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Hospital wastewater: reservoir of antibiotic resistant *Pseudomonas* strains in Ibadan, Nigeria

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Abstract

Transmission of nosocomial infections becomes debilitating when caused by multiple antibiotics resistant bacteria such as *Pseudomonas* species. *Pseudomonas aeruginosa*, notorious for both opportunistic and original infections, has multiple drug resistant (MDR) characteristics and thus a persistent threat to public health. This study was therefore carried out to determine the patterns of antibiotic resistance in *Pseudomonas* species isolated from the wastewater samples of a specialized private hospital. Wastewater samples were collected from four different points within and outside the hospital environment; namely, theater, laboratory, drainage and the receiving river. Isolation of *Pseudomonas* species was performed using the standard pour plate method. The isolates were identified using standard biochemical and sugar fermentation tests while the antibiotic susceptibility test was done using the disc diffusion method. A total of 97 strains of *Pseudomonas* species were isolated comprising 44 (45.4%) *Pseudomonas aeruginosa*, 41 (42.2%) *P. fluorescens* and 12 (12.3%) other *Pseudomonas* species. While all the isolates were resistant to piperacillin, 96 (99%) of the isolates were resistant to tetracycline, 93 (95.9%) to nitrofurantoin and 92 (94.8%) were resistant to aztreonam with cumulative total resistance of 4.1%. This study showed that antibiotics resistant *Pseudomonas* strains were present in hospital wastewater. Therefore, Federal/State Hospital Management Board and other regulatory government agencies should ensure that hospital effluents are properly treated before its disposal into the environment.

Keywords antibiotics resistance, environment, hospital wastewater, *Pseudomonas* species

Introduction

Increased production and discharge of hospital wastewater in recent decades as a result of improvement in medical services and products can be very debilitating [1]. Hospital wastewater has been assayed and reported to contain hazardous chemicals, dangerous pathogenic microorganisms and environmental pollutants at different concentrations and further reported the lack of global best practices in some hospital settings where processing and management of hospital wastewater are grossly lacking, especially in the developing countries of the world [2-3]. Meanwhile, antibiotics resistance by microbial pathogens is a major problem in clinics and public health, affecting the majority of total population [4]. Moreover, Magiorakos et al. [5] also observed that hospitals provide an enabling environment conducive for development of MDR bacteria, making the limited treatment options somewhat expensive. For the public health professionals, dissemination of antibiotics resistant bacteria from the wastewater treatment plants of hospitals is of great concern. There are studies that have previously shown that multidrug resistant *P. aeruginosa* is the main pathogen that is found in the discharge of the hospital wastewater treatment plants. This is an indication that the bacterium may play a major role in the transmission and the spread of the antimicrobial resistance, which globally is an increasing healthcare problem [6].

However, the human opportunistic pathogen, *Pseudomonas aeruginosa*, an ubiquitous Gram-negative bacterium has been reported to be spreading in hospitals especially *via* hands of healthcare workers and also when contact is made with hospital surfaces that are not properly cleaned as well as equipment's on which it can persist for a long period of time [7-8]. The bacteria remains the most common species of *Pseudomonas* isolated from hospital wastewater. This bacterial species is one of the major causes of mortality among critically ill patients. It has the highest fatality case among all Gram-negative bacteria infections. It causes nosocomial infection and responsible for about 10% of all hospital acquired infections. *Pseudomonas aeruginosa* infections are often life-threatening with intrinsic high resistance to numerous antimicrobial agents and posterior development of increased multi-drug resistance in health care settings [9-10]. The problem of antibiotics resistance in *Pseudomonas aeruginosa* is their increase due to denovo emergence of resistance after exposure to certain antimicrobials. Accumulation of resistance features after the exposure to various antibiotics and cross resistance between agents may result in multidrug-resistant (MDR) *Pseudomonas aeruginosa*. These MDR strains of *P. aeruginosa* may be transmitted among patients in hospitals and sometimes, these pathogens may lead to outbreak among cystic fibrosis patient attending the same clinic [11]. Since most hospital wastewaters are released into water bodies without treatment; there is plausible proliferation of drug resistant pathogens in water bodies with subsequent adverse public health effect. This study was hence carried out to determine the antibiotics resistance pattern of *Pseudomonas* species isolated from a specialized private hospital wastewater in Ibadan, Nigeria.

Methodology

Study Site

This study was carried out in a private Specialized Hospital located in Yemetu, Ibadan North Local Government Area of Oyo State, Nigeria. The hospital sections have pipes from which wastewater disposed were channeled into the drainage which terminated into a nearby river.

Sampling Points Geographic Positioning System (GPS)

The GPS reading of sampling points three and four were obtained and recorded, they were: Latitude- 7^{O}23.6149′N and Longitude- 3^{O}54.3061′E and Latitude- 7^{O}23.6219′N and Longitude-3^{O}54.2985E respectively.

Sample Collection

The hospital wastewater samples were collected at four different sampling points that include: the theatre (sampling point 1), laboratory (sampling point 2), drainage/pipe (sampling point 3) and the receiving river (sampling point 4). The samples were collected aseptically using standard microbiological method and transported to the laboratory on ice pack for immediate analyses.

Isolation and characterization of the Pseudomonas species

The *Pseudomonas* species were isolated using the method that was previously described [12]. In the test, duplicates of 1 ml aliquots of the raw sample (10^0), 10^{-1} and 10^{-2} of the wastewater samples were dispensed into labeled Petri-dishes; 10ml of already prepared Pseudomonas Centrimide Agar cooled to cheek tolerant temperature was poured unto the aliquots, gently rocked and allowed to solidified were inverted and incubated at 37^oC for 24-48 hrs. The isolates were sub cultured and characterized using standard biochemical and sugar fermentation tests.

Antibiotics susceptibility Testing

Antibiotics susceptibility test was carried out using the disk diffusion method recommended by Cockerill and CLSI [13]. A 16-18 hours old pure culture of *Pseudomonas* isolates standardized to 0.5 McFarland turbidity standards were swabbed on Mueller-Hinton agar according to the standard operational procedure. With the use of sterile forceps, the antibiotics were placed on the inoculated Petri dishes which were inverted and incubated at 37^{o}C for 18 to 24 hours. The zones of inhibition were measured in millimeters (mm), recorded and interpreted based on interpretive criteria of CLSI [13] and BSAC [14]. The antibiotics used for the susceptibility test includes ceftazidime 30µg, gentamicin 10µg, ciprofloxacin 5µg, ofloxacin 5µg, piperacillin 30µg, tetracycline and aztreonam 30µg and were obtained from Oxoid, UK.

Results

A total of ninety-seven (97) isolates of *Pseudomonas* species were obtained from the wastewater samples of the hospital. These include: *P. aeruginosa* 44 (45.4%), *P. fluorescens* 41 (42.3%) and other *Pseudomonas* species 12 (12.4%). The isolates obtained from different sampling points are as shown in Table 1. At sampling point 1, *P. aeruginosa* had the highest 18 (18.6%) rate of occurrence, while the least rate of occurrence (3.1%) was at sampling point 2. The highest rate of occurrence of *P. fluorescens* which was 14 (14.4%) was at sampling point 3, while the least rate of occurrence (4.1%) was at the sampling point 2. Furthermore, the highest number of other *Pseudomonas* isolates 9 (9.3) was obtained at sampling point 1.

Table 1. Number and frequency of *Pseudomonas* strains isolated from the sampling points n (%)

Sampling points	*P. aeruginosa* n=44	*P. fluorescens* n=41	Other *Pseudomonas* spp. n=12	Total n=97
Point 1 (theater)	18(19.8)	13(13.4)	9 (9.3)	40 (37.4)
Point 2 (Laboratory)	3(3.1)	4(4.1)	-	7 (7.7)
Point 3 (Drainage/pipe)	9(9.3)	14(14.4)	1 (1.0)	24 (26.4)
Point 4 (receiving river)	14(14.4)	10(10.3)	2 (2.1)	26 (28.6)
TOTAL	**44 (45.4)**	**41 (42.3)**	**12 (12.4)**	**97 (100)**

The pattern of the antibiotics susceptibility of the *Pseudomonas* species shown in Table 2 revealed that all the isolates exhibited complete resistance to piperacillin and aztreonam. Similarly, resistance of the isolates to tetracycline (95.9%) was very high. However, the least resistance that was observed was to ofloxacin (12.4%). Furthermore, while all the *P. aeruginosa* showed total resistance (100%) to both piperacillin and aztreonam, all the *P. fluorescens* were totally resistant to both piperacillin, aztreonam and tetracycline; while all the other *Pseudomonas* species exhibited total resistance (100%) to the following antibiotics: piperacillin, tetracycline and aztreonam. Interestingly, among the least resisted antibiotics were ofloxacin and ciprofloxacin with the resistant rate of 14.4% and 12.4% respectively. This was followed closely by ceftazidime and gentamycin with 15.5% resistance (Table 2).

Generally, the isolates exhibited multiple antibiotic resistance patterns which ranged between 3-7 antibiotics. The antibiograms of the *Pseudomonas* strains shown in Table 3 revealed the multidrug resistance (MDR) patterns of the isolates. Out of the total 97 isolates obtained in this study, 93 (95.9%) exhibited multidrug resistance comprising of 41 *P. aeruginosa*, 40 *P. fluorescens* and 12 other *Pseudomonas* species. Additionally, it was observed that 75 (82.4%) of the isolates showed resistance to a combination of three antibiotics (piperacilli-tetracycline-aztreonam) which include 33 *P. aeruginosa*, 32 *P. fluorescens* and 10 other *Pseudomonas* species. Furthermore, six of the isolates showed resistance to a combination of seven antibiotics (piperacillin-tetracycline-aztreonam-ceftazidime-gentamycin-ciprofloxacin-ofloxacin) including two (2) *P. aeruginosa*, three (3) P. fluorescens and one (1) other *Pseudomonas* species.

Table 2. Antibiotic resistant pattern of *Pseudomonas* strains isolated from hospital wastewater n (%)

Antibiotics	***P. aeruginosa*** **n=44**	***P. fluorescens*** **n=41**	**Other *Pseudomonas*** **spp. n=12**	**Total resistance n=97**
Piperacillin (30ug)	44 (100)	41 (100)	12 (100)	97 (100)
Tetracycline (30ug)	41 (97.7)	40 (100)	12(100)	93 (95.9)
Azetreonam (30ug)	44 (100)	41 (100)	12(100)	97 (100)
Ceftazidime (30ug)	7 (15.9)	6 (14.6)	2 (16.7)	15 (15.5)
Gentamycin (10ug)	7 (15.9)	6 (14.6)	2 (16.7)	15 (15.5)
Ciprofloxacin (5ug)	5 (11.4)	5 (12.2)	2(16.7)	12 (12.4)
Ofloxacin (5ug)	7 (15.9)	5 (12.2)	2 (16.7)	14 (14.4)

Table 3. Antibiograms of pseudomonas strains isolated from hospital wastewater n (%)

Antibiotypes	*P. aeruginosa*	*P. fluorescens*	Other species	
PRL-TET-ATM	33 (80.5)	32 (80)	10 (83.3)	75 (80.6)
PRL-TET-ATM-GEN	0 (0)	1 (2.5)	0 (0)	1 (1.1)
PRL-TET-ATM-CAZ	3 (7.3)	2 (5.0)	0 (0)	5 (5.4)
PRL-ATM-CAZ-GEN	1 (2.4)	0 (0)	0 (0)	1 (1.1)
PRL-ATM-CAZ-GEN-CPR	1 (2.4)	0 (0)	0 (0)	1 (1.1)
PRL-TET-ATM-CAZ-GEN-CPR	0 (0)	1 (2.5)	0 (0)	1 (1.1)
PRL-TET-ATM-GEN-CPR-OFL	1 (2.4)	1 (2.5)	1 (8.3)	3 (3.2)
PRL-TET-ATM-CAZ-GEN-CPR-OFL	2 (4.9)	3 (7.5)	1 (8.3)	6 (6.5)
Total	**41**	**40**	**12 (100)**	**93**

Out of the 44 *P. aeruginosa*, 41(93.2%) were multidrug resistant, while all (12) the other *Pseudomonas* species were also multidrug resistant. In addition, it was observed that five isolates were resistant to all the antibiotics tested including two *P. aeruginosa* and three *P. fluorescens*, four of which were isolated from sampling point 4 and one (*P. aeruginosa*) isolated from sampling point 1.

Discussion

The non-enforcement of mandatory wastewater treatment before discharge into the environment in Nigeria is prehistoric as the presence of bacteria in untreated wastewater above permissible limit is usually not fortuitous. Meanwhile, owing to ubiquity of microorganisms especially in natural environment, isolation of *Pseudomonas* from environmental sources such as soil samples [15-16] might be justified. However, isolation of *Pseudomonas aeruginosa* in this study, from hospital wastewater that had already been reported in several studies in Nigeria and elsewhere is of great concern [17-19]. Eluded to this is the detection of *Pseudomonas* species in clinical samples such as urine, pus, urine, ear, nose, wound and other infection sites [20-21].

Recently, multi-drug resistant *Pseudomonas* species from abattoir wastewater in Ibadan, Nigeria was reported [22]. The observation from the present study that showed all (100%) the *Pseudomonas* isolates are resistant to piperacillin is in line with the increasing antibiotics resistance globally. Furthermore, although a higher resistance rate to piperacillin was observed in this study, it is comparable to a high resistant rate (73.2%) to the same antibiotics at the same concentration in a study that was conducted on antibiotics susceptibility patterns of *P. aeruginosa* in a tertiary hospital in India [23]. Nevertheless, the total piperacillin resistance of *Pseudomonas aeruginosa* in this study is not in agreement with the 11.5% resistance of the same organism to piperacillin in wastewater samples in Saudi-Arabia [24].

The total resistance of *P. aeruginosa* and *P. fluorescens* observed in this study to aztreonam is not in line with the 51% reported in another study in Pakistan [25]. The reason for the disparity may be due to the type of studied samples. While the present study was on isolates from hospital wastewater, the latter study was on isolates obtained from fresh water spring contaminated with domestic sewage. However, the high resistance (95.9%) of *Pseudomonas* species to tetracycline in this study is comparably similar to the 99% resistance previously reported in another study [26]. This observation was however in contrast to the reported 50% [27] and 73% [25] of *Pseudomonas* species that were resistant to tetracycline. The reason for the differences may be due to the samples from which the organisms were isolated. Furthermore, resistance to gentamicin (15.5%) observed in this study is not in agreement with the report of Olayinka et al. [21]. In addition, resistance of the *Pseudomonas* strains in this study to tetracycline which were 97.7% (*P. aeruginosa*), 100% (*P. fluorescens*) and 100% (*Pseudomonas* spp.) were above the resistance to the same antibiotic with the same concentration in a recent study on *Pseudomonas* species isolated from abattoir wastewater in which resistance to the antibiotics by *P. aeruginosa* was 59.4%. However, for *P. fluorescens* and other *Pseudomonas* spp., they were 53.3% and 21.1% respectively [22].

Similarly, in contrast to Rabiu and Falodun [22] who had reported total effectiveness of ciprofloxacin (fluoroquinolones) as anti-Pseudomonas drug of choice, the studies of Ahmed et al. [28] and Akinpelu et al. [27] reported moderate resistance to ciprofloxacin which resonates with the current study but is different from the result obtained from research work done by Ansari et al. [29] where 73.3% of strains of *Pseudomonas* species studied were sensitive to ciprofloxacin. Other findings such as that of Anjum and Mir [26] and Kirecci and Kareem [30] had respectively progressively reported 38% and 45.3% of *Pseudomonas* species resistance to Ceftazidime though rather high than 14.4% observed in the current study.

Overall, the similarities and differences in this study and other studies may just depend on the following parameters: environmental condition, the different anthropogenic activities in and around the sampling sites and wastewater treatment technique practiced. Elizabeth and Vincent [31] had observed that multidrug resistant strains of *Pseudomonas* species is a growing menace and this may be due to altered target sites, bacterial efflux pumps, enzyme production or inhibition and loss of membrane protein and different mechanisms mediated by multidrug resistant strains of *Pseudomonas* species.

Conclusion

Hospital wastewater contains various pathogenic and multidrug resistance strains of *Pseudomonas* which are threat for global public health. This study showed that all strains of *Pseudomonas* obtained had resistance to three or more antibiotics. The hospital wastewaters thus harbour antibiotic resistant pathogens with plausible transference of resistance features horizontally to other competent bacteria. Disposal of the hospital wastewater to open rivers or stagnant water bodies seriously pose significant threat to public health. As such, proper measures should be put in place by the Federal and State health and environmental agencies by making sure that all the licensed hospitals should have a wastewater treatment plant.

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