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Review

Antibiotics and antibiotic resitant bacteria: from hotspots into the aquatic environment

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Abstract

There is a vast literature on the occurrence of antibiotics and antibiotic resistant bacteria (ARB) of human or animal origin in natural aquatic ecosystems as result of anthropogenic activities. Although hotspots for AR discharge in the environment were identified, that include: sewage and wastewater treatment plants (WWTP), industrial drug manufacurers, food and animal production, agriculture and aquaculture, and clinical settings such as hospitals, effcient current regulations to mitigate this pollution are lacking. The aim of this review is to provide insights regarding the spread of antibiotics and ARB from hotspots into aquatic environment and also an overview of current EU and UN regulations for combating AR pollution of our natural ecosystems.

Keywords

antibiotic resistant bacteria, wastewater treatment plant, antibiotic resistance

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Introduction

Infections due to antibiotic resistant-bacteria (ARB) are jeopardizing modern health care. Experts and world leaders of Global Research on Antimicrobial Resistance (GRAM) Project call for action against antibiotic resistance (AR) (Lancet 2022; 399: 629–55). It has estimated that more than 1.2 million people—died in 2019 as a direct result of ARB infections. Nowadays, AR is responsible for more deaths than HIV/AIDS or malaria and the strongest approach in our hands to control AR is the correct and judicious use of the available antibiotics in order to preserve their effectiveness, including the unnecessary use of antimicrobials in humans and animals (Giurazza et al., 2021). As highlighted by an ad hoc World Health Organization panel, very few antimicrobials that are in clinical development are exhibiting a novel mechanism of action (WHO, 2021). A secondary approach for controlling the AR is to limit the release of the antibiotics and ARB into environment (Bengtsson-Palme et al., 2018).

Several reports are indicating that wastewater systems are continuously discharging high amounts of ARB and ARG in surface waters, worldwide (Gao et al., 2012; Huijbers et al., 2015; Bougnom et al., 2018; Pärnänen et al, 2019; Adelowo et al., 2018;), suggesting that AR pollution is enriching the environmental resistance pool. As a result of the anthropogenic contamination, the levels of ARBs is extremely high in most of the river systems (up to 98% of the total detected bacteria), and lakes (up to 77% of the total detected bacteria) (Nnadozie and Odume, 2019). Understanding the role of AR pollution in the emergence and evolution of resistance is particularly important, as this could represent an important driver with major health consequences (Bengtsson-Palme et al., 2018). The aim of this review is to provide insights regarding the spread of antibiotics and ARB from hotspots into environment and also an overview of current public regulations for combating AR pollution of the natural ecosystems.

Hotspots for AR spread into environment

Until recently, the prevention and control of AR has been largely focused on improving the rational use of antibiotics in the hospital and community and surveillance programs for the emergence and spread of resistance in clinical settings. It was reported that effective stewardship programs can reduce antibiotic use by 20–40%, frequency of health-care associated infections (MRSA, Clostridium difficile), hospitalization, and bacterial resistance rates (Charani et al., 2019). Stewardship efforts are important; however they cannot win the war (Laxminarayan et al., 2013). The challenges of AR emergence and dissemination have advanced a One Health Approach that is recognizing that the health of people is connected to the health of animals and the environment (Kraemer et al., 2019).

The main anthropogenic sources of antibiotic residues and ARB are the human, animal and agricultural waste. It was estimated that a high amount of antibiotics and/or its metabolites (between 20 and 97%) are excreted through urine and feces (Jelic et al., 2015). The hospitals, urban, industrial, sewer systems are transporting these chemical pollutants and ARB of human and animal origin to the treatment facilities. However, the treatment technologies of these plants were reported to be unable to completely eliminate these pollutants, antibiotics ARB of human and animal origin being detected in the treated released water into the environment and in the animal sludge applied to fields with cultivated crops (Auguet et al., 2017). For example, the analysis of wastewater samples from a German urban wastewater treatment plants (WWTP) did not show differences in resistance between E. coli isolates taken from the inflow and from the outflow . Also, an integrated surveillance of European wastewaters that analyzed the influent and final effluent of 12 urban WWTPs located in seven countries (Portugal, Spain, Ireland, Cyprus, Germany, Finland, and Norway) has highlighted the strong relationship between clinical and environmental AR (Pärnänen et al, 2019).

Agriculture run-off, and process waters and wastewater from slaughterhouses are also considered hot spots for AR (Figure 1). The increased usage of antibiotics in livestock animals has led to the selection of ARB that can be transmitted directly from animal to human or indirectly via the food chain and eventually causing difficult-to-treat diseases in humans (Binsker et al., 2022). The direct entry of antibiotics, ARB and ARG in the environment takes place when cattle are out door or manure is used as fertilizer on nearby crop lands, where ARGs and surviving ARBs come into contact with the soil microbiome and could modify the profiles of ARB, ARGs and bacterial communities in receiving soils (Chen et al., 2018). Once in the soil, ARB and ARG may enter aquatic systems indirectly via surface runoff to surface water and/or by leaching to groundwater.

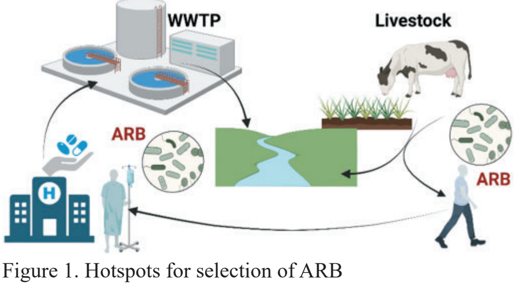

Figure 1. Hotspots for selection of ARB

The ARB discharged by the WWTP effluents and agriculture run-off into water bodies may be particularly prone to transfer AR genes to environmental bacteria via different horizontal gene transfer (HGT) mechanisms: conjugation, transformation, and transduction. Several researchers showed that conjugation take place in aquatic environments (Kimihiro et al., 2020). It has been showed that the incidence of the HGT is enhanced by low concentrations of antibiotics that are released into receiving water bodies (Aminov 2011). The possibility for HGT using MGEs (plasmids, transposons, insertion sequences, integrons, and integrative-conjugative elements) between ARB of human or animal origin and environmental bacteria is huge and it seems that it doesn't take bacteria much time to acquire the resistances markers (Sultan et al., 2018). Furthermore, the frequency of HGT is increased in biofilms due to the proximity of the cells, making biofilms in hot spots of antibiotic resistance (Aminov, 2011; Balcázar et al., 2015). However, despite the wealth of information about medical biofilms, the potential contribution of the environmental biofilms to the acquisition and spread of antibiotic resistance has not been fully investigated in aquatic systems (Balcázar et al., 2015). Environmental biofilms are a key component of ecosystem functioning, playing an essential role in the biogeochemical cycling of nutrients. Taken into consideration that biofilms are exposed to antibiotics used in veterinary and human medicine released into these waterbodies mainly through WWTP effluents and agricultural run-off, research is needed to investigate how such hot spots of AR may represent a concern for human health (Balcázar et al., 2015).

The dissemination of antibiotics ARB and ARG into environment

Antibiotics are considered the main driver of AR. Several researches have indicated a direct correlation between AR and the use of antibiotics (Olesen et al., 2018). Global estimates have indicated that similar amounts are used in humans and animals (118 mg/PCU and 133 mg/kg respectively) (Pokharel et al., 2020). Moreover, antibiotics used as plant protection products in some regions of the world (Pokharel et al., 2020). Residual antibiotics from agriculture run-off, poorly metabolized antibiotics from animal and human waste as well as antibiotics from drug manufacturer effluents are discharged in the environment. These antibiotic pollutants were already found in waterways and sediments worldwide (Table 1). They can accumulate in the environment and exert selective pressure on the soil and water microorganisms, enriching the environment AR pool.

Several reports have indicated connections between the enteric pathogens isolated from surface waters, impacted by human activity and active disease in the community (Yan et al., 2018; Yang et al., 2014). Bacterial isolates resistant to different antibiotic classes, were detected in surface water samples collected from lakes, rivers and sea (Lepuschitz et al. 2019, Zurfluh et al., 2013). As, lake and river waters is used for drinking water production, recreational activities and irrigation of crops, humans and animals are exposed to these ARB and could get colonized with such bacteria (Blaak et al., 2015). The antimicrobial susceptibility tests are showing that rivers and lakes are containing bacteria with high resistance rates to several classes of

Table 1. Antibiotics found in aquatic environments.

Antibiotic	Class	Max concentration ng/L	Type of environment	Location	References
Acetylsulfamethoxazole	Sulfonamid es	70	River water	United Kingdom	Ashton et al., 2004
Sulfadiazine	Sulfonamid es	209	River water	Pearl River, Guangzhou, China	Xu et al., 2007
Sulfamethoxazole	Sulfonamid es	259.6	River water	Huangpu River, Shanghai,China	Chen and Zhou, 2014
Amoxicillin	Penicillins	5,70	River water	River Arno, Italy	Zuccato et al., 2010
Cefalexin	B-lactams	12,60	Sediment	Dongjiang River, China	Chen et al., 2018
Chlortetracycline	Tetracyclin es	353400	Lagoon water	Iowa and Ohio, USA	Campagnolo et al., 2002
Oxytetracycline	Tetracyclin es	52.8	Sediment	Taihu Lake, China	Xu et al., 2014
Tetracycline	Tetracyclin es	107400	Lagoon water	Iowa and Ohio, USA	Campagnolo et al., 2002
Doxycycline	Tetracyclin es	11,30	River water	Huangpu River, Shanghai,China	Chen and Zhou, 2014
Azithromycin	Macrolides	72,1	Sediment	Mar Menor lagoon, Spain	Moreno-González et al., 2015
Clarithromycin	Macrolides	25,40	River water	River Arno, Italy	Zuccato et al., 2010
Erythromycin	Macrolidea	62.1	River water	Laizhou Bay, China	Zhang et al., 2012
Nalidixic acid	Quinolones	77	Sediment	Charmoise River, France	Dinh et al., 2017
Norfloxacin	Fluoroquinolones	267	Sediment	Baiyangdian Lake, China	Li et al., 2012
Ciprofloxacin	Fluoroquinolones	569	Sediment	Charmoise River, France	Dinh et al., 2017
Chloramphenicol	Chloramphenicol	127	River water	Pearl River, Guangzhou	Xu et al., 2007

antibiotics (Table 2). For example, in a study performed by Zurfluh et al. (2013), the enterobacteria collected from rivers and lakes in Switzerland were resistant to cefotaxime (77%), gentamicin (36.5%) kanamycin (28.4%), streptomycin 44.6%, nalidixic acid (59.5%) ciprofloxacin (44.6%), tetracycline (67.6%) chloramphenicol (23%), sulfamethoxazole (85.1%) and trimethoprim (74.3%) (Zurfluh, 2013). Through continously release of ARB into waterbodies, the environment could become a pool of AR at which human population, but also animals (livestock, wild life) may be exposed, therefore increasing the colonization rate.

The AR increase to fluoroquinolones and extended-spectrum cephalosporins due to the production of the ESBL CTX-M-15, was suggested to be responsible for the worldwide spread, on three continents, of Escherichia coli ST131, which is an extraintestinal pathogenic clone. Currently, this clone has been identified as the predominant E. coli in the gut of human healthy population. A public health problem of global dimension is the possibility of this clone to acquire MGE encoding carbapenemases. The colonization of human or animal gut with such bacteria could hamper medical treatment-options of severe infections caused by ESBL producing Enterobacteriaceae, consequently resulting in higher morbidity and mortality rates (Oteo et al., 2016; Yang et al., 2018). E. coli ST131 was found in various freshwaters ((Jiquiriçá and Brejões rivers (Brazil) (Bartley et al., 2019) (Furlan et al., 2020); Orontes River (Lebanon) (Moussa et al., 2021), rivers of Guadeloupe (France), (Guyomard-Rabenirina, 2017), River Thames, UK (Dhanji et al., 2011), river water in Spain (Colomer-Lluch et al., 2013); rivers and lakes, in Switzerland (Zurfluh et al., 2013.), river Ouche (France) (Bollache et al., 2019), river Inn (Austria), and marine environments (Adriatic Sea, Italy (Vignaroli et al., 2013), Salish Sea (Vingino et al., 2021)) i.e. rising questions about the health risks.

An important threat to public health of international concern are carbapenemase-producing Enterobacteriaceae, which are producing difficult to treat infections, being associated with high level of mortality. These bacteria have the potential for widespread transmission of resistance via MGEs. The current WHO guidelines are aiming to effectively prevent their occurrence and control their spread in acute health care facilities. In Europe, a total of 23 European countries reported a an increased prevalence of epidemiological situation of carbapenemase-producing Enterobacteriaceae between the years 2010 and 2018 (Brolund et al. 2019). These bacteria have been found to have a widespread distribution in waterways (Table 3) and sediments (Piedra-Carrasco et al., 2017), rising questions about the health risks. Their dissemination into surface water will increase the colonization level of human population with carbapenemase-producing Enterobacteriaceae, threatening our ablity to treat infections. Therefore, the prevalence carbapenemase-producing Enterobacteriaceae, especially those resistant to colistin is important to monitor in all the interconnected compartments: humans, animals and enviroment.

The worlwide rise in carbapenemase-producing Enterobacteriaceae has led to the reintroduction of colistion

Table 2. ARB detected in the environment.

Bacterial species	Class of antibiotics resistant to	Surface water, Country	References
Escherichia coli	β-lactams (cefotaxime) Lipopetides (Aminoglycosides (kanamycin, streptomycin, gentamicin) Nalidixic acid Quinolones Tetracyclines Phenicols (chloramphenicol) Folate pathway antagonists (sulfamethoxazole and trimethoprim)	Rivers and lakes, Switzerland	Zurfluh et al., 2013
Escherichia coli	β-lactams Aminoglycosides Quinolones	Rhine and New Meuse rivers	Blaak, H. et al, 2011

Table 3. Water bodies in which carbapenemase-producing *Enterobacteriaceae* were detected.

Water bodies	References
West Coast (California, USA)	Harmon et al., 2019
Midwest (California, USA)	Aubron et al., 2005
Vouga river basin (Portugal)	Tacão et al., 2015
River in Santo Tirso (Portugal)	Kieffer et al., 2016; Poirel et al., 2012
Lis river (Portugal)	Teixeira et al., 2020
River Rhine (Switzerland)	Zurfluh et al., 2013
River Mur (Austria)	Zarfel et al., 2017
River Danube (Germany)	Kittinger et al., 2016
River in Guangzhou (China)	Ye et al., 2017

in the therapy, as last line antibiotic for the medical care of infections in which these pathogens are implicated. However, data reports from EU/EEA countries, China and Japan are indicating an alarming increase of colistin resistance. An important contribution to the increase of bacteria resistant to colistin is represented by the use of this antibiotic in the livestock production. These selected ARB can be directly or indirectly spread from animals to humans though food chain. This health problem is reflected by the results of the environmental studies. Two reports, from South Africa and Tunisia have found Enterbacteriales resistant to colistin in wastewater (Ovejero et al., 2017; Adegoke et al. 2020; Hassen et al. 2020). The highest amount of ARB has been reported in the hospital effluents (Bardhan, Chakraborty and Bhattacharjee 2020). These findings are highlighting the importance of monitoring the colistin resistance in bacteria of animal and human origin, and the need for environmental studies for the elucidation of occurrence and distribution of these bacteria resistance to colistin in water bodies. These data will provide scientific evidence for the assessment of AR burden in clinical sector, community and environment and therefore the development of effective strategic interventions.

The impact of human activities on aquatic ecosystems is also highlighted by the occurrence and widespread distribution of AR genes of human or animal origins (Yang et al., 2017). Several ARGs have been identified in the freshwater biofilms receiving fecal contamination (Proia et al. 2013). Research studies have demonstrated significant correlations between several ARGs and corresponding antibiotics in water samples. Thus, low concentrations of antibiotics in freshwater play an important role in the maintenance and enrichment of AR pool (Xiong et al., 2015). Additional studies are necessary for a more complete understanding of human-based environmental reservoirs of ARB and ARGs. In particular, upstream and downstream urban WWTP sites would be useful for assessing and quantifying the influence of human contamination of freshwaters.

Current EU and UN regulations for combating AR pollution of the environment

Studies on environmental AR are growing and as emphasized in this paper, current research is aimed at investigating the health risks associated with the exposure of natural environments (soil and water) to ARB, AR genes and antibiotics. In recognition of the importance of tackling the AR, several international and national polices and regulations were developed. The United Nations Environment Program (Gaze and Depledge, 2017) emphasized that the spread of AR in the environmental is representing one of the most important environmental pollution problems of our time. The New EU One Health Action Plan against Antimicrobial Resistance (http://ec.europa.eu/health/amr/sites/amr/files/amr_summary_action_plan_2017_en.pdf) provides the framework for actions needed to decrease the emergence and dissemination of AR. National bodies of the EU need to elaborate and implement surveillance plans of AR in the environment and also to advance innovative and green technologies for waste removal containing AR from pharmaceutical companies, health care units, livestock and crop production sites, wastewater plants (https://www.who.int/news/item/02-03-2022-world-leaders-and-experts-call-for-action-to-protect-the-environment-from-antimicrobial-pollution). Standards and regulations are urgently required to monitor and control of AR release in the environment and countries of the EU should prioritize their development.

Conclusions

The extensive use of antibiotics had contributed to the exposure of natural environments (soil and water) to the detection of worrisome high levels of ARB and ARG of animal and human origin in different types of environmental samples collected from various ecosystems. These findings are of public health concern because the natural ecosystems are providing important services to the communities. Therefore there is of crucial importance to assess the baseline levels of AR in such environments in order to adequately address the health concerns. Additionally, the environmental microbial populations with essential roles in Earth ecosystems and knowledge about the way that are responding to the bacterial diversity and community to anthropogenic disturbances, in particular pathogenic ARBs, ARGs and antibiotics is of great significance. ARB from human sources have been found in sewage, in treated effluent and in sludge applied to farmland, these pollutants are discharged into the environment. Therefore, to stop the spread and enrichment of our environment with AR, innovative technologies for the treatment of sewage and agricultural waste are needed. The UN Environment Program and The New EU One Health Action Plan against Antimicrobial Resistance are providing the needed actions for combat AR. The EU countries should prioritize the elaboration and implementation of national standards for controlling the discharge of AR in the environment.

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