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# Role of pollution on the selection of antibiotic resistance and bacterial pathogens in the environment

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There is evidence that human activity causes pollution that contributes to an enhanced selection of bacterial pathogens in the environment. In this review, we consider how environmental pollution can favour the selection of bacterial pathogens in the environment. We specifically discuss pollutants released into the environment by human activities (mainly human waste) that are associated with the selection for genetic features in environmental bacterial populations that lead to the emergence of bacterial pathogens.

Finally, we also identify key pollutants that are associated with antibiotic resistance and discuss possibilities of how to prevent their release into the environment.

## Addresses

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## Introduction

Environmental pollution did increase significantly due to anthropogenic activities since the beginning of the industrial age [1<sup>\*</sup>]. Solid and liquid waste from urban settlements poses a global challenge in pollution management [2,3,4<sup>\*</sup>]. High loads of antibiotics, chemicals and micro-pollutants, that are released into the environment via anthropogenic waste, alter the biodiversity of microbial ecosystems [5–7]. Pollution derived xenobiotics in the environment act as stressors for bacterial populations. The natural response of bacterial populations to stress is the attempt to resist the stressors and/or to adapt by the

acquisition of novel genetic traits or resistance genes that allow them to persist and proliferate in the stressed environment [1<sup>\*</sup>,8,9]. While reducing biodiversity of terrestrial and aquatic microbial ecosystems [10], pollution has also been shown to increase the selection of bacterial pathogens in the environment [1<sup>\*</sup>,11,12,13<sup>\*\*</sup>]. Bacterial pathogens can sustain a significant part of their life cycle outside the host but once introduced they can present a severe public health threat since they can cause serious disease in plants, animals, and humans [14–16].

Antibacterial drugs represent one of the most important therapeutic advances in medical history [17]. However, bacterial resistance has increased dramatically during the last 2 decades [18] and multidrug-resistant bacteria are spreading worldwide and present a serious public health issue [19,20,21<sup>\*</sup>]. Notably, the emergence of antibiotic resistance is not limited to human medicine [22]. Antibiotic resistance may occur and spread within human populations, food, and farm animals, or via livestock derived food-products or animal waste, sewage, water, and the wider environment [10,21<sup>\*</sup>,23,24].

Antibiotic resistance genes (ARGs) are carried by bacteria among all phyla across various environmental niches [25–27]. Antibiotic resistance is a natural phenomenon that predates the modern selective pressure of clinical antibiotic use. Therefore, any niche populated by microorganisms can form a reservoir of ARGs, for which the term resistome has been proposed [28]. Antibiotic resistance genes encoding resistance mechanisms against clinically important antibiotics such as  $\beta$ -lactam antibiotics, tetracycline and vancomycin, have been identified in Beringian permafrost sediment samples that were estimated to contain ancient DNA, dating back approximately 30 000 years [25,26]. The role of antibiotics in nature is still not well understood. Antibiotics make up only a small fraction of bioactive molecules that are naturally produced by bacteria across several habitats ('The parvome' [17]). They are suggested to play an important role in bacterial–cell interaction and communication [25,29,30] and may be produced by bacterial strains to suppress growth of a neighbouring bacterial strain in a specific niche [31,32]. Notably, the natural concentrations of antibiotics are significantly lower and not comparable to the concentrations of antibiotics that are synthesized and applied in clinical and food production (agricultural and aquacultural) settings [29,33,34].

The use of antimicrobial agents (biocides and antibiotics) in clinical practice, veterinary medicine, agriculture and

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aquaculture has increased the selective pressure for antibiotic resistant bacteria and ARGs globally [35,36\*\*]. Selection and co-selection of ARGs in bacterial populations occurs during exposure to high, middle, or low levels of antibiotics and other xenobiotic compounds present in various environmental matrices [36\*\*,37,38,39\*].

### Sources of pollution: industrial, urban, medical, agricultural, and aquacultural waste – and their common nominator: (WASTE) – WATER

A significant proportion of urban, medical, and industrial waste is released into the environment via wastewaters (WW) [5,10,40–42]. Dependent on available infrastructure and national regulations, WW is released into the environment either treated, not sufficiently treated, or untreated, with the latter having the most detrimental impact in the receiving environment [10]. After WW has passed through wastewater treatment plants (WWTPs), the treated water is released into local surface waters or could be applied as an irrigation source in farmlands. The sludge of WWTP is sometimes used as fertilizers after specific treatment in agriculture [43–46]. WW and WW treatment plants have been proposed to serve as hot-spots for the selection and dissemination of bacterial pathogens and ARGs into the environment [40,47–49]. In fact, the most common entry routes for antibiotics, other xenobiotic compounds, pathogenic bacteria and ARGs in the environment are from sewage outfall [50–52], agricultural fertilisers and natural fertilizers such as pig slurry and cow manure [38,53,54] that contain resistance-selecting chemicals, for example, antimicrobials, metals and biocides. On a global scale, food production sites in both terrestrial and aquatic environments represent hot-spots and selection sites for ARGs and antibiotic-resistant bacteria (ARBs) since antibiotic and other antimicrobial agents are used during food production in various concentrations worldwide [38,55,56].

Particularly in lower income countries that lack specific WWT regulations, pharmaceuticals producing industry contributes significantly to the high loads of resistance-selecting chemicals in the WW receiving environment [4\*,20,57\*,58–60]. Recently, a link between non-antibiotic pharmaceuticals and the selection and transfer of ARGs has also been made, highlighting the potential of co-selection and cross-selection properties of commonly used pharmaceuticals such as ibuprofen, that are present in high concentrations in urban WW systems [61\*\*,62,63].

According to the UN world water development report of 2020, 80% of WW globally is released into the environment without treatment; only 8% of WW undergoes treatment in lower income countries, about 30% in lower-middle income countries and about 70% in high-income countries [60]. Fecal pollution contributes importantly to the burden of ARGs and ARBs present in the environment [40,42,64\*]. Human or animal gut bacteria often contain high loads of ARGs and bacterial pathogens

[65–67]. Fecal pollution is particularly high in urban settlements that lack sanitation and WW treatment systems [20,68\*\*]. Hospitals are also highlighted as hot spots for the selection of ARB and the dissemination of antibiotics, ARGs and pathogens via hospital WW [40,48,69\*]. It is evident that policies and improved WW treatment options need to be implemented urgently and on a global scale that aim to reduce the chemical and pharmaceutical pollution burden through WW [24,41,60,68\*\*].

### Types of pollutants

The main anthropogenic pollutants present in the environment associated with the selection for resistant pathogens are pharmaceutical residues (including antibiotics), biocides, surfactants, heavy metals, pesticides, potentially toxic substances (PTEs), and microplastics.

Recently, commonly prescribed pharmaceuticals such as nonsteroidal anti-inflammatories, ibuprofen, naproxen, diclofenac, gemfibrozil, and the  $\beta$ -blocker propranolol, have been shown to enhance natural transformation in bacterial populations and thereby to enhance the uptake of exogenous ARG [61\*\*]. Antimicrobial agents other than antibiotics, like quaternary ammonium compounds (QACs), find a wide application in clinical, domestic, and industrial settings, where they are mainly used as disinfectants [70]. However, non-susceptibility to for example QACs has emerged in bacteria, including in some nosocomial pathogens [32,71,72]. The most reported resistance mechanisms to QACs are due to active multi-drug efflux pumps (encoded by *qac* genes), that can also confer resistance to antibiotics, such as tetracycline [73,74]. The emergence and spread of resistance to disinfectants is not only a concern on its own but is feared to contribute to the simultaneous rise of antibiotic resistance by favouring cross-selection and co-selection of antibiotic resistance [75,76]. Cross-resistance is described as resistance towards multiple antimicrobial agents that act via a common pathway or the same target, whereas co-resistance refers to multiple resistance genes that are jointly present on mobile genetic elements such as plasmids, transposons or integrons [75,77]. Other disinfectants and surfactants for medical but also household and hand hygiene disinfection such as triclosan and chlorhexidine for example have also been reported to select for resistance against these agents in bacterial pathogens, and to enhance co-selection and cross-selection of ARGs [78–80]. Pesticides are applied on a global scale for crop management during intensive agriculture. The negative impact of pesticides on ecological biodiversity of insects and plants has been reported since the early 1960s [81]. Recent *in vitro* and *in situ* studies have also established a link between the application of pesticides and the rise of antibiotic resistance. It was found that the exposure of pesticides to *in vitro* *Escherichia coli* cultures did induce the evolution of antibiotic resistance in these bacteria. In another study, the increase of normalized

Table 1

**Summary table for the main sources of pollution that are associated with the selection for antimicrobial resistant bacteria in the environment**

Source of pollution	Environmental niches impacted	Putative mode of selection for antimicrobial resistance	Dissemination routes
Pharmaceutical residues (including antibiotics)	Human/animal gut microbiota, waste waters, recreational waters, sediments, and soils	Selection of ARGs and ARB	Water exposure and use, animal/human contact
Biocides, surfactants	Human/animal gut microbiota, waste waters, recreational waters, sediments, and soils, plants	Co selection and cross selection of ARGs in human/animal microbiota and environmental bacteria	Application of biocides in clinical and domestical settings, application of biocides to food crop and plants
Heavy metals,	Soils, sediments, recreational and wastewater	Co selection and cross selection of ARGs in human/animal microbiota and environmental bacteria	Water exposure and use, via the food chain
Potentially toxic substances (PTEs)	Soils, sediments, industrial sites and estuarian sites	Co selection and cross selection of ARGs in soil microbiota and environmental bacteria	Water exposure and use, via food chain and soil
Microplastics	Aquatic and terrestrial environment globally	Support for biofilms that accumulate pollutants, ARB and ARGs	Waste chain impacting terrestrial and aquatic environments

abundance of tetracycline, erythromycin and aminoglycoside resistance genes in agricultural soil exposed to pesticides was observed, highlighting that the presence of pesticides in the environment and in agricultural soils can select for antibiotic resistance [82,83].

Heavy metals are highly prevalent due to anthropogenic activities (mainly mining and other industrial and agricultural activities) and bioaccumulate in nature [84,85]. They have been particularly associated with the co-selection of ARGs in the environment [39,86,87] and are also referred to as potentially toxic elements (PTEs) that are frequently derived from 'old' industrial pollution [1]. Sediments from lakes, riverbeds and estuaries have been found to be enriched with heavy metals or PTEs particular in industrial zones [1,88,89] and their accumulation and dissemination in the environment is mainly linked with industrial pollution.

Recently and in the context of antimicrobial resistance, microplastics have been found to represent hubs enriching antibiotic-resistant bacteria and pathogens in municipal activated sludge and other aquatic and terrestrial environments [90,91,92]. Microplastics were shown to accumulate pollutants and serve as support for biofilms that are enriched in ARGs and bacterial pathogens-providing optimal conditions for the transfer and evolution of ARGs within bacterial populations. Indeed, biofilms are the preferred lifestyle of bacteria in the natural environment that give support to high densities of bacteria and are known to facilitate gene transfer [56,63] and to be enriched with ARGs in polluted environments (Table 1).

#### Impact at a one health scale

The environment constitutes a large reservoir of ARGs and there is evidence that at environmentally relevant concentrations, some antibiotics, and other chemical

stressors, mediate horizontal gene transfer (HGT) between bacteria [56,78,93]. We know that the emergence of antimicrobial resistant bacteria is not limited to humans or human medicine only, but is influenced by multiple direct and indirect pathways, that form a complex web of interactions that contribute to the selection and spread of resistance in the human/animal microbiota and the environment [10].

When confronted with antibiotic and chemical selection pressure or stress, bacteria can adapt very rapidly by mutating or by acquiring new genetic elements [94–97]. Bacteria possess a variety of complex mobile genetic elements (MGEs) such as bacterial plasmids, transposons or cassettes of integrons that enable horizontal transfer of ARGs [98]. Antibiotics themselves can induce the transfer of several MGEs [99].

There is evidence that selection for ARGs and ARB frequently occurs in clinical settings where concentrations of antibiotics are extremely high for bacteria in the human or animal microbiota. Recent *in silico* analysis showed that the majority of ARGs identified on MGEs originate from proteobacteria that were isolated from infection sites in humans or domestic animals [100]. On the other hand, there is evidence that low and sub lethal concentrations of antibiotics in the environment can also select for ARGs [37]. The dissemination of ARGs from antibiotic producers to pathogens, hence from the environmental ARG reservoir to pathogenic bacteria has been described as well, further highlighting the environmental bacterial resistome as an important reservoir for the dissemination of antibiotic resistance [101,102]. More focus needs to be put on the study, sampling, and sequencing of environmental matrices to assess which environmental niches, and to which extent, contribute to the emergence of antimicrobial resistance in bacterial

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pathogens. However, an increase of the resistance gene pool in environmental bacteria due to pollution is likely to increase the probability of the transmission of these genes into pathogenic bacteria.

Another important aspect in the evolution or rise of human bacterial pathogens are virulence factors, that allow bacteria to infect its hosts more successfully or circumvent its hosts defence mechanisms. There is evidence that the selection for ARGs is associated with the selection for virulence factors that are equally present on ARG-carrying MGEs [103–105]. Subsequently, there is an advantage for the selection of ARGs and virulence genes in bacterial populations that are constantly exposed to pollutants and ‘stress’, highlighting the importance of pollution management and removal in the environment to suppress the selection and dissemination of human bacterial pathogens in the environment.

The putative impacts of pollution and the associated emergence of bacterial pathogens for human, animal and environmental health (plants, water and soils) is significant and depends highly on the social and economic context as consequences differ and will further differ between high, middle or low-income countries [20,60,68\*\*]. Infectious diseases due to ARB are predicted to increase significantly and will severely impact human and animal health, compromising modern medicine as we know it, and limiting the food production chain [13\*\*,106,107]. Environmental health will be increasingly compromised [108] and disease due to soil pathogens affecting plants and food production are more likely to occur [109].

Pollution due to human activity has devastating consequences on the environment. Reduced biodiversity and climate change are suggested to further contribute to an increase in the emergence and transmission of pathogens and infectious diseases [110\*,111]. How exactly reduced biodiversity increases the risk of zoonosis and the emergence of resistant bacterial pathogens needs to be investigated further and should be a priority subject in research agendas. However, there is reason to assume that the exponentially growing global population, an increasingly dense and expanded urban infrastructure, and the associated increasing environmental pollution could increase the likelihood of pandemic outbreaks of infectious diseases as we are currently facing with the COVID-19 pandemic. Global pandemics due to novel zoonotic events and the subsequent global health management responses may in turn impact the environment in such a way that may pave the ground for the putative emergence of novel pathogens. For example, the impact of COVID-19 patient management on the emergence of antimicrobial resistance is currently discussed and warrants further attention as it is likely that increased antibiotic therapies and antimicrobial disinfection world-wide may favour the

emergence of antimicrobial resistance and bacterial pathogens in the environment [112].

Global policies need to be unified and inevitable when it comes to environmental protection and preservation, demanding significant investments in improving sanitation systems and wastewater management, as well as investing in alternative and more sustainable food production on a macro scale. To assure the effectiveness of antimicrobial treatments and to prevent the emergence of antibiotic resistance and bacterial pathogens in the environment, evidence-based policies should be developed. For example, policies should comprise the reduction of the use of antimicrobials in human and animal health and the management of already polluted environments. Overall, solutions to the actual and global public and environmental health crisis(es) that include social, industrial, scientific, and medical approaches, are complex and should be reviewed on its own to prioritize tasks and further highlight urgent research priorities.

#### Conflict of interest statement

Nothing declared.

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