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Antibiotic Susceptibility Patterns of *Pseudomonas* Species Isolated From Clinical and Environmental Samples in Awba Dam, Ibadan

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Abstract

Pseudomonas species are common pathogenic Gram negative bacteria frequently found in both environmental and clinical samples. They are opportunistic pathogen and could be multidrug resistant (MDR); their presence in the environment signifies threat to the public health. Water from Awba dam is used as municipal water supply for drinking, fishing and agricultural purposes in the University of Ibadan which gives reason for proper monitoring. The study was designed to isolate and characterize *Pseudomonas* species present in Awba dam reservoir and also to compare its antimicrobial resistance pattern with clinical isolates. *Pseudomonas* was isolated using Centrimide agar (Oxoid) and identification was done to species level using classical method. Disk diffusion method was applied to test antibiotic sensitivity according to clinical and laboratory standard guidelines. A total of 200 bacteria isolates were obtained from water and sediment, of which 30 were found positive for *Pseudomonas* giving a prevalence of 15.0% (30/200). Eighteen clinical samples were collected from University College Hospital with about 50% from urine, 27.8% (Ear), 11.1% (wound) and 5.6% each from pleural effusion and nasopharyngeal tube. Two species of *Pseudomonas; aeruginosa* and *fluorescens* were identified. Out of the 10 antibiotics used, environmental isolates showed no resistance to gentamicin, amikacin, ciprofloxacin, tetracycline and imipinem while clinical isolates showed 83.3, 16.7, 83.3, 100 and 11.1% resistance to the antibiotics respectively. Amikacin and Imipinem was the most effective drug for both environmental and clinical *Pseudomonas* isolates. High rates of MDR *Pseudomonas* (94.4%) from clinical strains and 13.3% of the environmental strains were observed. In conclusion, the result shows that *Pseudomonas* isolates from the clinical specimens are more exposed to antibiotics, and MDR isolates in the water system can be transferred to humans and animals.

Keywords: Antibiotic resistance, water, multidrug resistance (MDR), *Pseudomonas aeruginosa, Pseudomonas fluorescens*

Introduction

Pseudomonads are a large group of free living bacteria that live primarily in soil, seawater, and fresh water. *Pseudomonas* is the most common genera in crustaceans, marine fish and bivalves

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(Alexopoulos *et al.*, 2011). *Pseudomonas* is one of the most common gram negative pathogenic bacteria, frequently found in different environmental samples such as soil, sediments. *Pseudomonas* species have been incriminated in a number of waterborne outbreaks including those associated with use of recreational waters (Mena and Gerba, 2009); showers, hot tubs and swimming pools (Pirnay *et al.*, 2005); thus making the pathogens of growing public health concern.

P. aeruginosa is an opportunistic pathogen amongst the Pseudomonads that is most commonly associated with many infectious diseases seen in humans (Silby *et al.*, 2011). They are often associated with infections of the urinary tract, respiratory system, soft tissue, bone and joint, gastrointestinal infections, dermatitis, bacteremia, and a variety of systemic infections, particularly in patients with severe burns, cancer and AIDS (Pirnay *et al.*, 2005). These bacteria are widely distributed in nature and can survive on a wide variety of surfaces as it found its entry into the hospital environment either through visitors and patients or goods that enter in hospital. Contact transmission or vehicle transmission is common mode of transmission in hospital (Todar, 2011; Murray *et al.*, 2002). *P. aeruginosa* is an opportunistic nosocomial pathogen that resists several antimicrobial agents (Brooks *et al.*, 2011; Meenakumari *et al.*, 2011). A major factor in its importance as a pathogen is its intrinsic resistance to antibiotics and disinfectants (Campisano *et al.*, 2008). They represent a threat to public health because of their status as multidrug resistant bacteria. Multidrug-resistant (MDR) *P. aeruginosa* phenotype are known as those that are resistant to one anti-microbial agents in three or more anti-pseudomonal anti-microbial classes (carbapenems, fluoroquinolones, penicillins/cephalosporins and aminoglycosides) as stated by Centre for Disease Control and Prevention (CDC) (Magiorakos, 2011). *Pseudomonas* is one of the most common pathogenic bacteria, frequently found in different environmental samples. Their presence in water also poses the risk of transferring resistance to other pathogenic bacteria present in the human body (Mavridou *et al.*, 1994). *Pseudomonas aeruginosa* is an emerging opportunistic pathogen in healthcare settings and is amongst the leading causes of nosocomial pneumonia associated with high mortality rates (Rossolini and Mantengoli, 2005). Many of the antibiotic resistance bacteria that have been detected are of clinical origin i.e. hospital settings, environmental isolates have recently been implicated in many cases of antibiotic resistance. Water from Awba dam is used in municipal water supply for drinking, fishing and agricultural purposes. It experiences human inputs of complex mixture of domestic effluents from halls of residences in the University of Ibadan which could exert more selective pressure on bacteria populations in Awba stream and Reservoir. Therefore, there is need to study the antimicrobial resistance pattern present in Awba Dam to determine the level of resistance of the species to different classes of antibiotics determining multiple antibiotic resistance (MAR) index and compare with that of clinical isolates.

Materials and Methods

Sample Collection

Water was collected aseptically in sterile glass bottles from different sampling points by directly dipping the bottles into the surface of the water by means of canoe. The collection was done at different station points from one end to the other in each sampling station. Each sample A and B were made up of composite samples from the three stations. The samples were labelled properly and transported on ice to the laboratory for analysis.

Sediments collection was also made up of composite samples from three different stations. Samples were collected using a Van Veen grab sampler by means of canoe, emptied into pre-labelled polythene bags and taken to the laboratory for sorting and analysis.

Statistical analysis

Relevant data were collectively documented using Microsoft Excel data analysis tool. Values were expressed in means and percentage.

Results

A total of two hundred bacteria isolates were obtained from water and sediment. Among the total

samples, 30 isolates were identified as *Pseudomonas* giving a prevalence of 15.0% (30/200). Out of the 30, 11 (36.6%) were isolated from sediment and 19 (63.3%) from water (Table 1). Eighteen clinical samples were collected from University College Hospital with about 50% from urine, 27.8 (Ear), 11.1% (wound) and 5.6% each from pleural effusion and nasopharyngeal tube. The distribution is shown in Table 2.

Table 3 showed different *Pseudomonas* species isolated from both clinical and environmental samples. The results showed more *Pseudomonas fluorescens than aeruginosa* in sediment than water for environmental samples (80% and 20%, respectively) while clinical samples showed more *Pseudomonas aeruginosa* than *fluorescens* (55.5% and 44.4%, respectively). The susceptibility tests of *Pseudomonas* species against 10 different antibiotics is de ss shown in Table 4. Results showed that all environmental isolates showed no resistance to gentamicin, amikacin, ciprofloxacin, tetracycline and imipinem while clinical isolates showed 83.3, 16.7, 83.3, 100 and 11.1% resistance to the antibiotics respectively. Also, environmental isolates were highly susceptible to ceftazidime, ofloxacin, cefepime, ticarcillin and chloramphenicol in the following order: 93.3, 86.7, 70.0, 60.0 and 26.7 while the highest sensitivity observed from clinical isolates was 33.9% to ticarcillin. Results showed that isolates from the clinical specimens were more resistant to antibiotics than those obtained from Awba dam. Table 5 shows percentage of isolates that were able to resist more than three antibiotics. 13.3% of the environmental strains and 94.4% of the clinical strains showed multiple antibiotic resistance.

Table 1: Frequency of *Psedomonas* isolates in water and sediment of Awba Dam

Sample type	No	%
Water	19	63.3
Sediment	11	36.6
Total	30	100

Table 2: Distribution of *Pseudomonas* isolates among the patients specimens

Source/Site	No of Isolates	%
Urine	9	50
Ear	5	27.8
Wound	2	11.1
Pleural Effusion	1	5.6
Nasopharyngeal tube	1	5.6
TOTAL	18	100

Table 3. *Pseudomonas aeruginosa* and *fluorescens* isolated from environment and clinical samples

Source/Site	No of isolates	*Pseudomonas aeruginosa* No (%)	*Pseudomonas fluorescens* (No/%)
Environment	30	6 (20)	24 (80)
Clinic	18	10 (55.5)	8 (44.4)

Table 4: Antibiotics resistance patterns of *Pseudomonas* Isolates

ANTIBIOTIC	CLINICAL ISOLATES (18) RESISTANT NO (%)	ENVIRONMENTAL ISOLATES (30) RESISTANT NO (%)
GENTAMICIN	15 (83.3)	0 (0.0)
AMIKACIN	3 (16.7)	0 (0.0)
CIPROFLOXACIN	15 (83.3)	0 (0.0)
OFLOXACIN	15 (83.3)	2 (6.6)
CEFTAZIDIME	18(100)	2 (6.6)
CEFEPIME	15(83.3)	4 (13.3)
TICARCILLIN	12(66.7)	11(36.6)
TETRACYCLINE	18 (100)	0 (0.0)
IMIPINEM	2 (11.1)	0 (0.0)
CHLORAMPHENICOL	15 (83.3)	20 (66.6)

Table 5: Multiple antibiotic resistance (MAR) pattern of different *Pseudomonas* species isolated from Awba Dam samples

Type of Specimen	*Pseudomonas aeruginosa*		MAR		*Pseudomonas fluorescens*		MAR	
	NO	%	NO	%	NO	%	NO	%
WATER	6	100	-	-	13	54.2	2	15.4
SEDIMENT	-	-	-	-	11	45.8	2	18.2
TOTAL	6/30	100	-	-	24/30	100	4/24	16.7

Table 6: Multiple antibiotic resistance (MAR) pattern of different *Pseudomonas* species isolated from Clinical samples

CLINICAL SOURCE	*Pseudomonas aeruginosa*		MAR		*Pseudomonas fluorescens*		MAR	
	NO	%	NO	%	NO	%	NO	%
URINE	7	70	7	100	2	25	1	50
EAR	1	100	1	100	4	50	4	100
WOUND	1	100	1	100	1	100	1	100
PLEURAL EFFUSION	-	-			1	100	1	100
NASOPHARYNGEAL TUBE	1	100	1	100	-	100	-	-
TOTAL	10		10/10	100	8		7/8	87.5

Discussion

In the present study, more than 200 bacteria were isolated from water and sediment samples obtained from different points of Awba Dam. Among them 30 samples were found to be positive for *Pseudomonas* species with about six of them as *P. aeruginosa* and the remaining as *P. fluorescens*. The isolation rate of *P. aeruginosa* in water (20%) and 80% of *P. fluorescens* in water and sediment was observed. All *Pseudomonas* species were subjected to ten antimicrobial agents which are (penicillins) ticarcillin; (carbapenem) imipenem; (cephems) ceftazidime, cefepime; (aminoglycosides) gentamicin, amikacin; (quinolones) ciprofloxacin, ofloxacin; (phenicols), chloramphenicol; and tetracycline. The reason for choosing this antimicrobial was their wide use in the hospital as antipseudomonal agents. The studies on the antibiotic sensitivity of environmental isolates of *Pseudomonas* species are very scanty in literature. So the present study was carried out to find out the proportion of the resistance isolates in Awba dam and to compare their antibiotic resistance pattern with that of clinical samples. *Pseudomonas* is one of the major pathogens in healthcare associated infections (HAI) (Olayinka *et al.*, 2009). This is not only because they cause infections that are associated with significant morbidity and mortality but also because of their increasing rates of resistance which make them more difficult to be treated with inexpensive antibiotics. Emerging and increasing resistance to newer and otherwise efficacious antibiotics may compound the whole problem (Okeke and Sosa, 2003). *P. aeruginosa* isolated from water were susceptible to all

antibiotics while *P. fluorescens* in both water and sediment were resistant to one or more antibiotics used in the study. Among the ten antibiotics, complete sensitivity was found with imipenem and amikacin in environmental isolates. This is in consonance with the studies of (Marufa *et al.,* 2015) who found complete sensitivity with imipenem in *Pseudomonas aeruginosa* isolated from surface water. Isolates from environmental samples (Water and Sediment) demonstrated high level of susceptibility to imipenem 30 (100%), gentamicin 30 (100%), amikacin 29 (96.7%) and ciprofloxacin 29 (96.7%) and Amikacin 15 (83.3%), Imipenem 16 (88.9%) for clinical isolates. This finding is consistent with that of other studies which reported high level of resistance for the fluoroquinolones (ciprofloxacin) and aminoglycosides (amikacin and gentamicin) respectively (Olayinka *et al.,* 2004; Olayinka *et al.,* 2009; Oduyebo *et al.,* 1997).

The observation is contrary to a previous report suggesting that fluoroquinolones have lost their effectiveness against *P. aeruginosa* strains due to resistance (Jalal and Wretlind, 1998). Also in agreement with the observation in this study, Navon-Venezia *et al.*, 2005 reported considerable *Pseudomonas* resistance to the aminoglycosides (including gentamicin) and fluoroquinolones (including ofloxacin) in clinical isolates; while Lateef, 2004 observed high resistance to both antibiotics in *Pseudomonas* strains isolated from pharmaceutical effluents. The observations were not surprising as clinical environments tend to exert more selective pressure (leading to antibiotic resistance) on bacterial populations than non-clinical/non-pharmaceutical (e.g., municipal effluent) environments (Iwane *et al.*, 2001; Schwartz *et al.*, 2003). However the difference in resistance rates in these works may be explained by the increasing trend that had been noted globally (Okeke and Sosa, 2003). This may have implications on the effectiveness of the local and probably national hospital infection and antibiotic resistance control programs.

Conclusion

Human inputs of complex mixture of domestic effluents from halls of residences and other sources in the University of Ibadan should be reduced in order to reduce the number of bacteria population present in the dam.

The development of resistance to antimicrobials is a natural process, which cannot be stopped. Resistance means that people cannot be successfully treated and they remain ill for longer period of time. Also, epidemics are prolonged because of resistance and thus that there is a greater risk of infection to others. Results indicate that the resistance of *Pseudomonas* species in water may be caused by the uncontrolled disposal of effluents that may contain chemicals and antibiotic in the environment. Proper implementation of antibiotic policies and guideline must be there in every hospital to local susceptibility pattern. This study demonstrated that MAR *Pseudomonas* species were prevalent in clinical samples and environmental samples. Since the emergence of MAR *Pseudomonas* species is a public health issue, this work support the need for regular and consistent monitoring of municipal sewage effluents with a view to preventing the dissemination of these pathogens into the environment.

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