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Antibiogram profile of *Pseudomonas aeruginosa* isolated from some selected hospital environmental drains

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ABSTRACT

Objective: To isolate, identify and characterize *Pseudomonas aeruginosa* (*P. aeruginosa*) from hospital drains using culture-based and PCR methods.
Methods: Wastewater samples were obtained from hospital drains between August and October, 2015, using standard culture-based methods for isolation of *P. aeruginosa*. The isolates were further confirmed by specie-specific primer sets. Antimicrobial susceptibility testing of the isolates was conducted using the Kirby-Bauer disc diffusion method.
Results: The mean *P. aeruginosa* population densities were expressed ranging between ($1.7 \times 10^5 \pm 0.1$) and ($6.1 \times 10^5 \pm 0.2$) CFU/mL. The antimicrobial susceptibility profile of the *P. aeruginosa* isolates revealed that all the isolates 96/96 (100%) were resistant to penicillins (amoxicillin and cloxacillin) as well as penicillin/β-lactamase inhibitor (augmentin) while sensitivity was observed in carbapenems [imipenem 96/96 (100%)]. The multiple antimicrobial resistance index of the *P. aeruginosa* to the antimicrobials used ranged from 0.33 to 0.89 while the multidrug resistant profile revealed resistance to augmentin, amoxicillin, cloxacillin.
Conclusions: The present study reveals that hospital drains are potential reservoirs of multiple antibiotic resistances of *P. aeruginosa*.

1. Introduction

Nosocomial infection is an added burden to the patient in the hospital for one illness or the other and is caused by pathogens that are widespread within the hospital environment[1]. Within the hospital, microorganisms are found everywhere, and can get to the patient through various means which include water, air, contaminated equipments, food, catheters, linen, ventilators, scopes, preparations used for treatment and other contaminated disinfectants, infected patients, and visitors[2].

Pseudomonas aeruginosa (*P. aeruginosa*) is a Gram-negative rod shaped bacterium, which has a remarkable ability to adapt and thrive in a variety of environments, such as water, soil, occupational places, clinical settings, hospital, municipal wastewater and industrial effluents[3,4]. *P. aeruginosa* is widely distributed in nature, but has a higher prevalence in hospital environment. The characteristic features of *P. aeruginosa* isolates that allow their persistence in hospital environment include its ability to acquire resistance to varieties of antibiotics, withstanding physical conditions like temperature, high concentration of salts and antiseptics[5,6].

P. aeruginosa has been reported to be resistant to antibiotics such as fluoroquinolone[4] and it is naturally resistant to many antibiotics due to the low permeability barrier afforded by its outer membrane, the action of multidrug efflux pumps and its tendency to colonize surfaces in a biofilm form making the cells impervious to therapeutic concentrations of antibiotics[7]. The simple and rapid detection of *P. aeruginosa* is essential in environmental monitoring and assessment and by extension, the protection of public health. This is especially true in hospital and clinical settings where there is an elevated risk of contamination, growth, exposure, spread and infection of susceptible individuals[8]. Methods that are more practical and rapid will lead to more prevalent monitoring and

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earlier detection, so that intervention strategies can be implemented in the reduction of human exposure and infection. PCR has become the widely utilized technique because of its reliability and sensitivity[6]. For the genotypic detection and characterization of *Pseudomonas* species for their antimicrobial resistance and virulent gene signatures via PCR-based methods, various target genes have been reported[3,7].

P. aeruginosa have been isolated from adult bacterial meningitis with underlying disease in Tokyo, Japan[9], a tertiary care teaching hospital, India[1], a 5-year surveillance of wound infections in Okada, Edo State[10], from ear, nose and throat among patients attending Aminu Kano Teaching Hospital, Nigeria[11], a tertiary care hospital of Jhalawar District, India[12], and sinks in the patient rooms and a retrospective case-control study in Germany[13]. In addition, studies on the influence of hospital waste water discharge and its potential microbial hazards have been investigated in Brazil[14], and increasing frequency of *P. aeruginosa* infections in Turkey was also investigated[15]. In this paper, we report on the characterization of antibiotic-resistant *P. aeruginosa* from hospital drains, as part of our larger study on the reservoirs of antibiotic resistance determinants in the environment.

2. Materials and methods

2.1. Sample collection

The wastewater samples were collected between August and October, 2015. The untreated effluent flowing through hospital drains was sampled respectively from five hospitals [Central Hospital, Benin City; University of Benin Teaching Hospital (UBTH), Benin City; Our Lady's Hospital Oghara; Sapele Central Hospital Delta State and St. Philomena Hospital, Benin City]. Wastewater samples were collected using sterile 250 mL glass bottles and transported to the laboratory in a cooler with ice packs for analysis within 6 h of collection.

2.2. Isolation of P. aeruginosa

The untreated effluent samples were serially diluted (10^1–10^5). This was carried out by the method described in Igbinosa *et al.*[4]. An aliquot of 1 mL was respectively cultivated from each of the test tubes on tryptone soy broth (Merck, Germany) and incubated for 18 h. Thereafter, an aliquot of 100 µL was spread plated from the incubated enriched broth on glutamate starch phenol red agar (Merck, Germany) and cetrimide agar (Merck, Germany) in triplicate and incubated for 18–24 h. The population densities of the *P. aeruginosa* on cetrimide agar were enumerated as colony forming per unit (CFU/mL). Pink colonies were then selected from glutamate starch phenol red agar and streaked on cetrimide agar and incubated for 18–24 h to obtain presumptive isolates of *P. aeruginosa*.

2.3. Biochemical identification of P. aeruginosa

The purified *P. aeruginosa* isolates from cetrimide agar were further identified using morphological (Gram staining, motility), and biochemical tests (catalase, oxidase, urease, citrate, indole, Voges-Proskauer, and sugar fermentation test) as previously described by Ghane and Azimi[16].

2.4. Phenotypic characterization of the P. aeruginosa

The inoculated plates with growth on nutrient agar were viewed under UV light at 254 nm for fluorescence production[17]. For pyocyanin production, bacterial isolates were cultured on nutrient agar, pyocyanin agar and cetrimide agar and incubated in 37 ˚C for 24 h. Change of colour to the green-blue colour was indicative of pyocyanin production[17]. *P. aeruginosa* isolates were further assayed for protease production ability using skimmed milk agar. Test isolates were streaked on the 2% skimmed agar and incubated at 37 ˚C for 24 h. The incubated plates were checked for halo regions around streaks, and a zone of clearance of more than 1 mm around the streaks was recorded as positive for protease production[17]. The ability of the pseudomonads to elaborate pectinases was evaluated using spot inoculation procedure. Viable *P. aeruginosa* cultures were streaked respectively on Vincent agar plates containing pectin and incubated at 37 ˚C for 24 h. Thereafter, the incubated plates were flooded with iodine solution. The development of clear zone after iodine flooding was recorded as positive for pectinase production[18,19].

2.5. Determination of the antibiotic susceptibility of the P. aeruginosa isolates

Antimicrobial susceptibility testing was carried out by adopting the Kirby-Bauer disc diffusion method in accordance with the criteria of Clinical and Laboratory Standards Institute[20] with modification. A loop full of the test bacteria was inoculated into 3.5 mL of normal saline. The suspension of the test isolate was then adjusted to 0.5 McFarland turbidity standards (10^6 CFU/mL). With the aid of sterile swab sticks, each of the test suspension was streaked on Mueller-Hinton agar plates. Antibiotic disc obtained from Mast Diagnostics, Merseyside, United Kingdom was aseptically impregnated in the streaked agar plates. The discs utilized were phenicols [chloramphenicol (30 µg)], penicillins [amoxicillin (30 µg), cloxacillin (5 µg)], aminoglycosides [gentamicin (30 µg)], carbapenems [imipenem (30 µg)], tetracyclines [tetracycline (30 µg)], folate pathway inhibitors [cotrimoxazole (25 µg)], clindamycin [erythromycin (5 µg,)] and penicillin/β-lactamase inhibitor [augmentin (30 µg)]. The seeded agar plates were allowed to absorb for 10 min and incubated at 37 ˚C for 24 h. The diameter of the inhibitory zone was measured using transparent metre rule and interpreted as resistant, intermediate or sensitive according to the Clinical and Laboratory Standards Institute guidelines[20].

2.6. Extraction of genomic DNA

Bacterial genomic DNA was extracted following methods previously described by Igbinosa *et al.*[6] with modification. A loop full of the presumptive *P. aeruginosa* was suspended in 5 mL

tryptone soy broth and incubated in a shaker for 18 h at 37 °C. The bacterial suspension (200 μL) was subjected to lysis using a dry bath (MK200-2, Shanghai, China) at 100 °C for 10 min. This was followed by centrifugation at 11 000 r/min for 2 min using a mini centrifuge (Mini 14k, Zhuhai, Guangdong, China). The resulting cell debris were removed while the pellets were washed twice with 0.9% sterile physiological saline buffer solution, homogenized with 200 μL of lysosome solution and incubated for 90 min at 37 °C. The suspension was treated with 20 μL each of RNase and proteinase K, and incubated at 55 °C for 60 min. The lysate was treated with 200 μL of ethanol and transferred into the new binding column and centrifuged at 3 900 r/min for 1 min. The content was washed twice and the eluate was discarded. The column was put into a new 1.5 mL tube and the DNA was collected with elution buffer solution. The genomic DNA was stored at –20 °C until used directly as the template DNA.

2.7. Species-specific identification using PCR procedure

The extracted bacterial genomic DNA was amplified using *P. aeruginosa* specific primers: pa722F (5'-GGC GTG GGT GTG GAA GTC-3' and pa899R (5'-TGG TGG CGA TCT TGA ACT TCTT-3' amplicon size of 199 bp[21]. PCR procedure was carried out in a final reaction mixture of 25 μL in 200 μL PCR tube. The mixture in the PCR tubes was tapped gently and spun briefly at 10 000 r/min. The PCR tubes with all the components were thereafter transferred to Peltier-Based Thermal Cycler (MG96+/Y, Hangzhou, Zhejiang China). After amplification, the expected PCR product was verified by gel electrophoresis (CLS-AG100, Warwickshire, United Kingdom) containing ethidium bromide 0.5 mg/L for 1 h at 100 V in 0.5× TAE buffer (40 mmol/L Tris-HCl, 20 mmol/L Na-acetate, 1 mmol/L ethylene diamine tetraacetic acid, pH 8.5) and visualized under an UV transilluminator (Vilber Lourmat, EBOX VX5, France).

2.8. Statistical analysis

The ANOVA of the respective mean *P. aeruginosa* cell density obtained from the wastewater samples was conducted ($\alpha = 0.05$). Duncan's multiple range test was employed to locate the cause of any significant differences in the analysed mean cell counts.

3. Results

3.1. Population density of P. aeruginosa from different hospital drain environment

The *P. aeruginosa* mean cell counts for effluents collected from UBTH ranged from ($3.7 \times 10^5 \pm 0.1$) CFU/mL in the month of October, to ($6.1 \times 10^5 \pm 0.2$) CFU/mL in the month of August (Table 1). *P. aeruginosa* mean counts for wastewater collected from Central Hospital and St. Philomena Hospital, both located in Benin City ranged from ($2.7 \times 10^5 \pm 0.4$) CFU/mL in the month of August to ($3.4 \times 10^5 \pm 0.6$) CFU/mL in the month of September and ($1.7 \times 10^5 \pm 0.1$) CFU/mL in the month of August to ($2.0 \times 10^5 \pm 0.9$) CFU/mL in the month of September, respectively (Table 1). *P. aeruginosa* mean counts for wastewater sourced from Sapele Central Hospital and Our Lady's Hospital ranged from ($4.4 \times 10^5 \pm 1.6$) CFU/mL in the month of October to ($5.5 \times 10^5 \pm 0.1$) CFU/mL in the month of August and ($2.7 \times 10^5 \pm 0.4$) CFU/mL in the month of September to ($5.1 \times 10^5 \pm 1.8$) CFU/mL for October, respectively (Table 1).

Table 1
Population density of *P. aeruginosa* from different hospital drain environment.

Samples	10^5 CFU/mL			*P* value
	August	September	October	
A	6.1 ± 0.2^a	4.1 ± 1.3^{ab}	3.7 ± 0.1^b	0.014
B	2.7 ± 0.4^b	3.4 ± 0.6^a	3.0 ± 0.3^a	0.017
C	1.7 ± 0.1^b	2.0 ± 0.9^a	1.7 ± 1.2^b	0.012
D	5.5 ± 0.1^a	4.8 ± 1.5^b	4.4 ± 1.6^b	0.014
E	4.4 ± 0.5^b	2.7 ± 0.4^c	5.1 ± 1.8^a	0.001

Values are presented as mean ± SD. Values carrying different alphabets across rows show significant difference. A: UBTH; B: Central Hospital, Benin City; C: St. Philomena Hospital, Benin City; D: Sapele Central Hospital, Delta State; E: Our Lady's Hospital, Oghara, Delta State.

3.2. Frequency of occurrence of P. aeruginosa in hospital drains

The frequency of occurrence of the *P. aeruginosa* isolates revealed that 25/96 (26.04%) was isolated from UBTH; 20/96 (20.83%) was isolated from Central Hospital, Benin City; 14/96 (14.58%) was isolated from St. Philomena Hospital, Benin City; 18/96 (18.75%) was isolated from Sapele Central Hospital, Delta State; while 19/96 (19.79%) was isolated from Our Lady's Hospital Oghara, Delta State (Table 2).

Table 2
Frequency of occurrence of *P. aeruginosa* in hospital drains [*n* (%)].

Samples	August ($n = 37$)	September ($n = 27$)	October ($n = 32$)	Total ($n = 96$)
A	11 (29.73)	8 (29.63)	6 (18.75)	25 (26.04)
B	8 (21.62)	4 (14.82)	8 (25.00)	20 (20.83)
C	5 (13.51)	6 (22.22)	3 (9.38)	14 (14.58)
D	8 (21.62)	6 (22.22)	4 (12.50)	18 (18.75)
E	5 (13.51)	3 (11.11)	11 (34.38)	19 (19.79)

A: UBTH; B: Central Hospital, Benin City; C: St. Philomena Hospital, Benin City; D: Sapele Central Hospital, Delta State; E: Our Lady's Hospital, Oghara, Delta State.

3.3. Phenotypic characterization of the P. aeruginosa isolates

The phenotypic characterization of the *P. aeruginosa* isolates revealed that all the isolates exhibited fluorescence, protease, and pyocyanin activity while none of the isolates were able to exhibit pectinase activity.

3.4. Antimicrobial susceptibility profile of P. aeruginosa isolates

The antimicrobial susceptibility profile of the *P. aeruginosa* isolates (Table 3) revealed that all the isolates 96/96 (100%) were resistant to penicillins (amoxicillin and cloxacillin) as well as penicillin/β-lactamase inhibitor (augmentin). High level resistance

was also observed in folate pathway inhibitors [clotrimazole 95/96 (99%)], tetracyclines [tetracycline 95/96 (99%)], macrolides [erythromycin 92/96 (96%)], and phenicols [chloramphenicol 80/96 (83%)]. High level sensitivity was also observed with carbapenems [imipenem 96/96 (100%)].

Table 3
Antimicrobial susceptibility profile of *P. aeruginosa* isolates.

Antibiotic class	Antibiotic	*P. aeruginosa* isolates (n = 96)		
		Sensitive	Intermediate	Resistant
Phenicols	Chloramphenicol (30 µg)	1 (2)	15 (24)	80 (83)
Aminoglycosides	Gentamicin (30 µg)	40 (42)	35 (36)	21 (22)
Macrolides	Erythromycin (5 µg)	1 (3)	3 (5)	92 (96)
Carbapenems	Imipenem (30 µg)	96 (100)	0 (0)	0 (0)
Penicillins	Amoxicillin (30 µg)	0 (0)	0 (0)	96 (100)
	Cloxacillin (5 µg)	0 (0)	0 (0)	96 (100)
Penicillin/β-Lactamase inhibitor	Augmentin (30 µg)	0 (0)	0 (0)	96 (100)
Tetracyclines	Tetracycline (30 µg)	0 (0)	1 (2)	95 (99)
Folate pathway inhibitors	Clotrimazole (25 µg)	0 (0)	1 (2)	95 (99)

Values in parenthesis represent percentage.

3.5. Multiple antimicrobial resistance (MAR) index and multidrug resistant profile of the P. aeruginosa isolates

The MAR index of the *P. aeruginosa* to the antimicrobials used ranged from 0.33 and 0.89 (Table 4). The multidrug resistant profile of the *P. aeruginosa* revealed that 20/96 (20.8%) was resistant to chloramphenicol, augmentin, amoxicillin, cloxacillin, gentamicin, cotrimoxazole, erythromycin, tetracycline; 80/96 (83.3%) was resistant to chloramphenicol, augmentin, amoxicillin, cloxacillin, cotrimoxazole; while all the isolates in this study [96/96 (100%)] were resistant to augmentin, amoxicillin, cloxacillin (Table 5).

Table 4
MAR index of the *P. aeruginosa* isolates (n = 96).

No. of isolates	Antibiotics	MAR index
80	CHL, AUG, AMX, CXC, COT	0.56
96	AUG, AMX, CXC	0.33
80	CHL, AUG, AMX, CXC	0.44
72	CHL, AUG, AMX, CXC, COT, ERY, TET	0.78
74	CHL, AUG, AMX, CXC, COT, ERY	0.67
20	CHL, AUG, AMX, CXC, GEN, COT, ERY, TET	0.89
78	CHL, AUG, AMX, CXC, COT, TET	0.67

CHL: Chloramphenicol (30 µg); AMX: Amoxicillin (30µg); GEN: Gentamicin (30 µg); TET: Tetracycline (30 µg); CXC: Cloxacillin (5 µg); COT: Cotrimoxazole (25 µg); ERY: Erythromycin (5 µg); AUG: Augmentin (30 µg).

Table 5
Multidrug resistant profile of *P. aeruginosa* (n = 96).

Antimicrobial class	Number of antibiotics	Resistance phenotype	No. of isolates
7	8	CHL, AUG, AMX, CXC, GEN, COT, ERY, TET	20 (20.8)
6	7	CHL, AUG, AMX, CXC, COT, ERY, TET	72 (75.0)
5	6	CHL, AUG, AMX, CXC, COT, ERY	74 (77.1)
5	6	CHL, AUG, AMX, CXC, COT, TET	78 (81.3)
4	5	CHL, AUG, AMX, CXC, COT	80 (83.3)
3	4	CHL, AUG, AMX, CXC	80 (83.3)
2	3	AUG, AMX, CXC	96 (100.0)

CHL: Chloramphenicol (30 µg); AMX: Amoxicillin (30 µg); GEN: Gentamicin (30 µg); TET: Tetracycline (30 µg); CXC: Cloxacillin (5 µg); COT: Cotrimoxazole (25 µg); ERY: Erythromycin (5 µg); AUG: Augmentin (30 µg). Values in parenthesis represent percentage.

4. Discussion

P. aeruginosa isolates were selectively recovered from the examined hospital effluents from different hospital drains using phenotypic and genotypic methods. As such, 96 *P. aeruginosa* isolates were detected from the effluent which could be attributed to the nutritional richness of the effluents and further characterized for their antibiogram profile. In our recent publication, Igbinosa *et al.*[4] reported that PCR amplification of *P. aeruginosa* is a sensitive and specific method for the detection of *Pseudomonas* strains isolated from environmental samples in Benin City, Nigeria. Magalhães *et al.*[14] described hospital wastewater as a mixture of effluents emanating from different services: kitchen, internal laundry, heating and cooling systems, laboratories, radiology department, outpatients department, transfusion centres and wards.

P. aeruginosa been a frequent inhabitant in the hospital environment could attribute to a number of factors ranging from its capacity to thrive in diverse environmental conditions to its intrinsic ability to resist the action of different antimicrobials. The frequency of *P. aeruginosa* occurrence in the present study ranged from 25/96 (26.04%) isolated from UBTH to 14/96 (14.58%) isolated from St. Philomena Hospital, Benin City. Similar investigation by Davane *et al.*[2], revealed that 26 (52.0%) was found positive from the different environmental samples examined. A study by Ulu-Kilic *et al.*[15] revealed that two hundred and seventy-eight (23.8%) of the patients investigated had *P. aeruginosa* infection during their ICU stay. Fifty-nine patients (21.2%) in the case group received tigecycline before developing *P. aeruginosa* infections, which were found to be significantly more frequent than in the controls ($P < 0.01$)[15].

Antimicrobial resistance is an enigmatic problem of public health importance. The ability of *P. aeruginosa* to resist the action of potent antimicrobial agents that were initially designed to treat ailment resulting from their pathogenicity resulting in difficulty to treat infection thereby elongating the stay of patients in the hospital is of public health concern[6]. In our present study, it was observed that 40/96 (41.67%) of the *P. aeruginosa* cultures exhibited sensitivity towards the aminoglycoside (gentamicin). This observation was slightly different from an earlier report by Tanvir *et al.*[22] which indicated a sensitivity rate of 69.5% for *P. aeruginosa* isolates cultured from clinical specimens obtained from a hospital in Karachi, Pakistan. The sensitivity pattern exhibited by the pseudomonad against gentamicin in the present study was slightly similar to a report by Sivaraj *et al.*[23] which observed a 52% susceptibility rate for *P. aeruginosa* isolates recovered from both environmental and clinical sources in Tamil Nadu, India. About 21/96 (22%) of the effluent associated *P. aeruginosa* cultures exhibited resistance towards gentamicin (Table 3). Odjadjare *et al.*[5] outlined several general mechanisms through which *P. aeruginosa* could develop resistance. These included lack of permeability of the outer membrane to these drugs and the activity of aminoglycoside-modifying enzymes.

About 96% of the pseudomonad was resistant to erythromycin (Table 3). This trend differed slightly from an earlier observation by Sivanmaliappan and Sevanan[24] which reported maximal resistance to erythromycin by 100% of the *P. aeruginosa* isolates cultured

from swabbed 270 diabetic foot ulcers in tertiary care hospitals in and around Coimbatore, India. However, the almost dominant resistance patterns displayed by the pseudomonad effluent from the hospital settings contrasted with an earlier report by Odjadjare *et al.*[5] which indicated that 90% of the *P. aeruginosa* isolates from several treated municipal effluents were sensitive to erythromycin. Majority of the *P. aeruginosa* isolates were resistant to phenicol (chloramphenicol), tetracyclines (tetracycline) and penicillins (amoxicillin and cloxacillin) (Table 3). Igbinosa *et al.*[6] and Igbinosa *et al.*[3] reported that *P. aeruginosa* has been known to possess intrinsic resistance to β-lactams, including broad-spectrum cephalosporins, quinolones, chloramphenicol and tetracyclines, mainly because of the very low permeability of their cell wall. It has also been reported that *P. aeruginosa* was naturally resistant to narrow-spectrum penicillins, first- and second-generation cephalosporins, trimethoprim, and sulphonamides[7].

All the *P. aeruginosa* isolates in the present study were susceptible to imipenem (Table 3). This observation is in tandem with earlier reports by Kireçci and Kareem[25] as well as Mohammed and Mohammed[26] which indicated the 100% sensitivity rate of clinically sourced *P. aeruginosa* isolates. The sensitivity of *P. aeruginosa* towards imipenem might be due to the inability of the pseudomonad to elaborate carbapenem resistance mechanisms such as decreased outer membrane permeability, increased efflux systems, alteration of penicillin binding proteins and the production of carbapenem hydrolyzing enzymes (carbapenemases). These mechanisms were earlier outlined by Atti *et al.*[27] as well as Igbinosa *et al.*[6] as attributes possessed by carbapenems resistant *P. aeruginosa*. However, the sensitivity of the effluent associated *P. aeruginosa* isolates towards imipenem in the present study contrasted with a report by Moazami-Goudarzi and Eftekhar[28] which observed 94.7% resistance to imipenem from burn wound associated *P. aeruginosa* isolates collected from Shahid Motahari Burn Hospital, Tehran, Iran.

The MAR index in the present study ranged from 0.33 to 0.89 (Table 4). The high MAR index in the present study represents the health risk associated with the spread of drug resistance in the hospital drains investigated. Findings in the present study are similar to the findings of Igbinosa *et al.*[4] from abattoir and aquaculture environments where the MAR index ranged from 0.4 to 0.8. Similar findings were also observed by Odjadjare *et al.*[5] on MAR index between 0.26 and 0.58 from wastewater effluent. However, in totality, no clear pattern of MAR in line with isolate origin was observed.

The isolation of multiple drug resistance *P. aeruginosa* from the hospital wastewater (Table 5) was in agreement with a report by Bédard *et al.*[29] which indicated that hospital derived wastewater was a source of drug resistant bacteria. Similarly, Igbinosa and Obuekwe[7] reported the isolation of multidrug resistant *P. aeruginosa* strains from an abattoir setting in Benin City. The authors opined that multidrug resistance in environmental isolates might be linked to the uncontrolled disposal of antibiotics and chemicals into the environment which created a selective pressure on these drugs. Ustun *et al.*[30] noted that commonly used healthcare disinfectants such as triclosan and quaternary ammonium compounds act as substrates for the efflux pump systems of *P. aeruginosa*, which has been recognized as playing a critical role in non-enzymatic mechanisms of acquired drug resistance. As these products are present in high concentrations in hospital effluents, selective pressure for more highly resistant *P. aeruginosa* strains may therefore be exerted.

Magalhães *et al.*[14] reported that approximately one quarter of the total antibiotics used in human therapy are administered in hospitals. Hospital wastewater therefore represented a significant contributor to the total load of these antibiotics in municipal wastewater[29]. Although the possible presence of antibiotics in the examined wastewater was not investigated, an earlier research by Tuc *et al.*[31] indicated the detection of antibiotics at high concentrations in hospital effluents. Thus, the capacity of the resident organisms to proliferate in such environment gives it inherent capacity to resist the action of multiple antimicrobials. This could in turn result in the selective proliferation of resistant strains in the environment. Subsequently, when these resistant strains express themselves in pathogenic forms, they tend to cause difficulty to treat infections due to their capacity to resist the antibiotics intended to treat the ailment. Therefore, continuous monitoring and assessment of the environment is imperative to circumvent the tendency of these multidrug resistant organisms presenting themselves in high densities. More so, the use of antimicrobials should be regulated and disposal of antimicrobial compounds or derivatives should be properly treated before release into drains and the environment to avoid the possibility of proliferation of antimicrobial resistant strains in the surroundings.

The presence of multidrug resistant *P. aeruginosa* in hospital drains increases the possibility of these organisms to disseminate potential resistance and virulence genes within bacterial populations. Further studies on antibiotic resistance and virulence gene determinants using specific primers or probes for clonality and genetic profiles of multidrug resistant *P. aeruginosa* are the current subject in our research group.

Conflict of interest statement

We declare that we have no conflict of interest.

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