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Occurrence and persistence of antibiotic resistance genes in wastewater treatment plants in Aracaju/SE/Brazil

Ocorrência e persistência de genes de resistência a antibióticos em estações de tratamento de esgoto em Aracaju/SE/Brasil

P. R. C. M. Trindade[1]; S. S. Dolabella[1,2]; S. Jain[1]; L. F. de J. Nascimento[1]; A. G. Sacramento[2]; F. H. F. Barbosa[2]; A. A. T. Barbosa[1,2*]

[1]*Universidade Federal de Sergipe, Programa de Pós-Graduação em Biologia Parasitária, 49107-230, São Cristóvão, Sergipe, Brasil*
[2]*Universidade Federal de Sergipe, Departamento de Morfologia, 49107-230, São Cristóvão, Sergipe, Brasil*

**ana.barbosaufs@yahoo.com.br*
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Antibiotic resistance is one of the greatest threats to public health in the 21st century and the presence of antibiotic resistant determinants in wastewater may reflect the current local clinical resistance situation. The objective of this study was to detect the presence of antibiotic resistant genes (ARGs) in Wastewater Treatment Plants (WWTPs) localized in Aracaju/SE/Brazil and verify the role of WWTPs in limiting the spread of these contaminants in the environment. Samples of raw and treated sewage were collected from four WWTPs, and subjected to DNA extraction, PCR amplification and sequencing to detect ARGs for the most consumed antibiotics by the local population. The ARGs *bla*TEM, *gyr*A, *sul*1, aacC2, *qnr*S, *erm*B and *tet*M were detected in 100% of the raw sewage samples, while in the treated samples their prevalence was 100% for *bla*TEM, *gyr*A and *sul*1, 75% for *aac*C2 and 50% for *qnr*S, *erm*B and *tet*M. This study concluded that the consumption of antibiotics is intrinsic to the prevalence of ARGs in WWTPs. Considering that the treated effluent is released into nature and that the WWTPs were not designed to remove these emerging contaminants, the need for permanent monitoring and investments in efficient removal technologies is needed. Effluent analysis is an important surveillance tool for determining strategies to contain the environmental spread of antibiotic resistance.
Keywords: resistance spread, sewage, surveillance.

A resistência a antibióticos é uma das maiores ameaças à saúde pública no século 21 e a presença de determinantes de resistência a antibióticos em águas residuais pode refletir a atual situação de resistência clínica local. Os objetivos deste estudo foram detectar a presença de genes de resistência a antibióticos (GRAs) em Estações de Tratamento de Esgoto (ETEs) localizadas em Aracaju/SE/Brasil e verificar o papel das ETEs em limitar a disseminação desses contaminantes no meio ambiente. Amostras de esgoto bruto e tratado foram coletadas de quatro ETEs e submetidas à extração de DNA, amplificação por PCR e sequenciamento de GRAs para os antibióticos mais consumidos pela população local. Os GRAs *bla*TEM, *gyr*A, *sul*1, *aac*C2, *qnr*S, *erm*B e *tet*M foram detectados em 100% das amostras de esgoto bruto, enquanto nas amostras tratadas sua prevalência foi de 100% para *bla*TEM, *gyr*A e *sul*1, 75% para *aac*C2 e 50 % para *qnr*S, *erm*B e *tet*M. Este estudo concluiu que o consumo de antibióticos é intrínseco à prevalência de GRAs em ETEs. Considerando que o efluente tratado é liberado na natureza e que as ETEs não foram projetadas para remover esses contaminantes emergentes, justifica-se a necessidade de monitoramento permanente e investimentos em tecnologias eficientes de remoção. A análise de efluentes constitui ferramenta importante de vigilância para determinação de estratégias de contenção da disseminação ambiental da resistência a antibióticos.
Palavras-chave: propagação da resistência, esgoto, vigilância.

1. INTRODUCTION

Antibiotic resistance is one of the greatest threats to public health in the 21st century. The reduced efficacy of the available drugs to treat the infectious diseases results in prolonged hospital stays, increased health care costs and treatment failures [1, 2].

Inappropriate use of antibiotics is the main factor contributing to the spread of antibiotic resistance [3]. Excessive use in animal production, agricultural activities and clinical settings has

resulted in widespread dissemination of antibiotic resistance determinants in soil and water bodies causing significant environmental and health concerns [4-8].

Antibiotics, antibiotic resistant bacteria (ARB) and antibiotic resistance genes (ARGs) are antibiotic resistant determinants, considered as emerging environmental contaminants, and are directly related to the spread of antibiotic resistance in nature [9]. ARGs, even in their extracellular form, can remain stable in nature for long periods and disseminate to other pathogens via horizontal gene transfer (HGT). The set of ARGs in a given environment constitutes its resistome. Soil, sediments, surface waters, sludge, animal waste and sewage are important reservoirs of ARGs [9, 10].

Antibiotics, ARB and ARGs eliminated by the population in the cities, have been reported to be concentrated in wastewater treatment plants (WWTPs) [10-12]. Thus, WWTPs which have the objective of removing organic and inorganic contaminants, pathogenic bacteria and environmental dissemination of diseases, also function as important reservoirs of antibiotic resistance determinants [3, 10, 12, 13]. High nutrient availability, high oxygen concentration, and suitable pH and temperature support bacterial growth in the WWTPs, also favoring selective pressures and the horizontal transfer of ARGs [13, 14]. WWTPs are in general not designed to eliminate ARGs, and thus are a source for their release into nature [10, 15-17].

Considering the relevance of the topic, the objective of this research was to analyze samples of raw and treated wastewater from municipal WWTPs located in Aracaju-SE/Brazil, to check the presence of ARGs for the classes of antibiotics most consumed by the local population.

2. MATERIAL AND METHODS

2.1 Study Area

This research was carried out in Aracaju city, capital of the state of Sergipe, located in the northeast region of Brazil. The municipality has an area of 182,163 km², with 42 neighborhoods and an estimated population of 672,614 people for the year 2021 [18].

As for sanitary sewage, the city has a service rate of 53.5%, with 117.218 sewage connections, a sewage network with a total length of 1,123.56 km and four stations called Sewage Treatment Station (STS) or Quality Recovery Station (QRS). The samples analyzed in this study were collected from the four stations (North QRS, South QRS, West QRS and Orlando Dantas STS) [19]. North QRS is the largest station (255.7 km of extension), serving a population of 374,000 people. Its treatment capacity is 540 L/s using stabilization ponds, and the treated effluent is released into the Salt River. South QRS, West QRS and Orlando Dantas STS (with 70.7, 51.7 and 53.0 km of extension, respectively) serve 58,000, 63,000 and 24,000 people respectively. The treatment process of South QRS and West QRS include Anaerobic Digesters of ascending flow, Aerobic Reactor of activated sludge and chlorination. The treated wastewater of these WWTPs is released into Pitanga and Poxin River, respectively. Orlando Dantas STS uses Aerobic Reactor of activated sludge and chlorination as the treatment process, and the treated effluent from this WWTP is released into Samambaia River.

2.2 Sample Collection and Preparation

Sewage samples were collected between March 15 and April 5, 2021, from four different stations. Two liters of raw and treated sewage samples were collected per station using sterile flasks in the raw sewage inlet channel and in the treated sewage outlet channel, respectively, totalling eight samples. The samples were stored and transported in refrigerated thermal boxes and kept at 4 ºC for a maximum of 24 hours until the beginning of the analysis. On each sampling day, composite samples were collected over 12 hours (6:00 am to 5:00 pm), with subsamples taken every 60 minutes (n = 24). Each sample (raw and treated) was homogenized and submitted to physical-chemical and microbiological analysis. A volume of 200 ml from each sample was filtered through common filter paper, and then 90 ml of the filtered volume was centrifuged at

8000 x g for 12 minutes at 4 °C. The supernatant was discarded, and the pellet was stored at -20°C and later used for DNA extraction.

2.3 Physical-Chemical and Microbiological Analysis

To characterize the samples and evaluate the efficiency of the treatments at the stations in removing pollutants, physical-chemical and microbiological analysis were carried out. Temperature and pH were measured using a portable digital multiparameter meter – HQ440D (HACH, USA). Sedimentable Solids (SS) were determined by the Imhoff cone method. Chemical Oxygen Demand (COD), Biochemical Oxygen Demand (BOD5,20), Total Coliforms (CT) and Thermotolerant Coliforms (CTL) were analyzed according to the methodology described in the Standard Methods for the Examination of Water and Effluents at the Wastewater Treatment Station.

2.4 Molecular Analysis

Total bacterial DNA was extracted from pellets obtained from concentrated raw and treated sewage samples using the commercial Wizard® Genomic DNA Purification kit (Promega, USA) following manufacturer's recommendations. The concentrations (ng/µL) and the quality of the samples were also determined by spectrophotometry.

The extracted DNAs were initially amplified by the polymerase chain reaction (PCR) using the universal primers (27F 5'-AGAGTTTGATCCTGGCTCAG-3') and 1492R (5'-GGTTACCTTGTTACGACTT-3') for amplifying the bacterial 16S rRNA gene as a positive control. The amplification reaction was performed using 10 µL of Master Mix (Promega), 2 µL of DNA (50 ng), 1 µL of each primer (10 µM) and 6 µL of ultrapure water to complete a final volume of 20 µL per sample. The conditions for the reaction consisted of initial denaturation at 95 °C for 5 minutes, 20 cycles of denaturation at 94°C for 1 minute, annealing at 55°C for 30 seconds, and elongation at 72°C for 1 minute, followed by a final extension at 72°C for 7 minutes. The amplified samples were subjected to electrophoresis in a 1.5% agarose gel stained with ethidium bromide and examined in an UV transilluminator.

The extracted DNA samples were later analyzed for the presence of ARGs, also using PCR. The ARGs to be analyzed, were selected based on the most consumed classes of antibiotics in Aracaju/SE between 2014 to 2020 [20] according to the National Controlled Products Management System (SNGPC) of the National Health Surveillance Agency (ANVISA): *bla*TEM (beta-lactams), *qnr*S and *gyr*A (quinolones); *erm*B (macrolides), *aac*C2 (aminoglycosides); and *sul*1 (sulfonamides). Although tetracyclines were not among the most consumed classes of antibiotics in the city of Aracaju, the *tet*M gene, which confers resistance to this class, was also tested. The primers used and the PCR conditions are listed in Table 1.

Table 1. Primers used to detect antibiotic resistance genes in raw and treated wastewater samples.

Gene		Primer Sequence (5′→ 3′)	Annealing Temperature (°C)	Amplification length (bp)	Reference
*bla*TEM	FW	CATTTCCGTGTCGCCCTTATTC	60	800	[21]
	RV	CGTTCATCCATAGTTGCCTGAC			
*qnr*S	FW	TTGCCCATCAAGTGAGTAATCG	58	341	[22]
	RV	AGGATAAACAACAATACCCAGTGC			
*gyr*A	FW	AGCGACCTTGCGAGAGAAAT	60	330	[22]
	RV	GGAACCGAAGTTACCCTGACC			
*erm*B	FW	TAACGACGAAACTGGCTAAAATAAG	60	419	[22]
	RV	AACATCTGTGGTATGGCGGG			
*aac*C2	FW	TAGAGGAGATATCGCGATGC	62	896	[23]
	RV	ATTATCATTGTCGACGGCCT			
sul 1	FW	CGCACCGGAAACATCGCTGCAC	58	163	[22]
	RV	TGAAGTTCCGCCGCAAGGCTCG			
*tet*M	FW	TTTATCTGTATCACCGCTTCCG	60	154	[22]
	RV	ACAATCCGTCACATTCCAACC			

2.5 Sequencing

The PCR products obtained after gene amplification were purified using the Wizard® SV Gel and PCR Clean-Up System (Promega, USA), according to the manufacturer's recommendations, quantified and sent to the Sequencing Service of the Federal University of Pernambuco (UFPE). After sequencing, the sequences obtained were analyzed in silico by aligning with data present in GenBank using the BLAST-Basic Local Alignment Search Tool (NCBI - National Center for Biotechnology Information) algorithm to confirm the identity of the detected genes.

3. RESULTS

3.1 Physical-Chemical and Microbiological Analysis

The chemical oxygen demand (COD) indicates the total amount of organic matter (biodegradable and non-biodegradable) present in a sample of water or sewage. In the crude samples, the concentration of organic matter ranged from 381.00 mg/L to 625.00 mg/L, while in the treated samples it was between 316.00 mg/L and 41 mg/L. Considering the concentrations of organic matter in the samples from all seasons, the average of the crude samples was 503.75 mg/L and of the treated ones 170.25 mg/L. The removal efficiency of organic matter was higher in the Orlando Dantas STS (93.20%) and lower in the North QRS (22.17%), with the overall average COD removal efficiency at the stations equal to 66.20% (Table 2).

Biochemical oxygen demand (BOD), which corresponds to the amount of oxygen needed to oxidize biodegradable organic matter by aerobic microbial decomposition, ranged from 360.00 mg/L to 175.00 mg/L with a general average of 287.75 mg /L in the raw samples, while in the treated samples the lowest concentration was from the Orlando Dantas STS, with 20.00 mg/L, and the highest from the West QRS (107.00 mg/L), with the average concentration in the treated samples being equal to 65.75 mg/L (Table 2).

Regarding the analysis of coliforms, in the crude samples the count ranged from $9x10^9$ and $4x10^8$ for CT and from $5x10^8$ to $1.2x10^7$ for CTL. The treated samples that showed the highest densities for CT ($1.3x10^7$) and CTL ($8x10^5$) came from the North QRS, whereas the lowest were detected at the Orlando Dantas STS with CT and CTL equal to $1.60x10^4$ and $2.2x10^2$, respectively. The temperatures of all samples varied between 28.8°C and 32.1°C, while the pH range was between 7.08 and 7.89. As for sedimentable solids, the highest concentration was equal to 4.00 ml/L in a raw sewage sample, while in samples of treated sewage from all stations the concentrations were equal to 0.00 ml/L (Table 2).

Table 2. Physical-chemical and microbiological characterization of samples collected in sewage treatment stations serving the city of Aracaju - SE.

Parameters	Treatment Stations			
	North QRS	South QRS	West QRS	Orlando Dantas STS
COD – RS (mg/L)	406,00	625,00	381,00	603,00
COD – TS (mg/L)	316,00	124,00	200,00	41,00
Removal efficiency DQO (%)	22,17	80,16	47,51	93,20
BOD – RS (mg/L)	175,00	359,00	257,00	360,00
BOD – TS (mg/L)	80,00	56,00	107,00	20,00
Removal efficiency DBO (%)	54,29	84,40	58,37	94,44
CT – RS (NMP/100mL)	$1,7x10^9$	$5\ x10^8$	$4\ x10^8$	$9\ x10^9$
CT – TS (NMP/100mL)	$1,3\ x10^7$	$5\ x10^5$	$9\ x10^6$	$1,6\ x10^4$
CTL – RS (NMP/100mL)	$5\ x10^8$	$2,2\ x10^8$	$1,2\ x10^7$	$5\ x10^8$
CTL – TS (NMP/100mL)	$8\ x10^5$	$1,4\ x10^4$	$1,3\ x10^5$	$2,2\ x10^2$
Temperature – RS (°C)	31,3	32,1	31,7	31,0
Temperature – TS (°C)	28,8	30,1	31,3	29,9
pH – RS	7,39	7,15	7,08	7,66
pH – TS	7,89	7,61	7,18	7,41
Sedimentable solids – RS (mL/L)	1,30	4,00	1,00	3,00
Sedimentable solids – TS (mL/L)	0,00	0,00	0,00	0,00

COD: chemical oxygen demand; BOD: biochemical oxygen demand; RS: raw sewage; TS: treated sewage; CT: total coliforms; CTL: thermotolerant coliforms: QRS: Quality Recovery Station; STS: Sewage Treatment Station.

3.2 Molecular analysis

All ARGs investigated (*bla*TEM, *qnr*S, *gyr*A, *erm*B, *aac*C2, *sul*1 and *tet*M) were detected in 100% of the raw sewage samples analysed. In contrast, the treated sewage samples showed the following detection percentages for each resistance gene: *bla*TEM (100%), *gyr*A (100%), *sul*1 (100%), *aac*C2 (75%), *qnr*S (50%), *erm*B (50%) and *tet*M (50%). In general, the ARGs for the analysed classes were detected in 100% of the raw samples and 75% of the treated samples. The most prevalent ARGs in treated sewage samples were *bla*TEM, *gyr*A and *sul*1 (Table 3).

Among the WWTPs, North QRS was the one that presented the lowest detection of ARGs in the treated sewage samples (3 of the 7 genes analysed), followed by Orlando Dantas STS (4 of the 7 genes analysed). However, in the South and West QRS, all ARGs analysed were present in both raw and treated samples (Table 3).

Table 3. Selected antibiotic resistance genes detected in raw and treated wastewater samples from Wastewater Treatment Plants located in Aracaju city/SE.

Antibiotic Class	Gene	Wastewater Samples							
		NRS	NTS	SRS	STS	WRS	WTS	ORS	OTS
Beta-lactams	bla_{TEM}	+	+	+	+	+	+	+	+
Quinolones	*qnr*S	+	-	+	+	+	+	+	-
Quinolones	*gyr*A	+	+	+	+	+	+	+	+
Macrolídes	*erm*B	+	-	+	+	+	+	+	-
Aminoglycosídes	*aac*C2	+	-	+	+	+	+	+	+
Sulfonamides	*sul* 1	+	+	+	+	+	+	+	+
Tetracycline	*tet*M	+	-	+	+	+	+	+	-
	16S RNA	+	+	+	+	+	+	+	+

NRS: North QRS (Quality Recovery Station) Raw Sewage; NTS: North QRS Treated Sewage; SRS: South QRS Raw Sewage; STS: South QRS Treated Sewage; WRS: West QRS Raw Sewage; WRS: West QRS Treated Sewage; ORS: Orlando Dantas STS (Sewage Treatment Station) Raw Sewage; OTS: Orlando Dantas STS Treated Sewage. (+) Amplified (-) Not amplified

The identity of the amplified genes was confirmed after sequencing and comparison with the GenBank database. The ARGs *qnr*S, *sul*1 and *tet*M showed 100% identity with the resistant genes present in *Shigella flexneri* (KU848190.1), *Pseudomonas aeruginosa* (MF135190.1) and *Actinobacillus pleuropneumoniae* (MG920812.1) genomes, respectively. The *bla*TEM, *erm*B and *aac*C2 gene sequences reached 99% identity, respectively, with resistant genes present in *Escherichia coli* (KU892718.1), *Nocardia farcinica* (KM194594.1) and *Proteus mirabilis* (CP055009.1) genomes, respectively. Finally, the amplified *gyr*A gene fragment obtained 96% identity with the resistant gene present in *Escherichia coli* (CP048609.1) genome (Table 4).

Table 4: Result of sequencing analysis of antibiotic resistance genes amplified from sewage samples using the GenBank database.

Gene	GenBank Access Number	Reference strain: species and source	Identity (%)
*aac*C2	X51534.1	*Enterobacter cloacae*, *aac*C2 gene plasmid for aminoglycoside-(3)-N-acetyltransferase isoenzyme II (AAC(3)-II).	98
	CP055009.1	*Proteus mirabilis* STIN_74 chromosome, complete genome.	99
*gyr*A	CP048609.1	*Escherichia coli* estirpe STEFF_1, chromosome, complete genome.	96
*erm*B	KM194594.1	*Nocardia farcinica* CNM20080087 erythromycin resistance methylase gene (*erm*B), partial cds.	99
	MT337736.1	*Microbacteria sp*. SI58 adenine(2058)-N(6))-methyltransferase gene (*erm*B), partial cds.	99
*bla*TEM	KU892718.1	*Escherichia coli* ETEC beta-lactam resistance protein (*bla*TEM) gene, partial cds.	99
	EF125012.1	*Shigella flexneri* beta-lactamase TEM-1 gene, complete cds.	99
*qnr*S	KU848190.1	*Shigella flexneri* M11560 quinolone resistance pentapeptide repeat protein gene QnrS1 (*qnr*S), qnrS1 allele, complete cds.	100
	CP058876.1	*Escherichia coli* estirpe STLEFF_47 unnamed plasmid2, complete sequence.	100
sul 1	MF135190.1	*Pseudomonas aeruginosa* Ps270 In1342 class 1 integron, partial sequence.	100
	CP095616.1	*Escherichia coli* dm382b plasmid p_dm382b_NDM5, complete sequence.	100
*tet*M	MG920812.1	*Actinobacillus pleuropneumoniae* 1144 TetM gene tetracycline resistance protein (tetM), partial cds.	100
	AP024523.1	*Streptococcus sp*. DNA TP1632, complete genome.	100

4. DISCUSSION

The presence of ARGs in both raw and treated wastewater poses a major pathway for the dissemination of antibiotic resistance in the environment [3, 11, 12, 24]. ARGs can exist as extracellular genetic elements (eARGs) or within the chromosomes of antibiotic-resistant bacteria (ARB), both of which can facilitate the spread of resistance through horizontal gene transfer (HGT) [25, 26]. HGT involves the transfer of genetic material between bacterial strains, with eARGs contributing through transformation, where bacteria take up free DNA from the environment, and ARB through conjugation, a process in which genetic material is directly transferred between bacteria via physical contact [25, 26]. These mechanisms accelerate the

spread of resistance across microbial populations, potentially allowing ARGs to reach pathogenic microorganisms, further complicating treatment options and public health outcomes.

This issue is further complicated by the fact that most wastewater treatment plants (WWTPs) are not effective in fully eliminating ARGs [12, 27]. In fact, research suggests that WWTPs often act as concentrators, contributing to the spread of ARGs in the environment [10, 12, 16]. Even though the treated effluents from all WWTPs in this study met the physical-chemical and microbiological standards set by environmental regulations, ARGs were still detected in both raw and treated samples. As these ARGs are released into the environment, they can enter the food chain, potentially reaching humans and animals, heightening the risk of widespread antibiotic resistance.

This inefficiency has been observed globally. Studies in countries like Germany [28], Canada [29], China [24], Denmark [30], Spain [31], Finland [32], and Portugal [33] have used conventional PCR to detect ARGs in raw and treated wastewater, evaluating the effectiveness of treatment systems. Findings consistently show that certain ARGs, like *sul*1 and *bla*TEM, persist even after treatment. For example, Liu et al. (2019) [34] observed that *sul*1 remained the most abundant gene, with minimal reduction, a pattern mirrored by *bla*TEM in other studies [35, 36]. In our study, *sul*1, *blaT*EM, and *gyr*A genes exhibited similar persistence, highlighting the stability of certain ARGs despite treatment. This reinforces the need for deeper investigation into their environmental behavior and potential impacts.

It is reasonable to assume that the most frequently detected ARGs in WWTPs correlate with the antibiotics most consumed by the local population [37]. Based on this premise, we selected the ARGs for this study according to the most commonly used antibiotics between 2014-2020 in Aracaju/SE: beta-lactams, quinolones, macrolides, aminoglycosides, and sulphonamides [20]. Unsurprisingly, ARGs corresponding to these antibiotic classes were detected. However, despite the low consumption of tetracyclines during this period (less than 5%), the *tet*M resistance gene was found in all raw sewage samples, with a detection frequency comparable to other dominant ARGs. This may be explained by the persistence and mobility of tetracycline-resistant genes, which are stable and widely present in the environment. Furthermore, HGT likely contributes to their spread, indicating that other ARGs related to less consumed antibiotics could also be prevalent but were not tested in this study.

Interestingly, the North QRS treatment plant, which uses stabilization ponds, showed lower detection of ARGs in the treated effluent. Stabilization ponds, being natural systems, allow for longer retention times, enabling processes like the sedimentation of organic matter and its subsequent decomposition by anaerobic microorganisms, which could help reduce ARGs. Neudorf et al. (2017) [29] also found that stabilization ponds performed better in removing ARGs such as *sul*1, *erm*B, *bla*TEM, and *qnr*S compared to mechanical systems. Our results confirmed the absence of *gnr*S, *erm*B, *aac*C2, and *tet*M in treated wastewater from North QRS, suggesting that this system may be more effective in ARG removal.

Despite the advantages of stabilization ponds, activated sludge systems are more commonly adopted due to their smaller space requirements [38]. In this study, the South QRS, West QRS, and Orlando Dantas STS treatment plants all utilize activated sludge technology. However, the high bacterial density within activated sludge aeration tanks can promote HGT, thereby facilitating the spread of ARGs [16, 24]. Furthermore, extracellular ARGs (eARGs) can bind to solids, which may be released into the environment when the sludge is recycled or used as fertilizer, further contributing to ARG dissemination [39].

It has been pointed out that sludge fractions with lower sedimentation have greater potential for transferring ARGs. Improved decantation optimizes operating conditions, inhibiting the proliferation and dissemination of these genes in the environment [40]. In this aspect, we can infer that the significant removal of ARGs observed in the effluents treated in the stabilization ponds of the North QRS can be attributed, among other factors, to the greater sedimentation of the sludge. On the other hand, in the reactors of the South and West QRS, mechanical aerators constantly revolve the sludge and the settling time is minimal, causing a greater disposal of ARGs in the treated sewage.

Given that these WWTPs discharge treated effluents into important water bodies used for fishing, recreation, and water supply, the release of ARGs into these environments could allow them to persist and spread. This emphasizes the urgent need to develop more effective

technologies for eliminating ARGs in WWTPs to mitigate the environmental and public health risks associated with antibiotic resistance [41]. The continued release of ARGs into water bodies increases the likelihood of transfer to commensal or pathogenic bacteria, posing a significant threat to human health. Future research should evaluate the presence of ARGs in these receiving environments, as several studies have already shown that ARGs persist at discharge sites [9, 12, 37].

In summary, the findings of this study highlight a critical gap in current wastewater treatment practices and regulatory standards. The persistent presence of ARGs in treated effluent, despite meeting existing regulatory criteria, underscores the necessity for enhanced treatment technologies and regulatory frameworks that specifically address antibiotic resistance. Implementing comprehensive ARG monitoring and management strategies will be essential in reducing the environmental and health impacts of antibiotic resistance. Additionally, continued research into the resistome of WWTPs and the dynamics of ARG spread in aquatic environments is crucial for developing effective solutions to this growing public health challenge.

5. CONCLUSION

WWTPs receive wastewater from domestic, clinical and even industrial environments. Since they are not designed with the intention of combating bacterial resistance, native microbial communities can be found in these plants, capable of disseminating resistance genes associated with horizontally transferred genetic elements. WWTPs have been shown to serve as the main reservoirs and disseminators of ARGs, necessitating their monitoring to outline strategies to combat the spread of antibiotic resistance.

This research highlights the critical need for dedicated programs and ongoing microbiological monitoring that include antibiotic resistance aspects. Sharing information on antimicrobial resistance globally is essential for informed decision-making and international cooperation. Given the vast territorial expanse of Brazil, this study's focus on the resistome in Brazilian WWTPs is particularly significant, as few studies have addressed this issue in the region. By investigating the predominant resistome in Brazilian WWTPs, this research fills a crucial gap in understanding and managing the spread of antibiotic resistance, contributing valuable insights to global efforts in combating this pressing public health challenge.

Furthermore, the current lack of ARGs in standard quality parameters for treated effluent reveals a significant regulatory gap. This highlights the pressing need to integrate ARG monitoring into regulatory protocols to better manage the environmental risks associated with the spread of antibiotic resistance.

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