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Review

The potential contribution of aquatic wildlife to antibiotic resistance dissemination in freshwater ecosystems: A review[☆]

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ABSTRACT

Antibiotic resistance (AR) is one of the major health threats of our time. The presence of antibiotics in the environment and their continuous release from sewage treatment plants, chemical manufacturing plants and animal husbandry, agriculture and aquaculture, result in constant selection pressure on microbial organisms. This presence leads to the emergence, mobilization, horizontal gene transfer and a selection of antibiotic resistance genes, resistant bacteria and mobile genetic elements. Under these circumstances, aquatic wildlife is impacted in all compartments, including freshwater organisms with partially impermeable microbiota.

In this narrative review, recent advancements in terms of occurrence of antibiotics and antibiotic resistance genes in sewage treatment plant effluents source compared to freshwater have been examined, occurrence of antibiotic resistance in wildlife, as well as experiments on antibiotic exposure. Based on this current state of knowledge, we propose the hypothesis that freshwater aquatic wildlife may play a crucial role in the dissemination of antibiotic resistance within the environment. Specifically, we suggest that organisms with high bacterial density tissues, which are partially isolated from the external environment, such as fishes and amphibians, could potentially be reservoirs and amplifiers of antibiotic resistance in the environment, potentially favoring the increase of the abundance of antibiotic resistance genes and resistant bacteria. Potential avenues for further research (trophic transfer, innovative exposure experiment) and action (biodiversity eco-engineering) are finally proposed.

1. Introduction

Water is an essential resource for living organisms and the major transmission medium for emerging contaminants (ECs) into the environment (Vittecoq et al., 2016). Over the last 40 years, ECs such as pharmaceuticals (Bergh and Peoples, 1977; Lee and Arnold, 1983), personal care products, stimulant or illicit drugs (Parida et al., 2021) has been quantified in aquatic ecosystems and identified as one of the significant threats of our century. ECs reaching aquatic ecosystems come from different sources: domestic wastewater, hospital wastewater, industrial wastewater, livestock runoff, pit latrine or aquaculture (Gwenzi et al., 2023; Karkman et al., 2018). Wastewater are treated, but directly rejected in water bodies, but in the case of pit latrine and livestock uses, mechanism is different, spreading antibiotics into groundwater and surface water through hydrologically processes such as leaching (Gwenzi et al., 2023).

Among ECs, antibiotics are of particular concern because their

release has a double edge effect: ecotoxicity towards living organisms (Danner et al., 2019a; Orias and Perrodin, 2013; Perrodin and Orias, 2017) but also dissemination of antibiotic resistance (AR), even of last resorts such as colistin resistance genes, as a global health issue (Valiakos and Kapna, 2021). AR is a natural phenomenon occurring when microbes are under a selective pressure, chronically, or acutely (Bhullar et al., 2012), which poses a serious threat to human health when pathogens acquire AR (Koutsoumanis et al., 2021; European commission, 2017). Three main definitions of AR exist depending on the field and objectives (Martínez et al., 2015). Clinical definition is based on the reduction of efficiency of an antibiotic against a given pathogenic strain at Minimum Inhibitory Concentration (MIC) (the lowest concentration at which an antibiotic can inhibit visible growth of bacteria). Epidemiological definition is based on cut-off values for resistance which is based on the upper limit of the MIC values for a wild type population of a particular species. An operational definition, more gene-centric, is based on a pairwise comparison of parental strain with a

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mutant strain or with a strain containing a resistance gene that has been acquired by horizontal gene transfer (HGT) being resistant if the MIC of a compound is higher than its parental wild-type strain. This definition is more relevant for functional genomics and metagenomic studies of antibiotic resistance genes (ARGs) (Martínez et al., 2015), especially in the field of environmental sciences.

Antibiotic resistance (AR) is included within the broader definition of AMR. The inclusion or exclusion of Anti-microbial Resistance (AMR) criteria varies between publications and reports. For example, the CDC refers to resistance to antibiotics, including anti-fungal agents (Centers for Disease Control and Prevention, 2019). Some AMR-focused references include all forms of antimicrobials, including antibiotics, anti-fungals, and trace metal elements (Kariyawasam et al., 2022), in this review, AR will concern antibiotic resistance strictly. In 2017, the European commission estimated that 700 000 human deaths could be annually attributed worldwide to AMR (Anti-Microbial Resistance), approaching 33 000 deaths per year in the EU only (European commission, 2017). In the USA, the Centers for Disease Control and Prevention (CDC) reported 2.8 million antibiotic/anti-fungal resistant infections occur each year and more than 35 000 resulting deaths (Centers for Disease Control and Prevention, 2019). Based on a predictive statistical model developed by Antimicrobial resistance collaborators, 4.95 million deaths could be associated with bacterial AMR in 2019 and 1.27 million attributable to bacterial AMR (Murray et al., 2022). In 2050, it has been estimated that AMR could cause more deaths than cancer (CDC55(U.S.),2019a), thus reaching more than 10 million lives per year (O'Neill, 2016). In addition to the human damage, the actual European cost of AMR is estimated at 1.5 billion euros in terms of healthcare and productivity lost. By 2050, drug resistance could have an impact on the global economy estimated to 100 trillion USD cost worldwide (EFSA Panel on Biological Hazards (BIOHAZ) et al., 2021) and the World Health Organization (Robertson et al., 2021) emphasized the need for research and actions on the risks posed by AMR, in line with the objectives of the One Health approach (McEwen and Collignon, 2018).

Antibiotics are used to treat infections for human and veterinary medicine, in plant agriculture and in aquaculture, they are also massively used as growth promoters and prophylactically to compensate poor hygienic management of livestock (Chattopadhyay, 2014; Lulijwa et al., 2020; Van Boeckel et al., 2017). Antibiotics consumption never stopped to increase, reaching 40 165 million defined daily doses (DDD) estimated worldwide in 2018 with South Asia being biggest consumers (Browne et al., 2021). Between 2000 and 2015, worldwide consumption rate increased by 39 % (DDD per 1000 inhabitants) and the DDD increased by 65%. This increase has been driven by low/middle income countries. In fact, total amount of antibiotics consumed in high-income countries increased by 65% between 2000 and 2015, compared to 114 % in low income countries (Klein et al., 2018). Global consumption is expected to increase of 67 % between 2010 and 2030, from 63 151 tons to 105 596 tons (Van Boeckel et al., 2015). In Europe, the total consumption of antibiotic has been estimated at 11 382 tons in 2012 (European Centre for Disease Prevention and Control, 2017). The livestock consumption being twice higher than human one, it is estimated at 131 109 tons globally in 2013 and 200 235 tons are projected in 2030 for livestock uses (Van Boeckel et al., 2017). Between 2014 and 2018, a significant reduction of total consumption in 8 out of 30 EU/EEA countries occurred, but only one met WHO's target consumption (Robertson et al., 2021). Top 15 fish producing countries use 67 different antibiotics, and banned antibiotics are still found in water (Lulijwa et al., 2020).

When consumed by organisms, antibiotics are metabolized and excreted in urine and/or feces, depending on antibiotics and organisms. As in the case of humans, feces and urine are generally collected and treated in wastewater treatment plants (WWTPs) where different processes are applied to remove organic matter, nitrogen, phosphorus. This is generally not the case for livestock farming (cattle, pigs, sheep, lamb, chickens), where manure is usually used as fertilizer on soils, and

aquaculture where antibiotics residues enter directly water bodies (Vittecoq et al., 2016). The rates of antibiotics excreted depend on antibiotic class and organism. For example, after human consumption of clindamycin and erythromycin, 10 % of parent compounds are excreted in urine and feces whereas 87.7 % of ciprofloxacin are excreted (Jemba, 2006). In fishes, from 30 to 90 % of the parent compounds or metabolites can be excreted in feces or urines (Lulijwa et al., 2020). Environmental compartments (Soil, Hydrosphere, Sediment, Biota) are interconnected as a result of 'ecological connectivity'. The release and transfer of antibiotics poses a risk to humans, especially through water media and human/animal intermicrobiome communication (Baquero et al., 2019; Larsson et al., 2018).

In this narrative review, a comparative study of antibiotic and antibiotic resistance genes (ARGs) concentrations in freshwater and wastewater treatment plant effluents is presented, providing a new point of view in the up-to-date data. Then current literature about the interactions between AR and freshwater aquatic organisms is explored. Indeed, recent studies suggested that aquatic organisms' microbiomes could be vectors and/or reservoirs of AR, and even amplifiers of AR in the environment. This review aims to bring out an hypothesis on the implication of freshwater aquatic organisms in the AR. This hypothesis is built on the basis of monitoring data in wildlife, and lab exposure scenarios confirming/infirming hypothesis.

2. Global occurrence of antibiotics and antibiotic resistance genes in freshwater environment

Antibiotics represent approximately half of pharmaceuticals and personal care products contamination in aquatic environment (Yang et al., 2017). WWTP is one of the major source of antibiotics and ARGs in the environment and WWTP release effluents directly into water bodies (Zainab et al., 2020). A data collection of antibiotics and ARGs concentrations in freshwater river and WWTP have been done, following the methods detailed in supplementary text. The concentrations of antibiotics reported in treated water released by WWTP is influenced by processes and countries, and can reach up to 30 $\mu\text{g}\cdot\text{L}^{-1}$ in WWTP discharge and 484 $\mu\text{g}\cdot\text{L}^{-1}$ in freshwater (Fig. 1). While no significant difference in antibiotics concentrations were found in WWTP effluents across continents except for Africa, antibiotics concentrations in river freshwater were more contrasted, with highest and lowest median concentrations found respectively in Africa and Europe. Freshwater data could be explained by wastewater management dissimilarities because continents. In Africa, less WWTP systems are used and pit latrines are more common (Gwenzi et al., 2023). In addition, most studies were conducted close to cities, associated with higher economic development and thereby introducing a bias. A detailed overview of the antibiotics groups concentrations reported in literature is provided in Fig. 2 and a table is available in supplementary data (Table S2). Among the different groups represented, only concentrations higher than the limits of quantification and the samples clearly identified were considered, in line with previous studies (Danner et al., 2019b; Wang et al., 2020a). This could lead to overestimating antibiotic contamination in the environment and should be interpreted cautiously, in relation with detection frequencies. For example, in a study conducted by Wilkison et al. (2022), Sulfamethoxazole was at the highest detection frequencies (40%) while tetracycline was one of the least (2%), resulting in variable reliability on concentration data (Wilkinson et al., 2022). In literature concentrations from influent to effluent decrease, but antibiotics remain in effluent at low doses and are released (Michael et al., 2013; Pazda et al., 2019). Hypothetically, antibiotic concentration would decrease in the environment compared to treated wastewater due to dilution effect and/or degradation, and/or sorption to the sediments and a ten-fold factor decrease is expected (Larsson and Flach, 2022). Data collected for this review show more mixed results, with only a two-fold factor decrease in freshwater antibiotic concentration globally. These data must be interpreted with awareness of their limitations, including the variability of

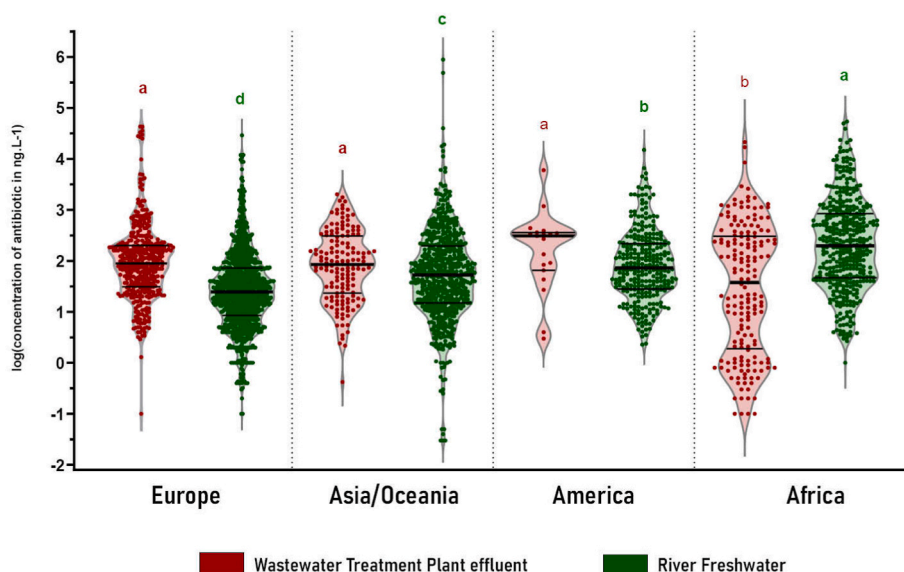


Fig. 1. Log concentration (in ng.L^{-1}) of antibiotic concentrations detected and measured in river water (green, $n = 2330$) and in treated water released by wastewaters (red, $n = 909$) per continents. Black line represents median of data. ANOVA test has been performed. Different letters means significant differences (p . value < 0.05). Antibiotic concentrations have been collected from 83 publications. Data collection method, detailed data table, and references used for data are available in Supplementary data. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

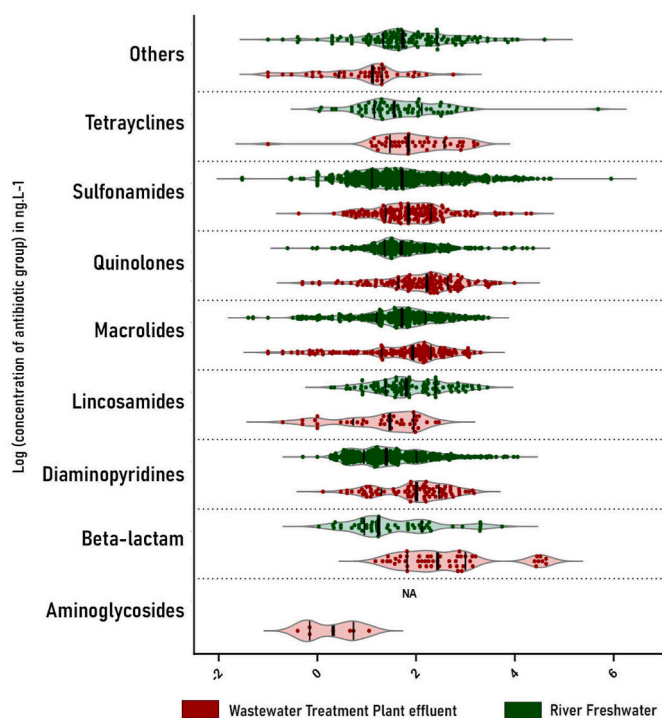


Fig. 2. Detailed log concentration (in ng.L^{-1}) of major antibiotic groups detected and measured in river water (green) and in treated water released by wastewaters (red). Middle black lines represent median of data. Antibiotic concentrations have been collected from 83 publications. Data collection method, detailed data table, and references used for data are available in Supplementary data. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

measurement methods between laboratories (Harris and Lucy, 2020), the imperfect spatial and temporal representativeness and publication bias (such as unpublished values below analytical limits). Despite these limitations, these data suggest chronic exposure to low doses of antibiotics for aquatic wildlife and microbes, which are therefore subjected to

constant selective pressure.

In addition to antibiotics release, WWTPs are also a source of AR. Indeed, WWTP processes seem to be favorable places for Horizontal Gene Transfer (HGT) and the selection of antibiotic resistant bacteria (ARB) because of high microbial density, high nutrient content and presence of ECs and other xenobiotics (Qiao et al., 2018). After treatment, a certain quantity of ARGs and/or ARB and/or Mobile Genetic Elements (MGEs) remains in water (Laht et al., 2014; Lekunberri et al., 2017; Maganha de Almeida Kumlien et al., 2021; Munir et al., 2011; Rizzo et al., 2013). Fig. 3 summarizes concentrations of ARGs groups in freshwater ecosystems and in effluent wastewater. WWTPs data are derived from different secondary or tertiary treatment processes. These tertiary treatments are constructed wetlands, chlorination, biological filter or UV, some of these disinfection system (such as chlorination and UV) can significantly reduce ARGs abundance in WWTP effluent (Fig. S1) (Barancheshme and Munir, 2018). Taken as a whole, this synthesis emphasizes that ARGs concentrations coming from WWTPs are 1.62 times higher than in the environment, suggesting a basal concentration of ARGs and/or other sources than WWTPs. As it was established that antibiotics concentration in WWTP effluent are positively correlated with ARGs occurrence in a recent worldwide review (Wang et al., 2020a), data into freshwater environment are more mixed and depending on many parameters (Liu et al., 2021; McKinney et al., 2010; Wu et al., 2010; G. G. Zhang et al., 2020) still being a gap in antibiotic resistance knowledge.

Antibiotics, other ECs, and ARGs constitute an exposome to which organisms are submitted during their lifetime (Hiller et al., 2019; Luo et al., 2014; Vineis et al., 2020). Mixtures of non-antibiotic pharmaceuticals can, for example, significantly induce HGT of ARGs (Wang et al., 2020b). Xenobiotics can have unpredictable synergistic effects on microbial communities (Zhang et al., 2022). Beyond antibiotics, the entire exposome contributes to selective pressure on micro-organisms and should be considered.

3. Aquatic organisms as reservoirs and vectors of AR

In this review, the concepts of **reservoir** and **vector** are contemplated with respect to AR dissemination (Fig. 4). The definition of a **reservoir** varies according to the publications; the one that will be used

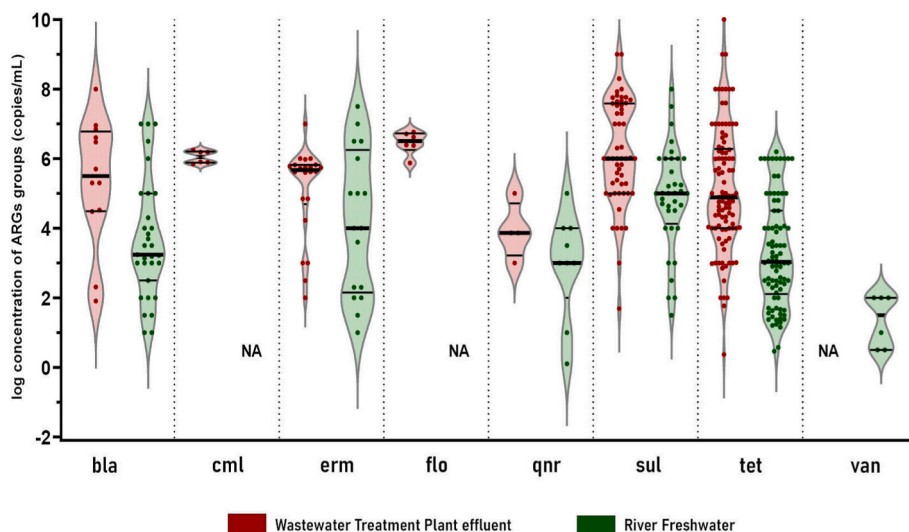


Fig. 3. Log concentration (in copies.mL⁻¹) of antibiotic resistance genes concentrations detected and measured in river water (green, n = 185) and in treated water released by wastewaters (red, n = 206). Middle black lines represent median of data. Antibiotic concentrations have been collected from 15 publications. Data collection method, detailed data table, and references used for data are available in Supplementary data. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

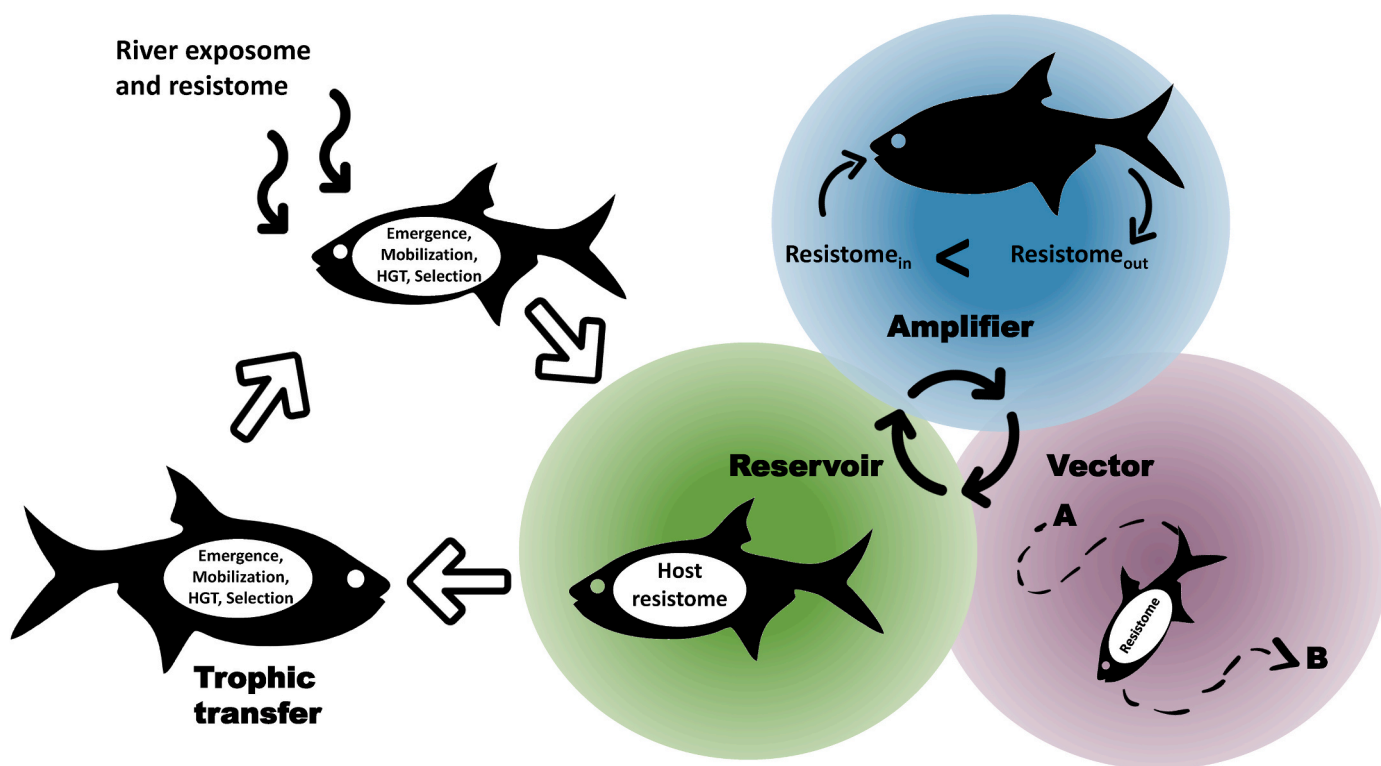


Fig. 4. Definition scheme of relationship and dynamics between vectors, disseminators, reservoirs, amplifiers and trophic transfer in freshwater ecosystems.

in this review is based on the arbovirus reservoir definition (Kuno et al., 2017). An antibiotic resistance **reservoir** is an environment or entity where antibiotic resistance genes can be stored in different forms, maintained and eventually available to other organisms. A reservoir has its defined resistome. A **vector** is a carrier (an organism, or can be physical such as microplastics) that moves AR from point A to point B (it can be intra or extra organisms) (Wilson et al., 2017) and will have the ability to contribute to disseminating this AR (Arnold et al., 2016). The ‘**emergence**’ of resistance is defined as the appearance of resistance in microorganisms and the ‘**mobilization**’ will be defined as appearance of

resistance associated with MGEs (Bengtsson-Palme et al., 2018).

AR can result from mutations or HGT, including transformations, transductions, or conjugation between bacteria, which are widely described in the review by Marti and collaborators (Davison, 1999; Marti et al., 2014). AR can be mobilize through (MGEs) such as plasmids, insertion sequences, insertion sequence common region (ISCR) elements, transposons, integrons, genomic islands, integrating conjugative elements or bacteriophages (Lood et al., 2017). These mechanisms can confer genes to bacteria’s genotype, providing resistance to antibiotics. Resistance mechanisms may counteract antibiotics mode of

action. These acquired resistance mechanisms include reducing membrane permeability, “immunity” genes and bypass (alternative metabolic pathway), antibiotic target modifications through mutation, or enzymatic inactivation of the medicine (Blair et al., 2015; Pazda et al., 2019).

In aquatic ecosystems, AR can be found in multiple biological compartments such as biofilms, or organisms’ microbiomes such as gut microbiota. Lehmann and collaborators conducted 16S rRNA gene sequencing to explore microbial diversity and quantitative PCR to assess class I integrons prevalence in a biofilm exposed to wastewater effluent. Diversity did not significantly change after 9 days of exposure to WWTP effluent, but the prevalence of class I integrons significantly increased compared to the control condition (Lehmann et al., 2016). In biofilm, HGT is favored by the high abundance, proximity and diversity of microorganisms (Balcázar et al., 2015a). In this case, biofilms should be reservoirs for AR dissemination (Abe et al., 2020a). Indeed, biofilms maintain the selective pressure (from antibiotics or other ECs) longer than in the water column because of their specific characteristics (charge, density, extracellular composition and porosity) (Taylor et al., 2011; Valdés et al., 2021). These conditions create a favorable environment for pathogens to acquire resistance, such as *Pseudomonas aeruginosa* and *Aeromonas hydrophila* with virulent factor in epilithic biofilm (Mao et al., 2021). Biofilms are ubiquitous in freshwater environments and continuously exposed to antibiotics in freshwater, and are thus considered as reservoirs for AR (Abe et al., 2020b; Balcázar et al., 2015b).

When present in biofilm or in the water column, ARGs can be transmitted to the gut microbiota of aquatic organisms through, respectively, biofilm grazing or water feeding (Eckert et al., 2016). Microbiota of animals can also be considered as another reservoir of AR, especially the gut microbiota of superior organisms such as vertebrates or invertebrates. Many publications report the presence of ARGs or ARB in the microbiota of wildlife, as recently reviewed (Abbassi et al., 2022). However, most publications focused on terrestrial animals. AR in the gut microbiota of birds, especially those feeding near aquatic environments such as waterfowl birds or seagulls, has been extensively explored, attesting to their contribution to the dissemination of AR in the environment (Carroll et al., 2015; Dec et al., 2020; Martín-Maldonado et al., 2020; Merkeviciene et al., 2017; Oravcova et al., 2013). Some of them having a significant impact locally on bacterial community just from their excretion (Klimaszuk et al., 2015). Stedt and collaborators found ESBL-producing bacteria in 28.7 % of gull’s fecal samples out of 3158, with 74.8% of Spanish cases (Stedt et al., 2015). In the same study, 13 variants of CTM-X genes have been found with *bla*_{CTX-M-1} and *bla*_{CTX-M-14} being the most abundant ARGs. While AR can occur naturally, it is crucial to compare these data between wild and captive animals, or at least animals with limited contact with humans. A study conducted by Lundbäck and collaborators showed that 3.2 % of fecal samples from wild *Eudypptula minor* had class I integron prevalence compared to 44.7 % in captive *Eudypptula minor* (Lundbäck et al., 2021). In another study, birds sampled at a WWTP carried the greatest resistance gene burden, suggesting that human wastes contribute to the spread of ARs in the environment (Marcelino et al., 2019).

Taken as a whole, the body of literature related to freshwater wildlife remains very scarce compared to terrestrial counterparts or marine aquaculture studies. However, several studies reported the occurrence of ARGs and MGEs in strictly aquatic wildlife, such as fish, with a total of $3.61 \cdot 10^{-1} - 9.86 \cdot 10^{-1}$ /16S rRNA copies (Jia et al., 2021; Xue et al., 2021a; Zhou et al., 2021) in gut microbiota, and $1.76 \cdot 10^{-1}$ /16S rRNA copies in skin microbiota (Hong et al., 2018). In this review, the focus is mainly on aquatic vertebrates with high relative gut length, implying a digestive system more isolated from the aquatic environment. This is the case for fish, amphibians and certain other organisms, but not for mussels or shrimps, for example (Dall, 1990; Stevens and Hume, 1995). An interesting study also explored the resistome in lower vertebrates using a metatranscriptomic approach (Chen et al., 2020). Chen and

collaborators found differences in ARGs transcripts between fish, amphibians and reptiles sampled in marine, freshwater, amphibious and terrestrial ecosystems. Marine fishes were carrying more ARGs (293.4 RPKM, reads per kilobase per million mapped reads, a method normalizing sequencing depth and length of genes) compared to freshwater fishes (79.1 RPKM). Fishes were carrying a greater percentage of ARGs encoded in MGEs than other animals, suggesting a higher frequency of HGT in aquatic population, including marine and freshwater, compared to terrestrial wildlife (Chen et al., 2020). In a study by Hong and collaborators, skin microbiota of 17 fish species (freshwater and marine) has been analyzed, revealing high detection frequencies of *floR*, *sul2*, *sul1*, *strB*, *aadA* and *tetS* genes. The abundance of ARGs was higher in freshwater fishes than in marine ones, such as *tetS* being 2.66 times more abundant in freshwater (0.08 ARGs/16S rDNA copies) than in marine fishes (0.03 ARGs/16S rDNA copies). (Hong et al., 2018). Two studies compared the guts of fishes (*Hemiculter leucisculus* (Xue et al., 2021a) and *Poecilia reticulata* (Jia et al., 2021)) from polluted area (water receiving domestic and industrial sewage and garbage, presence of Pb, Cu, Cr, past contamination) and ‘non polluted’ areas. These studies reported a significantly higher abundance of ARGs conferring resistance to tetracycline and quinolones, probably as a result of polluted area (Jia et al., 2021; Xue et al., 2021a). Another study reported *bla*_{TEM} genes in a low anthropized river (water supply and irrigation), and *sul1* and *qnrS* genes in highly anthropized river (small river, irrigation and recreational activities) (Marti et al., 2018). As Di Cesare and Manaia reported in their respective reviews, a positive correlation between water pollution and ARGs occurrence in aquatic environment exist, but the potential positive correlation with AR in gut microbiota still need to be establish (Di Cesare et al., 2016; Manaia et al., 2016). It is essential to acknowledge that literature is scarce regarding relationship between ARGs content in aquatic organisms’ gut and the antibiotics concentrations they are exposed to in freshwater river. Further research and effort are needed to include in different compartment measurement such Xue and collaborators did by measuring antibiotics and ARGs into gut and river to establish correlation and factor analysis (Xue et al., 2021b).

4. Organisms as amplifiers of AR?

An **amplifier** can be defined as a ‘genetic exchange reactor’ (Baquero et al., 2019; Kuno et al., 2017) meaning it not only hosts AR but promotes the emergence of resistance, the mobilization of resistance genes, the horizontal gene transfer and/or the selection of ARB, resulting in the increase of abundance and diversity of ARB, or ARGs, or MGEs (Kuno et al., 2017) (Fig. 4). High bacteria density matrices, such as the mammalian gut, or fish gut act as closed ecosystems with physical proximity where 10^5 to 10^{10} microorganisms.mL⁻¹ interact, which can result in enhanced HGT (Shterzer and Mizrahi, 2015; Taylor et al., 2011; Wang et al., 2018). Therefore, in addition to being a reservoir and a vector disseminating AR, organisms could globally act as **amplifiers** of AR in the freshwater environment (Arnold et al., 2016).

Recently, Loftie-Eaton et al. conducted experiments for demonstrating that HGT can occur in fish gut microbiota (2021). A donor bacterium carrying pB10gfp plasmid (multidrug resistance broad-host-range plasmid) was introduced into the zebrafish gut. The authors investigated the plasmid transfer to other bacteria within the zebrafish gut microbiota in controlled lab conditions (*in vitro*) and directly within the zebrafish (*in vivo*). *In vivo* experiments demonstrated that the plasmid was transferred to certain bacteria including *Achromobacter* (Loftie-Eaton et al., 2021). In another experiment, zebrafish were fed for 90 days (with sterilized brine shrimp particles) and *E. coli* K12 harboring RP4 plasmid with ampicillin, kanamycin, tetracycline and chloramphenicol resistance genes during a 120-day experiment. The prevalence of *traG* gene (required for RP4 conjugation) increased in feces compared to the control, suggesting that HGT occurred in the microbiota. The prevalence of *traG* and *cat* (chloramphenicol resistance gene) increased

in gut microbiota during feeding, and dropped when feeding stopped, indicating that bacteria were receiving plasmids from the added strains. The *trbBp* and *trfAp* transcripts (permitting conjugative transfer and spread of RP4) increased with feeding from *E. coli* strain, suggesting that exogenous ARB could proliferate and transfer ARGs in the microbiota through HGT (Fu et al., 2017). These two studies support the facilitation of HGT in gut microbiota.

Experiments conducted under the selective pressure of antibiotics and showing an increase in ARGs/ARB/MGEs in tissues (gut or skin) and water tanks after exposure are summarized in Table 1. For example, after 10 days of exposure to florfenicol, ARGs diversity and abundance significantly increased by a 3-fold factor in the gut of the freshwater fish *Piaractus mesopotamicus*; 80 ARGs were found with *mexD*, *mexQ*, *pmrE*, *macB*, *macA* and *crp* being the most abundant ones at 7 days exposure and *floR* (a resistant gene conferring resistance to florfenicol) was detected (Sáenz et al., 2019). Similarly, concentrations of *sul1*, *sul2*, *floR*, *tetA*, *tetG*, *clmA*, *qnrA* genes were significantly higher in the gut microbiota of the freshwater shrimp *Macrobrachium nipponense* after 8 weeks of exposure to sulfamethoxazole (SMX) (2.50 g/kg), or oxytetracycline (OTC) (2.00 g/kg) (Sun et al., 2020) or *sul1* in *Mytilus edulis* 96h exposed to SMX (100 µg.L) (Serra-Compte et al., 2019). Zhou and collaborators exposed tilapia fish for 100 days to duck manure containing ARGs (*tetG*, *tetM*, *tetO*, *tetX*, *sul1*, *sul2*, *tetA*, *tetC*, *EBC*, *CIT*, *MOX*, *DHA*, *FOX*, *TEM*) and CFX (0.1–3 mg.L⁻¹), and/or arsenic. Results showed that exposure to duck manure combined with CFX increased both diversity and the relative abundance of ARGs in fish intestines up to 7.25 times compared to the control (Zhou et al., 2020a). In another study, *Oreochromis niloticus* were fed for 8 days with 100 mg/kg/day of OTC followed by a 14-day withdrawal period. After exposure, an enrichment with the pathogen *Plesiomonas* and a decline in microbial diversity were observed in gut microbiota. The *tetA* and *int1* genes were present at day 0 and increased after antibiotic treatment, whereas *tetM* was more frequent in control fish than antibiotic-treated fish (Payne et al., 2021). In a recent study, adult *Xenopus tropicalis* were exposed to roxithromycin (ROX) and OTC alone (100 µg.L) or mixed during 10 and 20 days. The relative abundance of ARGs increased in single antibiotic exposure, but decreased in the mixed antibiotic test, suggesting that the combination was inhibiting the host of ARGs and *int1* in *Xenopus* gut (Lin et al., 2022). Consistent results has been found in Kayani and collaborators' study, where zebrafish were exposed for 120 days to low doses (1–5 µg.L⁻¹) of sulfamethoxazole (SMX) and OTC. Taxonomic composition was significantly affected with an increase in pathogens such as *Flavobacterium* (sp. 52, *johnsoniae*, sp.F1) in both conditions. But more noticeable is the increase of the relative abundance of ARGs. In particular, the *tet* and *sul* resistance genes were significantly higher in feces sample and showed a linear correlation with antibiotic concentrations (Kayani et al., 2021). Zebrafish were exposed for 200 days to OTC (0.1 and 10 µg mL⁻¹) and showed a higher relative abundance of *tetA* gene after 5 days exposure at 10 µg mL⁻¹ in the gut of zebrafish (Almeida et al., 2021). Another study showed, via bacteria culture and disk diffusion method, an acquisition of drug resistance in gut (>90%) and skin (>70%) microbiota of *Gambusia affinis* exposed to rifampicin at 25 µg mL⁻¹ for 7 days (Carlson et al., 2017).

In the same studies described above, findings were consistent for MGEs: the *floR* gene was detected in plasmid sequences (Sáenz et al., 2019). Moreover, phages' abundance, integrases, transposases and transposons also increased after antibiotic exposure. Additionally, a shift in the fish gut microbiome was observed, with a higher abundance of pathogens such as *Salmonella*, *Plesiomonas* and *Citrobacter* and a lower abundance of *Bacteroidaceae* (Sáenz et al., 2019). In Sun and collaborators' study, after exposure to SMX and OTC, *int1* gene was significantly correlated with other ARGs in prawn gut (Sun et al., 2020). The *int1* gene has also been reported in water, accompanying *sul1* genes (He et al., 2017). Integrons facilitate the acquisition and dissemination of antibiotic resistance genes (ARGs) across various environmental matrices through the horizontal gene transfer mechanisms of

conjugative plasmids and transposons, thereby significantly contributing to the proliferation of AR (Gillings, 2014; S. S. Zhang et al., 2020). In a study by Lin and collaborators, the exposure to mixed macrolides (ROX, OTC) enriched amphibians' gut with genes conferring macrolide resistance, but also many gene targets associated with cassettes and genetic mobility (Lin et al., 2022).

Despite ARGs or MGEs being below the limit of detection or quantification in different compartments, this review reports that ARGs found in gut microbiotas range between 4 and 9.4 log copies.mL⁻¹ which is generally higher than the median of ARGs concentration in rivers (3.5 log copies.mL⁻¹) and WWTP effluents (5.7 log copies.mL⁻¹) (Fig. 1). In some other studies, exposure water or organisms excretion have been collected and analyzed, showing an increase of ARGs and ARB (Fu et al., 2017; He et al., 2017; Zeng et al., 2019). This suggests that organisms not only host but could also release a resistome that is more diverse and abundant than in their living and feeding medium. In a plasmid-mediated ARGs transfer model in zebra fish (an experiment already described above), feces excreted by fish were found to be an important source of ARGs in aquatic environment, reaching 1 × 10¹⁰ cfu/(d.fish) excretion per day with 10⁸ cfu donor bacteria (Fu et al., 2017). In another study, *Carassius carassius* were exposed for 180 days to tetracycline (TET), cefotaxime (CFX), sulfanilamide (SMD), Cu and Zn, alone or mixed with increasing concentrations along time (0.1–3 mg.L⁻¹ TEC – CFX and 0.5–40 mg.L⁻¹ SMD). In single-substance antibiotic exposure, a significant increase of ARGs abundance in water was reported, with *tetC*, *tetA*, *tetG*, *tetE*, *tetB*, *sul1*, *sul2*, *sul3*, *bla_{MOX}*, *bla_{FOX}* and *bla_{DHA}* being detected. A synergic effect of TEC-CFX-SMD exposure was observed. Indeed, higher ARGs abundance and diversity were detected in water tanks with the 3 antibiotics compared to other conditions (He et al., 2017). In another study, water tanks hosting *Ictalurus punctatus* individuals were analyzed at days 0/10/25 treated with florfenicol at 10 mg kg during 10 days, and 15 days more without medication. The authors found a significant increase in phenicol-resistant genes after 10 and 25 days in water compared to day 0, and a significant correlation among ARGs that confer resistance to multiple drug classes (phenicol, aminoglycoside, penam, fluoroquinolone, tetracycline, glycolcycline, carbapenem, diaminopyrimidines, macrolides and rifamycin) indicating HGT of *floR* genes in these bacterial species (Zeng et al., 2019).

The remaining question is how the AR-inducing elements (ARGs, ARB, MGEs) are disseminated into the environment. What is the lifespan of transmissible resistome in different compartments? If transmissible, where is it occurring mostly? Which freshwater organisms contribute mostly to amplifying AR? From a public health point of view, what risks are humans exposed to? In an ecosystem devoid of the selective pressure of antibiotics, ARB would have no competitive advantage in maintaining a resistome. However, the data shows a constant low concentration of antibiotics in ecosystems (Figs. 1–2), which may lead to a competitive advantage of these ARB and their persistence in aquatic environments. At present, the risk of transmission from the environment to the human is considered low, compared with human-animal, animal-animal and human-human transmission. However, the exposome is present in bathing areas, through food intake (Zhou et al., 2020b), through irrigated vegetables (Uyttendaele et al., 2015), and probably in other unsuspected places. Mathematical models are not yet precise enough to understand environmental transmissions (Larsson and Flach, 2022). The consequences of this type of transfer can be multiple and unpredictable. As a result, the mechanisms of these transmissions need to be investigated (Larsson and Flach, 2022).

5. Trophic transfer of AR

Potential trophic transfer of AR through the food chain by ingestion of ARGs, MGEs or ARB also needs to be explored. Small organisms such as primary consumers (Eckert et al., 2016; Olanrewaju et al., 2019) can have a role in HGT occurrence in the aquatic environment. These organisms carrying AR elements will be ingested by higher taxa, and pass

Table 1

Experimental conditions and major AR changes in microbiota following exposure of aquatic organisms to antibiotics and antibiotic resistance.

Exposure conditions (days, concentration, substance)	Species exposed	Compartment analyzed	Change in microbiota and/or ARGs compared to control	Absolute concentration in copies.mL ⁻¹ or g ⁻¹	Relative concentration copies/16S	ARs detected	Reference
10 days exposure, 30 days depuration - florfenicol - 9.7 mg kg. day ⁻¹	<i>Piaractus mesopotamicus</i> (Pacu)	gut	↗ diversity of ARGs, 80 ARGs found: <i>mexD</i> , <i>mexQ</i> , <i>pmrE</i> , <i>macB</i> , <i>macA</i> and <i>crp</i> most abundant at 4d and 7d exposure and <i>floR</i> , compared to 16 at day 0 ↗ MGEs day 7 (transposases, phages integrases, resolvases, transposons) but decreased until day 26 comparable to day 0	–	0.60%	shift in microbiome to <i>Salmonella</i> , <i>Plesiomonas</i> , and <i>Citrobacter</i>	Sáenz et al., 2019
8 weeks - sulfamethoxazole - 2.50 g kg ⁻¹ or oxytetracycline - 2.00 g kg ⁻¹	<i>Macrobrachium nipponense</i> (Japanese shrimp)	gut	↗ <i>sul1</i> , <i>sul2</i> , <i>floR</i> , <i>tetA</i> , <i>tetG</i> , <i>clmA</i> , <i>qnrA</i> (both conditions)	1.10 ⁴ - 1.10 ⁸	–	–	Sun et al. (2020)
96h - sulfamethoxazole - 100 µg.L ⁻¹	<i>Mytilus edulis</i> (Common mold)	gut	↗ <i>sul1</i>	3.5.10 ⁶	7.08.10 ⁻³	–	Serra-Compte et al. (2019)
180 days - tetracycline - cefotaxime - 0.1–3 mg. L ⁻¹ - sulfanilamide - 0.5–40 mg.L ⁻¹ - Cu - Zn - mixed or separately, increasing over time	<i>Carassius carassius</i> (Crucian carp)	water tank	↗ <i>tetC</i> , <i>tetA</i> , <i>tetG</i> , <i>tetE</i> , <i>tetB</i> , <i>sul1</i> , <i>sul2</i> , <i>sul3</i> , <i>blaMOX</i> , <i>blaFOX</i> and <i>blaDHA</i> (single substances exposure) ↗ relative abundance of ARGs and diversity (mixed conditions, compared to single conditions)	–	2.10 ⁻³ - 1.7.10 ⁻¹	–	He et al. (2017)
10 days exposure, 15 depuration - florfenicol - 10 mg kg ⁻¹	<i>Ictalurus punctatus</i> (Channel cat-fish)	water tank	↗ phenicol-resistant genes (10d and 25d compared to d0) and significant correlation amongs ARGs that confer resistance to multiple drug classes	–	–	–	Zeng et al. (2019)
100 days - duck manure - 0.1–3 mg.L ⁻¹ - cefotaxime - and/or Arsenic	<i>Oreochromis niloticus</i> (Nile Tilapia)	gut	↗ diversity and relative abundance of ARGs (cefotaxime and/or arsenic conditions)	–	gut: 1.7.10 ⁻³ - 1.8.10 ⁻²	–	Zhou et al. (2020)
8 days–14 days depuration - oxytetracycline - 100 mg kg.day ⁻¹	<i>Oreochromis niloticus</i> (Nile Tilapia)	gut	↘ gut microbial diversity ↗ <i>tetA</i> and <i>intI1</i>	–	–	↗ <i>Plesiomonas</i>	Payne et al. (2021)
10 and 20 days - roxithromycin and oxytetracycline separately or mixed - 100 µg.L ⁻¹	<i>Xenopus tropicalis</i> (African clawed frog)	gut	↗ <i>ermF</i> , <i>tetB</i> , <i>tetS</i> , <i>tetE</i> , <i>tetO</i> , <i>tetX</i> (after 10d - ROX) <i>tetB</i> , <i>ermF</i> , <i>tetS</i> (after 20d - ROX) ↗ <i>tetA</i> , <i>tetB</i> , <i>tetE</i> , <i>tetS</i> , <i>ermF</i> , <i>mefA</i> , <i>ereB</i> , <i>mphB</i> (after 10 and 20d - OTC) ↘ ARGs global abundance and relative abundance of <i>intI1</i> , <i>intI2</i> , <i>intI3</i> in mixed exposure	–	1.15.10 ⁻⁴ - 1.18.10 ⁻²	–	Lin et al. (2022)
120 dpf - sulfamethoxazole - oxytetracycline - 1–5 µg.L ⁻¹	<i>Danio rerio</i> (Zebra fish)	stool	↗ relative abundance ARGs (<i>tet</i> , <i>sul</i>)	–	–	↗ <i>Flavobacterium</i> (sp. 52, <i>johnsoniae</i> , sp.F1) in both conditions	Kayani et al. (2021)
200 days - oxytetracycline - 0.1 and 10 µg mL ⁻¹	<i>Danio rerio</i> (Zebra fish)	skin, gut and water	↗ relative abundance ARGs (<i>tetA</i>) (after 5d - 10 µg mL - gut)	water: 3.31.10 ¹	gut: 2.5.10 ⁻¹	–	Almeida et al. (2021)
7 days - rifampicin - 25 µg mL ⁻¹	<i>Gambusia affinis</i> (Mosquitofish)	skin and gut	↗ acquisition of drug resistance in gut (>90%) and skin (>70%) microbiota	–	–	–	Carlson et al. (2017)

(continued on next page)

Table 1 (continued)

Exposure conditions (days, concentration, substance)	Species exposed	Compartment analyzed	Change in microbiota and/or ARGs compared to control	Absolute concentration in copies.mL ⁻¹ or g ⁻¹	Relative concentration copies/16S	ARBs detected	Reference
7 days - chironomus carrying E.coli with RK2 apramycin resistant gene	<i>Cyprinus carpio</i> (Eurasian carp), <i>Ctenopharyngodon idellus</i> (Grass carp), and <i>Mylopharyngodon piceus</i> (Black carp)	gut	↗ resistance gene <i>aac</i> (3)-IV	–	3.1.10 ⁻³ - 2.8.10 ⁻³ - 3.3.10 ⁻⁴	–	Ding et al. (2021)

through their own gut microbiome, thereby increasing the probability of HGT occurrence with their own gut microbiota, and potentially enhancing dissemination and release into the environment. Data from freshwater experiments are very scarce and very few studies were conducted. In this experiment, Chironomus larvae (*Protopharyngodon idellus*) has been inoculated with *E. coli* RK2 carrying the plasmidic apramycin resistance gene (*aac*(3)-IV gene), and fishes (*Cyprinus carpio*, *Ctenopharyngodon idellus*, and *Mylopharyngodon piceus*) were fed with the previous larvae. An enrichment of ARGs was evidenced in fish gut microbiota (Ding et al., 2021). As another example, ARGs in the gut microbiota of predatory mite *Hypoaspis aculeifer* were more abundant (especially *bla*_{SHV}, *fosX* and *aph6ia*) when mites were feeding on a primary consumer (collembolan *Folsomia candida*) that grew up in manure-contaminated soil, thus suggesting trophic transfer of ARGs and MGEs (Zhu et al., 2019). Studies of this type are very scarce and need to be repeated and extended in the aquatic environment to understand the fate of AR within a trophic chain. Undoubtedly, the issue of trophic transfer also concerns human food consumption, through animal-based food consumption. A significant link exists between human activities on natural habitats and the carriage of ARB by wildlife in terrestrial ecosystems, especially in anthropophilic and carnivorous species due to trophic transfer, the same strong link should be explored in the freshwater aquatic environment (Vittecoq et al., 2016). Further research is required to understand the risk for human health through the food chain.

6. Conclusion and perspectives

Current data exposed in this review are supporting the hypothesis that in addition to being reservoirs and/or a vector, aquatic organism microbiota could act as potential amplifiers of AR, especially organisms' gut. But literature remains scarce, and this hypothesis must be tested through innovative experiments with multiple stress exposure, combined assessment methods of AR, and multispecies experiments such as mesocosms experiment, offering a higher biological complexity (Stewart et al., 2013). Selective pressure such as antibiotics can increase AR through ARGs, ARB and MGEs. Although ARG diversity in gut, for example, decreased and returned to a 'basal' level at the end of antibiotic exposure in several experiments (Payne et al., 2021; Sáenz et al., 2019; Yassour et al., 2016), antibiotic contamination in the aquatic environment can be considered as chronic, and act as a constant selective pressure to (i) microorganisms present in the physical compartments (water, sediments) and (ii) organisms' microbiota.

The combined presence of other substances such as hormones, anti-inflammatories and antiviral drugs, heavy metals, microplastics, pesticides and more (Luo et al., 2014), needs to be explored to understand if a co-selective pressure for the potential amplification of AR and dissemination of ARB/ARGs/MGEs in the freshwater aquatic environment is occurring (Dolejska and Literak, 2019; Zhou et al., 2020a) as it has been demonstrated for heavy-metals (Seiler and Berendonk, 2012). However, wildlife vertebrates microbiota could be used as a new monitoring tool (Evariste et al., 2019). Experiments must consider all the steps of potential environment-human transmission, from the emergence of the AR, to potential transmission to organisms and their microbiota, to the

excretion of AR by organisms, including their dissemination into the environment afterwards. The monitoring of life span and dissemination of ARB/ARGs/MGEs is necessary to confirm the amplifier hypothesis or, on the contrary, demonstrate a mitigation effect. To fully understand AR fate and evolution, it is important to follow ecological traits favoring AR carriage by organisms and their characteristics (such as diet, life span, habitats, migration, etc.). This would help to predict their role in AR dissemination (Vittecoq et al., 2016). This systemic prism should integrate trophic transfer, direct/indirect interactions between freshwater aquatic species, impact of waterfowl birds in amplification cycle, link with functional traits, and the role of biodiversity. Little is known about the implication of biodiversity interaction with AR, especially in the era of biodiversity erosion (Butchart et al., 2010). A high biodiversity would mean a high diversity of coexisting reservoirs and a high number of interactions between these reservoirs, including trophic interactions. Research should be conducted to determine if a higher biodiversity would favor the dissemination of AR in aquatic ecosystems or if, on the contrary, it would mitigate dissemination. The latter could potentially result in an ecosystemic service provided by the aquatic ecosystems, calling for new approach to monitor and tackle AR. Finally, it is crucial to have an approach based on One Health to face AR problematic. Bridges need to be built between different fields to explore new areas of research. From a global point of view, political action to regulate the use of antibiotics, reducing global consumption in veterinary field and policy to evolve towards a plant-based diets (Van Boeckel et al., 2017) are essential and must be coupled with scientific knowledge presented in this review (Sadhukhan et al., 2020; Vittecoq et al., 2016). Research and the introduction of innovative and effective wastewater treatment processes must be pursued and promoted, given the priority in preventing the spread of antibiotics and AR (DIH, 2016; Ioannou-Ttofa et al., 2019; Lucas et al., 2016; Manaia, 2017; Mir-Tutusaus et al., 2017).

CRediT authorship contribution statement

Alexandre J. Thibodeau: Writing – review & editing, Writing – original draft, Methodology, Conceptualization, Data curation, Formal analysis, Investigation, Visualization. **Maialen Barret:** Validation, Supervision, Methodology, Conceptualization, Investigation, Resources. **Florence Mouchet:** Validation, Supervision, Project administration, Funding acquisition, Writing – review & editing. **Van Xuan Nguyen:** Resources, Writing – review & editing. **Eric Pinelli:** Validation, Supervision, Project administration, Methodology, Funding acquisition, Writing – review & editing.

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.

Data availability

Occurrence data are available in supplementary data.

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Appendix A. Supplementary data

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