biodegradation of an antibiotic depends on the presence of microbial populations which are resistant to its detrimental effects [132] and have developed the ability to degrade it during previous exposure to the compound [127,129]. Biodegradation makes it possible to completely remove a toxic compound from the environment if it is mineralized. Abiotic factors such as temperature, water content, soil texture and co-occurrence of other contaminants can affect a compound's bioavailability and consequently its biodegradation rate [26,132,135]. For example, the binding of quinolones to soil and sediments delays their biodegradation [136]. Oxygen, moisture, the presence/absence of alternative sources of carbon and nitrogen, and the presence of an acclimatized bacterial consortium are necessary for antibiotic biodegradation [132].

In water, biodegradation rates can increase or decrease depending on sunlight, salinity and anthropogenic contamination [135].

Specific bacterial groups or strains able to grow on antibiotics as a sole carbon source have recently been isolated from natural environments (soil or water). In some cases, they were also able to mineralize them [133,134,137–140].

As an example, the biodegradation of 18 antibiotics representing eight major classes of natural and synthetic origin was tested in soil by Dantas and co-authors [141]. The majority of the antibiotics tested (D-cycloserine, amikacin, gentamicin, kanamycin, sisomicin, chloramphenicol, thiamphenicol, carbenicillin, dicloxacillin, penicillin G, vancomycin, ciprofloxacin, levofloxacin, nalidixic acid, mafenide, sulfamethizole, sulfisoxazole and trimethoprim) belonging to different antibiotic classes, supported the growth of phylogenetically diverse clonal bacteria (selected from different soils) closely related to human pathogens. Furthermore, each antibiotic-consuming isolate was resistant to multiple antibiotics at clinically significant concentrations. This phenomenon suggests that this unappreciated reservoir of antibiotic-resistance determinants can contribute to increasing levels of multiple antibiotic resistance in pathogenic bacteria [141].

An *Alpha-Proteobacterium* (*Labrys portucalensis* F11) able to use a range of fluoroorganic compounds was found to be able to degrade the quinolones ofloxacin, norfloxacin and ciprofloxacin [134]. Another example of a bacterial strain capable of degrading antibiotics is the *Microbacterium* sp. strain C448. This bacterium was selected from an agricultural soil treated for many years with veterinary antibiotics simulating annual applications of manure from medicated swine. This strain was able to mineralize sulfamethazine; although the antibiotic concentration in the liquid media during the degradation experiment was quite high (50 mg L^{-1}), this study is very promising, considering that many antibiotics have high persistence.

It is well known that, if bacterial community diversity is high, the probability of biodegradation of a compound is also high and this is of critical importance in the testing of antibacterial compounds and the evaluation of test results.

Most of the studies performed used a single compound. It is known that antibiotics from the same or different groups may together have an additive effect, which affects their biodegradation [142,143]. For this reason, the impact of antibiotics in the environment is underestimated and the possibility of their biodegradation in the environment is currently not well studied.

4. Antibiotic resistance genes as pollutants

Antibiotic resistance can be divided into native resistance, which is normally found in bacteria in various natural environments, and acquired resistance, stemming from anthropogenic causes. Consequently, chromosomes of environmental bacteria normally contain antibiotic resistance genes (ARGs) [5,60–62]. These genes can also be found in

bacteria coming from isolated areas and without any direct interaction with pathogens, as in the case of *Paenibacillus* sp. LC231 found in a cave isolated from the surface for over 4 m years [5] or in the case of DNA coming from 30,000-year-old permafrost sediments [144]. Some of these genes harbor dozens of acquired resistance elements often conferring redundant protection against individual antibiotics [5]. In the presence of antibiotic pressure they can increase above usually occurring background levels [62] and, consequently, can be considered pollutants themselves. The presence of resistance genes in human pathogens in areas with low contamination by antibiotics [63] indicates that, once these elements are present in transferable genes, the probability of keeping them in natural ecosystems can be high.

Unlike antibiotics, contamination from ARGs is not necessarily due to a local and constant release of antibiotics; in fact, once these genes are in the environment, they can be spread among different bacterial species and habitats. ARGs can migrate between connected aquatic ecosystems [65], although it is unclear whether their presence is the result of a migration of bacteria resistant to antibiotics or transmission of resistance genes from plasmid Horizontal Gene Transfer (HGT) [66].

Pollution from ARGs can increase the likelihood of human pathogens acquiring resistance. For this reason, it has been suggested that hospital discharges, which contain human pathogenic infectious bacteria (resistant and susceptible) and antibiotics, be treated separately, to prevent the exchange of genetic material in purification [67]. Given the presence of resistance genes in environments without a history of antibiotic contamination, analyses of antibiotics and antibiotic genes in the environment are needed. In particular, quantitative data on the abundance of resistance genes in environments (soil, water) are necessary for their risk assessments [68,69].

5. Actions to decrease antibiotic and antibiotic resistance gene release into the environment

To minimize antibiotic resistance, intensified by the use of antibiotics in veterinary medicine, since 2006 the EU has banned the use of antibiotics as growth promoters [10] because administration at low doses (minimum inhibitory concentration) to modulate the metabolism of commensal bacterial flora can promote the spread of this phenomenon [21,145]. Unfortunately, in other countries (e.g. America, Canada and Asia), they are still widely used as growth promoters. Many countries have also restricted the use of antibiotics in aquaculture, especially of those antibiotics used in the treatment of human infections [146].

Although national programs to control antimicrobial resistance and to rationalize the use of antibiotics in humans are reducing the amount of antibiotics used in human therapy, their complete elimination is not feasible. It is therefore expected that the amount of antibiotics released into the environment from both human and veterinary medicine will continue to be at high levels in the future.

The effectiveness of reducing antibiotic use in decreasing the amount of resistance genes is controversial. Some authors have shown that the reduction of antibiotics in the environment can also decrease the amount of resistance genes and their transfer to humans, but others that, although the resistance is decreasing, its decline is slow and resistant populations persist [147]. Moreover, the fact that some resistance genes in human pathogens are found in environments that are not characterized by a previous contamination by antibiotics [64] suggests that ARGs can persist even in the absence of an antibiotic selective pressure. Consequently, quantitative and qualitative data on the abundance of resistance genes in different environments (soil, water) and a better understanding of the interactions between antibiotics and environmental bacteria are necessary for adequate risk assessments [67,68]. OECD and ISO tests generally applied for assessing chemical persistence and toxicity are not sufficient to evaluate the possible loss of microbial diversity and ecological functioning due to antibiotic occurrence. Ecological Risk Assessments of antibiotics, through the application of a form of toxic action approach, should therefore make more use of ecological endpoints targeting natural microorganisms (especially bacteria) and microbial communities [6].

6. Concluding remarks

The release of antibiotics and resistance genes into natural ecosystems is a recent event in evolutionary terms. There is a particular concern regarding their impact on non-target bacteria and their related ecological functions. These pollutants can directly (bactericidal and bacteriostatic effect with the disappearance or inhibition of some microbial groups involved in key ecosystem functions) or indirectly (selecting resistance, generating genetic and phenotypic variability, influencing various physiological activities) affect microbial communities.

National programs that limit the amount of antibiotics released into the environment have been performed for controlling antimicrobial resistance, although a complete elimination is not possible.

While a reduction in the spread of resistance has been reported following a discontinuation of therapy with a particular antibiotic, some suggest that the restoration of a total population to its former situation, including antibiotic sensitivity, is unlikely. To minimize the impact of resistance genes, isolation measures should be assessed to avoid, as much as possible, contact between the bacteria linked to the human sphere and environmental ones.

Both types of pollution (antibiotics and resistance genes) can affect the structure and function of environmental microbial populations. Since environmental microorganisms are the original source of resistance genes acquired through horizontal gene transfer to human pathogens, these changes are important for the future of human health. As regards resistance genes, the situation is more complex, since they are not “degradable pollutants” but auto-replication elements.

Owing to the key role of natural microbial communities in several ecosystem services, the need for more specific protection goals based on the ecosystem service concept in the ERA should make more use of ecotoxicological endpoints targeting microbial communities. In fact, the direct effects of antibiotics on natural microbial communities have been demonstrated, while the indirect effects of the presence of antibiotics on ecosystems are still largely unknown, although the long-term effects on ecosystem functions could be significant.

Moreover, in addition to controlling of the use of antibiotics, studies to improve their degradation in natural environments are needed to combat this type of pollution. In fact, the presence of microbial populations that are more or less efficient in the removal of these molecules can be considered a measure of the homeostatic capacity of an ecosystem. The biodegradation (and mineralization) of antibiotics have been observed (only for a few molecules), although it requires microbial adaptation and selection processes that occur over relatively long time periods of exposure in a pristine environment. However, the presence of specific populations does not exclude that there may be secondary effects on the structure and functions of microbial communities, in terms of exclusion of keystone species, alteration of functions, inhibition of specific activities, etc. Moreover, the composition of microbial groups could be sensitive and not immediately resilient to the presence of antibiotics (which could be considered a disturbance). Changes in composition are often associated with changes in ecosystem process rates. Changes in microbial communities due to disturbance may thus directly affect ecosystem processes.

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