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The occurrence of antimicrobial residues and antimicrobial resistance genes in urban drinking water and sewage in Southern Brazil

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Abstract

Antimicrobial resistance (AMR) is currently discussed as an important issue worldwide, and the presence of antimicrobial residues (ARs) and antimicrobial resistance genes (ARGs) in the environment, especially in the water sources, is a challenge for public health. This study was conducted to evaluate the occurrence and diversity of AR and ARG in water sources from an urban center, in Southern Brazil. A total of thirty-two water samples from drinking water treatment plants (24) and sewage systems (8) were collected during two annual samplings, winter and summer. The PCR was performed by 18 ARGs, and the detection of 47 ARs was performed by LC–MS/MS. All sewage samples presented carbapenemases, ESBL, and *mcr-1* genes as well as quinolones and sulfamethoxazole residues. In drinking water, we just detected bla_{TEM} and *tetB* genes and doxycycline residues in samples before treatment. This study provides data about AR and ARG in drinking water and sewage systems showing that these sources are important reservoirs of both. The limited effectiveness of wastewater treatment processes to remove mainly AR demonstrates the need to implement better protocols of disinfection, in order to limit the spread of AMR in the environment.

Keywords Contaminant emerging concern (CEC) · Drinking water treatment plant (DWTP) · Wastewater treatment plant (WWTP) · Antimicrobial resistance genes (ARG) · Antimicrobial residues (AR)

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Introduction

In the last years, antimicrobial resistance genes (ARGs) and antimicrobial residues (ARs) have been increasingly highlighted as contaminants of emerging concern (CECs) that affect human health [1, 2]. The emergence of these contaminants is a global concern [3–5] due to alarming projections of reaching 10 million deaths and a loss of 100 trillion USD in 2050 associated with antimicrobial resistance (AMR). The World Health Organization (WHO) recognizes AMR as one of the most urgent public health threats associated with widespread suffering and economic losses with devastating consequences on the human and veterinary healthcare systems. Similarly, the United Nations Environment Programme (UNEP) has designated AMR as one of the top six emerging environmental issues [3, 4].

Current scientific evidence also shows that the phenomenon of AMR worldwide has been favored by a scenario of self-medication, incorrect or unnecessary use of antibiotics

in humans (in nursing homes or community), shortage of health education, and especially overuse and misuse of antimicrobials in agribusiness, particularly as prophylactic in aquaculture [3, 6–8]. Moreover, the lack of law to determine quantification limits and monitorization of antimicrobials in drinking water and sewage in Brazil, as in other developing countries, substantially contributes to environmental contamination due to incorrect residues discarded, especially in urban areas near hospitals and health centers [9–11].

The antimicrobials used in different sectors, such as in the treatment of infectious diseases in both humans and animals, will pass the treated organisms unchanged or be excreted as metabolites or conjugates [12, 13]. These ARs will be discarded in the environment and will act as a selective pressure, furthering the transfer of ARGs by mobile genetic elements between pathogenic and nonpathogenic microorganisms increasing the dissemination of AMR [14–16].

The ARGs occur naturally in bacterial genomes and can be classified as intrinsic (when the bacteria produce proteins necessary for the resistance mechanisms) or acquired (when the bacteria acquired the gene involved in the resistance mechanism). This gene can be from other bacteria and acquired through horizontal gene transfer (HGT), or other mechanisms of transfer [17–19].

For these reasons, drinking water and wastewater are considered important reservoirs and hotspots for ARG and antimicrobial resistance bacteria (ARB) spread [20–22]. Although processes commonly used in wastewater treatment plants (WWTPs) are designed to reduce, remove, or inactivate pathogenic bacteria, ARB, ARG, and AR have been detected in surface waters near discharge sewage points [1, 23]. In addition, bacteriophages carrying ARG have also been identified in WWTPs, suggesting that common treatment processes may not degrade or remove these genes [24]. In this context, water bodies represent an important source of ARG dissemination, once the gene exchange between pathogenic and nonpathogenic bacteria happens easily in this environment [6, 15, 25, 26].

In this sense, this study aims to assess the occurrence and diversity of ARG and AR in drinking water treatment plants (DWTPs), sewage treatment plants (STPs), and sewage pumping plants (SPPs) in an urban center in Southern Brazil, as well as to compare the findings regarding seasonal variations.

Materials and methods

Sample collection and pre-treatment samples

This study was conducted in an urban area in Southern Brazil. The sampling covers most of its neighborhoods supplying almost 1,500,000 people, according to data from the Municipal Department of Water and Sewage. Sampling was performed in the winter and summer to assess seasonal differences. All locations of sampling (DWTPs, STPs, and SPPs) included in this study are shown in Supplementary information Fig. 1.

A total of 24 samples from drinking water treatment plants (DWTP) were sampled in pre-treatment/raw (DW-R) and post-treatment (DW-PT). All samples were obtained in the winter, 2018 ($n=6$ DW-R; $n=6$ DW-PT) and summer, 2019 ($n=6$ DW-R; $n=6$ DW-PT). DWTPs follow a standard protocol of water treatment briefly consisting of fast mixing/coagulation (aluminum sulfate), flocculation, sedimentation, filtration, disinfection (chlorine), fluoridation, and pH correction [27].

Furthermore, a total of 8 samples from sewage treatment plants (STPs) and sewage pumping plants (SPPs) were collected in the winter, 2019 ($n=2$ STPs; $n=2$ SPPs) and summer, 2020 ($n=2$ STPs; $n=2$ SPPs). STPs use the activated sludge method, thickening (centrifugation), digestion (anaerobic and aerobic), and dewatering (centrifugation) for sewage treatment [28]. The map localization of sampling is presented in Supplementary information Fig. 1.

We sampled a total of 2000 mL per site in pre-sterilized glass bottles. The total volume of each sample was divided into 1000 mL pre-cleaned glass bottles for ARGs analysis and AR quantification.

All samples were transported at 4 °C and processed within 24 h. The aliquot of 300 mL was filtered using common filter paper, followed by a 0.45-μm polyamide membrane filter and vacuum filtration apparatus through 0.22-μm cellulose acetate membrane filter (Sartorius, DE) to remove particulate matter. Subsequently, the samples were stored in a refrigerator at 4 °C ± 2 until the analysis. Sampling and processing were performed in triplicate.

DNA extraction

The polycarbonate membrane filters from DWTPs were cultured in 40 mL of brain heart infusion (BHI) broth at 37 °C for 24 h. This culture was used to prepare an inoculum of approximately 0.5 McFarland for DNA extraction by thermal lysis. DNA from STP and SPP samples were extracted from polycarbonate membrane filters by DNeasy PowerSoil Kit (QIAGEN, DE) due to the complexity of the matrix. After the extraction procedures, concentration and quality of the DNA were determined using a NanoDrop Lite Spectrophotometer (Thermo Fisher Scientific Inc., Waltham, USA). The extracted DNA was stored at −20 °C for further analysis.

Detection of antimicrobial resistance genes by PCR

A total of 18 ARGs from six different antimicrobial classes were searched by PCR assay: nine β-lactams resistance

genes (bla_{TEM}, bla_{SHV}, bla_{CTX-M} bla_{KPC}, bla_{NDM}, $bla_{OXA-48-like}$, bla_{GES}, bla_{VIM}, bla_{IMP}), one macrolide resistance gene (*ermB*), one polymyxin resistance gene (*mcr-1*), three quinolone resistance genes (*qnrA*, *qnrB*, *qnrS*), two sulfonamide resistance genes (*sul1*, *sul2*), and two tetracycline resistance genes (*tetA*, *tetB*). The primers and protocols used are described in Supplementary information Table 1. Positive and negative controls were included in all reactions.

Detection of antimicrobial residues by LC–MS/MS

We performed the targeted liquid chromatography coupled to mass spectrometry (LC–MS/MS) method using an Agilent 1260 Infinity liquid chromatography coupled to a Sciex API 5000 system. The chromatographic separation was carried out on an Agela Durashell RP (3 µm, 100 mm×2.1 mm; Agela, Torrance/CA). Mobile phases were water and acetonitrile, both containing 0.1% of formic acid. Flow rate of 300 µl min^{-1} and 3-min equilibration were applied. The gradient started with 2% B and was maintained for 2 min. From minute 2 to minute 5, % B increased from 2 to 95% maintained for 3 min, decreasing by 5% in 10 min, and then again by 2% in 11 min, maintained until 15 min of analytical running. Column temperature was maintained at 40 °C and the injection volume was 5 µl.

Multiple reaction monitoring (MRM) was used in mass spectrometer analysis. Mass spectrometry parameters specific for each compound were optimized to produce the strongest signals, such as the declustering potential, cell exit potential, entrance potential, and collision energy of the precursor and production. Measurement ranged a total of 47 antimicrobials belonging to sulfonamides, macrolides, fluoroquinolones, tetracyclines, cephalosporins, and dihydrofolate reductase inhibitors classes (Supplementary information Table 2).

Electrospray ionization was performed in positive mode based on the following source parameters: collision gas (CAD) 6; curtain gas (CUR) 20; ion source gas 1 (GS1) 50; ion source gas 2 (GS2) 50; ion spray voltage (IS) 5500, and temperature 400 °C. All data were treated on MultiQuant™ software (v.2.1.1, AB Sciex). A retention time window of 30 s was applied for automatic peak integration.

Results

From a total of 24 water samples analyzed from distinct sites (12 DW-R; 12 DW-PT), we detected the bla_{TEM} and *tetB* genes only in two DW-R samples collected in the summer (DW-R2 and DW-R4; Table 1). In the post-treated samples (DW-PT), the ARG was not detected.

Meanwhile, all 8 samples from STPs and SPPs presented ARGs from different antimicrobial classes, such as β-lactams, polymyxins, quinolones, sulfonamides, tetracyclines, and macrolides, regardless of collection date (Table 1).

We can highlight the presence of carbapenemase genes in different sites as follows: bla_{NDM-1} in samples collected in winter *(*STP1, SPP2, and SPP4) and samples collected in summer (SPP2 and STP3); bla_{KPC-2} in STP3, collected in winter; and bla_{IMP-1} in SPP4, collected in summer. In addition, all sewage samples carried the *mcr-1* gene that confers resistance to polymyxins (Table 1).

Among all AR analyzed in this study, doxycycline was detected and quantified in all DW-R samples (DW-R1 to DW-R6) collected in the summer, reaching a concentration > 1000 ng L^{-1} in four different treatment stations. The nalidixic acid was detected and quantified just in DW-R5, also sampled in the summer. No AR was detected in DW-R samples (collected in the winter) nor in DW-PT samples (Table 1).

In the sewage samples (STP and SPP), a wide variety of AR was detected in both seasons. Ciprofloxacin and azithromycin were detected and quantified in all samples assessed, with higher concentrations in winter (Fig. 1). Sulfonamides (sulfamethoxazole) and trimethoprim were detected in all samples but quantified only in the STP1, SPP2, and SPP4 samples (collected in the summer). Enrofloxacin was detected and quantified in the STP1 sample (collected in the winter) and the STP3 sample (collected in the summer). On the other hand, doxycycline was detected and quantified just in the SPP4 sample (collected in the summer) (Table 1).

Discussion

In this study, we detected doxycycline in all DW-R samples collected in the summer at high concentrations in most of them. These data are corroborated by studies showing that tetracyclines have been classified among the antibiotics frequently detected not only in drinking water, but in sewage, domestic wastewaters, surface, and groundwater resources, and sludge [21, 29, 31]. for their wide use in aquaculture. Besides that, tetracyclines are reported as more stable in the environment than other antibiotics, thus allowing them to persist for longer times, spread further, accumulate at higher concentrations, and contaminate water bodies and soils [32]. Furthermore, we detected ARG and AR in DWTP only during the summer, which could be explained because there are a few rainfalls in this period that impact water levels, reaching a high concentration of CECs including ARG and AR.

Our findings showed the presence of AR and ARGs in all sewage samples, indicating the ineffectiveness of treatment protocols in removing ARGs and AR. This fact points to the urgent need to improve the monitoring and regulation of sewage treatment. Once wastewater treatment systems are

Table 1 Antimicrobial resistance genes and antimicrobial residues identified in water and sewage samples

Sample	Antimicrobial resistance genes (ARGs)	Antimicrobial residues (AR)	Conc. [ng L^{-1}]
Collected in summer			
DW-R1[a]		Doxycycline	554.0
DW-R2[a]	bla_{TEM1}	Doxycycline	1338.0
DW-R3[a]		Doxycycline	1424.92
DW-R4[a]	bla_{TEM1}, tetB	Doxycycline	676.68
DW-R5[a]		Doxycycline	1525.64
		Nalidixic acid	833.96
DW-R6[a]		Doxycycline	1061.4
Collected in winter			
STP1	bla_{TEM1}; bla_{SHV2}; bla_{CTX-M}; bla_{NDM}; $bla_{OXA-48-like}$; bla_{GES}; mcr-1; qnrB; qnrS; sul1. sul2; tetA; tetB; ermB	Sulfamethoxazole	<LOQ[†]
		Ciprofloxacin	380.16
		Enrofloxacin	169.04
		Trimethoprim	<LOQ[†]
		Azithromycin	421.36
SPP2	bla_{TEM1}; bla_{SHV2}; bla_{CTX-M}; bla_{NDM}; bla_{GES}; mcr-1; qnrB; qnrS; sul1; sul2; tetA; tetB; ermB	Sulfamethoxazole	<LOQ[†]
		Ciprofloxacin	319.72
		Trimethoprim	<LOQ[†]
		Azithromycin	480.02
STP3	bla_{TEM1}; $bla_{SHV2;}$ bla_{CTX-M}; bla_{KPC}; $bla_{OXA-48-like}$; bla_{GES}; mcr-1; qnrB; qnrS; sul1. sul2; tetA; tetB; ermB	Sulfamethoxazole	<LOQ[†]
		Ciprofloxacin	374.4
		Trimethoprim	<LOQ[†]
		Azithromycin	691.48
SPP4	bla_{TEM1}; bla_{SHV2}; bla_{CTX-M}; bla_{NDM}; $bla_{OXA-48-like}$; bla_{GES}; mcr-1; qnrB; qnrS; sul1; sul2; tetA; tetB; ermB	Sulfamethoxazole	<LOQ[†]
		Ciprofloxacin	435.25
		Trimethoprim	<LOQ[†]
		Azithromycin	854.38
Collected in summer			
STP1	bla_{TEM1}; bla_{SHV2}; bla_{CTX-M}; mcr-1; qnrB; qnrS; sul1. sul2; tetA; tetB; ermB	Sulfamethoxazole	100.00
		Ciprofloxacin	169.2
		Trimethoprim	<LOQ[†]
		Azithromycin	189.6
SPP2	bla_{TEM1}; bla_{SHV2}; bla_{CTX-M}; bla_{NDM}; mcr-1; qnrB; qnrS; sul1; sul2; tetA; tetB; ermB	Sulfamethoxazole	277.00
		Ciprofloxacin	177.4
		Trimethoprim	<LOQ[†]
		Azithromycin	97.6
STP3	bla_{TEM1}; bla_{SHV2}; bla_{CTX-M}; bla_{NDM}; mcr-1; qnrB; qnrS; sul1; sul2; tetA; tetB; ermB	Sulfamethoxazole	<LOQ[†]
		Ciprofloxacin	164.00
		Enrofloxacin	118.4
		Trimethoprim	<LOQ[†]
		Azithromycin	136.8
SPP4	bla_{TEM1}; bla_{SHV}; bla_{CTX-M}; bla_{IMP}; mcr-1; qnrB; qnrS; sul1; sul2; tetA; tetB; ermB	Sulfamethoxazole	211.6
		Ciprofloxacin	142.8
		Trimethoprim	<LOQ[†]
		Doxycycline	82.2
		Azithromycin	94.8

ARGs antimicrobial resistance genes; *AR* antimicrobial residues; *DW-R* raw drinking water

STP sewage treatment plant; *SPP* sewage pumping plant; *LOQ* limit of quantification < 5.00 ng L^{-1}

[a]We just found ARG and AR in DW-R samples collected during the summer

[†]Some AR were detected below LOQ
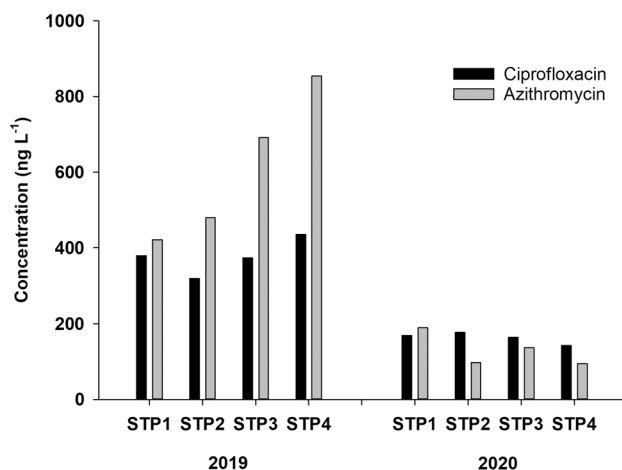
Fig. 1 Seasonal variation in antibiotic residues (ng L.$^{-1}$) of ciprofloxacin and azithromycin in the wastewater by continuous sampling. STP, sewage treatment plant; SPP, sewage pumping plant

regarded as important reservoirs for various AR and ARGs [33, 34], the effluent coming out need to be examined [34]. In our study, the sewage sampling included wastewater treatment plants that receive more than 90% of the hospital effluents in the city. This finding can be linked to the clinically relevant ARGs detected in our samples, such as bla_{KPC-2}, bla_{NDM-1}, and $mcr-1$. These genes confer resistance to carbapenems and polymyxins, antimicrobials restricted to the hospitals, that are the last resort to treat severe infections by gram-negative bacilli (BGN). According to data by the c(ANVISA), in Brazil, the resistance to carbapenems among BGN isolated from blood infection can reach 70% [35]. Then, the dissemination of carbapenemases and other genes such as $mcr-1$ into the environment poses a direct threat to public health as a potential reservoir for community-acquired infections once these genes can be transferred horizontally to human opportunistic pathogens such as *E. coli* and *P. aeruginosa*.

The sewage samples had a greater diversity of ARGs and a higher concentration of AR in the winter than in the summer. This is corroborated by results from a study conducted in Delhi, India [22], covering private retail pharmacies, public healthcare facilities, and private health clinics that involved a higher consumption of some antibiotics in the winter. The possible reasons for fewer concentrations of AR detected in sewage during the summer can be the high temperature, sunlight incidence, and increased microbial activity that promotes photo- and biodegradation of the antimicrobials, mainly beta-lactams [36–39]. In general, considering the variable weather conditions, mentioned earlier in the sampling protocol used in our study, the variation in AR is expected to occur.

The emergence and spread of ARGs and AR are complex, and predictions and prevention of AMR depend on environmental research investment, including water sources and sewage. Thus, it is important to point out that AMR is a multi-faceted issue and requires a special approach. Hence, it is essential to direct efforts towards monitoring a considerable scope of antimicrobials for human and animal use, in addition to accessing potential impacts of these residues on the environment and the AMR dissemination. Moreover, the effect of wastewater treatment on the dispersion of AR and ARGs in the environment should be clarified. Metagenomic approach to investigating the ARG profile in water bodies and analysis of plasmid types present in this environment can contribute to a better understanding of the dispersion of resistance [40].

Our results highlight the presence of AR and ARG in wastewater, even after treatment. The current evidence suggests that drinking water and sewage are an important source of AR and ARG, reflecting the environment's health in the urban centers.

Declarations

Conflict of interest The authors declare no competing interests.

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