



# Retrospective analysis of the global antibiotic residues that exceed the predicted no effect concentration for antimicrobial resistance in various environmental matrices

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## ABSTRACT

**Background:** Antimicrobial resistance (AMR) is a growing public health concern. Recent research has suggested that interactions between pathogens and antibiotic residues in various environmental matrices promote the development and spread of AMR in the environment. The levels of antibiotic residues in the aquatic environment have been analysed globally. Recently, Predicted No Effect Environmental Concentration (PNEC) values for many antibiotics have been suggested, based on their estimated minimal selective concentrations for selected bacterial species. The PNEC values can serve as a guide on the maximum levels of antibiotic residues in an environmental matrix, below which resistance is unlikely to develop.

**Aim:** We aimed to determine which of the antibiotics, considered as “priority antibiotics” by the World Health Organisation (WHO), most frequently exceeded their PNEC values in the global aquatic environment.

**Methods:** We obtained data from the German Environment Agency pharmaceutical database on means, medians or single values of 12 antibiotic types in five different environmental matrices [municipal wastewater treatment plant effluent, industrial wastewater effluent, hospital wastewater effluent, surface water, and drinking water] across 47 countries. We compared the mean levels of the 12 antibiotics in each environmental matrix to their suggested PNEC values to determine which antibiotic types exceeded PNEC and were most likely to select for resistance. We also determined which environmental matrices and countries had the highest burden of antibiotic residues.

**Results:** Our study revealed that 7.9% of all analyses of antibiotic residues performed in the environmental matrices globally exceeded PNEC. Ciprofloxacin and clarithromycin had the greatest proportion (> 30%) of residues exceeding PNEC. Hospital wastewater and industrial wastewater had the highest burden of antibiotic residues exceeding PNEC. No antibiotics exceeded PNEC in drinking water.

**Conclusion:** While most environmental monitoring studies have focused on municipal wastewater treatment plants, the limited number of studies on hospital wastewater and industrial wastewater revealed that a large number of antibiotic residues coming from these sources exceeded their PNEC values. Our study highlights the importance of implementing on-site treatment systems that aim to destroy antibiotics prior to discharging wastewater to surface waters. Attention needs to be focused on the role that environmental matrices, particularly our wastewater sites, play in promoting antibiotic resistance. Novel treatment technologies need to be developed and implemented to increase the removal efficiencies of treatment plants and from antibiotic manufacturing, and decrease the discharge of antibiotic residues into aquatic environments.

## 1. Introduction

Antimicrobial resistance (AMR) has emerged as a growing public health concern. The potential ramifications from a reduction in options to treat infectious diseases has been widely acknowledged. These

include an increase in hospital residence time and increased mortality from infection, reaching into other areas of health care, including oncology and surgery where antimicrobials are vital for prophylaxis and treatment of infectious complications. AMR is estimated to account for 700,000 deaths annually worldwide, which may increase to

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approximately 10 million deaths and US\$ 100 trillion annually by 2050 (Neill, 2016).

The development of AMR, or more specific to this article, antibiotic resistance, is a complex issue that can only be addressed using the One Health approach that recognizes the interconnection between humans, animals, plants, and the environment. Antibiotic resistance is expected to occur when the Minimal Inhibitory Concentration (MIC) of a given antibiotic for a specific bacterial organism is higher than the concentration needed to inhibit the growth of a corresponding parental wild-type strain, resulting in treatment failure in patients with infection (Martinez et al., 2015). The occurrence of genes that code for resistance is not a new phenomenon, with some environmental bacteria predating the antibiotic era being reported to carry resistance genes (Finley et al., 2016). Indeed, antibiotics and antibiotic resistance genes (ARGs) are considered natural elements of the environment. The concern is that human activities, including the misuse of antibiotics and inadequate disposal of them, have significantly influenced the distribution and proliferation of these resistance genes in the environment (Finley et al., 2016).

The role of environmental matrices, such as water and sewage sources, in driving the development and spread of AMR has become a significant concern among environmental scientists, engineers, health-care professionals and government agencies alike. Indeed, antibiotic residues and ARGs are considered as 'emerging contaminants' in the water and sanitation world. Novel ARGs develop through such processes as mutations, rearrangements and horizontal gene transfer between environmental and pathological bacteria, requiring bacteria to share a common habitat for this to occur (Bengtsson-Palme et al., 2017). Bengtsson-Palme et al. proposed the following steps in the development of antibiotic resistance, namely, the emergence of novel ARGs, mobilisation of these ARGs, the transfer of them to pathogenic bacteria, and the dissemination of resistant organisms (Bengtsson-Palme et al., 2017).

Antibiotics reach the environment through several pathways including direct excretion into water or land by humans or animals, land application of animal manure to fertilise crops, or discharge of effluents from municipal wastewater treatment plants, hospital wastes and manufacturing plants (WHO, 2017). The underlying concern is that in all areas where antibiotics are utilised, the end result is their release into the environment where they create a potential breeding ground for antibiotic resistance. Many studies worldwide have attempted to quantify the level of antibiotic residues present in the environment. The German Environment Agency/ Umweltbundesamt (UBA) reviewed 1016 original peer-reviewed publications and 150 review articles, published up until 2016, that reported environmental concentrations of pharmaceutical substances worldwide in surface water, groundwater, tap/ drinking water, manure, soil, sewage and other environmental matrices, in order to create a systematic database (UBA, 2016). Using this database, one can access a vast amount of data on the environmental levels of various antibiotic types.

Until recently, having access to data on environmental levels of antibiotic residues was meaningless as there were no published guidelines on the minimum levels that may result in the development of antibiotic resistance. Recently, however, Bengtsson-Palme et al. suggested predicted no effect environmental concentration (PNEC) values for several antibiotics (Bengtsson-Palme and Joakim-Larsson, 2015). They derived PNECs by estimating minimal selective concentrations (MSC's), using the EUCAST database on MIC values (European Committee on Antimicrobial Susceptibility Testing, 2014). Information obtained from the EUCAST database was used to estimate the lowest MIC's for each antibiotic on the species they were tested against. The majority of the antibiotics were evaluated against at least five different families, including *Staphylococcaceae* and *Streptococcaceae*, and *Enterobacteriaceae*. Based on this approach, the lowest MIC values ranged from 0.69 µg/L (predicted concentration for ceftriaxone in *Neisseria meningitidis*) to 32,000 µg/L (clavulanic acid in *Acinetobacter baumannii*)

(Bengtsson-Palme and Joakim-Larsson, 2015). The PNEC values obtained do not account for multidrug resistant bacteria.

The suggested PNEC values, based on the MIC of the antibiotic, are intended to guide environmental regulation and monitoring programmes in efforts to keep environmental antibiotic concentrations below a certain level that will not likely promote AMR development and spread. Although there is currently no standard process in place for treating sewage or drinking water to remove antibiotic residues and there are no regulatory limits implemented for any pharmaceutical residues in treated municipal or hospital wastewaters, the PNEC values can be valuable for prioritising what to monitor and to direct mitigation projects.

Many studies present results on levels of antibiotic residues in environmental matrices. We are not aware of any articles that compare these levels to the PNEC values of the various antibiotics. We believe that doing so would emphasise the burden of antibiotic residues in the environment and the risk they pose in driving AMR worldwide.

The aim of this article, therefore, is to a) determine which antibiotic residues most frequently exceed the PNEC in the five environmental matrices evaluated (municipal wastewater treatment plant effluent, industrial wastewater effluent, hospital wastewater effluent, surface water, and drinking water), and b) determine which countries represented in the database have the highest proportion of antibiotics whose environmental residues exceed the PNEC in different environmental matrices. The results from this data analysis can support decisions on what types of antibiotics need to be regulated in the aquatic environment, and which countries produce the highest burden of antibiotic residues in the environment. Knowledge on the distribution and occurrence of antibiotics that exceed PNEC should bring attention to the correct disposal of antibiotics and need for more efficient wastewater treatment systems to reduce the development and spread of AMR.

## 2. Methods

### 2.1. Data acquisition

Data were obtained from the UBA database on levels of pharmaceuticals in the environment. As mentioned above, the development of this database began in 2012 in order to assess the global occurrence of pharmaceuticals in the environment. The database consists of 123,761 entries, with 631 different pharmaceuticals analysed in 20 different environmental matrices across 71 countries (UBA, 2016). Each entry in the database includes the generic name of the active pharmaceutical ingredient and whether it is used in human or veterinary medicine, the environmental matrix sampled, the country and region that the samples were collected from, the number of samples analysed in each entry, the measured antibiotic concentrations given in standardised units (ug/L or mg/kg), the statistical description of the measured antibiotic concentrations (mean, median, minimum, maximum or single value), author details, and a rough rating of the quality of the study (good or poor). The database is free to access and allows users to direct their search to a particular region, environmental matrix, or pharmaceutical substance. We focused our search on 12 selected antibiotics, namely, amoxicillin, azithromycin, ciprofloxacin, clarithromycin, clindamycin, doxycycline, enrofloxacin, ofloxacin, oxytetracycline, sulphamethoxazole, tetracycline and trimethoprim. We chose these antibiotics as they are frequently used in human and veterinary medicine, are classified as priority antibiotics by the WHO and are the most frequently studied in the environment (WHO, 2016). All these antibiotics also have available PNEC values. We also filtered our search of the database to five environmental matrices, namely, treated wastewater treatment plant (WWTP) effluent or municipal wastewater, industrial wastewater (wastewater from industrial sites and pharmaceutical production sites), effluent from hospital wastewater, receiving surface water (river and stream), and drinking water. We chose these environmental matrices as they are most frequently influenced by human activities and will have

**Table 1**

The mean concentration and number of samples analysed for each antibiotic and environmental matrix across 47 countries.

Antibiotic	PNEC Value	Mean Concentration (in µg/L) <sup>a</sup>					No. of analyses exceeding PNEC (No. of Analyses Performed <sup>b</sup> )					Proportion of Analyses Exceeding PNEC Globally
		Municipal wastewater	Hospital wastewater	Industrial wastewater	Surface water (river/stream)	Drinking water	Municipal wastewater	Hospital wastewater	Industrial wastewater	Surface water (river/stream)	Drinking water	
Amoxicillin	0.250	0.1	0.1	–	0.0	0.0	3 (243)	0 (3)	–	0 (230)	0 (20)	0.6%
Azithromycin	0.250	0.2	*0.9	0.0	0.0	0.0	68 (322)	31 (69)	0 (3)	46 (964)	0 (120)	9.8%
Ciprofloxacin	0.064	*577.6	*6.5	*3548.6	*383.4	0.0	460 (1485)	212 (212)	11 (13)	86 (464)	0 (28)	34.9%
Clarithromycin	0.064	*0.2	*2.8	*0.1	0.0	0.0	499 (798)	68 (103)	3 (3)	45 (860)	0 (152)	32.1%
Clindamycin	1.000	0.1	0.4	0.0	0.1	0.0	0 (248)	0 (24)	0 (3)	1 (355)	0 (20)	0.2%
Doxycycline	2.000	0.2	0.1	0.3	0.0	0.0	3 (194)	0 (15)	0 (2)	0 (266)	0 (44)	0.6%
Enrofloxacin	0.064	*53.3	*1.5	*23.0	*87.5	0.0	64 (253)	3 (29)	6 (11)	11 (344)	0 (144)	10.7%
Ofloxacin	0.500	*3.7	*4.2	0.5	*2.0	0.0	104 (3594)	45 (168)	3 (5)	32 (488)	0 (2)	4.3%
Oxytetracycline	0.500	0.1	0.1	*23119.0	0.0	0.0	17 (2625)	4 (21)	17 (28)	0 (473)	0 (40)	1.2%
Sulfamethoxazole	16.000	0.3	2.8	*18416.8	0.1	0.0	0 (2833)	1 (170)	13 (32)	1 (2558)	0 (471)	0.2%
Tetracycline	1.000	0.2	0.0	*453.5	0.0	0.0	8 (294)	0 (57)	13 (26)	0 (491)	0 (165)	2.0%
Trimethoprim	0.500	*0.6	*1.4	*3078.7	0.0	0.0	81 (1538)	50 (76)	2 (11)	32 (1437)	0 (329)	4.9%
							1307 (14427)	414 (947)	68 (137)	254 (8930)	0 (1535)	2043 (25976)
							9.0%	43.7%	49.6%	2.8%	0.0%	7.9%

<sup>a</sup> Mean concentration calculated from all database entries that reported the measured environmental concentration as either a mean, median or single value.

<sup>b</sup> One compound analysed in one sample equals one 'analysis'.

\* Values that exceed the PNEC level for each antibiotic.

the most impact on human health. With these matrices, we were able to assess in which particular environmental matrix there is the highest likelihood of the development of antibiotic resistance. We included only those entries that were classified in the database as "good" literature credibility in order to exclude any entries of low quality. We also only looked at those entries in the database that assessed either mean/median or single values to avoid double counting of samples from the same study where maximum and minimum were also reported. PNEC values, in µg/L, for each antibiotic studied, were obtained from Bengtsson-Palme et al. (Bengtsson-Palme and Joakim-Larsson, 2015).

## 2.2. Data analysis

Using our new, filtered database, we obtained a mean value (in µg/L) of the level of each antibiotic residue in each environmental matrix (Table 1). We reported on the number of analyses performed (Table 1) by counting the number of samples analysed for each antibiotic compound (i.e. one antibiotic compound in one sample equals one 'analysis'). We calculated the proportion of analyses that exceeded PNEC per antibiotic type and environmental matrix by dividing the number of analyses that exceed PNEC with the total number of analyses performed. For example, 2202 analyses reporting a mean/median or single value were performed on ciprofloxacin across all the environmental matrices with 769 of those analyses exceeding PNEC; this gives a proportion of 34.9% of analyses that exceed PNEC (see Table 1, final column).

We calculated the proportion of countries with a mean antibiotic concentration that exceeded PNEC for each antibiotic type in each matrix and displayed the results in Fig. 1. Finally, we calculated the proportion of antibiotic types and the proportion of analyses performed in each country that had a mean environmental concentration that exceeded PNEC and presented the data visually in bubble graphs (Figs. 2–5). For example, Australia had data on nine of the antibiotic types in municipal wastewater, of which one (ciprofloxacin) had a mean concentration that exceeded their PNEC. In addition, Australia had data on 392 analyses performed in municipal wastewater of which 21 analyses exceeded PNEC. The raw data for Figs. 2–5 is included as supplementary information.

## 3. Results

Table 1 demonstrates that all antibiotic types had some analyses which cross the PNEC level globally (see final column). Results also show that 7.9% of antibiotic analyses performed exceeded the PNEC value. Hospital wastewater and industrial wastewater had the highest burden of antibiotic residues with 43.7% and 49.6% of analyses exceeding PNEC, respectively. Industrial wastewater had disproportionately high mean concentrations of ciprofloxacin (3548.6 µg/L), oxytetracycline (23119.0 µg/L), sulfamethoxazole (18416.8 µg/L) and trimethoprim (3078.7 µg/L) compared to the other matrices. Drinking water had no antibiotics with a mean concentration exceeding PNEC. Ciprofloxacin (34.9%) and clarithromycin (32.1%) had the highest proportion of analyses that exceed the respective PNEC values while amoxicillin (0.6%), clindamycin (0.2%), doxycycline (0.6%) and sulfamethoxazole (0.2%) had the lowest proportion of analyses that exceed PNEC.

Fig. 1 shows the number of countries (y-axis) that had studies on the selected antibiotics and environmental matrices. The proportion of countries (in %) where the mean level of antibiotic residues exceeded PNEC is indicated as the data label on top of each stacked column that represents a specific environmental matrix. For instance, it can be seen from Fig. 1 that no country had data on amoxicillin in industrial wastewater, only one country (7%) had levels of amoxicillin exceeding PNEC in municipal wastewater, while no country had mean amoxicillin concentrations exceeding PNEC in hospital sewage, surface water and drinking water. Not shown in the graph are country-specific data: for example, only China had a mean amoxicillin level that was higher than the PNEC value in municipal wastewater. Meanwhile, Fig. 1 reveals that 43 countries had data on sulphamethoxazole concentrations in surface water (river/stream), but none had mean sulphamethoxazole levels that exceeded its PNEC. On the other hand, three countries reported sulphamethoxazole in industrial wastewater, all of which exceeded PNEC.

There was a predominance of countries with studies assessing municipal wastewater and surface water, with a paucity in the other matrices. Depending on the antibiotic, nine or less countries had data on drinking water, and only three or less on industrial wastewater. No countries have a mean level of clindamycin that exceeded PNEC in any of the environmental matrices. No countries have a mean level of any antibiotic that exceeds PNEC in drinking water. Except in drinking

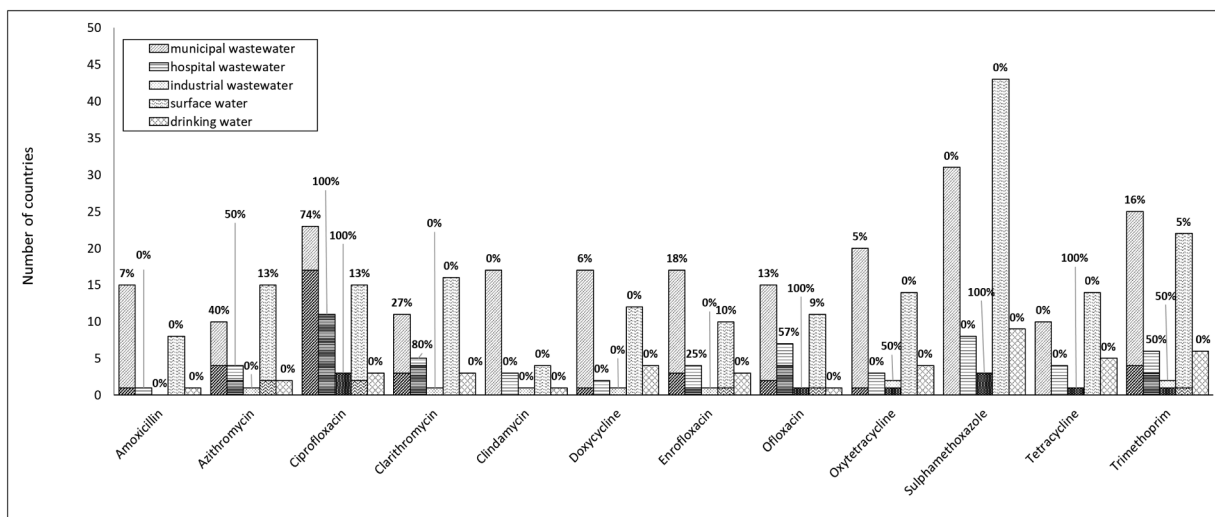


Fig. 1. Number of countries with data on antibiotic residues (Y axis) and proportion of countries (%) whose mean (of means, medians or single value data entries) level of antibiotic residues exceeds PNEC in various environmental matrices (as demonstrated by data labels).

water, Fig. 1 reveals the high burden of ciprofloxacin in all environmental matrices studied. In fact, ciprofloxacin has the highest average concentration in municipal wastewater (577.6 ug/L), hospital wastewater (6.5 ug/L), industrial wastewater (3548.6 ug/L) and surface water (384.3 ug/L) (Table 1). Clarithromycin was also detected at high frequency above its PNEC value in all the environmental matrices across the countries. While there were fewer countries with data on hospital wastewater and industrial wastewater, a high proportion of these countries had mean antibiotic values that exceeded PNEC in these matrices. For all countries with data, ciprofloxacin and clarithromycin levels exceeded PNEC values in hospital wastewater. The mean levels of ciprofloxacin, clarithromycin, ofloxacin, sulfamethoxazole and tetracycline exceeded PNEC in all countries with data for industrial wastewater.

Figs. 2–5 show the proportion of analyses performed on the antibiotic types which exceed PNEC for each of the individual countries per environmental matrix. The proportion of analyses that exceeded PNEC are represented on the y-axis while the size of bubble for each country is

indicative of the number of analyses performed in each country – the larger the bubble, the more analyses performed (exact numbers are presented in the supplementary data). The number in brackets next to the country name indicates the number of antibiotic types that each of the countries had data on. If one looks at Fig. 2 (municipal wastewater) it can be seen that the United Kingdom has the greatest number of analyses performed of which none exceed PNEC, but only data on four different antibiotic types. In contrast, Japan only has data on a single analysis performed which exceeded PNEC. India exceeded PNEC in 92.0% of analyses performed, while Croatia exceeded PNEC in 53.2%. Fig. 3 shows that Cote d’Ivoire, Sweden, China, Czech Republic and Vietnam all had 50.0% or more of their analyses exceeding PNEC in hospital wastewater. Cote d’Ivoire had data on 12 analyses performed for two antibiotic types of which 100% exceeded PNEC. Portugal had the greatest number of analyses performed (274) of which 45.6% exceeded PNEC. Fig. 4 shows that there were only three countries with data for industrial wastewater, namely, Kenya, Croatia and China. Kenya had data on six analyses performed for two antibiotic types of

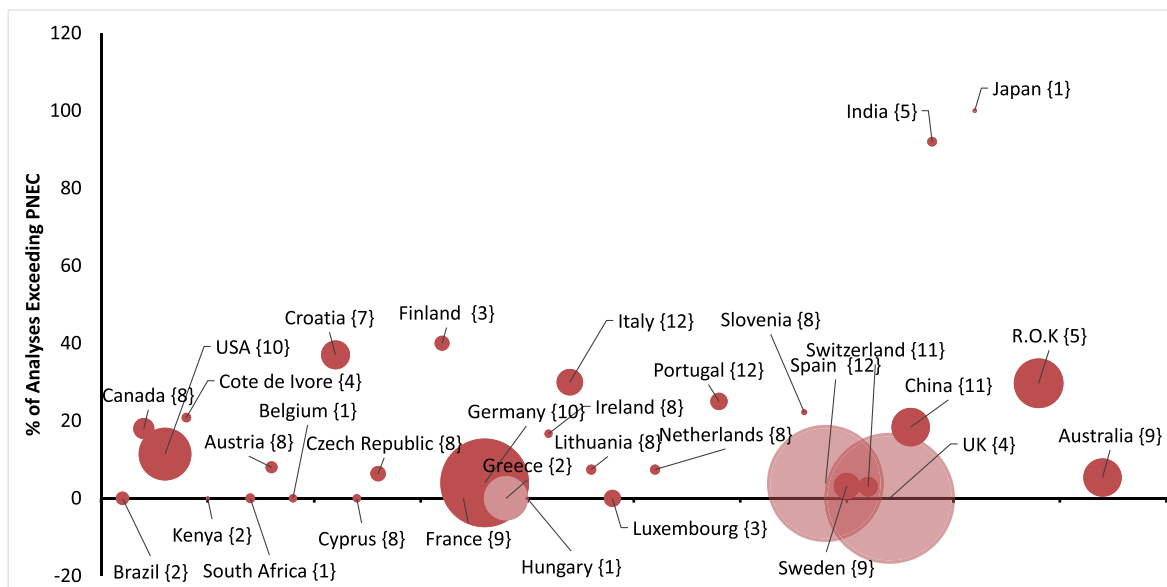


Fig. 2. Proportion of analyses performed exceeding PNEC per country in Municipal Wastewater (y-axis) (number of antibiotic types each country had data on is indicated in brackets). \*size of the bubble is indicative of the quantity of data available for each country.

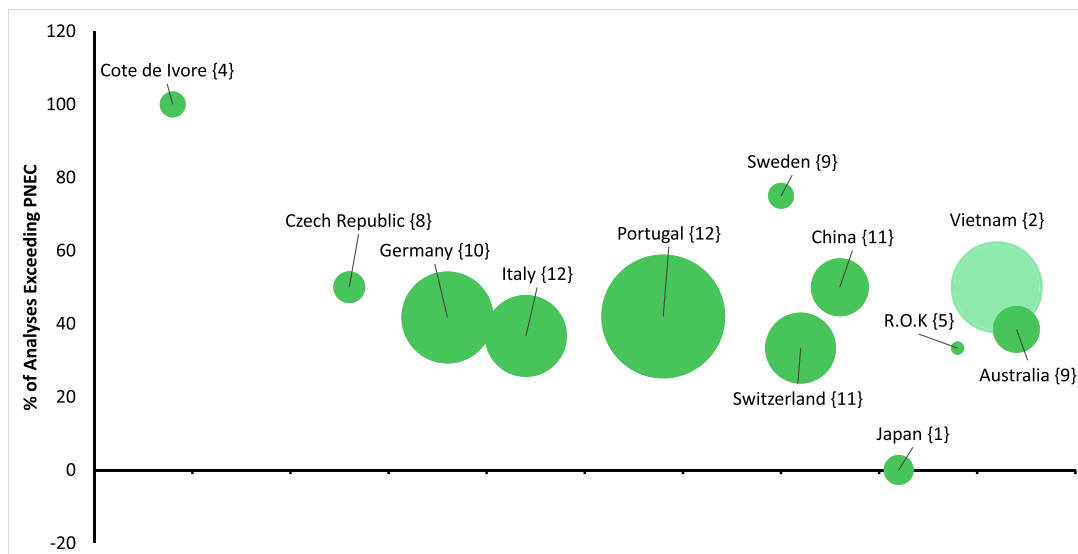


Fig. 3. Proportion of analyses performed exceeding PNEC per country in Hospital Wastewater (y-axis) (number of antibiotic types each country had data on is indicated in brackets). \*size of the bubble is indicative of the quantity of data available for each country.

which 100% exceeded PNEC, while Croatia and China exceeded PNEC in 50.0% and 46.7% of their analyses respectively, with China having data on the greatest number of analyses (105). Fig. 4 visualises 44 countries that had data on surface water (river/ stream). Kenya again, recorded the highest proportion of analyses that exceeded PNEC (66.7%) with India having data on 88 analyses performed on ten antibiotic types of which 46.6% exceeded PNEC. The remainder of the countries had proportions of less than 20% of analyses exceeding PNEC with the majority of countries having no analyses that exceeded PNEC. Germany had the data on the greatest number of analyses (1960) of which 0.4% exceeded PNEC. No countries had analyses that exceeded PNEC in drinking water (data not shown). China was the only country with analyses that exceeded PNEC in all environmental matrices, except drinking water, ranging from 14.4% in surface water to 50.0% in hospital wastewater (Figs. 2–5).

#### 4. Discussion

AMR is one of the most concerning health issues of the modern era. The environmental concentrations of antibiotics have become an important focus in research, as it has emerged that environmental matrices are a potential breeding ground for bacteria to develop resistance. The suggestion of PNEC values for antibiotics has arisen in an attempt to begin the discussion on effective reduction in antibiotic residues from our environmental matrices.

Our results show that in total, 7.9% of antibiotic analyses performed had a mean concentration that exceeded their various PNEC values across all the matrices and countries studied. These findings are concerning as it indicates that antibiotic residues are reaching our environment and water resources and may have the potential to promote AMR. The fluoroquinolone antibiotics (e.g. ciprofloxacin, enrofloxacin and ofloxacin) as well as the macrolides (e.g. clarithromycin) proved to be the most prevalent in the environmental matrices studied.

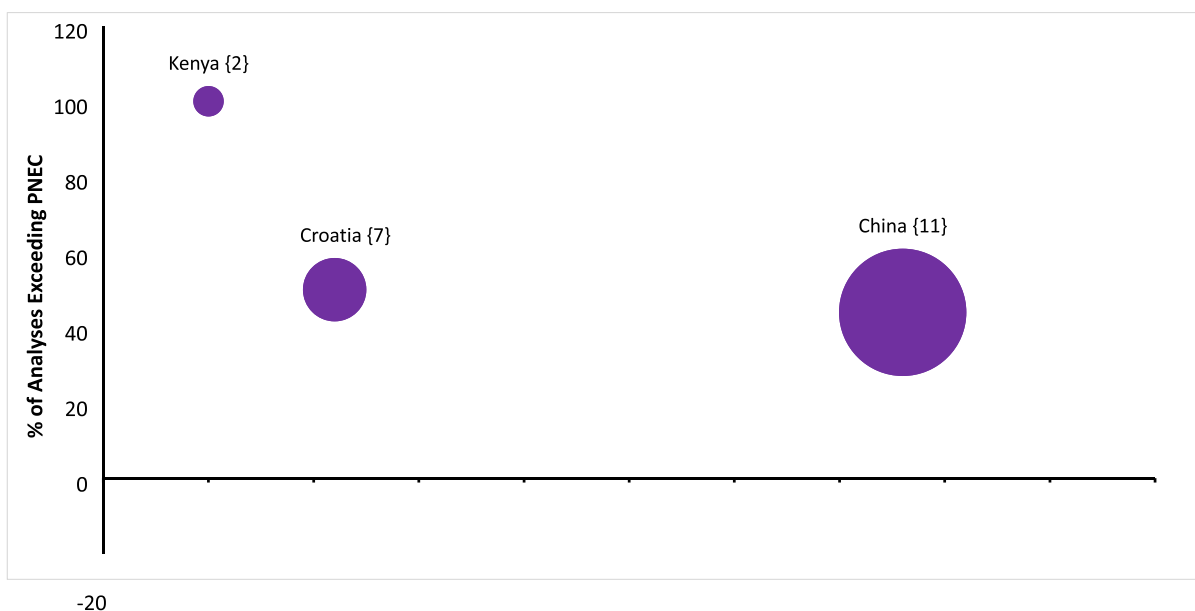


Fig. 4. Proportion of analyses performed exceeding PNEC per country in Industrial Wastewater (y-axis) (number of antibiotic types each country had data on is indicated in brackets). \*size of the bubble is indicative of the quantity of data available for each country.

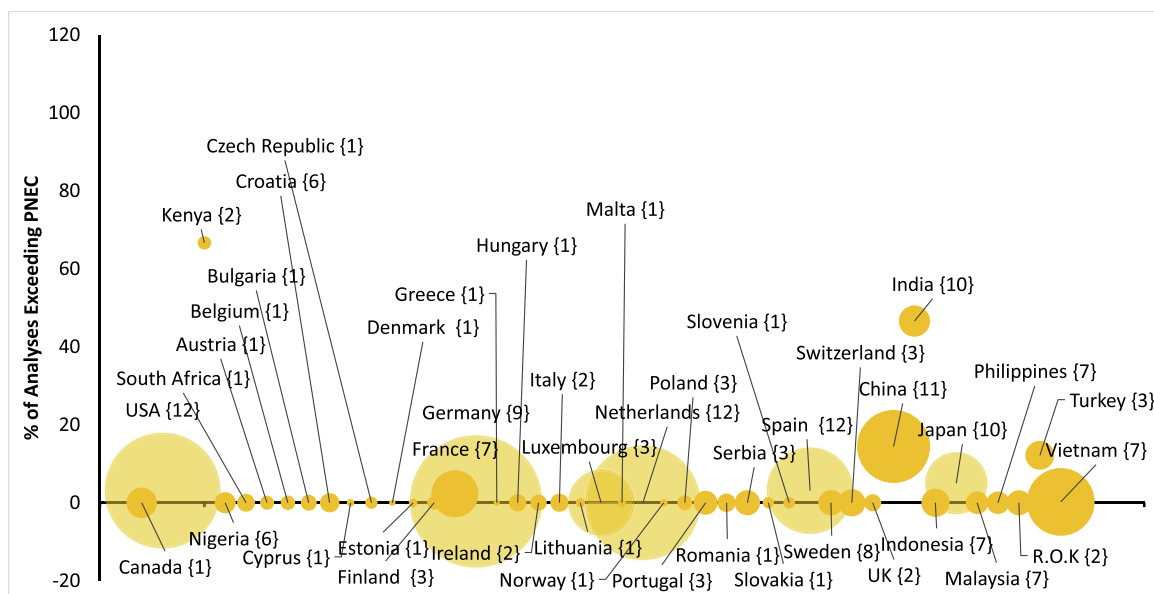


Fig. 5. Proportion of analyses performed exceeding PNEC per country in Surface Water (y-axis) (number of antibiotic types each country had data on is indicated in brackets). \*size of the bubble is indicative of the quantity of data available for each country.

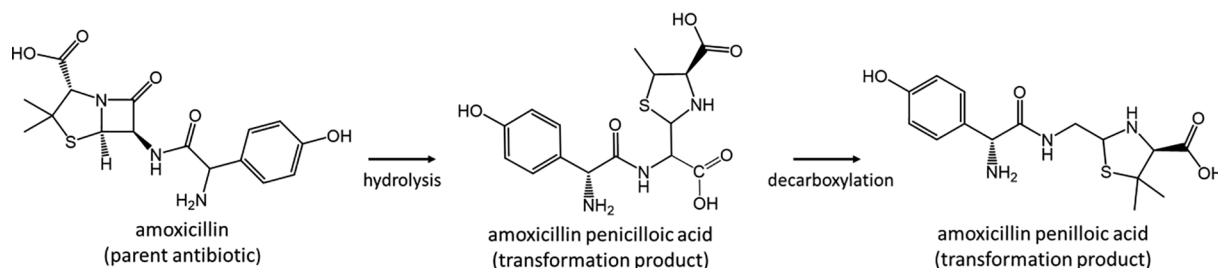


Fig. 6. Conversion of amoxicillin to its transformation products.

Fluoroquinolones are potent broad-spectrum synthetic antimicrobials that are used widely for both Gram-positive and Gram-negative bacteria. The high concentrations of these classes of antibiotics in the environment is likely a result of their high volume of use as well as their stability in the environment. In comparison, the WHO states that amoxicillin and co-amoxiclav, which are  $\beta$ -lactam antibiotics, are the most frequently prescribed antibiotics globally, because they are effective against common Gram-positive and Gram-negative bacteria (World Health Organ, 2018), and yet, the mean concentration of amoxicillin only exceeded the PNEC value in one country with data, namely China (municipal wastewater). The detection of amoxicillin in environmental water samples is rare most probably due to its unstable  $\beta$ -lactam ring that undergoes hydrolysis to form amoxicillin penicilloic acid (Fig. 6), which degrades further via decarboxylation to form amoxicillin penilloic acid (Hirte et al., 2016; Arsand et al., 2018). The prevalence of these amoxicillin metabolites in surface waters of Bangladesh has been reported recently; but most of the studies in the past do not monitor for these antibiotic metabolites (Angeles et al., 2020).

The exact amount of an antibiotic that reaches the environment varies depending on the route of application, its metabolism within the user and its route of excretion. For antibiotic classes such as  $\beta$ -lactams, fluoroquinolones and tetracyclines, excretion is usually more than 50% of the administered dose (Berkner et al., 2014). For macrolides, the excreted fraction is generally lower, but its stability in the environment is greater. Within the environment, antibiotics partition into different compartments according to their physico-chemical properties, and can be transformed by abiotic or biological processes. Fluoroquinolones for example, undergo poor biological degradation and thus accumulate in the environment, explaining at least in part why we found that

fluoroquinolones most frequently exceeded PNEC (Berkner et al., 2014). It is not surprising then that high cases of resistance to ciprofloxacin have been observed in clinical samples from patients (Iqbal et al., 1997; Redgrave et al., 2014).

From our study, we found that hospital wastewater and industrial wastewater were the matrices that had the most antibiotic residues exceeding PNEC. This is a logical finding given that hospitals are facilities in which antibiotic use is concentrated, and industrial wastewater are sites of collection of waste-products of pharmaceutical and other industrial production. Unfortunately, most of the data available in hospital wastewater were from developed countries so we were unable to draw conclusions between developed and developing countries. However, it must be noted that the majority of countries that had data on hospital wastewater are among the largest prescribers of antibiotics globally, likely explaining the large proportion of antibiotic samples that exceed PNEC in this matrix (<https://cddep.org/tool/antibiotic-prescribing-rates-country/>). It is also clear from our results that some antibiotic manufacturing industries were major sources of tetracycline and sulfamethoxazole contamination in the environment. These findings are concerning because ARGs conferring resistance to tetracyclines and sulphonamides are highly abundant in the environment, including drinking water (Guo et al., 2014). Although not tested as often as tetracyclines and sulphonamides in industrial water, the finding of relatively high proportions of fluoroquinolone analyses exceeded PNEC is equally concerning.

It may seem a comforting finding that no antibiotic types had mean concentrations that exceeded PNEC in drinking water. However, the number of countries that actually had data on drinking water was low (nine countries, all high-to-middle income) underlining the limitations

of this finding. Based on risk-assessment studies from three high-income countries, the WHO decided to prioritise other issues (e.g. waterborne pathogens) and advised not to implement costly monitoring of pharmaceuticals in drinking water unless specific circumstances dictated the need to (World Health Organization, 2012). Such circumstances may actually be present in areas where inappropriate waste management is practiced in antibiotic production facilities, especially if combined with a lack of safely managed drinking water in that area. Kenya is a clear example of this where analyses show a high proportion of antibiotic residues in its industrial wastewater sites as well as a high proportion exceeding PNEC in surface water, possibly demonstrating leakage into this environmental receiving system. It is unclear what levels of different antibiotics in drinking water are necessary for increasing the risk of antibiotic resistance in the human gut. On the other hand, there are indications that even small amounts of antibiotic may be harmful, especially in pregnant women where there is a risk of malformation of the developing fetus (Mahmood et al., 2019). One important question is how to capture the presence of major antibiotic resistant determinants within human populations exposed to low levels of antibiotics in drinking water, which may, at least be partly reflected by rates of asymptomatic carriage of antibiotic resistant bacteria and ARGs. Regular exposure of human gut microbiota to low levels of antibiotics may cause selection for antibiotic resistant microorganisms resulting in the prevalence of ARGs among commensal bacteria (e.g. *Bifidobacteria*) that colonize the intestine (Duranti et al., 2017). Intestinal colonization of ARGs may place the carrier at personal risk of infection by a resistant pathogen and contribute to the dissemination and maintenance of AMR within the community (Ljungquist et al., 2019). Antibiotic resistant bacteria carriage has been best studied for extended spectrum beta-lactamases (ESBLs). Community-based studies in Southeast Asia routinely find ESBL intestinal colonization rates of 50–80%, and the region is estimated to have more than 1 billion ESBL carriers (Karanika et al., 2016). Global prevalence of ESBL colonization is increasing by 5% annually (Woerther et al., 2013).

Our findings highlight the need to re-examine our water and sewage treatment policies in order to implement methods to control our environmental sources of antibiotic residues. Indeed, one would expect that treated municipal wastewater would have low levels of antibiotic residues as it has been through the three-step treatment process (physical, biological and chemical) (Joss et al., 2006). However, our findings indicate that many countries, including high-income countries, have samples of municipal wastewater with antibiotic concentrations exceeding PNEC. This suggests that even the most advanced countries have wastewater treatment plants that do not effectively remove many of the antibiotics assessed in this study, which subsequently enter the receiving surface waters. Indeed, 9.0% of the global analyses performed and 22 of the 32 countries with data on municipal wastewater had antibiotics with a mean concentration that exceeded PNEC. 92.0% of analyses performed in India exceeded PNEC in municipal wastewater which could be a result of improper waste management from antibiotic manufacturing combined with India's poor sanitation quality, as only 44% of the population have access only to basic sanitation (WHO, JMP, UNICEF, 2017). India also had a high proportion of analyses exceeding PNEC in surface water, likely also explained by leakage of antibiotic containing manufacturing waste, and human and animal waste into this environmental matrix.

Recently, there has been research on methods to eradicate antibiotic residues from the environment. Kurt et al. proposed that advanced oxidation processes can remove pharmaceuticals from waters (Kurt and Mert, 2017). Hu et al. demonstrated that flocculation, sedimentation and sand filtration were more effective than oxidation (Hu et al., 2018), while Burch et al. concluded that chlorination was the most effective method (Burch et al., 2019). All the studies on the removal of antibiotic residues from wastewater have made clear that no single method of wastewater treatment completely removes residues. However, a recent study reported that a combination of advanced treatment processes,

such as ozonation, followed by treatment with activated carbon, can remove more than 95% of pharmaceutical residues in wastewater (Angeles et al., 2020). Nevertheless, these types of treatment strategies can be expensive and may be cost-prohibitive, especially for low-income countries.

If one looks at which countries had antibiotic residue concentrations that exceeded the PNEC values in our study, it is clear that almost all countries that had data exceeded PNEC for at least one antibiotic, highlighting the fact that the occurrence of antibiotics in the aquatic environment is a global problem. China had antibiotic residue concentrations that exceeded PNEC in every environmental matrix examined, except drinking water. This might be a result of China's large volume of research on the subject as it was one of the only countries that had a significant amount of data on all the environmental matrices and antibiotics examined. Interestingly, despite the large population of antibiotic consumers and the high number of pharmaceutical manufacturing industries in China, it scores lower than some of the other countries in terms of proportion of antibiotics that exceed PNEC.

## 5. Strengths and limitations

As far as we are aware, this is the first study that compares average antibiotic concentrations in several environmental matrices to their PNEC. We used a large dataset of 25 976 analyses, including 48 different countries, 12 antibiotic types and five different environmental matrices. Our research is the first to quantify what proportion of the antibiotic residues in our environmental matrices globally may be likely to promote AMR. We also show which environmental matrices and which countries may have the highest burden of antibiotic residues.

The limitations of our study lie predominantly in the database used. Even though the number of analyses is seemingly high, 47 countries are certainly not all in the world, and many of the countries that had data, did not so in all the various environmental matrices resulting in gaps in information. As we filtered the database to only include those entries that reported on mean/ median or single values to avoid double counting studies which also reported on minimum and maximum values, we do acknowledge that this may have resulted in the exclusion of data from studies that only reported on minimum and maximum values. The database used also does not take into account the population served or the relative volumes of the various environmental matrices. While most of the studies included used the same principle of analysis and same units of measurement, we do acknowledge that studies may have differed in their sampling protocols which may lead to some margin of error. We also acknowledge that different countries have different infrastructure and methods of processing waste and that directly comparing matrices may not be accurate. However, we believe that results from this study emphasise the potential for AMR to develop in the environmental matrices where antibiotics frequently exceeded PNEC. Finally, while the Bengtsson and Palme PNEC values are the best estimate of prediction of development of resistance that we currently have, they are still estimates and have not been validated and therefore is open to some error as well.

## 6. Conclusions

In summary, we have demonstrated which antibiotics, which environmental matrices and which of the 47 countries included in the German database on pharmaceuticals in the environment have the highest burden of antibiotic residues. Our results provide insights on what antibiotics are most likely to result in the development of AMR, based on the frequency at which the antibiotics exceeded their PNEC values. We hope that the scientific and political community become alerted to the fact that the proportions of antibiotic residues in our environment, water resources, and wastewater effluents from municipal, hospital, and industrial facilities do exceed the levels that are likely required for AMR to develop and spread.

Future research should focus on novel and more efficient treatment systems for removal of antibiotic residues, as well as validation studies on antibiotic residues' PNEC values in the environment. We also recommend that countries with a deficit on antibiotic residues in the environment become involved in activities in order to reduce some of the gaps for more accurate risk assessment. Governments and industries need to become more active in controlling the consumption of antibiotics, in ensuring safer disposal of antibiotics wastes from hospitals and antibiotic manufacturing, and in advocating for awareness on the issue of AMR and its deleterious effects.

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## CRediT authorship contribution statement

**Amy Booth:** Conceptualization, Methodology, Formal analysis, Data curation, Writing - original draft, Visualization. **Diana S. Aga:** Conceptualization, Methodology, Validation, Resources, Writing - review & editing, Supervision. **Astrid L. Wester:** Conceptualization, Methodology, Validation, 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.

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## Appendix A. Supplementary material

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.envint.2020.105796>.

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