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Occurrence and spread of beta-lactamases-producing *Enterobacteriaceae* isolated from river receiving treated effluent of wastewater treatment plant

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ABSTRACT

Rivers receiving effluents from urban wastewater treatment plants are suspected to be among the main sources for the development and dissemination of multidrug-resistant bacteria into the environment. In the present study, we analyzed 15 river samples in order to assess the spread of extended-spectrum β-lactamase (ESBL)-producing *Enterobacteriaceae*. One hundred eighty-eight *Enterobacteriaceae* were identified and classified as members of the genera *Pantoea*, *Klebsiella*, *Escherichia*, *Enterobacter*, *Serratia*, *Yersinia*, *Providencia,* and *Shigella*. Based on susceptibility results, the most part of isolates were highly resistant to the tested β-lactams (AMX, TIC AMC, and ATM), first-generation cephalosporins (CL), and second generation (FOX). ESBL production was determined by different methods, concluding its presence in 31.38% of the isolates by the disc approximation method, 25% by double-disk synergy test, and 28.72% by double-disk test. Given this situation, there is an urgent need to make more attention to the contamination of urban river by ESBL-producing bacteria, which constitute the main source of community infection.

Keywords: Antibiotic resistance; ESBL-producing *Enterobacteriaceae*; Wastewater treatment plants; Influent; Effluent; River

1. Introduction

Since the early 1940s when drug resistance has been recognized, a great deal has happened and many kinds of research have started aimed to determine the cause of the rapid spread of extended-spectrum β-lactamase (ESBL)-producing *Enterobacteriaceae* in the environment [1]. Despite many national and international reports, including that of the World Health Organization, urging ways to curtail it, the problem continues to grow and to evolve from one decade to the next [2].

The rational use of antibiotics in human therapy (domestic and hospital use), food-producing animals, and agriculture purposes has intensively contributed to the release of these compounds into the environment [3,4]. During the last two decades, the number of studies focusing on the spread of antibiotics, antibiotic-resistant genes (ARGs) and antibiotic-resistant bacteria (ARB), into the environment is constantly increasing with the aim to bridge

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the various knowledge gaps associated with these issues [5,6]. Anthropogenic sources, such as urban wastewater treatment plants (WWTPs), are considered as one of the main 'hotspots' of potential evolution and spreading of antibiotic resistance into the aquatic environment [7].

WWTP effluents are considered the main anthropogenic sources for antibiotics, multidrug-resistant (MDR) bacteria, and ARGs release into the environment [8]. Nevertheless, the detection of MDR and ARGs in wastewater (WW) effluents has played an increasingly dominant role and has drawn attention away in the reuse of WW. In particular, MDR carrying antibiotic-resistance genetic material can be spread into the environments, which reduce the therapeutic potential against human and animal pathogens and, finally, poses a serious problem to public health [9,10].

The relationship existing between antibiotic consumption and the emergence and development of resistances is now well documented [11]. A major contributor to this increasing resistance is the production of inactivating enzymes, in particular ESBLs. There is no agreement on the exact definition of ESBLs. Nevertheless, ESBLs are commonly defined as beta-lactamases that confer resistance to the penicillins, cephalosporins, carbapenems, and monobactems by hydrolysis of these antimicrobials. In addition, these enzymes are inhibited by beta-lactamase inhibitors such as clavulanic acid. Resistances to β-lactams mediated by ESBL are especially relevant among *Enterobacteriaceae*. In Algeria, isolation of ESBL-producing *Enterobacteriaceae* from hospitalized patients and hospital environment has been reported [12,13]; however, the influx of this organisms in environment remains poorly studied.

WWTPs combine high complex and dynamic community of microorganisms that are associated with fecal pollution from diverse sources such as WW, agricultural fecal wastes, and wildlife fecal droppings [14]. Although WWTP process remove organic matter and substantially reduce levels of fecal bacteria, they release residual concentrations of antimicrobial compounds (antiseptics, disinfectants, heavy metals, etc.) and ARGs to downstream soil and aquatic ecosystems, in concentrations leading to the selective survival of resistant bacteria [15]. Agricultural irrigation is by far the most established among the applications of WW reuse in arid and semiarid regions at all development levels and in low-income countries [16]. For this purpose, Singer et al. [17] reported that the identification of the source ARB discharged into the river after treatment is important to establish proper risk assessment and abatement procedures for human and animal WWs. Until now, there is still lack of fundamental data on the spread and dissemination of ARB in aquatic system receiving treated effluent from WWTP especially in Algeria.

The aim of this study was to detect ESBL-producing *Enterobacteriaceae* in samples from the river water where the treated effluent of WWTP is discharged the effluents of a hospital sewage treatment plant. Fecal streptococci (FS), total coliforms (TC), fecal coliforms (FC), and sulfite-reducing anaerobic bacteria (SRAB) were also determined in order to evaluate the efficiency of the plant in removing these microorganisms.

2. Materials and methods

2.1. Plant description and study site

This study was carried out in the treatment plant located 1 km of the city of Sedrata (Wilaya of Souk Ahras), situated in northeast Algeria. The WWTP is operating with conventional activated sludge and was planned for a population estimated at 100,000 equivalent inhabitants expandable to 473,000 equivalent inhabitants in 2030. It not only receives an average daily flow of about 7,000 m^3 made up primarily of domestic WW but also receives WW from hospitals and industrial plants. The treated effluent is discharged into the Oued Charef River, pouring directly into the Oued Charef dam (Fig. 1).

At least ten visits were made to the treatment plant throughout the period of study (January 2015 to June 2017), collecting an independent 100-mL sample bottle. A total

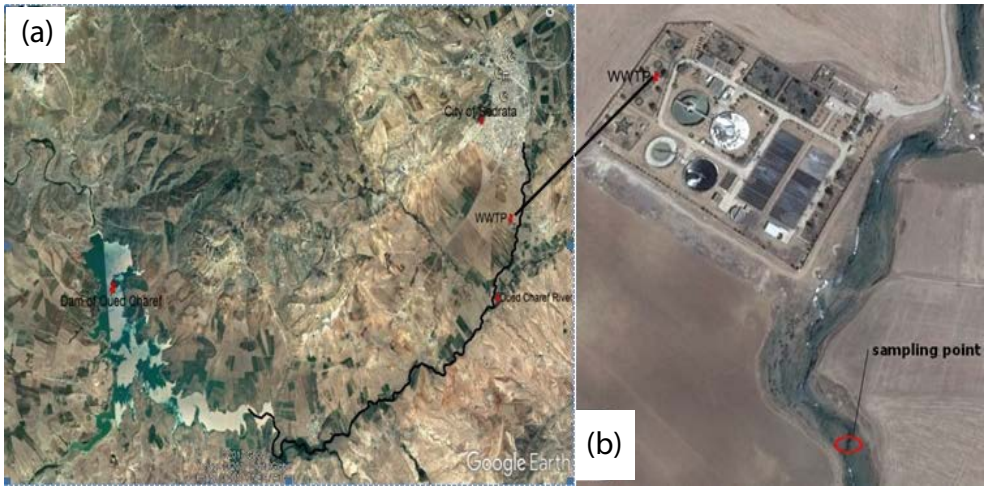

Fig. 1. Location of sampling sites in (a) the urban area of Sedrata city, WWTP, Oued Charf River, and Dam of Oued Charf and (b) sampling site, 200 m upstream the WWTP discharge.

of 30 samples were collected approximately 200 m downstream the discharge point of the treated effluent of WWTP. Sampling were made according to Guiraud [18]; the bottle is opened and immersed completely in an upturned vertical position by holding it at the bottom. It is then returned until the opening is slightly higher than the bottom and directed in the opposite direction of the current. The proximity of the surface must be at least 30 cm. Sample was transported to the laboratory at a temperature of 4°C and processed on the day of collection.

2.2. Environmental parameters

The physicochemical and microbiological parameters were pH, suspended matter, BOD_5 (biochemical oxygen demand over 5 d), and COD (chemical oxygen demand), as well as microbiological parameters. All these parameters were evaluated according to Standard Methods for Examination of Water and WW [19].

The removal efficiency of the various parameters was calculated by the following Eq. [20]:

$$\text{Removal efficiency} = \left(A - B\right) \times \frac{100}{A} \quad (1)$$

where A is the concentration (level) in the influent and B the concentration (level) in the effluent.

2.3. Enumeration and identification of bacterial strains

The river water samples were collected using sterile bottles. For quantitative analysis, a series of decimal dilutions (10^{-1}, 10^{-2}, 10^{-3}, and 10^{-4}, even up to 10^{-7} for influent WW) was prepared starting with 1 mL river water samples diluted in 9 mL of saline solution (0.9% NaCl). A volume of 100 μL from each well-homogenized dilution was inoculated onto the culture media. Each dilution was inoculated onto each of selective media: Eosin Methylene Blue Agar, Violet Red Bile Glucose, Mac Conkey agar (Oxoid), and Désoxycholate Citrate Lactose Succhrose. Plates were incubated at 37°C for 24–48 h after inoculation. Following incubation, typical of enterobacteria colonies on each plate were isolated. All isolates were identified by conventional techniques (API 20E). Strains were frozen at –30°C in nutrient broth with 15% glycerol until processed for further experimentation.

For the enumeration of TC, FC, and FS, we have used the most probable number (MPN) method consisting of inoculation of sample test portions and/or dilutions in a liquid culture medium. The media used are meat-liver Bright green bile lactose broth (BLBVB) for TC and FC, and Rothe broth and Litsky broth for FS. For SRAB, we have used the mass inoculation method (in tube) in the meat-liver agar.

2.4. Antibiotic susceptibility testing

Antimicrobial susceptibility tests were performed by using a disk diffusion method according to the CA-SFM (Committee of the Antibiogram of the French Society of Microbiology) standard guidelines [21] on Mueller-Hinton agar (Bio-Rad Laboratories). *Escherichia coli* ATCC 25922 and *Klebsiella pneumoniae* ATCC 700603 strains were used as quality controls for antimicrobial susceptibility and the ESBL screening tests, respectively. The susceptibility break points for all antimicrobials were those recommended by CA-SFM [21]. Isolates were considered as multiresistant when they exhibited resistance to three or more classes of antimicrobial agents [22]. On the other hand, isolates that exhibited zone diameters of ≤22 mm for ceftazidime, ≤25 mm for ceftriaxone, and ≤27 mm for cefotaxime and aztreonam were submitted to the ESBL detection tests.

2.5. ESBL detection

2.5.1. Double-disk synergy test

Double-disk synergy test (DDST) was carried out according to Jarlier et al. [23]. Third-generation cephalosporin disks, cefotaxime (CTX 30 μg), ceftazidime (CAZ 30 μg), ceftriaxone (CRO 30 μg), or aztreonam disk (ATM 30 μg) was placed 30 mm (center to center) from a central disk containing amoxicillin/clavulanic acid (AMC 20/10 μg). Enhancement of the zone of inhibition toward the amoxicillin/clavulanic disk after 24 h incubation at 37°C was considered indicative of a potential ESBL producer.

2.5.2. Disc approximation method

This test was conducted as described by Rahal [24]. Cefotaxime (30 μg) disks were placed at 15, 20, 25, and 30 mm center to center away from an AMC disk which is placed in the center of the plate. The test was considered positive if there is restoration of cefotaxime activity resulting by the appearance of synergy image between CTX and AMC.

2.5.3. Double-disk test

This test was performed as described by Rahal [25]. Disks of amoxicillin/clavulanic acid (AMC 20/10 μg) and third-generation cephalosporins (CTX 30 μ) were placed at 25 mm (center to center) on Mueller Hinton agar inoculated with the investigated strain. After 1 h of incubation at ambient temperature, the AMC disk was removed and replaced by CTX disk. The test was considered positive for ESBL production if the inhibition diameter of CTX disk applied after prediffusion of the AMC disk is ≥5 mm with respect to the diameter of CTX disk.

2.6. Statistical analysis

To determine if there is a statistically significant difference between the various parameters in sewage before and after treatment, *t*-test was performed. The difference were considered significant at $p < 0.05$. The Pearson's correlation analysis was performed to find out relationships among various characteristics.

3. Results and discussion

3.1. Environmental parameters

The mean values of BOD_5 and COD in influent sewage were 164.9 ± 7.17 and 215.29 ± 2.95 mg L^{-1}, respectively

(Table 1). However, the river samples collected showed moderate BOD_5 and COD mean values of 6.31 ± 1.59 and 21.52 ± 3.06 mg L^{-1}, respectively. These values are comparable with other studies reported from different parts of the world, in Portugal [26], Morocco [27], Tunisia [28], and Jordan [29]. The average decreases in BOD_5 and COD were 81.17% ± 0.92% and 75.48% ± 3.86%, respectively, with the average BOD_5 and COD values in treated WW were lower than the established range (120 and 35 mg L^{-1}, respectively) with regard to what is usually expected for domestic WW in Algeria [30]. This could be explained by the fact that the runoff waters are mixed with domestic WW, thus decreasing the concentration of COD and BOD_5 at the entrance of WWTP [31].

The microbiological analysis of raw WW and treated samples during the period from January 2015 to May 2017 showed significant differences ($p < 0.05$) in mean values of microbial load (TC, FC, FS, and SRAB) between WW (raw) and treated WW (final). Due to the variability of raw sewage quality, the influent contained higher average concentrations of TC, FC, and FS (73.13 ± 3.91) × 10^6, (34.29 ± 3.65) × $10^{6,}$ and (3.89 ± 0.47) × 10^6 MPN/100 mL, respectively, but relatively lower average concentration of SRAB (Table 1). Correspondingly, low concentrations of these microorganisms were detected in river water, with (13.39 ± 4.75) × 10^2 MPN/100 mL of TC, (0.20 ± 0.13) × 10^2 MPN/100 mL of FC, and (0.93 ± 0.11) × 10^2 MPN/100 mL of FS. The results have also shown that at the end of the treatment process, $\log_{10}$ reduction of TC, FC, FS, and SRAB were 2.17 ± 0.11, 2.27 ± 0.11, 1.53 ± 0.06, and 1.9 ± 0.9, respectively. Even the treatment showed greater removal efficiency rate of 97% ± 0.38% to 99.44% ± 0.13% in the levels of the organisms in each of these groups.

The present study demonstrates that although WW treatment processes reduce bacterial number in the sewage with removal rates close to 99%, some ARB may remain in the effluent WW. These results are not in agreement with those obtained by Prado et al. [32] which reported that the sewage treatment plant did not perform well in removing pathogenic microorganisms.

It is important to note that the removing efficiency could relate to many other factors that need to be taken into consideration such as dilution of raw WW by heavy rain, temperature of the WW, the hydraulic and solid retention time, environmental conditions, and characteristics of the raw influent, which may all play a role in the elimination of microorganisms in WW.

3.2. Isolation and identification of Enterobacteriaceae strains

In the present study, a total of 225 g negative bacilli representing different colony morphologies were recovered from 30 samples of river water. Among these, 188 isolates were identified as members of *Enterobacteriaceae* family. The distribution of the isolates from various samples is presented in Table 2. From the overall *Enterobacteriaceae* isolates, the relative abundance of each genus revealed that as expected, the most abundant isolates were members of *Klebsiella*, which was the most prevalent with 35 (18.62%) isolates followed by *E. coli* with 31 (16.49%), *Enterobacter cloacae* with 22 (%) isolates, *Serratia plymuthica* with 19 (10.11%), *Citrobacter freundii* 18 (9.57%), *Klebsiella oxytoca* with 16 (8.51%), and *Yersinia enterocolitica* with 15 (7.98%) isolates. In addition, other pathogenic genera have been isolated, but with lower frequencies: *Pantoea* spp 12 (6.38%), *Shigella* spp, and *Providencia rettgeri* with 10 (5.32%) for each.

The high prevalence of *Klebsiella* sp. in this study could be explained by the fact that these bacteria were more resistant to the treatment procedure than other coliforms (Silva et al. [33]). These causative agents of several kinds of infections and

Table 1
Physicochemical and microbiological parameters obtained on each sampling points from treatment plant

Parameters	Influent wastewater	Effluent wastewater	River water	Log10 reduction, average ± SD[e]	Removal (%), average ± SD
pH	7.14 ± 0.08	7.35 ± 0.25	7.12 ± 0.15	NA	NA[g]
SM[a] (mg × 10^3 L^{-1})	90.8 ± 6.87	3.0 ± 0.54	0.42 ± 0.27	NA	NA
[b]BOD_5 (mg L^{-1}) (mean ± SD[e])	164.9 ± 7.17	31.01 ± 1.97	6.31 ± 1.59	0.73 ± 0.02	81.17 ± 0.92
[c]COD (mg L^{-1}) (mean ± SD)	215.29 ± 2.95	52.79 ± 8.89	21.52 ± 3.06	0.62 ± 0.08	75.48 ± 3.86
Total coliforms (MPN[h]/100 mL)	(73.13 ± 3.91) × 10^6	(5.08 ± 1.13) × 10^5	(13.39 ± 4.75) × 10^2	2.17 ± 0.11	99.3 ± 0.18
Fecal coliforms (MPN/100 mL)	(34.29 ± 3.65) × 10^6	(1.9 ± 0.42) × 10^5	(0.20 ± 0.13) × 10^2	2.27 ± 0.11	99.44 ± 0.13
Fecal streptococci (MPN/100 mL)	(3.89 ± 0.47) × 10^6	(11.54 ± 0.66) × 10^4	(0.93 ± 0.11) × 10^2	1.53 ± 0.06	97 ± 0.38
Sulfite-reducing anaerobic bacteria (SRAB) (CFU[d]/100 mL)	(1.78 ± 0.38) × 10^5	(2.22 ± 0.41) × 10^3	(0.33 ± 0.2) × 10^2	1.9 ± 0.9	98.71 ± 0.28

[a]SM: Suspended matter.
[b]BOD_5: biochemical oxygen demand.
[c]COD: chemical oxygen demand.
[d]CFU: colony-forming unit.
[e]Mean and standard deviation (±SD).
[f]NT: not tested.
[g]NA: not applicable.
[h]MPN: most probable number.

Table 2
Detection of ESBL-producing *Enterobacteriaceae* using phenotypic methods

Species	No. of isolates (N = 188)	ESBL-production by					
		Double-disk synergy test (DDST)		Disc approximation method (DAM)		Double-disk test (DDT)	
		Positive (N = 47)	Negative (N = 141)	Positive (N = 59)	Negative (N = 129)	Positive (N = 54)	Negative (N = 134)
Pantoea spp	12	04	08	04	08	04	08
Klebsiella pneumoniae	35	10	25	12	23	10	25
Klebsiella oxytoca	16	04	12	07	09	06	10
E. coli	31	08	23	08	23	08	23
Enterobacter cloacae	22	07	15	07	15	07	15
Serratia plymuthica	19	05	14	06	13	06	13
Citrobacter freundii	18	04	14	06	12	05	13
Yersinia enterocolitica	15	02	13	04	11	04	11
Providencia rettgeri	10	01	09	01	09	01	09
Shigella spp	10	02	08	04	06	03	07
Total (%)	188 (100)	47 (25)	141 (75)	59 (31.38)	129 (68.62)	54 (28.72)	134 (71.28)

many of the nosocomial infections have been found to occur in both sewage and natural water sources. On the other hand, *Klebsiella* exhibits characteristics in cellular structure, including the presence of a capsular polysaccharide, which may permit these species to achieve better survival through the treatment procedure [26,34].

We have recorded high frequency of *E. coli* 31 (16.49%) in river water; this bacteria is commonly regarded as one of the first microorganisms of choice in water quality monitoring programs and serves as the primary indicator for water contaminated with fecal matter due to their prevalence in the gut of warm-blooded animals as well as high numbers excreted in both humans and animals [14].

3.3. Antimicrobial susceptibility

A total of 188 *Enterobacteriaceae* strains were isolated from river samples, and they were also screened for their antimicrobial susceptibility (Fig. 2). Results revealed high frequency of resistance to the β-lactam group, AMX (90%), AMC (88%), TIC (74%), ATM (42%), CL (72%), KF (54%), FOX (60%), and CTX (62%) and quinolones, OFX (62%), NA (60%), and FOS (58%), whereas the resistance against aminoglycosides was much reduced (0% against AK, 2.5% GM, and 18% TOB). Only amikacin retained its full activity on all tested isolates. Furthermore C, F, K, AK, GM, and IMP were effective against more than 82% of the isolates.

In recent years, many researchers have reported that WW treatment processes achieve variable and often incomplete removal of antibiotics and MDR bacteria, resulting in discharge of antibiotics into aquatic environment. The high β-lactam resistance might be explained by its intensive use and the selective pressure, which accelerated the emergence of resistance to β-lactam-β-lactamase inhibitor combinations [35].

Several surveys on antibiotic-resistant enterobacteria in treated WW are leading to divergent conclusions. Some researchers have reported that ARB have been less effectively eliminated than sensitive bacteria [36]. The abundance of ARB measured in this study closely agree with those of other studies investigating the behavior of these bacteria in WWTPs and confirmed that, in general, WWTPs efficiently remove ARB through the different treatment steps [37]. Furthermore, Silva et al. [33] suggested that WW treatment could reduce the total number of enteric bacteria in sewage but may increase the proportion of antibiotic-resistant coliforms in effluent water.

Goni-Urriza et al. [38], in their study on the impact of an urban effluent on the antibiotic resistance of *Enterobacteriaceae* in a riverine area in the north of Spain, have noticed high resistance frequency rate for quinolones (20%), tetracycline (18%), and β-lactams (13%), with higher percentages detected downstream from the WW discharge. In the present study, in addition to the high levels of resistance (>50%) to AMX, AMC, TIC, CL, KF, FOX, and CTX shown on the part of *Enterobacteriaceae* isolates, >58% of these isolates were resistant to quinolones OFX, NA, and FOS. Regarding resistance to quinolones, similar results were obtained by Ojer-Usoz et al. [39]. Our findings thus, together with those previously reported, suggest an increase in antibiotic resistance levels in WWTPs with high percentages of multiresistant strains. Moreover, our results reinforce the view that environmental compartments are directly affected by anthropogenic activities and reflect the alteration of water environments by human action [26].

Moreover, in their study, Silva et al. [33] have reported that a high percentage (89%) of multiresistant coliforms isolated from WWTPs could partially or completely transfer their resistance profiles to the recipient strain. It is evident from the data that the increase in resistance of coliforms isolated from treated WW can be explained by bacterial conjugation or other genetic mechanisms allowing the horizontal transfer genes of antibiotic resistance [40].

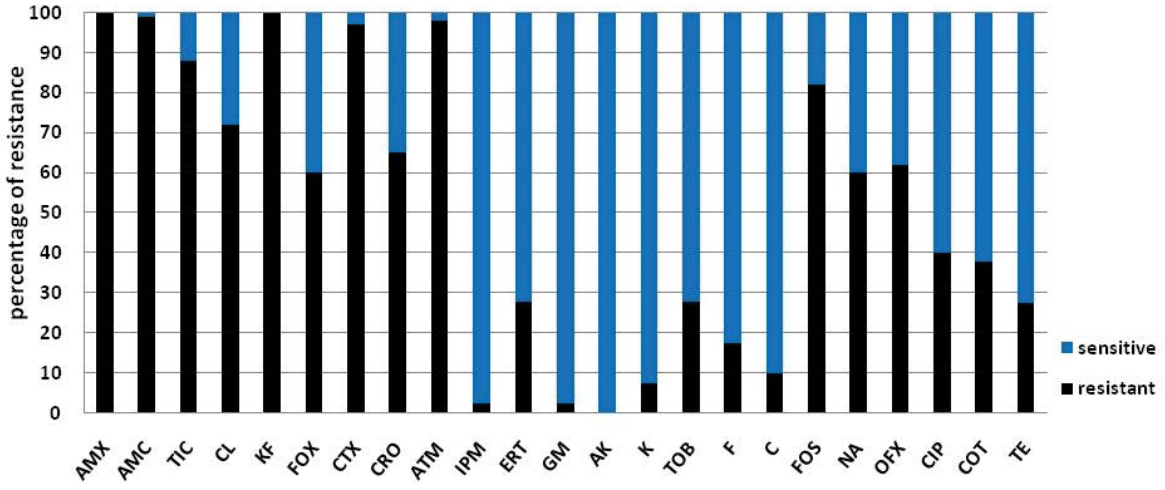

Fig. 2. Antibiotic resistance percentages for 188 isolated strains.
AMX: amoxicillin, AMC: amoxicillin/clavulanic acid, TIC: ticarcillin, CL: cephalexin, KF: cefalotin, FOX: cefoxitin, CTX: cefotaxim, CRO: Ceftriaxone, ATM: aztreonam, IPM: imipenem, ERT: Ertapeneme, GM: gentamicin, AK: amikacin, K: Kanamycin, TOB: tobramycin, F: nitrofurantoin, C: chloramphenicol, FOS: fosfomycin, NA: nalidixic acid, OFX: Ofloxacin, CIP: ciprofloxacin, COT: cotrimoxazol, TE: tetracyclin.

According to Picão et al. [41], the discharge of WW associated with MDR bacteria including ESBL-producing bacteria into an urban river is worrisome because these bacteria reach the open estuaries and could persist in the aquatic environment and act as opportunistic pathogens and/or resistance reservoirs that could accelerate the evolution of antimicrobial resistance in the community.

3.4. Detection of ESBL-producing strains

Among 188 *Enterobacteriaceae* strains isolated and screened for the production of ESBL, 54 (28.72%) were defined and selected as ESBL producers (Table 2). The DDST has to suspect the presence of ESBL in 47 (25%) strains, and the disc approximation method (DAM) was positive for 59 (31.38%) isolates, whereas the double-disk test with cefotaxime confirmed the presence of ESBLs in 54 (28.72%) strains.

Previous studies have pointed out that ARB, especially those producing ESBL, are partially eliminated in sewage treatment plants [42,43]. If they are not eliminated during the purification process, they pass through the sewage system and may end up in the environment, mainly in surface waters, groundwater, and sediments. Numerous studies have reported that WWTPs reduce the total number of bacteria, especially coliforms, in their final effluent [42]. Nevertheless, the treatment is not efficient enough to remove ARGs that are released downstream of the discharge of WWTP effluents to the receiving river [37]. In addition, by linking various environmental compartments, including municipal WW and surface water, WWTPs may facilitate horizontal transfer of resistance determinants among a rich diversity of commensals, environmental microorganisms, and clinically relevant pathogens [44]. In this regard, WWTP may contribute to the occurrence, spread, and greater abundance of both ARB and antibiotic-resistance determinants in the environment.

There are some studies indicating that the WW treatment process could be one of the routes leading to dissemination of ARB into the environment have reported that WW treatment processes achieve variable and often incomplete removal of antibiotics, resulting in discharge of antibiotics into surface waters [40,45].

The rate of ESBL-producing *Enterobacteriaceae* obtained in this study (71.28%) was higher than that reported in previous studies conducted on 221 strains isolated from WW in Rio de Janeiro, Brazil, by Chagas et al. [46], where 40% were characterized as ESBL producers. The most common ESBL-producing isolates were *Klebsiella pneumoniae*, *Enterobacter cloacae*, and *Escherichia coli*.

Levy and Marshall [47] have reported that the ESBLs, carried among *Enterobacteriaceae* such as *Enterobacter* and *Klebsiella*, destroy even the latest generations of penicillin and cephalosporins. The results obtained in this study suggest that WWTP may contribute to the dissemination of ARB, including ESBL-positive strains, from the WWTPs to the environment by emission of those bacteria from sewage into the water reservoirs such as lakes or rivers, which are receivers of WWTP discharges.

4. Conclusion

This study has demonstrated the presence and the dissemination of ESBL-producing *Enterobacteriaceae* strains in the river waters receiving treated effluent from WWTP. These may be the consequence of the anthropogenic activities, particularly in urban and clinical environments. Moreover, the dissemination of MDR bacteria may be due to the high possibility of horizontal gene transfer among strains of different *Enterobacteriaceae* genera, although WW treatment processes reduce bacterial number in the sewage with removal rates close to 99%.

Our finding has also demonstrated that despite the efficiency of removal of the organic burden by this system,

the municipal sewage continues to be a reservoir of ARB and probably ARGs causing an imminent threat to public health.

River water downstream of the treatment plant is used for irrigation, and increases in ARB concentrations may result in the contamination of agricultural products, which are generally sold in local markets. One of the solutions consist of other posttreatment processes or the application of disinfection processes that could minimize the spreading of ARB to the environment, preventing dissemination of multiresistant microorganisms and their genes of resistance into the environment, thus promoting prevention measures to protect public health.

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