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Environmental contamination in a high-income country (France) by antibiotics, antibiotic-resistant bacteria, and antibiotic resistance genes: Status and possible causes

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ABSTRACT

Antimicrobial resistance (AMR) is a major global public health concern, shared by a large number of human and animal health actors. Within the framework of a One Health approach, actions should be implemented in the environmental realm, as well as the human and animal realms. The Government of France commissioned a report to provide policy and decision makers with an evidential basis for recommending or taking future actions to mitigate AMR in the environment. We first examined the mechanisms that underlie the emergence and persistence of antimicrobial resistance in the environment. This report drew up an inventory of the contamination of aquatic and terrestrial environments by AMR and antibiotics, anticipating that the findings will be representative of some other high-income countries. Effluents of wastewater treatment plants were identified as the major source of contamination on French territory, with spreading of organic waste products as a more diffuse and incidental contamination of aquatic environments. A limitation of this review is the heterogeneity of available data in space and time, as well as the lack of data for certain sources.

Comparing the French Measured Environmental Concentrations (MECs) with predicted no effect concentrations (PNECs), fluoroquinolones and trimethoprim were identified as representing high and medium risk of favoring the selection of resistant bacteria in treated wastewater and in the most contaminated rivers. All other antibiotic molecules analyzed (erythromycin, clarithromycin, azithromycin, tetracycline) were at low risk of resistance selection in those environments. However, the heterogeneity of the data available impairs their full

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exploitation. Consequently, we listed indicators to survey AMR and antibiotics in the environment and recommended the harmonization of sampling strategies and endpoints for analyses.

Finally, the objectives and methods used for the present work could comprise a useful example for how national authorities of countries sharing common socio-geographic characteristics with France could seek to better understand and define the environmental dimension of AMR in their particular settings.

1. Introduction

1.1. Context

The development of resistance of bacterial pathogens to antibiotics has greatly complicated the treatment of life-threatening bacterial infections (Cassini et al., 2019; Furuya and Lowy, 2006; O'Neill, 2014). If antimicrobial resistance (AMR, specifically meaning resistance of bacterial commensals or pathogens to antimicrobial drugs) development proceed at the current rate, it is predicted that "if no action is taken", drug resistant infections will account for 10 million global deaths each year by 2050, outpacing those caused by cancers (O'Neill, 2014). Within this context, national and international authorities have developed and are deploying action plans to mitigate the development of AMR (Comité interministériel pour la santé, 2016; European Commission, 2017; WHO et al., 2020; World Health Organization, 2015). These action plans generally have four pillars: surveillance, stewardship, infection prevention and control, and research and innovation. Antibiotics are widely used in human and animal medicine and in food animal production (Klein et al., 2018; Van Boeckel et al., 2015). Under the pressure of antibiotic selection, AMR develops in medicated people and in food animals (Furuya and Lowy, 2006). Additionally, antimicrobial-resistant bacteria commensal or pathogenic for humans (ARB) are thought to be exchanged between humans and animals through direct or indirect transmission via the food chain or the environment (Hernando-Amado et al., 2019). The linkage between animals and humans, and the importance of the environment in facilitating this linkage, has been coined the One Health Framework (Hernando-Amado et al., 2019). Hotspots for AMR development and potential transmission routes of ARB across the One Health continuum are illustrated in Fig. 1.

It is generally recognized that, in order to be successful, strategies to mitigate the development and the transmission to humans of ARB will require concerted action across the One Health continuum, *i.e.* in human and animal sectors and the environment (Cassini et al., 2019; Comité interministériel pour la santé, 2016; European Commission, 2017; WHO et al., 2020; World Health Organization, 2015). The key objective in both human medicine and agricultural production is clearly to reduce the use of antibiotics to the minimum necessary, in order to reduce selection pressure and prevent AMR development in humans and food animals (Berendonk et al., 2015; Cantas et al., 2013; Hernando-Amado et al., 2019; Ter Kuile et al., 2016; Törneke et al., 2015). On the other hand, taking into account the role of the environment in the development and transmission of AMR is recent, and actions in the environmental sector are currently the least implemented in the framework of public policies (European Commission, 2019; Munkholm et al., 2021; Singer et al., 2016).

Within this context, the French Government commissioned a detailed report on the state of AMR in the aquatic and terrestrial environments of this country based on the One Health approach. The full report was released in November 2020 (Anses, 2020a). We slightly updated its conclusions due to the recent publication of PNEC values (Murray et al., 2020).

The overall objective of this report was to provide policy and decision makers with an evidential basis for recommending or taking future actions to mitigate AMR in the environment. We suggest that the objectives and methods used for this present work could comprise a useful example for how national authorities elsewhere could seek to better understand and define the environmental dimension of AMR in their

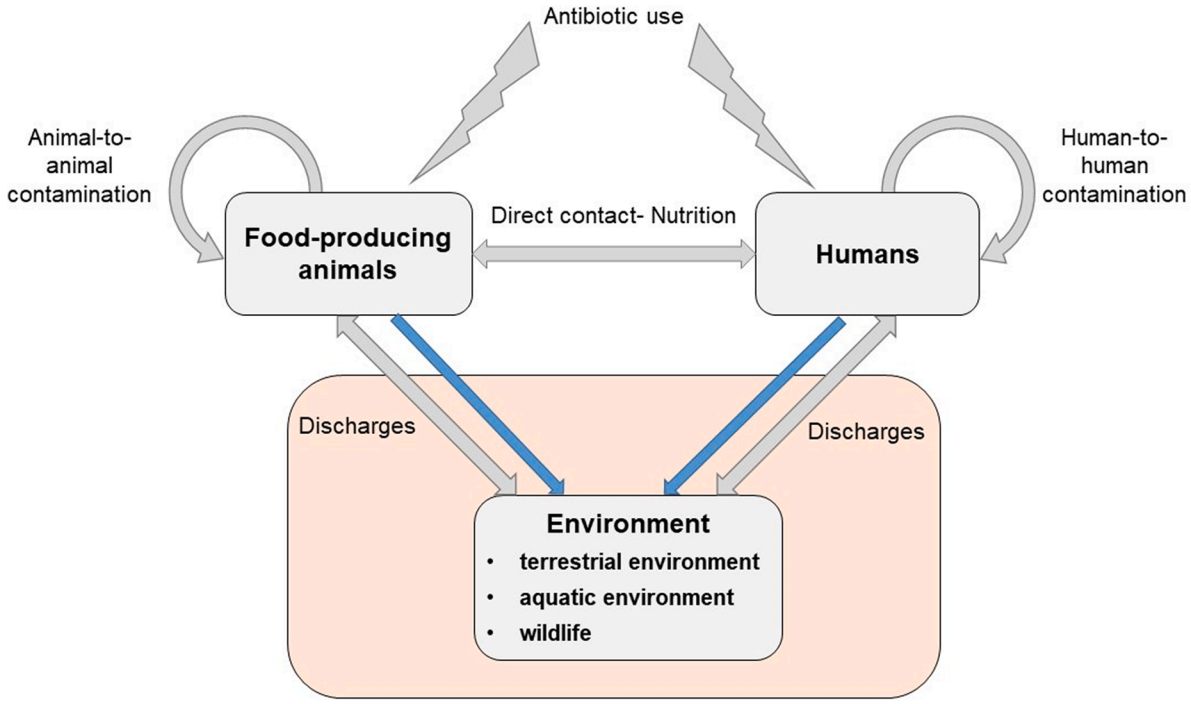


Fig. 1. The flow of antimicrobial resistance and antibiotics across the One Health continuum of food-producing animals, humans, and the environment. Grey arrows indicate flow of resistance genes and resistant bacteria; blue arrows indicate flow of antibiotic residues.

particular settings. Important characteristics of France that were taken into account in this review include climate, governance, management of waste streams, agriculture practices, determinants of health and high-income setting. Consequently, the French situation is applicable to countries that share similar attributes; the more variance from these characteristics, the less applicable the analysis will be.

1.2. Geographical and health characteristics of France

The significance of the environment for AMR development and transmission is influenced by climate and landscape, and numerous societal, cultural, economic, and regulatory factors (Collignon et al., 2018). These need to be taken into account in order to understand how far the conclusions reached in this national analysis can or cannot be extrapolated to other settings.

Metropolitan France is located in Western Europe with a surface area of about 641,000 km^2, about 0.86% of which is water. The climate is variable across the country with mean annual temperatures ranging from 0.2 °C to 28.3 °C and annual precipitation from 523 mm to 1,932 mm (data from Météofrance – see Appendix B).

In 2019, *per capita* income was about US$40494 (Worldbank, 2019). The country has 67 million citizens, most of whom reside in large urban centers. France is a high-income country with excellent water sanitation and hygiene infrastructure. Safe potable water is ubiquitous. Nearly 85% of households are connected to a wastewater treatment plant (WWTP), providing a total treatment capacity of 100 million-population equivalents (PE). Around 80% of WWTPs are of low capacity (<2000 PE), of which 62.5% are of very low capacity (<500 PE). In contrast, 6% of WWTPs have a capacity ≥ 10,000 PE and account for more than 80% of the total capacity of WWTP in France (Commissariat général au développement durable, 2019). Hospitals, health care institutions, and other public infrastructures are connected to these WWTPs, so that they are key elements for the discharge of AMR of human origin (Goulas et al., 2020). The rest of the population (15%) lives in houses connected to on-site wastewater treatment facilities.

In 2014, French agriculture represented 24% of the total European agricultural production (La Représentation permanente de la France auprès de l'Union Européenne, 2021) and was the world's third largest exporter of agricultural goods. Bovine, poultry, and swine production respectively accounted for 9.9%, 5%, and 4.3% of the total French agricultural production. The distribution of these three farming types is highly heterogeneous over the French territory, with intensive pig and poultry farming in the northwestern part of the country, and the rare occurrence of farms in the southeast region. Antibiotics sold to treat livestock reached around 117 tons for bovines, 140 tons for swine, and 73 tons for poultry, with global exposition of animals decreasing dramatically since 2011 (−25.5% for bovines, −60.5% for poultry, −54.0% for swine) (Anses, 2020b).

France has many characteristics that are shared by other European and North American countries. According to the State of Health in the EU initiative, the French healthcare system provides good access to high quality care. Preventable mortality by treatment is one of the lowest in the EU, which indicates that the health system protects people suffering from acute conditions. Antibiotic use in agriculture and in human medicine is regulated by national and European legislation (Journal officiel de la République Française, 2015, 2016). Notable common features are that access to antibiotics for both human and animal health is by prescription only with no over-the-counter sales. In 2019, antibiotic consumption in France for human medicine was among the highest in Europe, with 23.3 defined-daily doses of antimicrobials for systemic use per 1000 inhabitants and per day in the community (which ranged from 8.7 to 32.4 in Europe) (ECDC). There is currently essentially no domestic manufacturing of antibiotics.

1.3. Objectives

The objectives of this work were to gather available information concerning the current state and key drivers of AMR in the environment of France. First, we give an overview of the mechanisms underlying the emergence and persistence of AMR in the environment. Second, we drew up an inventory of current knowledge concerning the contamination of the terrestrial and aquatic environmental compartments by antibiotic residues and resistance to antibiotics (ARB, and antibiotic resistance genes – ARGs) and their spatial and temporal distribution. Third, we identified the antibiotics at risk to select AMR in the environment of France. Finally, we recommend avenues to improve and broaden our knowledge on the burden of AMR in the environment of this country.

2. Methods

2.1. Working group

In November 2016, the three Directorate Generals for Risk Prevention, Health, and Food Safety requested the advice of the French Agency for Food, Environmental and Occupational Health & Safety (ANSES) on the theme 'Antimicrobial resistance and the environment'. It is an administrative public establishment accountable to the French Ministries of Health, Agriculture, the Environment, Labor and Consumer Affairs. The Director General of ANSES created a working group to answer this question and has launched a call for interested experts. ANSES chose the experts based on their field of expertise and availability, seeking a balance of disciplines; fundamental, clinical, veterinary, environmental microbiology, and residue chemistry. The experts attested their independence through a public declaration of potential conflicts of interest, available on the website of the French Ministry of Health (dpi.sante.gouv.fr).

2.2. Data and method of expertise

The expertise was based on data and knowledge obtained from published scientific literature searched using PubMed and ScienceDirect, institutional reports on water, soils, and bacteria. Concerning more specifically the environmental concentrations of antibiotics, peer-reviewed literature, databases from research programs and surveillance databases on the French water resources were used (detailed in Appendix B).

The expertise was carried out in compliance with the standard NF X 50-110 "Quality in expertise - General requirements for expertise (May 2003)". This expertise focused on aquatic and terrestrial environments and their sources of contamination in metropolitan France and French overseas departments and regions. The environmental concentrations of antibiotics and the environmental distribution of ARB and ARGs of human health concern were considered, regardless of their method of quantification. Animal pathogens, environmental commensal bacteria, and genes conferring resistance to antibiotics that are not used in human medicine were not considered in the analysis. Where relevant, or to compensate for a lack of French data, environmental contamination measured elsewhere in Europe and in Canada was used for comparison. Aspects of the expertise concerning the conceptual basis of the spread of AMR drew on international literature. Likewise, this review does address conditions that could favor the transfer of ARGs from environmental bacteria to human pathogenic bacteria.

2.3. Investigated compartments

In high-income countries such as France, the main sources of environmental contamination with ARB and antibiotic residues are human and animal fecal waste inputs. Consequently, potential hotspots are treated wastewater effluents, fertilizers of human or animal fecal origin, and fish farm effluents (Gordon et al., 2007; McKinney et al., 2018;

Pruden et al., 2013; Quintela-Baluja et al., 2019). Leaking septic systems (Schaider et al., 2017) and effluent from landfill sites can also be a source of contamination but, in the absence of national data, these sources of contamination were excluded from the scope of this review.

Fig. 2 illustrates the sources and dissemination pathways of contamination in the main receiving environmental compartments considered; namely aquatic environments (including surface water, groundwater, and coastal water), terrestrial environments (including soils and plants), and wildlife.

3. Mechanisms underlying the emergence and persistence of antimicrobial resistance in the environment

Our understanding of the mechanisms involved in the emergence and persistence of AMR have generally been informed through gene mutation and transfer experiments carried out in the laboratory (Baharoglu and Mazel, 2011; Gullberg et al., 2014). Such experiments usually investigate the interactions of single antibiotics on single bacterial species, or bacterial mixtures of moderate complexity. Recently, the SELECT method (SELection End points in Communities of bacTeria) has been implemented to determine PNECs of antibiotics in complex microbial community (Murray et al., 2020). The findings are assumed to reflect what happens *in situ*, but the physical, chemical, and biological complexity of the terrestrial and aquatic environments cannot be fully replicated in the laboratory. In addition, nor can the effects of environmental exposure to complex and varying mixtures of selective and potential co-selective substances typical of waste streams be considered. Some of the challenges and unknowns this complexity represents are considered in the following sections.

3.1. At the scale of the bacterium

At the cellular level, the bacterial response induced by an antibiotic will vary according to the exposure dose and the bacterial species in question. It is generally accepted that maintenance of an ARG represents a fitness cost that puts the resistant bacterium at a competitive disadvantage relative to its susceptible parent (Melnyk et al., 2015). This disadvantage can be remedied through compensatory mutations that counterbalance the fitness cost (Andersson and Hughes, 2014) or external factors such as the presence of sub-inhibitory concentrations of antibiotics, *i.e.* concentrations below the minimum inhibitory concentration (MIC). These low concentrations may promote the emergence and persistence of ARGs by: (*i*) inhibiting the growth of antibiotic-susceptible populations and thus promoting the maintenance of antibiotic-resistant populations (defining the Minimum Selective Concentration, MSC); (*ii*) favoring the accumulation of mutations of low biological cost allowing bacteria to reach low-level resistance to different classes of antibiotics (Gullberg et al., 2011); (*iii*) inducing the SOS response, which in turn accelerates horizontal transfer of ARGs through transduction or conjugation (Jutkina et al., 2018; Ross and Topp, 2015) and decreases DNA replication fidelity, thus increasing the frequency of mutations affecting, among others, genes implicated in antibiotic resistance (Baharoglu and Mazel, 2014).

Data on the cumulative effects of simultaneous exposure to several selective agents are very scarce. However, a combination of biological effects of mixtures of selective agents present in the environment may be strongly suspected since: (*i*) co-selection of a bacterial clone co-resistant to several selective agents can be achieved by each of the selective agent to which the clone is resistant (Murray et al., 2019), and (*ii*) mechanisms

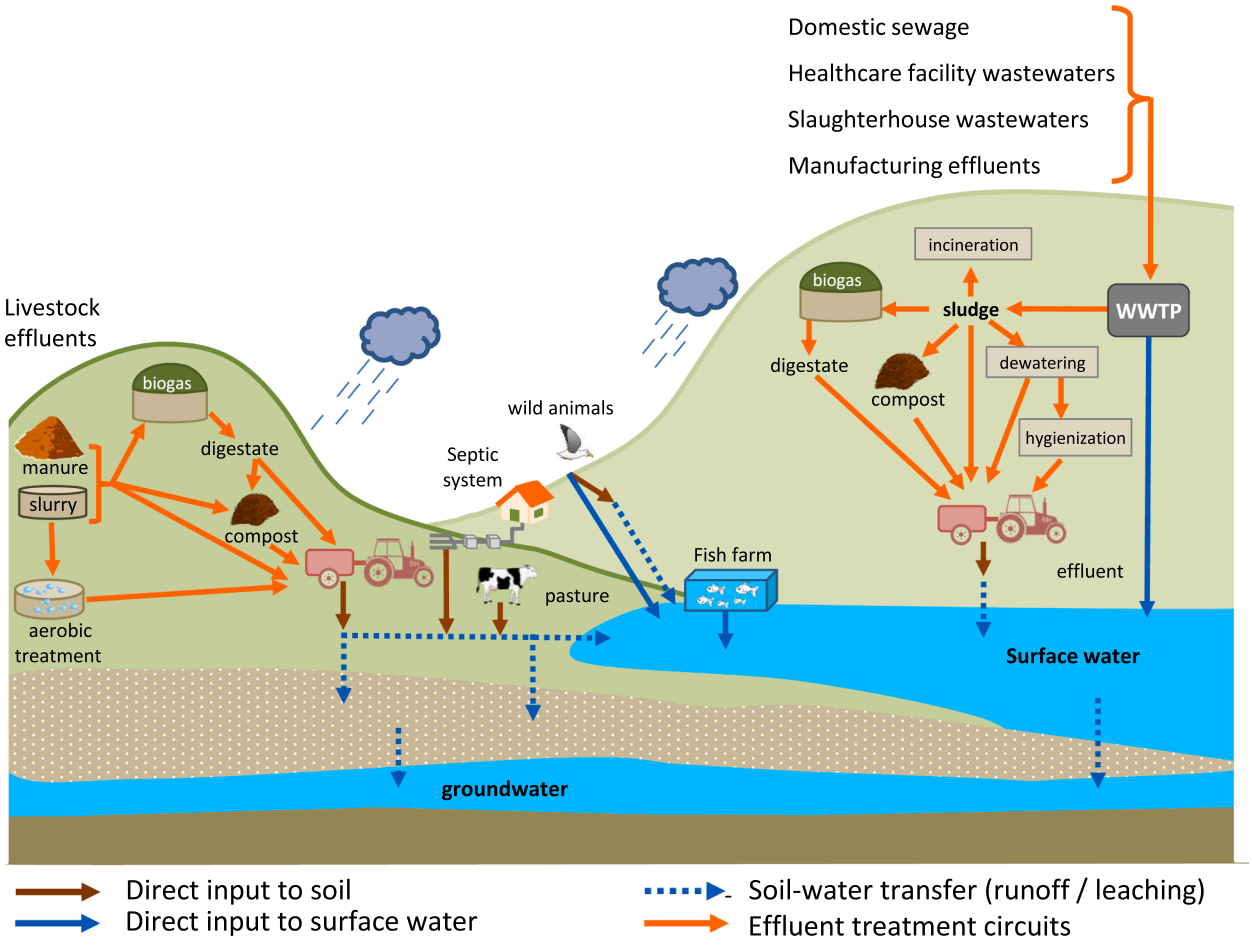


Fig. 2. Main sources and contamination routes of soil and water by antibiotic-resistant bacteria and antibiotic residues. WWTP, wastewater treatment plant.

of resistance to heavy metals and biocides can confer resistance to antibiotics (Nishino et al., 2007). This phenomenon is called cross-resistance.

3.2. At the scale of the bacterial community

Within a population or community, bacteria can cooperate to resist the actions of an antibiotic, for example through the formation of protective structures such as biofilms (Vega and Gore, 2014; Wright, 2005), whose formation can be favored by bacterial exposure to sub-inhibitory concentrations of antibiotics. It is generally accepted that the majority of bacteria in practically all environments are integrated into biofilms (Flemming et al., 2016). Biofilms can be extremely heterogeneous in structure, chemical and bacterial community compositions. Gradients of pH, oxygen and other electron acceptors and organic nutrients can be abrupt and highly dynamic, creating distinct microenvironments (Flemming and Wingender, 2010). Bacteria living in biofilms may be more resistant to antibiotics than the same bacteria in the planktonic state for several reasons (Flemming et al., 2016). First, the biofilm matrix constitutes a physical barrier to the penetration of antibiotics. Second, nutrient depletion in these microenvironments leads the bacteria to a viable but non-culturable (VBNC) or persistent state, which may reduce their sensitivity to antibiotics (Ayrapetyan et al., 2018; Fisher et al., 2017). Third, community cooperation could occur, for example allowing β-lactam-susceptible bacteria to survive in close proximity to individuals producing β-lactamases that hydrolyze these antibiotics. Within such multicellular structure, should at least one bacterial species of the community produces an enzyme that deactivates an antibiotic, bacteria susceptible to that antibiotic are protected by such 'public good' resistance mechanisms (Meredith et al., 2015). And finally, expression of quorum sensing can indirectly impact resistance to antibiotics (Hoffman et al., 2005; Nalca et al., 2006).

ARGs are often carried on plasmids, which intra- and inter-species dispersal can be spurred by the exposure to an antibiotic. Thus, even though the fraction of a community that carries a plasmid may be extremely small in the absence of antibiotic pressure, should antibiotic pressure be applied ARGs will be dispersed through the community after conjugative transfer of the plasmid (Lopatkin et al., 2017; San Millan and MacLean, 2017).

For all these reasons, the environmental risk assessment (ERA) of antibiotics in the environment and their potential to select for AMR cannot only rely on the testing of isogenic bacterial cultures with gradients of selective agents. Instead, the determination of PNECs must rely on methodological approaches dealing with mixed bacterial communities (Murray et al., 2021).

3.3. Mechanisms that govern bioavailability and bioaccessibility of antibiotic

Bioaccessibility expresses a constraint in time and/or space, linked to habitat, and has to be considered, in particular, in complex matrices such as soils or sediments, where the vast majority of bacteria are in biofilms which, among others, provide a physical barrier preventing the entry of antibiotics (see above) (Flemming et al., 2016). Bioavailability depends on intrinsic physicochemical properties of the active compound and is influenced by the environmental conditions and interactions with the surrounding matrix. For example, cation exchange and/or cation bridging mechanisms between the charged moieties of fluoroquinolones and cations result in a reduced bioavailability of these antibiotics. Fluoroquinolones form strong surface complexes with many transition metal ions through the carboxyl group and ring carbonyl oxygen (Gu and Karthikeyan, 2005). Also the presence of the divalent inorganic cations Mg^{2+} and Ca^{2+}, which are widely abundant in water, reduces the bioavailability of tetracycline by forming stable tetracycline-metal complexes (Werner et al., 2006; Zhang et al., 2018). Some macrolides are also affected by the presence of hydrated cations (Ca^{2+}, Na^{+}) that may act as proton-donors. Macrolides and tetracycline speciation are also strongly influenced by their association with dissolved organic matter (Chen et al., 2015; Sibley and Pedersen, 2008). In extremely complex matrix such as soil whose physicochemical properties (pH, content and nature of organic matter, cation exchange capacity) are highly variable, the possibility of predicting the concentration of bioavailable antibiotics and then exposure of living organisms is limited (Menz et al., 2018; Sidhu et al., 2019).

3.4. Transfer of ARGs to pathogenic bacterial species in the environment

Environmental microbial communities constitute a reservoir of ARGs, whose diversity and abundance remains largely unknown, and that have the potential to be acquired by pathogenic bacteria *via* mobile genetic elements (Nesme and Simonet, 2015). It is indeed known that several ARGs that have emerged in human bacterial pathogens, such as $bla_{CTX\text{-}M}$ and *mcr-1* genes, originated from environmental or commensal bacteria (Bonnet, 2004; Kieffer et al., 2017). Considering that the selection of a new ARB is more likely in an environment contaminated by one or more antimicrobial compounds, environments enriched with autochthonous bacteria, allochthonous potential pathogenic bacteria and selective antimicrobial compounds would be possible hotspots for the selection of new ARB equipped with new ARGs. The evolution of resistance to antibiotics in bacterial biofilm communities would be faster than in planktonic communities, since the physical proximity of the bacterial populations within the biofilm facilitates the horizontal transfer of genes by conjugation (Arias-Andres et al., 2018; Arias-Andres et al., 2019; Balcázar et al., 2015). While horizontal gene transfers are major drivers of bacterial evolution, their rates strongly depend on surrounding conditions; in that frame, transfers of new ARGs to pathogens and their subsequent maintenance and dissemination are likely rare.

Anthropogenic activities favor the diversification of ARGs and their dissemination in phylogenetically distant bacterial species. Fig. 3 summarizes conditions favoring the emergence of pathogenic bacterial strains carrying new ARGs of environmental or clinical origin in aquatic or terrestrial receiving environments. The probability of this emergence increases with the intensity of environmental contamination by pathogenic bacteria and depends on the presence of selective agents, which is

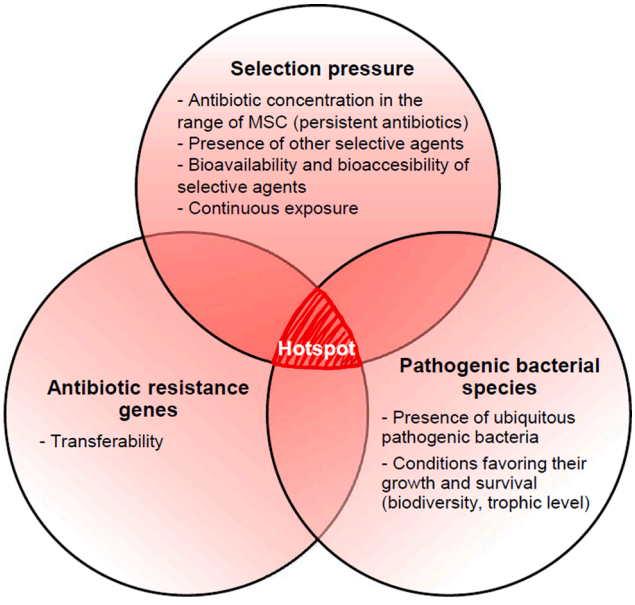


Fig. 3. Environmental conditions favorable to the transfer of antibiotic resistance genes to pathogenic bacterial species. MSC, minimal selective concentration.

discussed hereafter.

4. Environmental contamination by antibiotics in France

Surveillance of antibiotic residue concentrations in the environment of France has historically been limited in scope. Given the small number of environmental samples evaluated, heavily biased by the over-representation of surface water samples, and the few antibiotics analyzed, only a limited overview of the environmental contamination by antibiotics in France can be reported (Supplemental Table 1). Despite these constraints, the most frequently quantified antibiotics in soil, organic waste products (OWPs, including sewage sludges and animal fecal waste) and water in France belong to the quinolone, fluoroquinolone, macrolide, and sulfonamide families, which are environmentally persistent (Boxall et al., 2004). Though β-lactams are the most widely consumed antibiotics in human medicine (Moulin et al., 2008), they are rarely found in the environment due to their ready degradability (Supplemental Table 1).

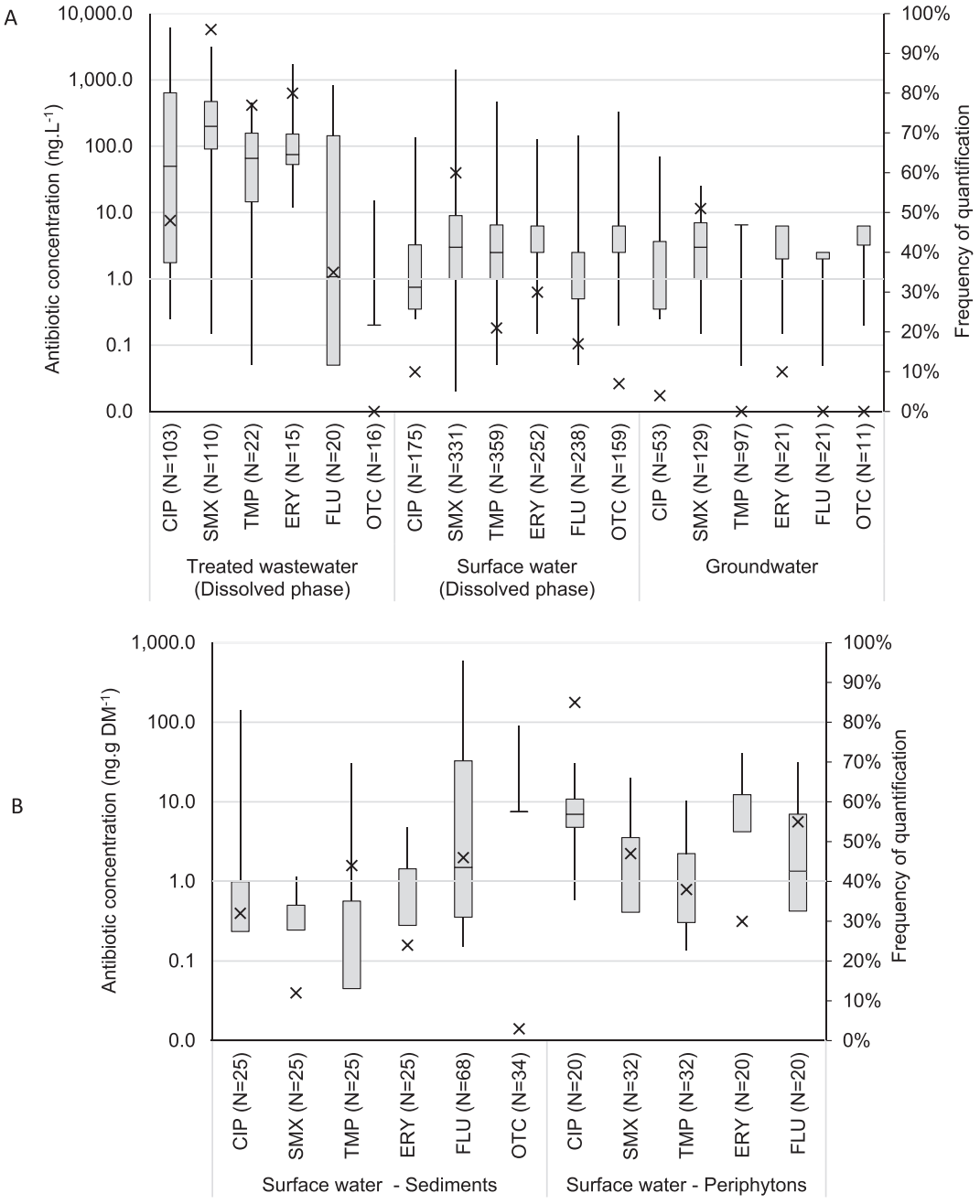


Fig. 4. Concentration of antibiotics from different pharmacological classes in the dissolved phases of treated wastewater, surface and groundwater (A) and in aquatic sediments and periphytons (B) in France. Box plots indicate min, P25, median, P75 and max of antibiotic concentrations (left y-axis). The crosses indicate the frequencies of quantification (right y-axis). The antibiotics considered are those for which sufficient data were available for all the matrices (we chose an arbitrary cutoff of 10 data points). We substituted results below the analytical limit with half of the detection limit value. CIP, ciprofloxacin (fluoroquinolone); SMX, sulfamethoxazole (sulfonamide); TMP, trimethoprim; ERY, erythromycin (macrolide); FLU, flumequine (quinolone); OTC, oxytetracycline (tetracycline); N, number of samples; DM, dry matter (Aubertheau-Laurent, 2013; Azimi et al., 2018; Camilleri et al., 2015; Capdeville, 2011; Chonova et al., 2016; Chonova et al., 2018; Coetsier et al., 2009; Dinh et al., 2017a; Feitosa-Felizzola and Chiron, 2009; Garcia-Armisen et al., 2013; Guibal et al., 2018; Joigneaux, 2011; Mailler et al., 2015; Tamtam et al., 2008; Wiest et al., 2018).

As observed in other countries, the antibiotics found in the environment come from anthropogenic sources, mainly treated or untreated wastewater, and agricultural discharges. Their continuous input into receiving environments and their persistence account for the ubiquity of some antibiotics. Nevertheless, in France, antibiotic concentrations are generally low in water, sediments and soils (Supplemental Table 1). This is not the case in countries devoid of or badly equipped with sanitation systems, and to industrial regions of antibiotic-producing countries (*e.g.* India, China) (ChangingMarkets, 2016).

4.1. Aquatic environments and sources of contamination

Fig. 4 presents the concentrations of the six most frequently analyzed antibiotics in treated wastewater and in the receiving compartments of aquatic environments as reported in the peer reviewed literature and collected by national study sites (SIPIBEL, PIREN; see Appendix B).

These data show that WWTP discharges are a major source of antibiotics in aquatic environments in France (Fig. 4A) (Capdeville, 2011; Oberlé et al., 2012; Ory et al., 2016; Perrodin et al., 2013). Beside this major human source of water contamination, spreading of OWPs as fertilizers (see 4.2 on terrestrial contamination) can also ultimately contribute to a more diffuse and incidental contamination of aquatic environments due to runoff, leaching and/or infiltration (Charuaud et al., 2019).

Concentrations of antibiotics in the dissolved phase of surface water decrease with distance from the WWTP discharge point due to dilution, degradation, and sorption to particulate matter. Concentrations can vary seasonally due to changes in river flow rates and water temperature, and from precipitation events that drive combined sewer outflows, releasing untreated wastewater into receiving bodies (Alygizakis et al., 2016; Baker and Kasprzyk-Hordern, 2013; Batt et al., 2006).

Antibiotic concentrations are usually less than 100 ng.g DM^{-1} in sediment and periphyton (Fig. 4B). The frequency of detection and median concentrations are generally higher in periphyton than in sediments, but the highest concentrations have been reported in sediments (Aubertheau et al., 2017; Labadie et al., 2019; Vivant et al., 2015). The concentrations of antibiotics in the sediment increase with proximity to sewage outflows (Labadie et al., 2019). Antibiotics stored in sediments may be remobilized during extreme hydrological events (Heß et al., 2018). The cooler and more reducing conditions in sediments are associated with poor biodegradation rates and the accumulation of persistent antibiotics such as fluoroquinolones, macrolides, and sulfonamides (Tamtam et al., 2011b; Thiebault et al., 2021). Indeed, along a sediment core corresponding to several decades of accumulation, antibiotic concentrations reflected the persistence properties of the antibiotics and the time of their market authorization (Tamtam et al., 2011b; Thiebault et al., 2021; Thiebault et al., 2017). Although traces of sulfamethoxazole have been measured in groundwater (Lopez et al., 2015; Vulliet et al., 2011), other antibiotics are only exceptionally quantified in this matrix (Supplemental Table 1).

Fig. 5 illustrates the decrease in the concentration of antibiotics and their frequency of quantification along the contamination continuum from a hospital. Taking ciprofloxacin and sulfamethoxazole as examples, we found that WWTPs reduce the concentrations of these antibiotics by 1.1–2.5 Log_{10} and that concentrations in surface water are 0.7–1.3 Log_{10} lower than in treated wastewater. Ciprofloxacin was rarely (≤1%) detected in the dissolved phase of surface water and groundwater. The detection of sulfamethoxazole in these matrices was more frequent (3–27%). Overall, the median concentrations of these antibiotics were ≤ 5 $ng.L^{-1}$ in the dissolved phase of surface water and groundwater.

4.2. Terrestrial environments and sources of contamination

Antibiotics are entrained into soil with the application of OWPs. Fig. 6 presents the concentrations measured for six antibiotics in France in WWTP sludge, solid manure, and soils amended with OWP. Each year, France produces 729 million tons (Mt) of OWPs. Of the 300 Mt of OWPs spread on fields yearly, a minority (1.1 Mt) are sludges produced by WWTPs and the vast majority (94%) are livestock effluents. Overall, 6.6 Mha of field crops and grasslands are amended with OWPs, representing 24.6% of the useful agricultural area in metropolitan France (Ademe, 2018). However, since the different farming types are heterogeneously distributed in France, soils are also heterogeneously amended over the territory (Houot et al., 2014).

Quinolones and fluoroquinolones are the most frequently quantified antibiotics and those with the highest concentrations in WWTP sludge (median concentrations between 2,600 and 3,000 ng.g DM^{-1} for each ofloxacin, ciprofloxacin, and norfloxacin). The other antibiotics are found at median concentrations lower than 62 ng.g DM^{-1} (Fig. 5 and Supplemental Table 1) (Bourdat-Deschamps et al., 2017).

The data on manure slurry, as commonly managed in the dairy and swine sectors, are scarce. Liquid OWPs seemed to be less contaminated with antibiotics than solid farm OWPs. Hence, the mean concentrations of ciprofloxacin were 241 ng.g DM^{-1} and 6 $ng.L^{-1}$ and those of doxycycline were 11,898 ng.g DM^{-1} and 505 $ng.L^{-1}$ in solid and liquid farm OWPs, respectively (Fig. 6).

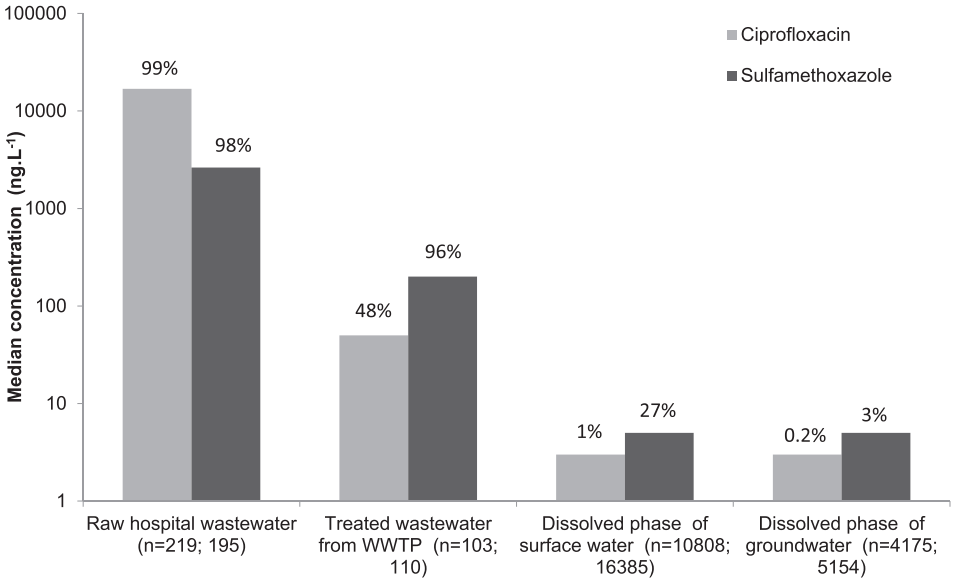


Fig. 5. Median concentrations of ciprofloxacin and sulfamethoxazole and proportion of samples above the limit of detection along the contamination continuum. The numbers of samples for ciprofloxacin and sulfamethoxazole are indicated in brackets, in this order). See Supplemental Table 1 for details. We substituted results below the analytical limit with half of the detection limit value. The labels on top of the bars give the proportion of samples above the limits of detection (LOD; 6 $ng.L^{-1}$ for ciprofloxacin and 10 $ng.L^{-1}$ for sulfamethoxazole (Patrolecco et al., 2018)).

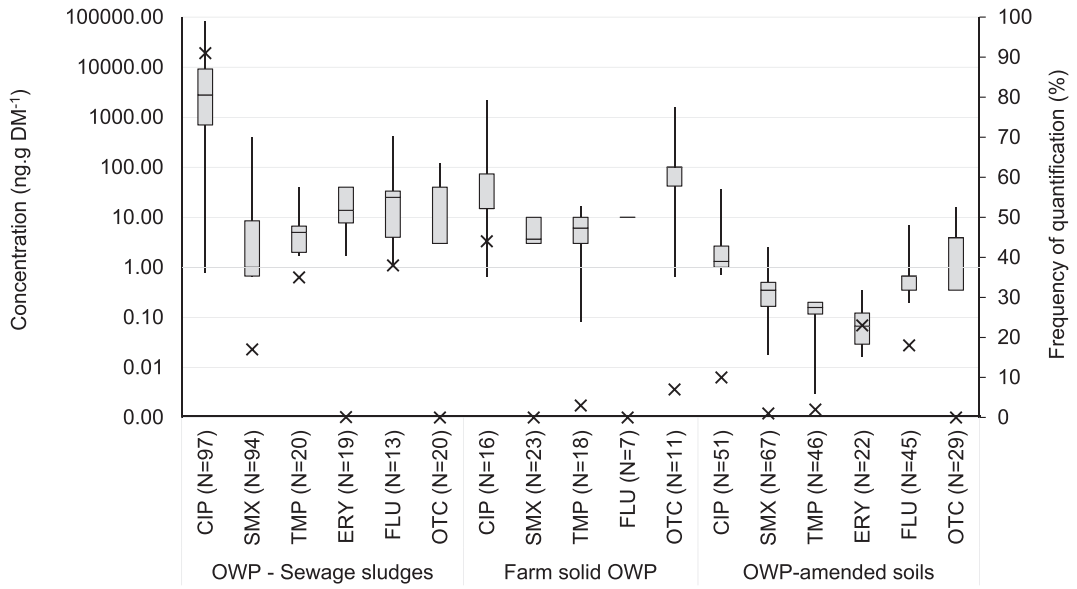


Fig. 6. Concentrations of six antibiotics from different pharmacological classes measured in OWPs (sewage sludges and solid farm products) and in OWP-amended soils in France. The antibiotics considered are those for which the largest amount of data was available in their pharmacological class, for all the considered matrices. Concentrations are presented as box plots (min, P25, median, P75, and max; left y-axis) and quantification frequencies as crosses (right y-axis). Results below the analytical limit were substituted by half of the detection limit value. All the dosage of ERY in solid farm product (n = 4) were below the LOD and not represented. CIP, ciprofloxacin (fluoroquinolone); SMX, sulfamethoxazole (sulfonamide); TMP, trimethoprim; ERY, erythromycin (macrolide); FLU, flumequine (quinolone); OTC, oxytetracycline (tetracycline); OWPs, Organic Waste Products; N, number of samples; DM, dry matter. For amended soils: (Bourdat-Deschamps et al., 2017; Dinh et al., 2017b; Nazaret, 2020a,b; Salvia et al., 2015; Salvia et al., 2012; Tamtam et al., 2011a); for farm solid OWP: (Algros and Jourdain, 2007; Bourdat-Deschamps et al., 2017; Moraru et al., 2012; Nazaret, 2020a,b; Pourcher et al., 2014; Simonet and Gardeur-Algros, 2012); for sewage sludges: (Algros and Jourdain, 2007; Bourdat-Deschamps et al., 2017; Simonet and Gardeur-Algros, 2012; Tamtam et al., 2009).

The median concentrations for antibiotics quantified at least once in OWP-amended agricultural soil do not exceed 2.1 ng.g DM^{-1}, with the nature of the antibiotics depending on the type and source of OWPs. The temporal variations of concentrations depend on the periodicity of enrichment with OWPs. Surface application of manure generally results in higher antibiotic levels in runoff than do injection or incorporation (Joy et al., 2013). However, periods of more than two weeks between spreading and rain events reduce antibiotic levels in runoff (Barrios et al., 2020).

Although the overall consumption of antibiotics is known in human (728 tons/year in 2018) and animal (471 tons/year in 2018) health (Santé Publique France et al., 2019), the estimation of the amount of antibiotics reaching the environment is nearly impossible. Hence, the *in vivo* metabolism, the *ex vivo* stability, the solubility of the antibiotic compounds are too diverse to allow any trustable estimation. The difference in the nature and the duration of treatments of OWPs prior to spread on fields, and the fact that only a fraction of the OWPs are used as fertilizers further complicates the task.

5. Contamination by ARB and ARGs

In France, as in all industrialized countries, ARB and ARGs are detected in all terrestrial and aquatic environments subjected to anthropogenic pressures (Buelow et al., 2020; Kümmerer, 2004). The vulnerability of water and soil to contamination is primarily related to exposure to fecal waste streams (Servais and Passerat, 2009; Zhu et al., 2019). Surface water is prone to contamination from both point sources (*e.g.* WWTP discharges) and non-point sources (*e.g.* runoff from OWP-amended fields) (Bréchet et al., 2014; Buelow et al., 2020; Dinh et al., 2017a; Drieux et al., 2016; Henriot et al., 2019; Labadie et al., 2019; Laroche et al., 2010; Oberlé et al., 2012; Ratajczak et al., 2010).

There is a paucity of French data for certain sources (*e.g.* manure, sludge, aquaculture) and for some environmental compartments (*i.e.* aquifers, coastal zones, soils, rhizosphere, phyllosphere, wildlife).

5.1. ARB and ARGs in wastewater effluents

Contamination of water by ARB and ARGs of human and animal origin can be reduced by natural attenuation. Their dissipation rate in the water environment will depend on biotic or abiotic parameters including the dilution in the receiving environment as well as protozoan predation, viral lysis, and ability of ARB to overcome environmental stresses (Rozen and Belkin, 2001; van Elsas et al., 2011). However, this attenuation is no longer observed in areas subject to continuous and significant inputs of ARB, ARGs, and selective agents, such as sediments or biofilm at close proximity to a WWTP discharge (Aubertheau et al., 2017; Fairbairn et al., 2016; Leclercq et al., 2013; Oberlé et al., 2012). In these polluted areas, the persistence of AMR could be favored by the high concentrations of selective agents (Fig. 4) so that the rate of AMR contamination could, at least in part, compensate that of dissipation.

Globally, most WWTPs only use primary and secondary treatment, without downstream treatments such as ultrafiltration or UV treatment. These WWTPs reduce the concentration of fecal bacteria and the concentration of ARGs in treated effluent by about 2 Log_{10} (Stalder et al., 2014). Indeed, the Log_{10} reduction of ESBL-producing *E. coli* and vancomycin-resistant enterococci are 1.5 and 2.5 Log_{10}, respectively, consistent with the Log_{10} reduction of their susceptible parents (Bréchet et al., 2014; Galvin et al., 2010; Gouliouris et al., 2019; Leclercq et al., 2013; Oberlé et al., 2012; Servais and Passerat, 2009; Slekovec et al., 2012; Varela et al., 2013). The few available data on slaughterhouse effluents in France come from beef slaughterhouses. Effluents from calf slaughterhouses are more concentrated in antibiotic-resistant *E. coli* than those from adult cattle slaughterhouses (Um et al., 2016). The functioning and efficiency of slaughterhouse WWTPs are comparable to urban WWTPs (Bibbal et al., 2014).

In order to assess the spread of AMR independently from culture methods, *intI1* concentration has been proposed as a proxy for anthropogenic contamination (Gillings et al., 2014). Hospital effluents contain about 10 times more copies of *intI1* ($\sim 10^{11}$ copies.L^{-1}) than urban wastewater. Although their volume is very much lower than that of the

urban community in which they are collected (Stalder et al., 2014), hospital effluents have much more abundant ARB than household sewage (Buelow et al., 2018; Buelow et al., 2020). There is a larger diversity of ARGs in domestic raw sewage than in hospital wastewater (Buelow et al., 2020), the latter are enriched in genes encoding resistance to hospital-specific antibiotics, for example *aacA4* and *aadB* that confer resistance to aminoglycosides (Stalder et al., 2014).

5.2. ARB and ARGs in surface and ground water

The vast majority of fecal ARB found in aquatic environments come from anthropogenic sources. The presence of ARB and ARGs in aquatic environments depends on recurrent emissions of fecal bacteria from WWTP discharges. For instance, concentrations of ESBL-producing *E. coli* increased from 20 CFU.L^{-1} upstream to 1,300 CFU.L^{-1} downstream of the WWTP of a 120,000 inhabitant city (Bréchet et al., 2014). In addition, runoff from agricultural areas amended with OWPs and overflow from combined sewage collection systems during heavy rainfall can also contribute to the contamination of surface water. Spatial dynamics in contamination by fecal ARB and their corresponding ARGs, could be observed in water column, biofilm or sediment sampled along a river continuum, from the effluent discharge point to downstream. For instance, the proportion *intI1*/16S rRNA reached 1.89.10^{-1} in the River Seine downstream the city of Paris, while went back to 10^{-3} at the estuary of the river (Aubertheau et al., 2017; Dinh et al., 2017b; Fairbairn et al., 2016; Leclercq et al., 2013; Oberlé et al., 2012; Petit et al., 2017). Indeed, studies reported the disappearance of specific populations of fecal ARB (*E. coli* and *Enterococcus faecium*) in favor of other populations of the same bacterial species, less resistant to antibiotics but probably better adapted to the aquatic environment (Berthe et al., 2013; Martak et al., 2020). In groundwater, mainly karst hydrosystems, the occurrence of ARB of fecal origin is depending on the hydrological conditions and the land use (pasture and manure application). Overall, antibiotic-resistant *E. coli* were rarely detected at the drilling of this groundwater used for the production of drinking water, except during major rainfall events (Laroche et al., 2010; Ratajczak et al., 2010; Ribeiro et al., 2012).

5.3. ARB and ARGs in organic waste products

Two French studies have quantified ARB or ARGs in OWPs and evaluated how they vary according to the treatment. These studies were focused only on manure and confirmed observations from other countries where OWPs underwent similar treatments (Le Devendec et al., 2016; Moraru et al., 2012). For example, Moraru *et al.* in France and Sharma *et al.* in Canada observed a decrease in concentrations (>3 Log_{10}) of antibiotic-resistant *E. coli* during chicken litter storage and during composting of cattle manure, respectively (Moraru et al., 2012; Sharma et al., 2009).

Le Devendec *et al.* reported that broiler manure composting has little impact on the relative abundance of some ARGs (*e.g. sul2*), but significantly decreased (3.7–4.7 Log_{10}) the abundance of others (*e.g. tet*(M), *erm*(B)) (Le Devendec et al., 2016). Canadian studies reported that treatments applied to manure (composting, dewatering) or to WWTP sludge (lime stabilization, thermal treatment) reduced the abundance of ARGs, reducing the risk of transmission of ARGs to crops or water resources (Lau et al., 2017; Murray et al., 2019; Tien et al., 2017). Clearance of ARGs depends on the treatment applied to the matrix. Hence, the concentration of eight ARGs in heat-treated pellets were 1–1.5 Log_{10} lower than in anaerobically-digested sewage sludge and 1.3–2.2 Log_{10} lower than in aerobically digested biosolids (Lau et al., 2017). Similarly, by testing ten ARGs, Tien *et al.* found that digested, dewatered, and composted manure contained concentrations of ARGs 0.8–3 Log_{10} lower than in raw manure (Tien et al., 2017).

5.4. ARB and ARGs in soil

Very few studies measured soil contamination by ARB and ARGs in France, with most of them being unpublished. In agreement with data reported in other countries, these studies described the common presence of ARB and ARGs within the bacterial communities of agricultural, corroborating the status of these soils as an environmental reservoir. As an illustration, Hartmann *et al.* detected isolates of CTX-M−1−producing *E. coli* both in cultivated soil in Burgundy amended one year earlier with manure, and in grassland soil sampled in the vicinity of dairy farms (Hartmann et al., 2012). Some isolates shared the same genotype than those found in animals from these farms, suggesting the long-term persistence of the isolates from animal origin after manure application. An on-going French study showed that the persistence of ARGs depends on many factors (*i.e.* climate, topology of the amended fields, soil type, frequency of application, characteristics of the manure applied, and composition of the soil bacterial communities). However, the same study found that the absolute abundance of ARGs (*i.e. sul*, *intI1*, *tet*) (*i*) increased by 1 to 2 Log_{10} one month after bovine manure application, and (*ii*) generally did not increase over the long term in amended plots despite repeated applications (personal data, S. Nazaret). In field experiments conducted in Canada, the kinetics of ARG dissipation in soil after land spreading was similar regardless of pre-application treatment (Lau et al., 2017; Murray et al., 2019; Tien et al., 2017). Consequently, manure and sludge pre-application treatments generally reduce but do not eliminate ARB and ARGs, so that spreading treated manures and sludges may still represent a potential risk of contamination of adjacent water resources through runoff or soil leaching.

5.5. Wildlife as a reservoir for ARB and ARGs

Wildlife, mainly birds, mammals but also invertebrates, are suspected to be involved in the spread of ARB in the environment. ARB and ARGs have been detected in the intestinal microbiota of terrestrial vertebrates (*e.g.* wild boars, wolves, rodents) with omnivorous or carnivorous animals being more often contaminated with ARB than herbivores (Vittecoq et al., 2016). In the freshwater environment, fish feeding on periphyton close to WWTP discharges were contaminated with ESBL-producing *E. coli* (Bollache et al., 2019). Finally, birds have been repeatedly described as ESBL carriers, and the frequency of ARB carriage by seabirds increases with proximity to humans (Bonnedahl et al., 2009; Haenni et al., 2020; Vittecoq et al., 2017). Moreover, antimicrobial contamination of migratory birds raises further questions on the capacity of these animals to disseminate ARB and ARGs over very long distances. Globally, almost all of these studies showed that the occurrence of ARB in aquatic or terrestrial wildlife is reflecting both the diet of the wildlife and the impact of human activities (Ramey and Ahlstrom, 2020).

6. Potential (co)selection of antimicrobial resistance in the environment in France

6.1. Do antibiotic concentrations in treated wastewater and receiving bodies favor antibiotic resistance dissemination?

In order to assess the risk of antibiotics to select for or to favor the persistence of AMR in French waters, we compared antibiotic concentrations quantified in treated wastewater, receiving water, and groundwater with PNEC values (Fig. 7). Murray *et al.* (Murray et al., 2021) gave PNEC-R (PNEC for resistance) and PNEC-P (PNEC for persistence) based on experimental data with mixed bacterial communities. Experimental data were available for eleven antibiotics (cefotaxime, azithromycin, clarithromycin, erythromycin, ciprofloxacin, chloramphenicol, gentamicin, kanamycin, streptomycin, tetracycline, and trimethoprim) which span seven antibiotic classes. We had robust data (with MECs being assessed > 10 times) for all these compounds,

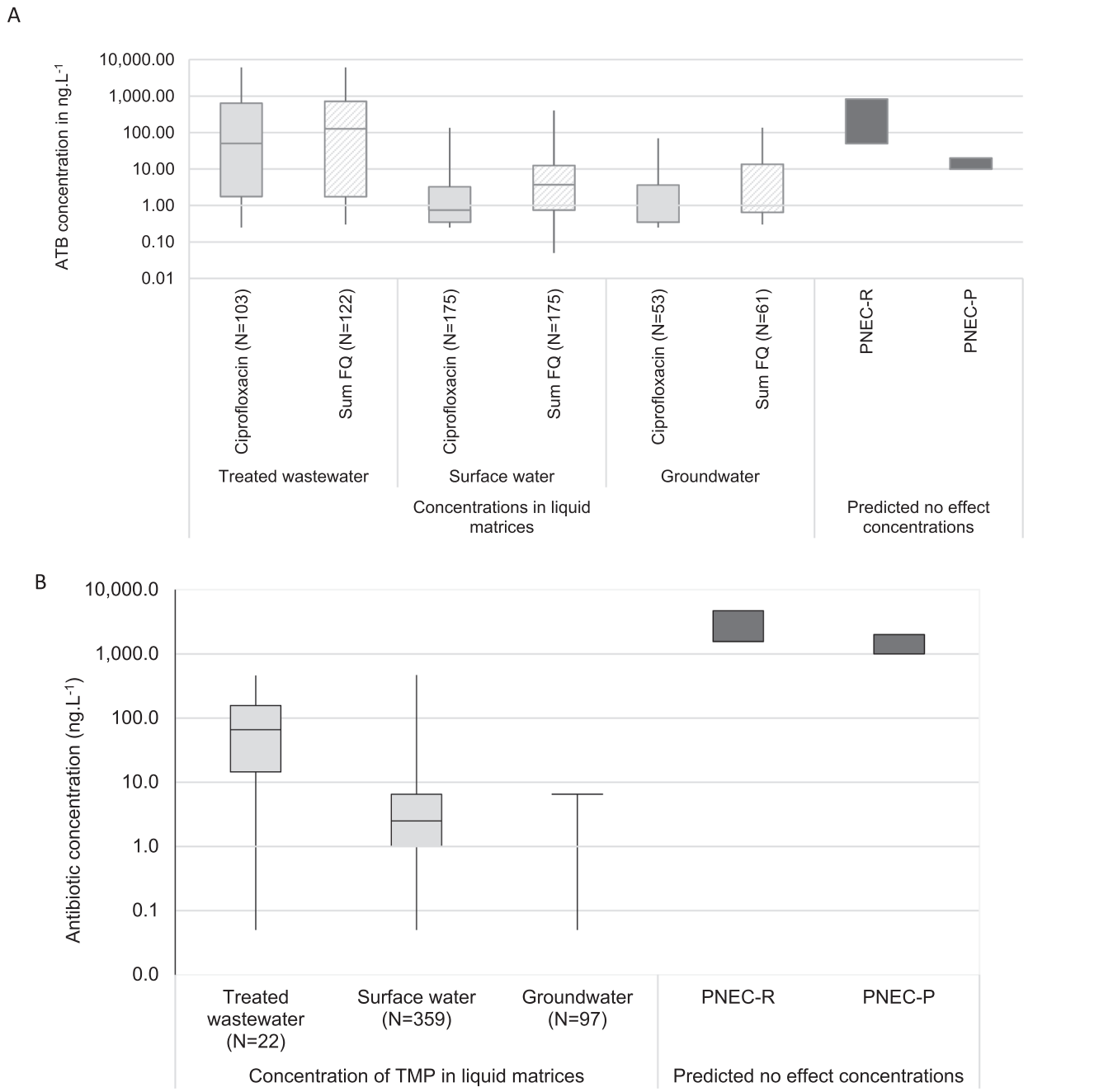


Fig. 7. Antibiotic concentrations in the dissolved phase of various liquid environmental matrices (antibiotic alone in light gray; sum of all antibiotic residues from the same class in dashed grey) and the predicted no effect concentration for resistance (PNEC-R) and for persistence (PNEC-P) (dark gray) for ciprofloxacin (panel A), trimethoprim (panel B), macrolides (panel C) and tetracycline (panel D). FQ, fluoroquinolones; TMP, trimethoprim; ML, macrolides; TC, tetracyclines; N, number of samples; PNEC values were taken from (Murray et al., 2021). The PNEC values were those of ciprofloxacin (panel A; PNEC-R, 50–780 ng.L^{-1} ; PNEC-P, 10 ng.L^{-1}), trimethoprim (panel B ; PNEC-R: 1,560–3,130 ng.L^{-1} ; PNEC-P: 1,000 ng.L^{-1}), azithromycin, clarithromycin, erythromycin (panel C ; PNEC-R: 50,000–12,500,000 ng.L^{-1} ; no PNEC-P yet defined), tetracycline (panel D ; no PNEC-R yet defined ; PNEC-P: 10 ng.L^{-1}).

except aminoglycosides for environmental liquid matrices in France (see Supplemental Table 1). Of note, cefotaxime and chloramphenicol were detected in $\leq$ 3% of the tested surface water. PNECs were still unknown for penicillins, carbapenems, sulfonamides, and colistin (Murray et al., 2021).

Only concentrations in the dissolved phases of the liquid matrices of treated wastewater, surface water and groundwater were considered because of strong uncertainties in concentrations and bioavailability in other environmental matrices. For example, concentrations reported on a dry weight basis in matrices that have high moisture contents such as periphyton or sediments will be an overestimation of the concentration that microorganisms are exposed to (Jonker, 2021). Likewise, the bioavailability of antibiotics in complex matrices such as soils and sediments will be constrained by sorptive processes that will vary widely with the antibiotic and the physical and chemical composition of the matrix (Al-Khazrajy et al., 2018; Li et al., 2021).

Considering that antibiotics of the same class can induce a biological response at similar concentrations, we used the individual antibiotic concentrations and the sum of the concentrations of antibiotics of the same class to consider a potential additive effect.

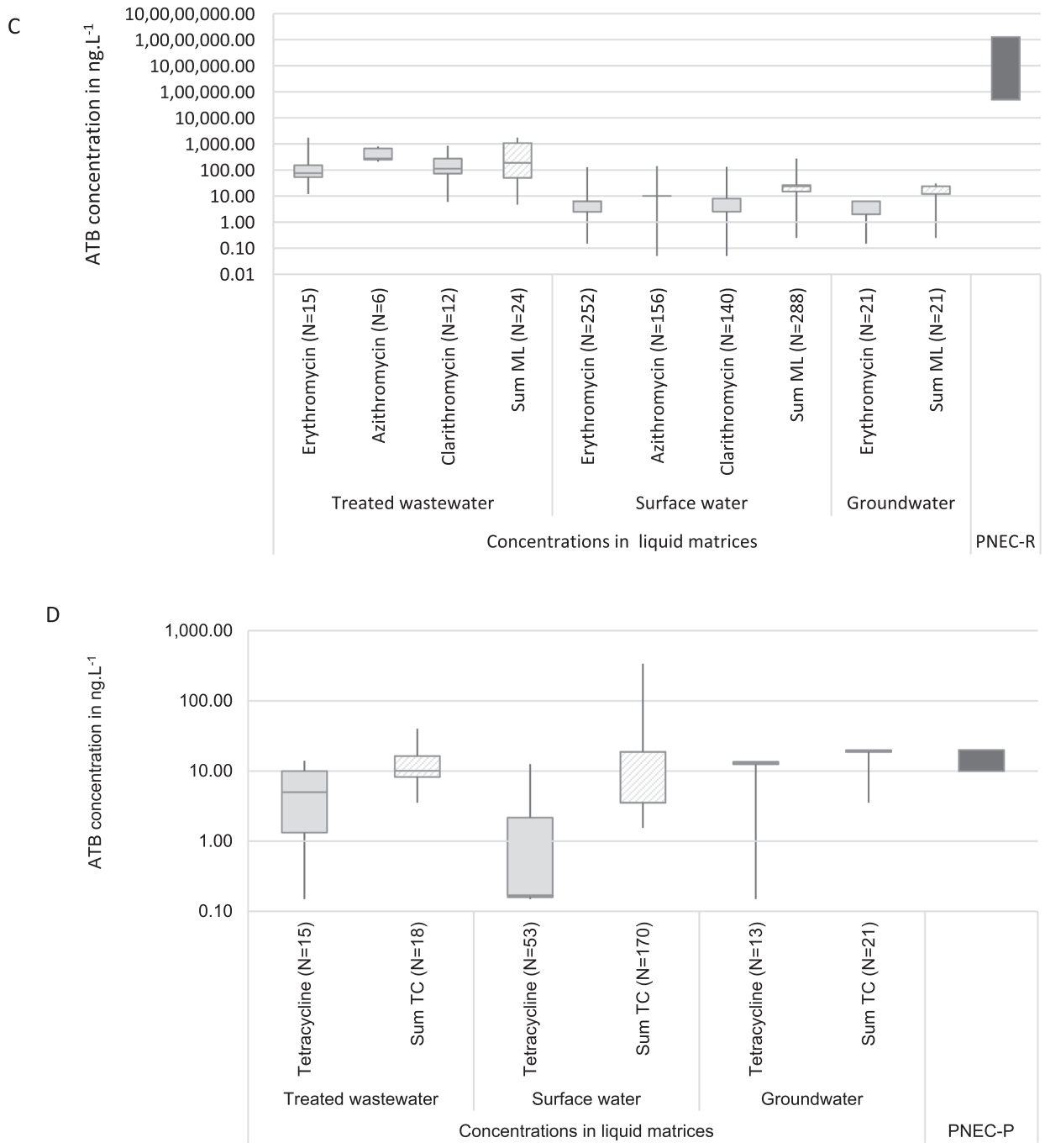


Fig. 7. (*continued*).

The ciprofloxacin concentrations in treated wastewater and in the most polluted surface water could favor the emergence of resistance. The PNEC-P of ciprofloxacin (10 ng.L^{-1}) was low enough to be reached in all the liquid matrices tested (Fig. 7A). In contrast, the cumulated concentrations of macrolides and the concentration of trimethoprim did not reach their respective PNECs (Fig. 7B and 7C). The concentrations of tetracycline in treated wastewater and in surface water reached the PNEC-P of the compound (10 ng.L^{-1}) (Fig. 7D). Fig. 8 displays the risk quotients (RQs) for aquatic environments in France calculated from PNEC-R and MECs. Overall, the antibiotic likely to pose the greatest risk to the French environment is ciprofloxacin, and by extension all fluoroquinolones. These observations are in agreement with those derived from international data on fluoroquinolone MECs (Chow et al., 2021). However, the application of *in vitro* results to predict effects that the fluoroquinolone MECs may exert (Fig. 7A) must be tempered by the potential bioaccessibility and bioavailability of residues *in situ* (see Section 3.3). Although trimethoprim concentration does not reach the PNEC, this antibiotic is at medium risk in the most polluted wastewater and receiving rivers.

6.2. *Associations between environmental concentrations of selective agents and antimicrobial resistance*

Knowledge about the impact of selective agents on antibiotic resistance in the environment is still patchy. However, based on studies carried out *in situ* and the analysis of the information outlined above, some conclusions can be drawn.

An environment contaminated by bacteria resistant to a certain class

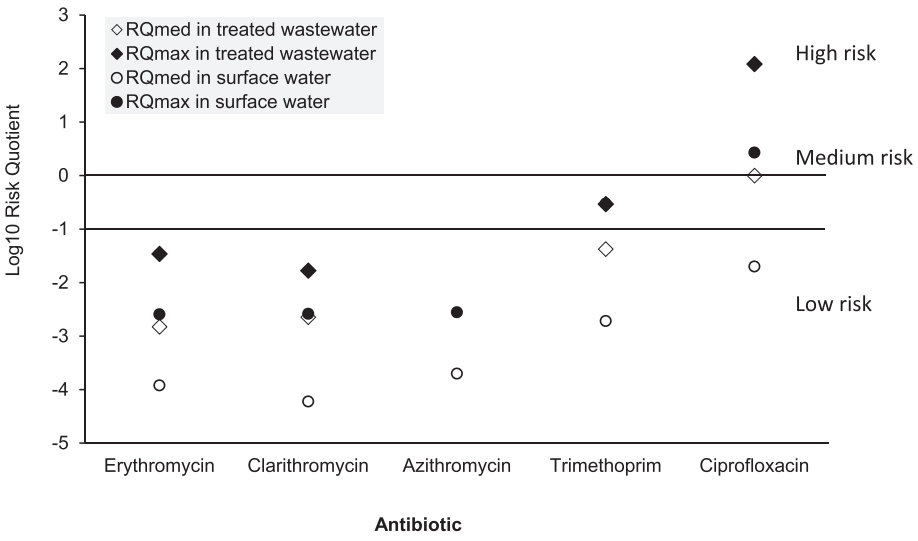


Fig. 8. Risk quotients (RQs) for aquatic environments (treated wastewater and surface water) in France. RQ_{max} (RQ maximum) and RQ_{med} (RQ median) were based on maximum and median measured environmental concentrations (MECs) divided by the PNEC-R determined experimentally with the SELECT method (Murray et al., 2020). MECs were extracted from Supplemental Table 1. RQs were represented on a logarithmic scale. High, medium, and low risks were defined by Log_{10} RQ values > 0, -1 to 0, and < -1, respectively. We kept for analysis antibiotics whose MEC was assessed > 10 times and which were above the detection limit in > 10 % of the cases.

of antibiotics, for example β-lactams, may not contain antibiotics in that class if the antibiotics are not inherently persistent. For instance, the presence of penicillin-resistant *E. coli* in a river in which penicillins were undetectable suggests that resistance was acquired in the digestive tract of individuals (human and/or animal) receiving antibiotics and not in the environment (Oberlé et al., 2012; Petit et al., 2012). Sampling multiple sites upstream and downstream of the wastewater treatment plant in the Vienne catchment area (Rhône river) failed to reveal associations between antibiotic concentrations in river periphytons and the abundance of *intI1* (Aubertheau-Laurent, 2013; Aubertheau et al., 2017). This is in line with non-French studies showing that, with the exception of pharmaceutical industry discharges, the abundance of ARGs in aquatic environments seems due to fecal contamination by ARB rather than selection of resistant environmental bacteria (Ahmed et al., 2018; Karkman et al., 2019; Milaković et al., 2019).

Terrestrial or aquatic environments are potentially contaminated by mixtures of selective agents, among which heavy metal and biocides can contribute to the maintenance of AMR in the environment by exerting a co-selection pressure (see Section 3.1) (Pal et al., 2017). However, there is little data available to indicate if this happens *in situ* (Berg et al., 2010; Carey and McNamara, 2014; Han et al., 2019; Middleton and Salierno, 2013; Tandukar et al., 2013; Zhao et al., 2019), and the effects (synergistic, additive, or antagonistic effects between different compounds) of mixtures is difficult to anticipate (Gullberg et al., 2014; Vos et al., 2020). Although it is reasonable to speculate that different antibiotic classes targeting the same bacterial component (*i.e.* macrolides and tetracyclines) would be additive in mixtures, there are no data in the literature on this subject.

7. Perspectives and recommendations

This review revealed numerous knowledge gaps and limitations in the comparison and generalization of the available data for the context of France. We faced several difficulties including the absence or paucity of data for several sources, the overall lack of metadata and the lack of coherent and comparable sampling strategies in time and space. To compensate for a lack of French data, environmental contamination measured in countries sharing socio-geographic characteristics similar to France, *i.e.* Europe and Canada, was used for comparison. Likewise, conclusions of this review are applicable to countries that share similar attributes; the more variance from these characteristics, the less applicable the analysis will be. We thus recommend six main avenues to globally improve and broaden information on the burden of AMR in the environment.

7.1. Harmonization of sampling, methods and indicators

In contrast to ARB and ARGs, databases already exist concerning the monitoring of environmental contamination by antibiotics (see Appendix B). However, comparisons between studies are difficult, due to the diversity of the measured parameters. Likewise, sampling and analytical methods need harmonization for all chemical and microbial contaminants of water resources. We thus recommend:

- to optimize and sustain data acquisition by providing a minimal list of common indicators (ARB and ARGs) required to monitor environmental contamination (Table 1). These indicators, chosen based on current knowledge and available analytical methods, will allow a spatial and temporal comparison of the environmental contamination dynamics between studies, but also in the different environmental compartments. Such a minimal list of common indicators was initiated by the WHO, who proposed a simple and broadly implementable protocol (called "Tricycle") to detect ESBL-producing *E. coli* in the three human, animal and environmental sectors (WHO, 2021).
- to define priority antibiotics to be monitored in the aquatic environment. Where available, validated analytical methods that comply with national and/or international standards must be used to facilitate inter-study comparisons. In France, we propose to monitor representatives of five antibiotic classes: fluoroquinolones (ciprofloxacin), trimethoprim, sulfonamides (sulfamethoxazole, sulfamethazine), tetracyclines (tetracycline), and aminoglycosides (gentamicin). Compounds which have a low RQ (*e.g.* macrolides), limited usage (*e.g.* chloramphenicol, colistin), and those that are rapidly dissipated in the environment (*e.g.* β-lactams) will be excluded.
- to monitor the resistance patterns of ubiquitous pathogenic bacterial species able to live in the environment and humans (*e.g. Klebsiella pneumoniae, Stenotrophomonas maltophilia, Aeromonas hydrophila*).
- to monitor the spatial and temporal dissemination of antibiotics, ARB and ARGs in distinct receiving environments, and to evaluate the variability over time of these contaminant concentrations in receiving waters in study-sites and/or instrumented sites representative of the geographic diversity and land use of the French and European territory. Ideally, studies should cover the parameters listed above (antibiotics, ARB, ARG) for WWTP effluents, receiving surface water $>$ 1 km downstream of the effluent pipe, and groundwater (comprehensive sampling). In smaller-scale studies, the number of samples might first be reduced to receiving surface water

Table 1
Proposed indicators of antimicrobial resistance in the environment.

Proposed indicator	Method	Results and units	Advantages	Limitations
E. coli resistant to 3rd generation cephalosporins or ESBL-producing *E. coli* [a]	Culture on selective media	– Absolute value (CFU.L $^{-1}$ or CFU.g^{-1}) preferable – Relative value (relative to abundance of total *E. coli*)	– Relatively simple tracking – Indicators of environmental exposure – Present in soils, water, sediments – Assess the anthropogenic impact on AMR in the environment – Identify hotspots and/or periods at risk of fecal contamination – Indicators shared by human and veterinary practices – Indicator proposed by the WHO Tricycle protocol	– Do not allow the description of new ARB – Quickly lose their cultivability in the environment
intI1 [b]	qPCR	– Relative abundance (gene copy / 16S rRNA) – Number of copies.L $^{-1}$ for liquid matrices – Number of copies.g DM $^{-1}$ for solid matrices	Relatively simple trackingIndicators of anthropogenic pressureCan be coupled with the ARG search to evaluate their mobility	– Does not identify ARGs present in the matrices – Method partially standardized – Possible problem of extraction and/or amplification inhibitors in some contaminated environmental matrices – Limit of quantification ~ 10^3 gene copies – Since *intI1* is widespread, there is a need of "blank" data on pristine environments in order to infer trends in *intI1* abundance.

[a] These two parameters partially overlap. Currently, resistance to 3rd generation cephalosporins in *E. coli* is predominantly mediated by ESBL production.
[b] Future recommendations for qPCR targets will be based on the outcomes of metagenomic surveys of municipal and agricultural effluents.

with all parameters tested (intermediate sampling). Ultimately, parameters might be restricted to the quantification of *E. coli* and ESBL-producing *E. coli* in receiving surface water (limited sampling).

7.2. Placing the One Health concept on a global scale

We recommend the creation of a long-term integrated surveillance network using a One Health approach, which connects ARB, ARGs, and antibiotic residues data from environmental, human, and animal compartments. Each surveillance system must take into account the distribution of the different farming types over the territory to prioritize regions with the highest risk of contamination. We also recommend open centralized repositories for surveillance data and metadata at the national and European levels. The success of a national surveillance initiative requires the use of internal controls and standardized techniques. However, such guidelines are still lacking nationally and internationally for the indicators proposed in Table 1. Laboratories must then use procedures guaranteeing the quality and comparability of the associated data and metadata. Moreover, a reconciliation of the various regulatory monitoring tools (environmental and sanitary) would facilitate the management of AMR in the environment. The four antibiotics (amoxicillin, ciprofloxacin, trimethoprim, and sulfamethoxazole) on the 3rd version of the Water Framework Directive's Watch List (Gomez Cortes et al., 2020) included the one that is likely to pose the greatest risk to the French environment (*i.e.* ciprofloxacin). However, ciprofloxacin only represents ~ 30% of the prescription of fluoroquinolones in France (Santé Publique France, 2017). Since, the PNECs of other major fluoroquinolones (*i.e.* levofloxacin and ofloxacin) are likely similar to those of ciprofloxacin, the next Watch List should also include these compounds.

In the future, these recommendations should be adapted based on the epidemiology of ARB and ARGs in human and animals, and on the development of new methods. Applications of metagenomics in surveillance of wastewater effluents and agricultural effluents is an example of evolving approaches that hold promise.

7.3. Broaden the panel of matrices and sources tested

In the French context, it is necessary to increase the range of matrices studied notably to consider the particulate fraction of liquid matrices, sediments and the periphyton. Monitoring of contamination levels should be extended to other potential sources and receiving matrices. Sources of contamination for which data are sorely needed include sewerage, installations producing or formulating antibiotics, and old landfill sites. Receiving bodies for which there is very little information include fish farms, coastal waters, soils from terrestrial environments such as urban, *peri*-urban and agroforestry and polluted sites, and soil meso- and macrofauna such as earthworms and arthropods which can both constitute hotspots and play a role of dissemination vectors.

7.4. Anticipate climate change impacts on AMR in the environment

Climate change in Europe is likely to modify the environmental contamination with AMR and antibiotics. In the near future, the expected increase in the frequency of extreme rainfall events (EASAC, 2018), and the expected sharp variation of river flows will increase the risk of contamination by ARB, ARGs, and antibiotics. Hence, both the combined sewer overflows and the resuspension of sediments during extreme rainfall events can enhance the release of antibiotics, ARB, and ARG into the aquatic compartment (Olds et al., 2018; Pérez-Valdespino et al., 2021). Finally, lower minimum flows imply less volume for dilution and, hence, higher concentrations downstream of point discharges such as WWTP.

7.5. Clarify the impact of antibiotics on the development of antimicrobial resistance and its dissemination

There is a need to determine the sub-inhibitory concentrations of antibiotics, on their own and in mixtures, that favor the persistence, dissemination, selection and the emergence of AMR through *in vitro* experimentation in complex media or through experiments at different scales (*e.g.* at the laboratory with biofilms and mesocosms, or *in situ* with field plots). Likewise, it is necessary to identify the environmental conditions allowing the persistence and dissemination of AMR and to study of the expression of panel of ARGs under anthropogenic pressure. The determination of PNEC-R and/or PNEC-P values for highly-prescribed antibiotics (tetracyclines, sulfonamides, azoles) using experimental approaches with bacterial community should be a priority (Murray et al., 2021).

7.6. Change the scale to study interactions between antibiotics and antibiotic resistance

We must also increase our knowledge on the actual exposure of bacterial communities to antibiotics, by a better characterization of the bioavailability at a microenvironment scale considering the interactions between bacterial populations and all the constituents of its environment. Hence, the impact of anthropogenic activities on environmental microbial communities is generally assessed on a macroscopic scale. However, the environment in which microorganisms live, given their size, is at the μm scale (Nunan et al., 2001; Ranjard and Richaume, 2001). The concepts of microbial ecology and of microbial ecotoxicology have not been sufficiently taken into account for the study of AMR, particularly on the scale of these microenvironments (Banerji et al., 2019).

The impact of selective agents in the environment has mainly been studied in liquid matrices. In solid matrices, the bioavailability and bioaccessibility of antibiotics remain difficult to quantify (Menz et al., 2018; Sidhu et al., 2019). Studies on antibiotics in soils are mostly carried out using destructive approaches by homogenizing samples. This neglects the notions of microbial habitats and likely gives biased views on the exposure levels of soil aggregates.

At a macroscopic scale, the development of digital big data processing tools (*e.g.* machine learning and deep learning based on artificial intelligence) could analyze environmental monitoring data to assess the risk of AMR retention in different situations. New numerical prediction tools that could anticipate potential evolutions (climate change, changes of water use) could help policy-makers (Doorn, 2021).

7.7. Plan and act using a 'one health' approach

Taking all the above recommendations into consideration, the "One Health" approach will necessarily be multidisciplinary and will bring together microbiologists of all backgrounds (basic science, environmental or clinical microbiology), veterinarians, physicians, pharmacists, chemists, but also mathematicians, computer scientists, big data analysts, and specialists in the social sciences. Their results would orient research and investment priorities to reduce environmental contamination with AMR. The importance of global transfers of goods, animals and people reminds us that AMR does not stop at borders. It is therefore fundamental to consider AMR on a global scale.

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Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. List of acronyms

AMR	Antimicrobial resistance
ARB	Antimicrobial-resistant bacteria commensal or pathogenic for humans
ARG	Antibiotic resistance genes and associated genes (*e.g. intI1*)
DM	Dry matter
LOD	Limit of detection
MIC	Minimum inhibitory concentration
MSC	Minimal selective concentration
OWP	Organic waste product
PE	Population equivalent
PNEC-P	Predicted no effect concentration for persistence
PNEC-R	Predicted no effect concentration for resistance
VBNC	viable but non-culturable
WHO	World Health Organization
WWTP	Wastewater treatment plant

Appendix B List of databases

Name	Description	Web site
PIREN	Database on the hydrographic network of the Seine river	piren-seine.fr
SOKARST	Network of karst groundwater monitors	sokarst.org
ADES	Groundwater database	ades.eaufrance.fr
NAIADES	Continental water database	naiades.eaufrance.fr

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Name	Description	Web site
QUADRIGE	Coastal water database	quadrige.eaufrance.fr
SIPIBEL	Surface water, groundwater, wastewater database	graie.org/sipibel
METEOFRANCE	French weather database	meteofrance.com

Appendix C. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.envint.2021.107047.

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