

Antibiotic resistance of *Pseudomonas aeruginosa* isolated from hospital wastewater in the Czech Republic

Nikola Roulová ^{*}, Petra Mot'ková, Iveta Brožková  and Marcela Pejchalová

Department of Biological and Biochemical Sciences, Faculty of Chemical Technology, University of Pardubice, Studentská 573, Pardubice 532 10, Czech Republic
^{*}Corresponding author. E-mail: nikola.roulova@student.upce.cz

 NR, 0000-0002-7670-0615; IB, 0000-0003-2440-8265

ABSTRACT

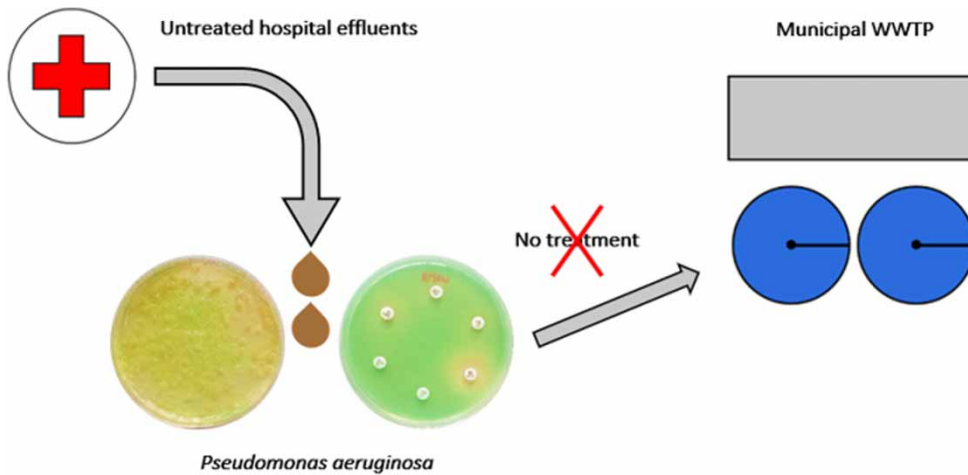
Resistant bacteria may leave the hospital environment through wastewater. The opportunistic pathogen *Pseudomonas aeruginosa*, due to its intrinsic resistance to many antibiotics and its ability to easily acquire antibiotic resistance determinants, poses a significant threat to public health. The aim of this study was to evaluate the antibiotic resistance profiles of cultivated *P. aeruginosa* in untreated hospital effluents in the Czech Republic. Fifty-nine *P. aeruginosa* strains isolated from six hospital wastewaters were tested for antimicrobial susceptibility through the disc diffusion method against seven antimicrobial agents. Resistance was found in all antibiotics tested. The highest resistance values were observed for ciprofloxacin (30.5%), gentamicin (28.8%), and meropenem (27.2%). The *P. aeruginosa* isolates also exhibited resistance to ceftazidime (11.5%), amikacin (11.5%), piperacillin-tazobactam (11.5%), and aztreonam (8.5%). Seventeen strains of *P. aeruginosa* (28.8%) were classified as multidrug-resistant (MDR). The results of this study revealed that antibiotic-resistant strains are commonly present in hospital wastewater and are resistant to clinically relevant antipseudomonal drugs. In the absence of an appropriate treatment process for hospital wastewater, resistant bacteria are released directly into public sewer networks, where they can serve as potential vectors for the spread of antibiotic resistance.

Key words: antibiotic resistance, hospital wastewater, multidrug resistance, *Pseudomonas aeruginosa*

HIGHLIGHTS

- *Pseudomonas aeruginosa* is commonly present in untreated hospital wastewater.
- The isolates presented resistance to clinically relevant antipseudomonal drugs.
- 28.8% of *P. aeruginosa* strains were identified as multidrug-resistant (MDR).
- Resistant strains are released with untreated hospital effluents into public sewer networks where antibiotic-resistant strains can easily spread.

GRAPHICAL ABSTRACT



INTRODUCTION

Healthcare facilities, where the use of antibiotics is more frequent and intensive and where there is high selective pressure in the bacterial community, are regarded as important hotspots for the selection of resistant bacteria (Varela *et al.* 2014; Tesfaye *et al.* 2019). Hospitals also play a significant role in the dissemination of antibiotic-resistant bacteria into the environment. Antibiotic-resistant bacteria may leave hospitals on colonized patients, but also via wastewater (Hocquet *et al.* 2016). Hospital effluents pose a special category of waste that is highly hazardous to public health and ecological balance due to their infectious and toxic characteristics (Fuentefria *et al.* 2011; Rodriguez-Mozaz *et al.* 2015; Proia *et al.* 2018). Hospital effluents contain large amounts of pathogenic bacteria and a variety of substances with antimicrobial activity, including not only antibiotics, but also disinfectants, heavy metals, and nonmetabolized pharmaceuticals (Chagas *et al.* 2011; Miranda *et al.* 2015; Santoro *et al.* 2015; Hocquet *et al.* 2016). Large amounts of antimicrobial compounds present in hospital wastewater exert a continuous selective pressure able to promote the development of antibiotic resistance (Hocquet *et al.* 2016; Lien *et al.* 2017). The effect of selective pressure consists in the progressive elimination of antibiotic susceptible bacteria and favoring the proliferation of resistant strains (Varela *et al.* 2014; Hocquet *et al.* 2016; Pazda *et al.* 2019). Resistant bacteria can react to selective pressure and adapt to new environmental conditions and are potential vectors for the spread of antibiotic resistance (Moges *et al.* 2014; Osińska *et al.* 2017). Moreover, selective pressure may also induce innate organisms to a rapid adaptation to selective conditions by acquiring new genetic traits (Santoro *et al.* 2015).

Despite their specific nature, hospital effluents are very often directly discharged into the public sewer network, collected at municipal wastewater treatment plants (WWTPs) and co-treated with urban wastewater (Verlicchi *et al.* 2010; Santos *et al.* 2013). In WWTPs, antibiotic-resistant bacteria, antibiotic-resistant genes, and the environmental microbiota are continuously mixed with antibiotics and their residues, and other substances with potential selective pressure that can come from various sources (Rizzo *et al.* 2013; Santoro *et al.* 2015; Manaia *et al.* 2016; Proia *et al.* 2018). Furthermore, the biological wastewater treatment process based on activated sludge, widely used in municipal WWTPs, provides optimal conditions for the transfer of antibiotic resistance genes, the development of new antibiotic resistance bacteria, and for the creation of hotspots for the spread of resistant bacteria and genes into the environment (Manaia *et al.* 2016; Nnadozie *et al.* 2017; Osińska *et al.* 2017). Wastewater offers an abundance of nutrients, high microbial concentration, and close interaction between bacteria, capable of facilitating the transfer of antibiotic-resistant genes between bacterial communities (Manaia *et al.* 2018). The final effluents, which are discharged from municipal WWTPs, contain antibiotic-resistant bacteria, sometimes at higher percentages than in raw wastewater (Novo *et al.* 2013). The worst fear apprehended for public health is the transfer of resistance genes between environmental bacteria and human pathogens (Moges *et al.* 2014; Asfaw *et al.* 2017). In addition, the accumulation of resistance features after exposure to various antibiotics and cross-resistance between agents may result in multidrug-resistant (MDR) bacteria (Israel Falodun *et al.* 2019; Krzeminski *et al.* 2019). MDR pathogens present a significant threat to global public health and are of intense clinical concern (McLain *et al.* 2016; Nnadozie *et al.* 2017). The occurrence and spread

of multidrug antibiotic resistance among bacterial pathogens may have serious consequences for human health (Pazda *et al.* 2019). In the future, most of the presently available antibiotics in medicinal practise may be ineffective against resistance bacteria and the infections caused by MDR pathogens will be completely untreatable (Asfaw *et al.* 2017; Pazda *et al.* 2019).

Although hospital effluents usually constitute a minor part of the raw influent that is drained into municipal WWTPs, they are the main source of antibiotic residues. Hospital wastewater also brings with it an enormous number of antibiotic-resistant bacteria and their gene pool (Korzeniewska & Harnisz 2012). For this reason, hospital effluents represent one of the most serious pollutants that discharge into the environment (Asfaw *et al.* 2017).

The opportunistic human pathogen *Pseudomonas aeruginosa* is a ubiquitous microorganism, able to persist in many niches but preferring moist environments. It is a common hospital-acquired pathogen responsible for severe nosocomial infections, especially in critically ill and immunosuppressed patients. *P. aeruginosa* is the major pathogen of cystic fibrosis and is also involved in a variety of infections, including respiratory and urinary tract infections, wound and soft tissue infections, and infection of patients with thermal injuries (Fuentefria *et al.* 2011; Slekovec *et al.* 2012; Santoro *et al.* 2015; Rostami *et al.* 2018).

P. aeruginosa is intrinsically resistant to many antibiotics and is capable of easily acquired antibiotic resistance determinants (Feng *et al.* 2017; Imanah *et al.* 2017). Furthermore, *P. aeruginosa* has a high potential to evolve multidrug resistance phenotypes (Golle *et al.* 2017). The presence of different resistance mechanisms has a significant clinical impact, since it limits the therapeutic options for *P. aeruginosa* infections, compromises the efficacy of antipseudomonal agents, and makes *P. aeruginosa* infection very difficult to treat (Feng *et al.* 2017; Golle *et al.* 2017; Azam & Khan 2019; Rocha *et al.* 2019). *P. aeruginosa* strains have been reported to be resistant to a wide range of currently available antimicrobial agents, such as fluoroquinolones, but also carbapenems and third-generation cephalosporins, which are preferred options in the therapy of serious infections caused by MDR strains (Imanah *et al.* 2017; Azam & Khan 2019).

Resistant strains of *P. aeruginosa* originating from patients and the hospital environment can be discharged with untreated hospital effluents into municipal sewer networks. This distribution of *P. aeruginosa* from hospitals to the environment can increase the occurrence of antibiotic resistance and contribute to the transfer of antibiotic resistance genes in the bacterial community. The aim of this study was to evaluate the occurrence and diversity of antibiotic resistance of *P. aeruginosa* strains isolated from untreated hospital wastewater. Monitoring and characterization of antibiotic-resistant pathogenic bacteria in hospital effluents is necessary to assess their potential threat to human health. In addition, knowledge of the resistance of pathogenic bacteria to current antibiotics is useful for improving the therapeutic efficacy of antibiotics in clinical practice.

METHODS

Sample collection

Untreated hospital wastewater samples were collected from six different hospitals in the Czech Republic. The healthcare facilities differed in capacity. The largest facility is Hospital 1 (1,100 beds), followed by Hospital 6 (750 beds) and Hospital 5 (550 beds). Hospitals 2, 3, and 4 have 200 beds. All hospitals are not equipped with WWTP or other wastewater treatment systems (e.g. chlorination), and untreated hospital wastewater is directly discharged into the municipal sewer network and treated at central municipal WWTP. The samples were collected at the central points of discharge of wastewater into the municipal sewer network and contained wastewater from the entire hospital area. Half-liter wastewater samples were collected by submerging sterile bottles at the sampling points, using a telescopic sampling stick. The pH of the wastewater samples was measured immediately after sampling. A total of six samples were collected from January 2020 to March 2020.

Sample processing, isolation, and identification of *P. aeruginosa*

Immediately after sampling, the samples were transported to the laboratory in a cooling box and processed within 2 h after collection. A 10 mL aliquot of wastewater was transferred to 90 mL of Tryptone Soya Broth (Soyabean Casein Digest Medium, Himedia, India) and incubated aerobically at 37 °C for 24 h. After incubation, a 100 µL aliquot of serial ten-fold dilutions of bacterial suspension was streaked on Cetrimide agar (Cetrimide Agar Base, HiMedia, India). The plates were incubated aerobically at 37 °C for 24–48 h. After incubation, presumptive large, flat blue-green pigmented colonies with irregular margins were subcultured on non-selective blood agar (Blood Agar Base No. 2, HiMedia, India). From each positive Cetrimide agar plate, five presumptive colonies were transferred on blood agar or all colonies if less than five were present. Suspect colonies of *P. aeruginosa* on blood agar with typical metallic sheen and hemolysis were identified by Gram staining and biochemical tests, catalase, oxidase, and motility. The final identification was performed using the NEFERMtest[®] 24 kit

(Erba Lachema s.r.o., Czech Republic) based on bacterial biochemical properties, according to the manufacturer's instructions.

Antimicrobial susceptibility testing

A standard disk diffusion method was used to determine the antimicrobial susceptibility profiles of *P. aeruginosa* isolates according to the recommendations of the European Committee on Antimicrobial Susceptibility Testing (EUCAST 2021a). A 24 h old pure culture of isolates standardized to 0.5 McFarland turbidity standards was swabbed on Mueller-Hinton agar (Mueller-Hinton Agar, HiMedia, India) according to the standard operational procedure. Incubation was performed at 35 ± 2 °C for 18 ± 2 h. The zones of inhibition were interpreted according to the current EUCAST Breakpoint Tables (EUCAST 2021b). The isolates were classified as 'susceptible, standard dosing regimen' (S), 'susceptible, increased exposure' (I), and 'resistant' (R) according to the recommendations of the EUCAST (EUCAST 2019). The panel of seven antimicrobial agents (Oxoid, USA) was selected for *P. aeruginosa* isolates, including piperacillin-tazobactam (30/6 µg), ceftazidime (10 µg), ciprofloxacin (5 µg), meropenem (10 µg), gentamicin (10 µg), amikacin (30 µg), and aztreonam (30 µg). When an isolate was resistant to at least one antimicrobial agent in three or more different classes of antibiotics, it was considered MDR. The reference strain *Pseudomonas aeruginosa* (ATCC 27853) was used for quality control.

RESULTS

P. aeruginosa isolates in hospital wastewater

The pH of the hospital wastewater samples analyzed ranged from 6 to 7.5. A total of 96 presumptive *Pseudomonas* spp. isolates were obtained from six hospital wastewater samples. All isolates were biochemically identified as *P. aeruginosa*. The number of *P. aeruginosa* isolates in different hospital wastewater samples is shown in Table 1. The highest occurrence of *P. aeruginosa* isolates was observed in wastewater from Hospitals 3 and 4 (24 strains), while in wastewater from Hospital 2 only three *P. aeruginosa* isolates were detected. The pH of the wastewater at this sampling point had the highest value of all wastewater samples (7.5); however, the pH was still within the range that enabled the growth of *P. aeruginosa*.

Antimicrobial susceptibility profiles

Of the 96 *P. aeruginosa* strains obtained from untreated hospital effluents, 59 strains were selected for antibiotic susceptibility testing against a panel of seven antimicrobial agents. Resistance was found for all antibiotics tested in this study. The highest resistance rates were observed for ciprofloxacin (30.5%, 18/59), followed by gentamicin (28.8%, 17/59) and meropenem (27.2%, 16/59). Resistance to piperacillin-tazobactam was detected in seven of *P. aeruginosa* isolates (11.9%, 7/59). The same levels of resistance were observed both against ceftazidime (11.9%, 7/59) and amikacin (11.9%, 7/59). Resistance to aztreonam was detected relatively rarely (8.5%, 5/59). A significant proportion of strains were categorized as 'susceptible, increased exposure' (I). All *P. aeruginosa* strains were classified either resistant (R) or susceptible, increased exposure (I) to piperacillin-tazobactam, ciprofloxacin, ceftazidime, and aztreonam. No *P. aeruginosa* strains were characterized as 'susceptible, standard dosing regimen' (S) to these antimicrobial agents. Furthermore, six *P. aeruginosa* strains were classified as susceptible, increased exposure (I) to meropenem (Table 2).

Table 1 | Number of *Pseudomonas aeruginosa* strains isolated from wastewater in different hospitals

Sampling points	<i>Pseudomonas aeruginosa</i> (n=96) Number of strains (%)
Hospital 1 (hospital wastewater 1)	16 (16.7)
Hospital 2 (hospital wastewater 2)	3 (3.1)
Hospital 3 (hospital wastewater 3)	24 (25)
Hospital 4 (hospital wastewater 4)	24 (25)
Hospital 5 (hospital wastewater 5)	21 (21.9)
Hospital 6 (hospital wastewater 6)	8 (8.3)
Total	96 (100)

Table 2 | Resistance of *Pseudomonas aeruginosa* strains isolated from six hospital wastewater samples

Antibiotic class	Antibiotic (concentration)	Number of <i>Pseudomonas aeruginosa</i> strains (%) (n=59)		
		S	I	R
Penicillins	Piperacillin-tazobactam (30/6 µg)	0 (0)	52 (88.1)	7 (11.9)
Cephalosporins	Ceftazidime (10 µg)	0 (0)	52 (88.1)	7 (11.9)
Fluoroquinolones	Ciprofloxacin (5 µg)	0 (0)	41 (69.5)	18 (30.5)
Carbapenems	Meropenem (10 µg)	37 (62.7)	6 (10.2)	16 (27.1)
Aminoglycosides	Gentamicin (10 µg)	42 (71.2)	0 (0)	17 (28.8)
	Amikacin (30 µg)	52 (88.1)	0 (0)	7 (11.9)
Monobactams	Aztreonam (30 µg)	0 (0)	54 (91.5)	5 (8.5)

S, susceptible, standard dosing regimen; I, susceptible, increased exposure; R, resistant.

Twenty-five isolates (42.4%, 25/59) were resistant to at least one antibiotic tested. Resistance to three or more antimicrobials was observed in 28.8% (17/59) of *P. aeruginosa* isolates and all of these *P. aeruginosa* isolates (28.8%, 17/59) were classified as MDR. Generally, *P. aeruginosa* isolates exhibited seven multidrug resistance patterns that ranged from three to six antimicrobial agents (Table 3). Most of the MDR isolates were resistant to three antimicrobial agents (52.9%, 9/17). Four isolates (23.5%, 4/17) showed resistance to the six antibiotics tested. Two isolates (11.8%, 2/17) were resistant to five antimicrobial agents and two isolates (11.8%, 2/17) were resistant to four antimicrobial agents. The most common resistance pattern was ciprofloxacin-meropenem-gentamicin, observed in eight isolates (47.1%, 8/17).

Although resistant strains of *P. aeruginosa* were detected in all hospital wastewater samples, a significant difference in their presence was observed. High levels of resistant strains were detected in wastewater of Hospital 4, where 63.6% of *P. aeruginosa* isolates were resistant to at least one antimicrobial agent. Similarly, strains isolated from wastewater of Hospital 3 showed high resistance values. The percentage of isolates resistant to at least one antibiotic was 53.8%. On the contrary, the occurrence of resistance strains in wastewater of Hospitals 5 and 6 was insignificant (Table 4). MDR *P. aeruginosa* strains were detected in four hospital effluents. The prevalence of MDR patterns was varied in different hospital wastewater samples. Hospital 3 wastewater contained only the MDR pattern ciprofloxacin-meropenem-gentamicin. In addition, the resistant pattern piperacillin-tazobactam-ciprofloxacin-ceftazidime-meropenem-gentamicin-amikacin was found exclusively in wastewater from Hospital 4.

DISCUSSION

In this study, antibiotic-resistant profiles of cultivable *P. aeruginosa* were analyzed in samples of untreated hospital effluents. *P. aeruginosa* resistant strains were detected in all samples studied, indicating that these strains can be disseminated from the hospital environment via the wastewater system. However, the different prevalence rates of resistant strains in hospital effluents were revealed. The diversity in the occurrence of *P. aeruginosa* resistant strains in individual samples was significant. The

Table 3 | Frequency of resistant patterns of multidrug-resistant *Pseudomonas aeruginosa* strains

Multidrug-resistant pattern	Multidrug-resistant <i>Pseudomonas aeruginosa</i> strains (n=17) Number of strains (%)
MEM-CIP-CN	8 (47.1)
CAZ-CIP-CN	1 (5.9)
CIP-CN-AK-ATM	1 (5.9)
TZP-CAZ-MEM-CIP	1 (5.9)
TZP-MEM-CIP-AN-AK	1 (5.9)
TZP-CAZ-MEM-CIP-AK	1 (5.9)
TZP-CAZ-MEM-CIP-CN-AK	4 (23.5)

TZP, piperacillin-tazobactam; CAZ, ceftazidime; MEM, meropenem; CIP, ciprofloxacin; CN, gentamicin; AK, amikacin; ATM, aztreonam.

Table 4 | Frequency of resistant strains of *Pseudomonas aeruginosa* in different hospital wastewater samples

Resistant pattern	Number of <i>Pseudomonas aeruginosa</i> resistant strains (n=25)					
	HW1 n=12	HW2 n=3	HW3 n=13	HW4 n=11	HW5 n=13	HW6 n=7
ATM	2	–	–	–	–	2
CIP	1	–	–	–	–	–
CN	1	–	–	–	1	–
MEM	–	1	–	–	–	–
MEM-CIP-CN	–	–	7	1	–	–
CAZ-CIP-CN	–	–	–	–	1	–
CIP-CN-AK-ATM	–	–	–	1	–	–
TZP-CAZ-MEM-CIP	1	–	–	–	–	–
TZP-MEM-CIP-AN-AK	–	–	–	1	–	–
TZP-CAZ-MEM-CIP-AK	–	–	–	1	–	–
TZP-CAZ-MEM-CIP-CN-AK	1	–	–	3	–	–
The percentage of resistant strains	50	33.3	53.8	63.6	15.4	28.6

TZP, piperacillin-tazobactam; CAZ, ceftazidime; MEM, meropenem; CIP, ciprofloxacin; CN, gentamicin; AK, amikacin; ATM, aztreonam; HW, hospital wastewater.

percentage of resistant strains was independent of the size of the hospital since the highest occurrence rate (63.6%) was observed in Hospital 4 with a capacity of 200 beds. In contrast, the lowest prevalence of resistant strains (15.4%) was observed in Hospital 5, with a capacity of 550 beds. The different occurrence of resistant strains in wastewater from different hospitals may be due to differences in the focus of medicinal activity and the current composition of patients hospitalized at the time of sample collection in the hospital.

In the present study, resistance to ciprofloxacin was detected the most frequently. Since ciprofloxacin plays an irreplaceable role in the treatment of *P. aeruginosa* infection, resistance to this antibiotic can cause serious complications in the therapeutic strategy. Ciprofloxacin is the most effective fluoroquinolone against *P. aeruginosa* and, for this reason, is also one of the most widely used antibiotics against this bacterium (Rehman *et al.* 2019). Exposure to high concentrations of fluoroquinolones in the hospital environment can contribute to the development of resistance to ciprofloxacin through overexpression of the efflux pump or by acquisition of ciprofloxacin-resistant genes through horizontal gene transfer (Xu *et al.* 2021). The occurrence of ciprofloxacin resistance in *P. aeruginosa* water isolates is closely associated with hospital wastewater. In the present study, resistance to ciprofloxacin was detected in 30.5% of *P. aeruginosa* strains. Also, Magalhães *et al.* (2016) reported a high resistance to ciprofloxacin in *P. aeruginosa* isolates obtained from raw and treated hospital effluents. The percentage of isolates resistant to ciprofloxacin was 78% (7/9) and 63% (6/8), respectively. On the contrary, no ciprofloxacin-resistant *P. aeruginosa* strains were detected in receiving river water. According to the previous study, Fuentesfria *et al.* (2011) observed resistance to ciprofloxacin only among *P. aeruginosa* strains isolated from hospital wastewater. In superficial water, no ciprofloxacin resistance was detected. Similarly, resistance to ciprofloxacin was not present in 27 *P. aeruginosa* strains isolated from municipal wastewater (Luczkiewicz *et al.* 2015). Another study also reported lower levels of resistance to ciprofloxacin in *P. aeruginosa* strains obtained from municipal wastewater and surface water. The percentage of isolates resistant to ciprofloxacin was 9.4% (5/53) (Govender *et al.* 2021).

Resistance to the carbapenem antibiotic meropenem was observed relatively often. Resistance to this antibiotic was determined with a frequency of 27.2%. This finding is consistent with the increasing worldwide occurrence of carbapenem resistance in *P. aeruginosa* strains (Rostami *et al.* 2018; El-Mahdy & El-Kannishy 2019). Moreover, these strains are frequently isolated from patients with hospital-acquired infection and could be transferred with hospital effluents to municipal WWTPs (Govender *et al.* 2021; Pérez-Corrales *et al.* 2021). Haller *et al.* (2018) detected significant resistance levels to carbapenems meropenem and ertapenem in *Pseudomonas* spp. obtained from hospital effluents in Singapore. Similarly, a high percentage of resistance to carbapenems presented *P. aeruginosa* strains isolated in wastewater from two hospitals located in Rio Grande do Sul, Brazil (Fuentesfria *et al.* 2011). Vaz-Moreira *et al.* (2016) also found resistance to meropenem in *P. aeruginosa* strains isolated from hospital effluent. Nevertheless, resistance to meropenem was less common,

with prevalence values of 33.3% (12/36). Resistance to carbapenems is a serious threat to global public health, since carbapenems constitute the most effective antibiotics for the treatment of MDR *P. aeruginosa* infections (Rostami *et al.* 2018; El-Mahdy & El-Kannishy 2019). Carbapenem-resistant *P. aeruginosa* was recognized as a critical priority pathogen that poses the utmost threat to human health by the World Health Organization (WHO). This implies that effective therapy is not available and new antibiotics are urgently needed (Balkhair *et al.* 2019; Govender *et al.* 2021).

A significant difference in aminoglycoside resistance rates (gentamicin and amikacin) was observed among the tested *P. aeruginosa* isolates. Resistance to gentamicin was found in 28.8% (17/59) of *P. aeruginosa* strains and was the second most frequently detected resistance, while resistance to amikacin was observed relatively rarely (11.7%, 7/59). The high resistance to gentamicin in *P. aeruginosa* strains is consistent with the EUCAST recommendation that this bacterium is not a good target for the therapy of gentamicin. Amikacin can still be used in the therapy of *P. aeruginosa* infection. Nevertheless, for the treatment of systemic infection, amikacin must be used in combination with other active therapy (EUCAST 2021b). In another study, the diversity of aminoglycoside resistance in *P. aeruginosa* hospital effluent isolates was also detected. Forty-one *P. aeruginosa* isolates were obtained from hospital wastewater in Rio de Janeiro. The percentage of isolates resistant to gentamicin was 15%, while resistance to amikacin was the least common and was determined with a frequency of 2% (Miranda *et al.* 2015). According to a previous study, a difference in aminoglycoside resistance levels was found in *P. aeruginosa* isolates obtained from raw and treated hospital wastewater. Seven of nine isolates (78%, 7/9) in raw hospital wastewater were resistant to gentamicin, while only four isolates showed resistance to amikacin (44%, 4/9). In treated hospital effluent, the resistance rate to gentamicin was even higher than in raw wastewater (88%, 7/8). Resistance to amikacin in treated hospital wastewater was 25% (2/8) (Magalhães *et al.* 2016). Resistance to gentamicin was less common in 94 *Pseudomonas* spp. isolates from hospital effluent in Cluj-Napoca, Romania. Eighteen isolates exhibited resistance to gentamicin (17.02%, 18/94) (Butiuc-Keul *et al.* 2021). Similarly, low resistance to gentamicin presented *P. aeruginosa* strains obtained from hospital wastewater in Nigeria. Resistance to this antibiotic was detected in seven isolates (15.9%, 7/44) (Israel Falodun *et al.* 2019).

Resistance to the third-generation cephalosporin ceftazidime (11.9%, 7/59), as well as to the combination of piperacillin with tazobactam (11.9%, 7/59), was observed rarely. In the case of aztreonam, only five isolates (8.5%, 5/59) were resistant to this antimicrobial agent. Santoro *et al.* (2015) also found high susceptible values to piperacillin-tazobactam and ceftazidime in 27 *P. aeruginosa* strains isolated from hospital wastewater in the city of Rio de Janeiro, Brazil. Piperacillin-tazobactam were effective against all isolates and resistance to ceftazidime showed five isolates (18.5%, 5/27). In contrast, the highest proportion of resistance was detected for aztreonam (62.9%, 17/27). Comparable resistance rates to ceftazidime, piperacillin-tazobactam, and aztreonam were detected in 27 *P. aeruginosa* strains obtained from municipal wastewater in northern Poland. The majority of isolates showed resistance to aztreonam (70.4%, 19/27), while resistance to ceftazidime was observed in five isolates (18.5%, 5/27). No *P. aeruginosa* strains were resistant to piperacillin-tazobactam (Luczkiewicz *et al.* 2015). In the other study, in which the resistance profiles of the *P. aeruginosa* strains from WWTPs effluents were determined, the highest proportion of these isolates was resistant to ceftazidime (37.1%, 23/62). Also, resistance to piperacillin-tazobactam was commonly observed (30.6%, 17/62) (Golle *et al.* 2017).

Aside from resistance to a single antibiotic, the most significant public health problem is the accumulation of resistance features, which may result in MDR strains. In the present study, 17 *P. aeruginosa* strains (28.8%, 17/59) were classified as MDR. Israel Falodun *et al.* (2019) reported the high presence of multidrug *P. aeruginosa* strains in hospital wastewater in Nigeria. The percentage of MDR strains was 93.2% (41/44). Similarly, a significant proportion of *P. aeruginosa* strains in hospital wastewater in Rio de Janeiro presented MDR profiles (83%; 34/41) (Miranda *et al.* 2015). In contrast, only six of the 27 *P. aeruginosa* strains had multidrug resistance profiles and were classified as MDR (22.2%, 6/27) in hospital wastewater in Rio de Janeiro (Santoro *et al.* 2015). Nevertheless, as reported by Luczkiewicz *et al.* (2015), the real prevalence of multidrug resistance in the bacterial community is not well established, due to differences in the definition of this phenomenon.

CONCLUSION

Resistant *P. aeruginosa* strains were found in all hospital wastewater samples studied. Almost 30% of *P. aeruginosa* strains were resistant to three or more antibiotics and classified as MDR. The results revealed considerable resistance to important antimicrobial agents such as ciprofloxacin and meropenem, which are the antibiotics of choice for the therapy of *P. aeruginosa*. Increasing resistance to currently effective antimicrobial agents brings complications to the treatment process

of human *P. aeruginosa* infection. This situation is worsened by the fact that hospital effluents, which commonly contain resistance bacteria, are frequently discharged into public sewer networks without any treatment aimed at reducing bacterial contamination. The release of *P. aeruginosa* resistant strains through hospital wastewater to municipal wastewater systems could result in extensive genetic exchange by horizontally transferring resistance features to other competent bacteria. For these reasons, it would be appropriate to create reference standards and specific treatment methods to manage hospital effluents.

DATA AVAILABILITY STATEMENT

All relevant data are included in the paper or its Supplementary Information.

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