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RANDOM AMPLIFIED POLYMORHIC DNA TYPING OF MULTIDRUG-RESISTANT CLINICAL AND ENVIRONMENTAL *Pseudomonas aeruginosa* STRAINS FROM ABEOKUTA, NIGERIA.

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ABSTRACT

Pseudomonas aeruginosa, a multidrug-resistant organism is responsible for most opportunistic infections. Genetic relatedness between clinical and environmental strains has always been limited to hospital settings. This study utilized RAPD-PCR typing method to evaluate genetic relatedness between multidrug-resistant *P. aeruginosa* strains from diverse water samples (bottled water, tap water, sachet water, well water, hospital storage tank, and swimming pool water) and clinical strains (wound, blood, urine, eye and ear swab) collected from different locations in Abeokuta, Nigeria. Polymorphic DNA bands with sizes ranging between 250 and 3000bp were generated from both clinical and environmental strains. Within each population, both clinical and environmental strains were divided phylogenetically into two groups of *Pc1* and *Pc2* at 55% and *Ps1* and *Ps2* at 70% respectively. Genetic similarities between clinical and environmental strains yielded a total of 7 unique fingerprints. Cluster 2 (51.2%) had the largest number of strains in which strains from wound, blood and ear, clustered with strains from hospital storage tank, tap water, swimming pool water, sachet water and well water. These fingerprints proof genetic relatedness between clinical and environmental strains in Abeokuta, southwest Nigeria which is of public health significance, particularly, for immunocompromised individuals.

KEY WORDS: *Pseudomonas aeruginosa*; multidrug resistance; genetic relatedness; water quality; RAPD-PCR.

INTRODUCTION

Pseudomonas aeruginosa, a gram-negative rod, ubiquitous in nature (Finnan *et al*., 2004), accounts for significant proportion of nosocomial infections (Micek *et al*., 2005; Taheri *et al*., 2008). It causes different infections such as, urinary tract, respiratory system, soft tissue, bone and joint, gastrointestinal and a variety of systemic infections, dermatitis and bacteremia particularly in patients with severe burns, cancer and AIDS patients (Oni *et al*., 2002). *Pseudomonas aeruginosa* infection is significant particularly in immunocompromised patients (Fichtenbaum *et al*., 1997; Asboe *et al*., 1998; Kiewitz and Tummler, 2000; Lang *et al*., 2004; Andualem, 2012) such as HIV/AIDS and cancer. It is an opportunistic bacterium responsible for chronic lung infection in cystic fibrosis patients, as well as the leading cause and death

in cystic fibrosis (Savaþ *et al*., 2005; Kidd *et al.,* 2011).

Pseudomonas aeruginosa is found in moist environment including surface water, drinking water, distilled water, swimming pool and spas. Its ability to thrive in a broad range of environment is in parts caused by the fact that it possesses a large and diverse genome (Stover *et al*., 2000). The pathogenicity of *P. aeruginosa* is enhanced by production of both extracellular (cyanide, proteases) and cell-associated (pili, flagella, lipopolysachharide, Type III system effector proteins, type III secretion system and alginate) virulence factors (Finnan *et al*., 2004). Environmental strains of *P. aeruginosa* can serve as the source for human infection (Wolfang *et al*., 2003). The role of tap water outlets and patients in hospital environment has been a subject of investigation by various research such as Cholley *et al*., 2008; Trautmann *et al*., 2009; Jiun-Ling *et al*., 2009; Salama *et al*., 2012 with serious implications of water contributing to nosocomial infections.

Studies on the origin and clonal diversity of *P. aeruginosa* have become very important because of increasing incidence of infections caused by this organism worldwide. Genetic relatedness between clinical and environmental Pseudomonas with the use of Random Amplified Polymorphic DNA-Polymerase Chain Reaction (RAPD - PCR) has been proven to be highly discriminatory (Bogiel and Gospodarek, 2010; Deligianni, 2010; Mansefeild *et al*., 2010; Kidd *et al*., 2011; Maatallah *et al*., 2011; Fazeli *et al*., 2012; Gawish *et al*., 2013).

This study was therefore, designed to examine isolates of multidrug resistant *P. aeruginosa* from diverse clinical and environmental sources for genetic relatedness using RAPD-PCR and to determine if water is a significant source of clinical infections in Abeokuta, Nigeria.

MATERIALS AND METHODS

Source of bacterial culture

A total of 43 clinical *P. aeruginosa* strains from wound (16), ear swab (9); urine (9); blood (5); and eye swab (4) were obtained from two hospitals (Sacred Heart Hospital and Federal Medical Centre). A total of 41 environmental strains from bottled water (1); sachet water (5); tap water (6); well water (12); swimming pool (9) and hospital storage tanks (8) were isolated from diverse locations in Abeokuta, Nigeria. The isolation procedures have been described previously.

Genomic DNA Extraction

Genomic DNA was extracted as described by Abd-El-Haleem *et al.* (2003). Total bacterial DNA was prepared using the boiling approach. Bacterial cells were pelleted by centrifugation, resuspended in 50µL of TE buffer and then lysed by boiling for 10 min. The lysate was centrifuged and the supernatant was transferred to a new tube. The crude cell lysate was used directly for PCR.

RAPD- PCR Amplification

The genomic DNA was amplified with a RAPD-2 arbitrarily primer sequence forward 5'CCGTCAGCA 3' and backward 3'GGCAGTCGT 5'. PCR was performed in 20 µl of a reaction mixture containing DNA (200ng), a master mix containing (1 X PCR Buffer, 12.5Mm MgCl2, 1mM dNTP, 2.5 U of DNA Taq polymerase), 25µMol (each) primer, and sterile distilled water. Thermal cycling was conducted in Eppendorf vapoprotect (Germany thermal cycler). After an initial denaturation for 5min at 95°C, 40 amplification cycles were performed (30 s at

95ºC, 1min at 28ºC and 2 min at 72ºC). The final cycle was extended at 72ºC for 10 min. A reaction mixture containing sterile water was included as a negative control and a purified DNA mixture of the targeted bacteria was included as a positive control. The amplified PCR products were analysed by gel electrophoresis in 2% agarose gels stained with ethidium bromide, and then visualized and photographed using a photo documentation system under a short wave ultraviolet light trans illuminator.

Phylogenetic Analysis of RAPD fingerprints

Positions of unequivocally scorable RAPD bands were transformed into a binary character matrix ("1" for the presence and "0'' for the absence of a band at a particular postion). Pairwise distance matrices were complied by the Numerical Taxonomy System (NTSYS) 2.0 software (RohIf, 2000) using the Dice coefficient of similarity. Phylogenetic relationship was created by the unweighted pair-group method arithmetric (UPGMA) average cluster analysis (Jako *et. al.,* 2009).

RESULTS

Banding patterns of RAPD

DNA banding pattern of various *P. aeruginosa* obtained from clinical and environmental sources shows molecular weight range between 250 and 3000bp using RAPD-2 primer. Plate 1 shows the representative electrophoresis gel banding pattern.

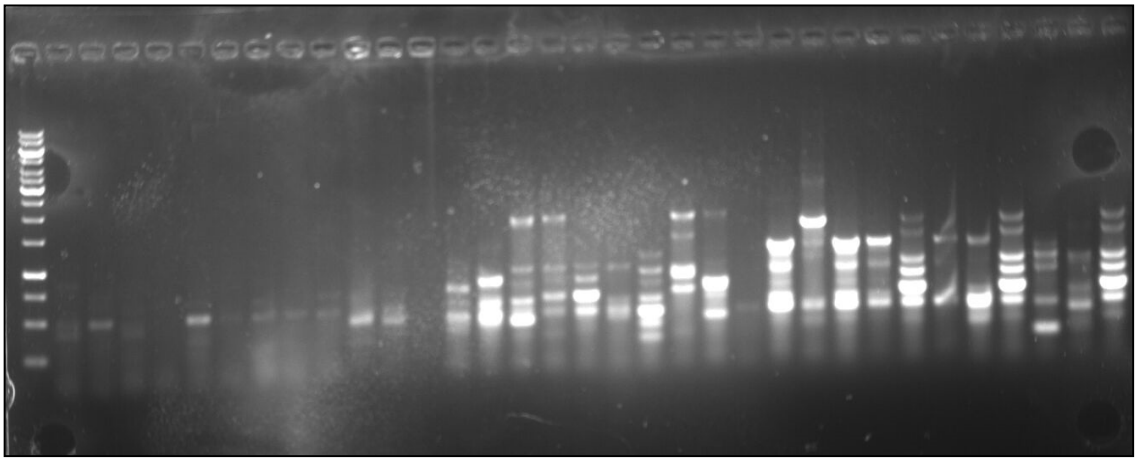
Phylogenetic analysis of multidrug resistant clinical P. aeruginosa strains

Table 1 shows the phylogenetic analysis of clinical isolates of *P. aeruginosa* obtained at 55% similarity dividing strains into *Pc1* and *Pc2*. *Pc1* constitutes 9.3% while the remaining belonged to Pc2. *Pc2* was subdivided into *Pc2a* (9.3%), *Pc2b* (11.6%), *Pc2c* (18.4%) and *Pc2d* (51.2%) at 70% similarity.

Phylogenetic analysis of multidrug resistant environmental P. aeruginosa strains

Figure 2 shows the dendogram while Table 2 shows the phylogenetic analysis of multidrug resistant environmental *P. aeruginosa*. Isolates were classified into two groups at 70% phylogenetic similarity, which consist of *Ps1* (19.5%) and *Ps2* (80.5%). *Ps1* comprises of three sub-groups: *Ps2a* (53.7%), *Ps2b* (16.3%) and *Ps2c* (9.3%) at 75% genetic similarities.

Figure 3 and Table 3 show genetic polymorphism and sub-clonal diversity of *P. aeruginosa* obtained from clinical and water samples. All the isolates were grouped into seven clusters: cluster 1 (9.5%), cluster 2 (51.2%), cluster 3 (17.8%), cluster 4 (1.2%), cluster 5 (11.9%), cluster 6 (3.6%) and cluster 7 (4.8.%)



Lane M: molecular marker: lane 34 to 43: environmental DNA; lane 44 to 65: clinical DNA

Plate 1: Banding patterns determined by RAPD showing the genetic relatedness of some multidrug-resistant clinical and environmental *Pseudomonas aeruginosa* strains.

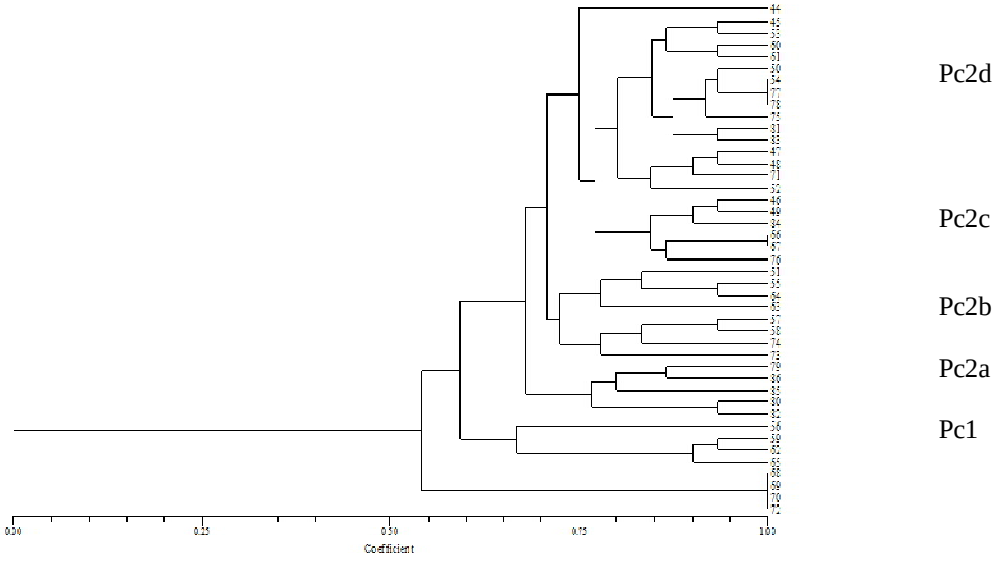


Figure 1. Dendogram determined by RAPD showing the genetic relatedness of multidrug-resistant clinical *Pseudomonas aeruginosa* strains.

Table 1. Genetic relatedness of multidrug resistant clinical *Pseudomonas aeruginosa*

Clonal group	Clonal sub-groups	percentage (%)	Wound		Blood		Ear swab		Urine		Eye swab	
			No of isolates	Percentage (%)	No of isolates	Percentage (%)	No of isolates	Percentage (%)	No of isolates	Percentage (%)	No of isolates	Percentage (%)
Pc1	Pc1	9.3	0	0.0	0	0.0	4	9.3	0	0.0	0	0.0
	Pc2a	9.3	2	4.7	1	2.3	1	2.3	0	0.0	0	0.0
	Pc2b	11.6	0	0.0	0	0.0	0	0.0	3	7.0	2	4.7
	Pc2c	18.4	4	9.3	2	4.7	1	2.3	1	2.3	0	0.0
	Pc2d	51.2	10	23.3	2	4.7	3	7.0	5	11.6	2	4.7
			16	37.2	5	11.6	9	20.9	9	20.9	4	9.4

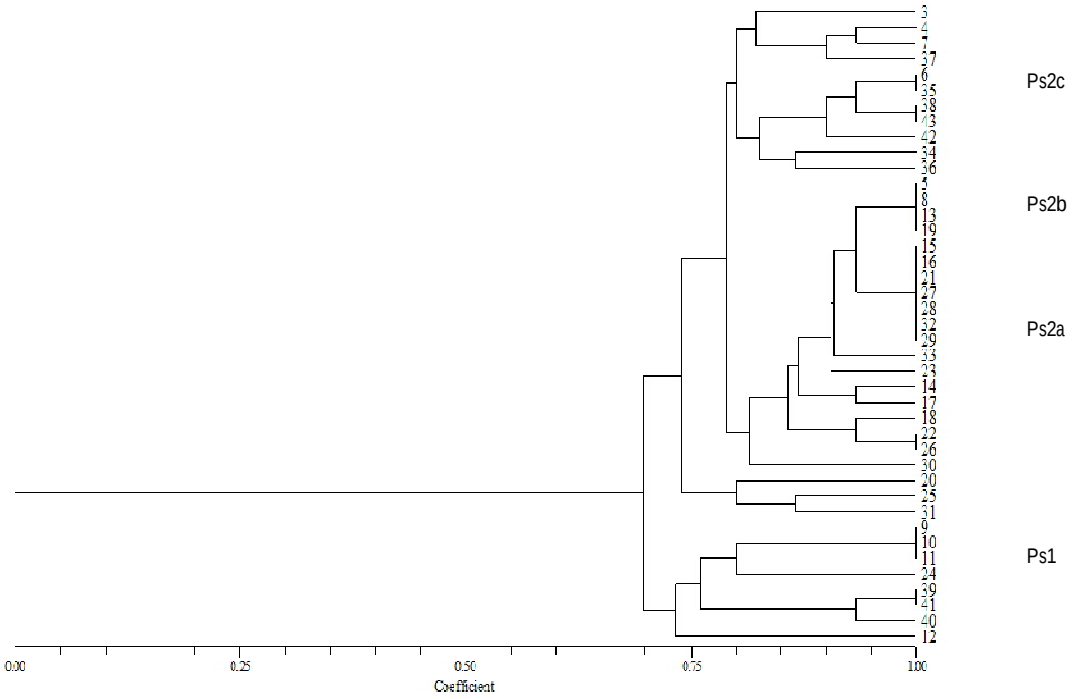


Figure 2. Dendogram estimated by RAPD-RAPD showing the genetic relatedness of multidrug-resistant environmental *Pseudomonas aeruginosa* strains.

Table 2. Genetic relatedness of multidrug resistant environmental *Pseudomonas aeruginosa*

			Hospital storage tank		Tap water		Swim-ming pool		Bottled water		Well water		Sachet water	
Clonal	Sub-clonal	Percentage (%)	No of isolates	Percentage	No. of isolates	Percentage	No of isolates	Percentage	No of iso-	Percentage	No of iso-	Percentage	No of isolates	Percentage
Ps1	Ps1	19.5	2	4.9	2	4.9	1	2.4	0	0.0	0	0.0	3	7.3
Ps2	Ps2a	53.7	2	4.9	4	9.8	8	19.5	1	2.4	7	17.1	0	0.0
	Ps2b	17.1	1	2.4	0	0.0	0	0.0	0	0.0	4	9.8	2	4.9
	Ps2c	9.7	3	7.3	0	0.0	0	0.0	0	0.0	1	2.4	0	0.0
			8	19.5	6	14.7	9	21.9	1	2.4	12	29.3	5	12.2

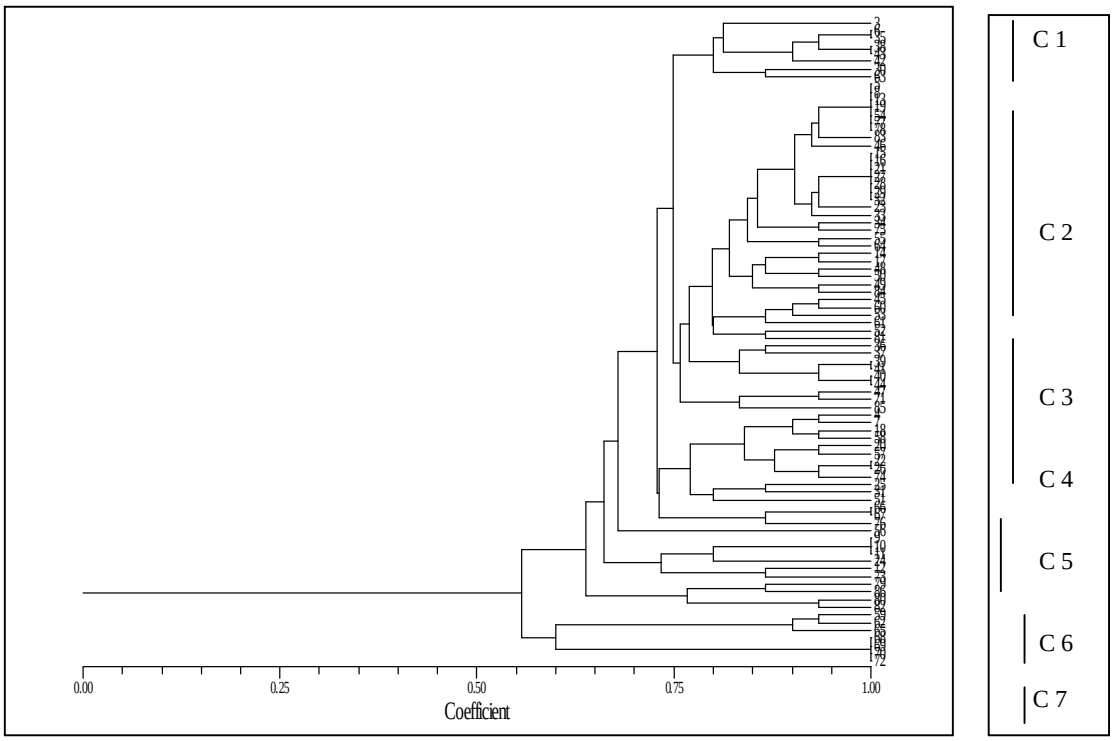


Fig. 3. Dendogram estimated by RAPD showing the genetic relatedness of multidrug-resistant clinical and environmental *Pseudomonas aeruginosa* strains.
Key: C = Cluster group

Table 3. Genetic relatedness of multidrug resistant clinical and environmental *Pseudomonas aeruginosa*

Cluster groups	Total Isolate	Wound		Blood		Ear swab		Urine		Eye swab		Hospital tank		Tap water		Swim-Ming Pool		Bottled Water		Well Water		Sachet Water	
		No of isolates	Percentage (%)	No of isolates	Percentage (%)	No of isolates	Percentage (%)	No of isolate	Percentage (%)	No of isolates	Percentage (%)	No of isolates	Percentage (%)	No of isolates	Percentage (%)	No of isolates	Percentage (%)	No of isolates	Percentage (%)	No of isolates	Percentage (%)	No of isolates	Percentage (%)
C 1	8	0	0.0	1	1.2	0	0.0	0	0.0	0	0.0	2	2.4	0	0.0	0	0.0	0	0.0	2	3.6	2	2.4
C 2	43	11	13.1	3	3.6	1	1.2	4	4.8	3	3.6	2	2.4	4	4.8	4	4.8	0	0.0	8	9.6	3	3.6
C 3	15	3	3.6	0	0.0	2	2.4	2	2.4	0	0.0	2	2.4	0	0.0	4	4.8	1	1.2	1	1.2	0	0.0
C 4	1	1	1.2	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.00
C 5	10	0	0.0	0	0.0	1	1.2	3	3.6	1	1.2	2	2.4	2	2.4	1	1.2	0	0.0	0	0.0	0	0.0
C 6	3	1	1.2	1	1.2	1	1.2	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
C 7	4	0	0.0	0	0.0	4	4.8	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
	84	16	19.1	5	5.9	9	10.7	9	10.7	4	4.8	8	9.8	6	7.2	9	10.7	1	1.2	1.2	14.3	5	0.0

DISCUSSION

Multidrug resistant *Pseudomonas aeruginosa* has been previously reported from both hospital and water environment from across the nation, Nigeria. This study investigated genetic relatedness between clinical and diverse environmental *P. aeruginosa* strains using RAPD-PCR typing method which has been proven to be highly discriminatory. A very high polymorphism with banding sizes ranging from 250-3000bp generated was used to construct the dendogram and phylogenetic relationship in order to infer clonality among strains. The polymorphism observed in this study is supported by the fact that *Pseudomonas aeruginosa* is said to exhibit an epidemic population structure with horizontal transfer of DNA resulting in high frequency of genomic islands (Morales-Espinosa *et al*., 2012).

The phylogenetic tree for clinical strains classified the strains into two major groups of *Pc1* with 4 (9.3%) strains and *Pc2* with 39 (81.7%) strains at 55% phylogenetic similarity expressing their high diversity. *Pc1* comprised only strains from ear swab whereas *Pc2* was further differentiated into four subgroups at approximately 65% similarity. *Pc2a* (9.3%), comprised strains from wound (2), blood (1), and ear (1) while *Pc2b* (11.6%), consists of strains from urine (3) and eye swab (2) only. *Pc2c* (18.4%), clustered strains from wound (4), blood (2), ear swab (1), and eye swab while *Pc2d* (51.2%) clustered strains from all clinical samples from wound (10), blood (2), ear swab (3), urine (5) and eye swab (2). Similar results of diversity by RAPD-PCR typing of clinical strains from Nigeria with unique fingerprints were observed by Akanji *et al.* (2011) on nosocomial infections and Smith *et al*. (2012) on wounds.

The dendogram for the environmental strains revealed two major clonal groups indicated as *Ps1* and *Ps2* at 70% genetic similarities. Out of 41 strains, only 8(19.5%) belonged to *Ps1* group while the remaining 33 (80.5%) belonged to *Ps2*, implying that all the environmental strains have relative genetic diversity. Though, *Ps2* was further divided into sub-group (*Ps2a, Ps2b and Ps2c*) at 82% similarity, there is little diversity in their genetic polymorphism, showing that despite their different sources, they are more closely related than clinical strains. Strains from hospital storage tank spread across all the genotypes with 4.9% in *Ps1* and 14.6% in *Ps2* showing clonality.

In this study, the combined dendogram of both the clinical and environmental strains of *P. aeruginosa* classified them to seven different clusters. In cluster 1 (8 strains), strains from blood (1), clustered with hospital storage tank (2), well water (3) and sachet water (2). Cluster 2 with 43 (51.2%) strains had the largest number whereby wound (11), blood (3), and ear (3), clustered with strains from hospital storage tank (2), tap water (4), swimming pool (4), sachet water (3) and well water (8). It is notable that in cluster 3 with 15 (17.6%) strains, wound (3), ear (2), urine (2), clustered with strains from hospital storage (2), swimming pool (4), bottled water (1), and well water (1). From this result, it is observed that multidrug resistant *P. aeruginosa* from the environment is clonally related with clinical strains similar to Gad *et al*. (2007), Ndip *et al*. (2007), El-Bialy *et al*. (2008), Taheri *et al*. (2008), and Salimi *et al*. (2010).

Though, cluster 4 (1.2%) and cluster 7 (4.8%) had stand alone wound (1) and ear swab (4) strains respectively, while cluster 6 had 3 clinical strains only, each respectively from wound, blood and ear, the results re-

flect the genetic uniqueness of these strains. This study revealed genetic relatedness between clinical and environmental multidrug-resistant *P. aeruginosa* by RAPD- PCR typing suggesting little variation in virulence, infection types and environmental survival. Gawish *et al*. (2013) observed that virulence genes in genome of *P. aerugino-sa* strains regardless of their origin whether clinical or environmental were conserved, as detected by whole-genome DNA microarray, and this lead to the suggestion that the environmental strains possess the ability to cause human infections despite the low probability of encountering a human host. Similarly, Wolfang *et al*. (2003) studied clinical and environmental *P. aeruginosa* using a whole genome microarray, which revealed no correlation between genome content and infection type and concluded that the organism possesses a highly conserved genome that encodes genes important for survival in numerous environments which allows it to cause a variety of human infections. Finnan *et al*. (2004) observed that phylogenetically, isolates from cystic fibrosis patients and that of hospital environment, clustered together with one another on the *mdh* gene tree.

The finding from this study implies that environmental strains could contribute to clinical infections and that water could serve as a major route of dissemination of infections within hospitals and other community. This is significant for other types of *P. aeruginosa* infections that were not considered in this study such as diarrhea particularly for immunocompromised patients, because *P. aeruginosa* is also one of the opportunistic bacterium in HIV/AIDS patients with diarrhoea (Fichtenbaum *et al*., 1998; Osazuwa *et al*., 2011, Andualem, 2012). Also, in respiratory tract infections, once established, eradication of *P. aeruginosa* from the respiratory tract of HIV-seropositive individuals with advanced immunosuppression is problematic and a chronic infective state appears common (Asboe *et al*., 1998).

Prior to this study, there have been few studies on genetic relatedness of *Pseudomonas aeruginosa* from clinical infections in Nigeria. This is the first report on molecular typing of multidrug resistant clinical and environmental *P. aeruginosa* in Nigeria.

CONCLUSION

The fingerprints of *P. aeruginosa* provide genetic relatedness between clinical and environmental multidrug resistant strains which is of public health significance, particularly, in immunocompromised patients.

RECOMMENDATIONS

There is a need to evaluate virulence and resistance genes in environmental *P. aeruginosa* strains as well as evolutionary pathways between clinical and environmental strains. Also, in order to evaluate the full burden of multidrug resistant *P. aeruginosa* on immunocompromised individuals such as HIV in Nigeria, there is a need to investigate its co-infection in south western Nigeria.

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