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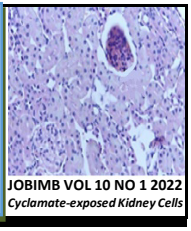

Molecular Identification of Biofilm-forming *Pseudomonas aeruginosa* Isolates from Noncritical Surfaces of a Tertiary Healthcare Center in Abia State, Southeast Nigeria

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ABSTRACT

The presence of biofilm-forming *Pseudomonas aeruginosa* on noncritical surfaces in a hospital has been attributed to healthcare-associated infections (HAIs) leading to multidrug-resistant (MDR) infections. This study is aimed at identifying the molecular characteristics of Biofilm forming *Pseudomonas aeruginosa* found on non-critical surfaces in a hospital environment. Samples were collected using the swab technique from different noncritical surfaces surrounding hospitalized patients in different wards namely sphygmomanometers, thermometers, bed rails etc. Bacterial analysis was performed by using conventional microbiological techniques, biochemical tests and Microbact 24E assay. Biofilm forming *Pseudomonas aeruginosa* were visualized using Crystal Violet staining assay and then estimated by measuring the Optical Density through Spectrophotometer. Four hundred and fifty (450) positive samples of bacterial isolates from noncritical surfaces of six selected wards of the hospital were obtained from one thousand three hundred and fourteen (1314) samples. One hundred and thirty-nine (139) isolates of *Pseudomonas* spp. were obtained, out of which 115 (82.73%) were identified as biofilm formers. Of these 115 biofilm formers, 83(72.17%) belonged to *Pseudomonas aeruginosa*, 30(22.09) *Pseudomonas fluorescens,* 20(17.39%) *Pseudomonas putida* and 6(5.22%) belongs to *Pseudomonas chlorophis*. The final characterization of Biofilm forming *Pseudomonas aeruginosa* isolates was verified by 16S rDNA sequencing. Sequence analysis showed similarity at 99.5% Pearson`s correlation coefficient (r) between 16S rDNA of the representative standard isolate (from gene library) and Biofilm forming *Pseudomonas aeruginosa* strain obtained from the Tertiary health facility, Federal Medical Centre (FMC), Umuahia. The nucleotide sequence of the experimental isolates expressed genetic mutation which represents the point of involvement of Pel B gene and is involved in the biofilm formation character of *Pseudomonas aeruginosa* in this study. There is an urgent need to take nosocomial transmission from noncritical surfaces serious in FMC Umuahia.

INTRODUCTION

Pseudomonas aeruginosa, albeit widespread in almost all habitats, is occasionally discovered in specialized reservoirs within the hospital environment, such as sinks. This organism's mode of transmission has not been fully explored, but it is evident that direct contact with contaminated surfaces and indirect contact routes, such as insufficient hand hygiene on the part of healthcare professionals, are potential routes of infection. *Pseudomonas aeruginosa* can survive in the environment for

many days to six months on surfaces, which may contribute to cross-contamination in a healthcare setting [1–3]. Although airborne transmission of this organism has been proposed, scientific proof on the subject is lacking. Different biofilm morphologies can be generated depending on *Pseudomonas aeruginosa* strains and/or nutritional circumstances. For example, in glucose basal medium, the biofilm lifestyle cycle of *Pseudomonas aeruginosa*, PAO1, a derivative of the original Australian PAO isolate that is a standard isolate for research distributed worldwide to laboratories and strain collections, can be subdivided into five major phenotypic steps depicting the typical formation stages of microbial biofilms. These stages can be sub-divided into five major phenotypic steps portraying the pretty standard formation phases of microbial biofilm. The process begins with the reversible adherence of planktonic bacteria to a suitable surface for growth, proceeded by the irreversible attachment of bacteria, which forms micro colonies in the EPS matrix. Bacterial micro-colonies increase gradually, and their confluences result in a more organized phenotype. Non-colonized gaps are then colonized by bacteria, which eventually cover the entire surface. Finally, bacteria are disseminated from the sessile structure and re-enter the planktonic stage, where they can spread and colonize new surfaces [4,5].

Pseudomonas aeruginosa produces at least three polysaccharides (alginate, Pel, and Psl) that are important for the biofilm structural stability [6]. *Pseudomonas aeruginosa* mucoid and non-mucoid strains differ in the qualitative composition of their polysaccharides in the biofilm matrix, which is primarily alginate or Psl/Pel, respectively [7]. Alginate, a linear unbranched polymer consisting of D-mannuronic acid and L-glucuronic acid, contributes to biofilm structural stability and protection, as well as water and nutrient retention [8]. Pel polysaccharide is mostly a glucose-rich matrix material with an unknown composition [9], whereas Psl is a repeating pentasaccharide composed of D-mannose, L-rhamnose, and D-glucose [10].

Pel and Psl are engaged in the early phase of biofilm formation and can act as a major structural scaffold for biofilm development [7]. Extracellular DNA (eDNA) is a critical functional component of the *Pseudomonas aeruginosa* biofilm matrix. Its involvement in Biofilm formation is very significant, for instance;
(i) DNase inhibits *Pseudomonas aeruginosa* biofilm formation [11]; (ii) eDNA-deficient biofilms are more sensitive to some detergents [12]; (iii) eDNA facilitates twitching motility-mediated biofilm widening by preserving coherent cell alignments [13]; (iv) eDNA has been proposed to play an important role in the initial and early development of *Pseudomonas aeruginosa* biofilm.
(v) Finally, during hunger, eDNA serves as a nutrition source for bacteria [15].

Pseudomonas aeruginosa extracellular appendages such as flagella, type IV pili, and cup fimbriae, which are also called matrix components, have sticky functions in cell-to-surface interactions (irreversible attachment) as well as microcolony development in biofilms. When compared to wild-type strains, mutants faulty in flagella-mediated movement and mutants defective in the synthesis of the polar-localized type IV pili do not produce microcolonies [16]. The primary goal of this research is to identify the molecular characteristics of Biofilm forming *Pseudomonas aeruginosa* identified from non-critical hospital surfaces.

MATERIALS AND METHODS

The Tertiary Health facility where the study was carried out is 800 beds capacity Federal Medical Center, Umuahia, Abia State, Southeast, Nigeria after obtaining approval from the Research and Ethics committee of the Hospital. Samples were collected using the swab technique from different noncritical surfaces surrounding hospitalized patients. Sampling was carried out in six busy wards of the hospital. Sample collection was repeated three (3) times per critical surface. One hundred and thirty-nine (139) isolates of *Pseudomonas* species on noncritical surfaces in hospital wards were isolated using the swab technique from 1314 samples. These non-critical surfaces include tap heads, sinks, bed rails, tabletop and medical equipment like sphygmomanometer and Thermometer. Bacterial analysis was performed by using conventional microbiological techniques, routine biochemical tests and the Microbact 24E assay. Developed biofilms were visualized with crystal violet staining method and then estimated by measuring the optical density through spectrophotometer [17].

Motility and Preliminary biochemical tests techniques

Motility is the main physiological character of interest here and was determined by the hanging drop method, while preliminary biochemical tests like Sugar reaction, mainly oxidation of glucose, were determined by phenol red broth base (10g/L peptone, 1g/L yeast extract, 5g/L sodium chloride,0.018g/L phenol red, with the addition of filter-sterilized sugar solution at a final concentration of 0.5%). The reaction time was within 24 hours at an optimum temperature of 37°C. Other biochemical tests like the methyl-red test were performed by the addition of an indicator (0.1g methyl red test per 300ml 95% ethanol) to cultures grown for 48h in 4ml of MR-VP broth. Voges Proskauer test was performed by the addition of 40% potassium hydroxide in water and 5%1-naphthol in 95% ethanol to cultures grown for 24h in MR-VP broth. Indole production test was determined by Kovacs reagents (5g p-dimethylaminebenzaldehyde, 25ml HCL and 75ml sulphanillamide pentanol-1-ol). The nitrate reduction test was carried out by the addition of 1% of 1M HCL and 0.02% N-1 naphthalene diamine HCL in water to nitrate test broths[18].

Microbact 24E biochemical identification assay for *Pseudomonas* spp.

All *Pseudomonas* isolates identified by conventional biochemical tests were again subjected to the Microbact test [19] in line with the manufacturer`s instruction for confirmation. The Microbact 24E system (**Fig. 1**) is a miniaturize pH dependent and substrate utilization identification test system for Enterobacteriaceae and other miscellaneous Gram-negative bacilli

Crystal Violet Assay

Biofilm forming *Pseudomonas aeruginosa* was confirmed using the Crystal Violet Assay technique [19]. Crystal violet Assay is a staining method (**Fig. 2**) which helps in the quantitation of biofilm mass though, does not give information on biofilm viability as it stains both the bacteria cells and the extracellular matrix [20].

Molecular Identification

Conventional primed Polymerase Chain Reaction (PCR) was used for the molecular identification of biofilm-forming *Pseudomonas aeruginosa* isolates for confirmation of their true identity according to Spike *et al.,* [21]. Steps involved in molecular characterization of biofilm-forming *Pseudomonas aeruginosa* by Polymerase Chain Reaction include DNA extraction, PCR amplification, Gel Electrophoresis and sequencing.

DNA extraction

Biofilm-forming *Pseudomonas aeruginosa* isolates were sub-cultured and grown in Mueller Hinton Broth (MHB) at 37ºC for 24h. Then 1ml of the liquid culture was transferred into a 1.5 ml volume microfuge tube. Bacteria cells were harvested by centrifugation at 12,000 rpm for 5 min. The supernatant was discarded, and the pellet was washed twice with ultrapure water and re-suspended in 1 ml ultrapure water. DNA extraction was carried out (ethanol precipitation method) by adding 0.1 vols. 3M Sodium acetate 2.5- vols. ice cold 100% ethanol, this is vortexed to mix thoroughly, and precipitated at -20°C for I hour. After centrifugation, the clear supernatant containing the DNA was transferred to a new microfuge tube and used directly in a specific Polymerase Chain Reaction amplification step for the determination of the Genus and Specie of the isolate.

Amplifications and cycling.

PCR amplifications were performed on a thermocycler (A&E Laboratories, UK Model Cyl-005). The reaction volume was 25µl and consisted of 10X PCR buffer, 25 mM $MgCl_2$, 10 mMdNTP's mixture, 5 U/µl of Taq DNA polymerase (Fermentas, USA), 10 pmol of each primer set, and 5ng of extracted bacterial DNA. Amplifications were performed following an initial denaturation at 96°C for 5 min; 35 cycles of 96°C for 60 s, 55°C for 60 s followed by 72°C for 60 s; and a final period of extension at 72°C for 10s according to manufacturer's instruction.

Gel Electrophoresis

Amplified products (10 µl) were separated using 1.5 % agarose gel electrophoresis in TAE buffer (40 mM Tris-acetate, 2 mM EDTA, pH 8.3) performed at 70 V for 1 hour. Gels were stained with 0.5 µg/ml of ethidium-bromide for 45 min and destained with water for 20 min. In a photo documentation system, stained gels were evaluated using an ultra-violet (UV) transilluminator (Clinix, Japan). In the study, a major band matching to the expected band size in comparison to the DNA mass ladder was taken into account.

Sequencing

The amplified product was then purified using MinElute UF plates (Qiagen) following the manufacturer's protocol before being used in a sequencing reaction. Sequencing was carried out on each DNA strand with Big-Dye Terminator Ready Reaction Mix v3.1 (PE Bio-systems, Foster City, US) under the following conditions: initial denaturation at 96°C for 1 min, 30 cycles of 10 sec at 96°C, 5 sec at 51°C and 2 min at 60°C. Unincorporated dye terminators were removed by precipitation with 95% alcohol. The reaction products were separated and detected on an ABI PRISM genetic analyzer 3100 (PE Bio-systems) using a standard sequencing module with a Performance Optimized Polymer and 5 cm array. The universal Primer pair used for sequence analysis was 27F-1492R primers [22]. The sequencing protocol optimized using this universal primer helps reduce the reaction turn-around time (TAT) and very cost-effective.

Molecular Identification of biofilm-forming *Pseudomonas aeruginosa*

Molecular identification of biofilm-forming *Pseudomonas aeruginosa* isolates was carried out using conventional primed polymerase chain reaction (PCR). Bands that were clearly in alignment with the molecular mass ladder were subsequently chosen as positive for either genus or species of isolates, having separated during gel electrophoresis to the same position of the ladder. PCR products were digested before sequencing using protease. Of the eighty-three (83) samples of Biofilm forming *Pseudomonas aeruginosa,* isolates identified from Microbact 24E biochemical and crystal violet assay, Forty (40) samples proceeded to Sequencing after conventional PCR Analysis. Sequence analysis identified two groups of biofilms forming *Pseudomonas aeruginosa* isolates with the following nucleotide sequence (**Fig. 3**) compared to the control strain obtained from gene library.

RESULTS

The majority of *Pseudomonas* species isolated in this study exhibited characteristic biochemical characters as confirmed by Microbact biochemical identification assay (**Table** 1). Out of 139 isolates of *Pseudomonas* spp. 115(82.73%) were identified as biofilm formers among biofilm forming bacteria (**Tables 2** and **3**). Of this number of biofilm formers, *Pseudomonas* spp. discovered from noncritical surfaces classified further as 83 (72.17%) belonged to *Pseudomonas aeruginosa*, 30 (22.09%) *Pseudomonas fluorescens,* 20 (17.39%) belong to *Pseudomonas putida* and 6 (5.22%) belong to *Pseudomonas chlorophis* (**Table 4**). The crystal violet assay technique was suitable for quantitation of *Pseudomonas aeruginosa* biofilm formation as it is suitable for both motile and non-motile bacterial isolates [23]. The former adheres to both the bottom and walls of the reaction wells while the latter can only be found at the bottom of the well thus making it easy for biofilm identification and quantitation (**Fig. 2**).

Table 1. Motility and biochemical identification reactions of diverse bacterial species in Hospital environment**.**

Sample Code	Colonial Characteristics	Motility	Gram reaction	Catalase	Urease	Coagulase test	Indole	Oxidase	Citrate utilization	Methyl red	Voges prausker	Bile solubility	Glucose	Sucrose	Lactose	Maltose	Mannose	Probable organism
SE, IE,	Translucent and mucoid colonies, 1-2mm in diameter. In young cultures, colonies are raised but later become flattered with raised edge, giving them a ringed appearance (draughtsman appearance)	-	Gram-positive cocci in chains	-	-	-	-	-	-	-	-	+	-	-	-	-	-	*S. pneumonia*
OE, SE	Slightly raised edge colony, 1-2 mm in diameter	-	Gram-positive cocci in clusters	+	+	-	-	-	-	-	-	-	-	+	+	-	+	*Coagulase negative S. aureus*
WFE, SE	Convex, smooth and white colony with entire edge	-	Gram-positive cocci in Clusters	+	-	+	-	-	-	-	+	-	-	-	+	-	+	*S. aureus*
EE, IE	Smooth, high colony with irregular edge	+	Gram-negative short rods	+	-	-	-	+	+	-	-	-	A	-	-	A	-	Pseudo-monas spp.
IE, WFE	Swarming, flat colony with fishing odour	+	Gram-negative rods	-	-	-	-	-	+	-	-	-	-	A G	A G	A G	-	AG *Proteus* spp.
SE, QE	Mucoid, colony, 1-4mm in diameter	+	Gram-negative rod	-	-	+	-	-	-	-	+	-	-	A G	A G	A G	-	*E. coli*
PW, RT	Moderate sized smooth and gummy colony	-	Gram-negative rod	-	-	+	-	-	-	+	-	-	-	-	-	-	-	*K. pneu-moniae*

Note: A=Acid, G=Gas, + (Positive), - (Negative) CoNStaph = Coagulase negative Staphylococcus

Table 2. Classification of Biofilm forming bacterial isolates.

S/N	Bacterial Isolates	Total Isolates obtained	№ that Formed Biofilm	% Biofilm occurrence in each isolate
1	*Staphylococcus aureus*	79	13	16.46
2	*Pseudomonas* spp.	139	115	82.73
3	*Escherichia Coli*	117	21	17.94
4	*Klebsiella pneumonia*	27	6	22.22
5	*Streptococcus pneumonia*	42	4	9.52
6	Proteus spp.	20	3	15.00
7	Coagulase negative *Staphylococcus aureus*	26	1	3.70

Table 3. Microbact 24E biochemical identification assay of *Pseudomonas* spp. isolates.

Test				
Oxidase	+	+	+	+
Motility	+	+	+	+
Nitrate	+		+	-
Lys	-	-	-	-
Orn	-	-	-	-
H2S	-	-	-	-
Glu	+	-	-	-
Mann	-	+	-	-
Xyl	-	+	-	-
ONPG	-	-	-	-
Ind	-	-	-	-
Ure	-	-	-	-
V-P	-	-	-	-
Cit	+	+	+	-
TDA	-	-	-	-
Gel	-	-	-	-
Mal	-	-	-	-
Ino	-	-	-	-
Sorb	-	-	-	-
Rha	-	-	-	-
Suc	-	-	-	-
Lac	-	-	-	-
Ara	-	-	-	-
Raff	-	-	-	-
Sal	-	-	-	-
Arg	+	-	-	-
No of probable organism	83	30	21	6 139
Pseudomonas species	*P. aeruginosa*	*P. fluorescens*	*P. putida*	*P. chlorophis*

Key: H_2S-Hydrogen sulphide production, ONPG-β-Galactosidase, VP- Voges Proskauer reaction, TDA- Tryptophan deaminase,Oxidase, oxidase reaction, NIT-Nitrate production, LYS-Lysine decarboxylase, ORN- Ornitine decarboxylase, GLU- Glucose, MANN- Mannitol,XYL-Xylose, IND-Indole production, URE- Urea hydrolysis, CIT- Citrate utilization, GEL-Gelatin liquefaction, MAL-Malonate utilization, INO-Inositol, SORB-Sorbito, RHA-Rhamnose, SUC-Sucrose,LAC-Lactose, ARA-Arabinose, RAFF- Raffinose, SAL-Salicin, ARG-Arginine dihydrolase.

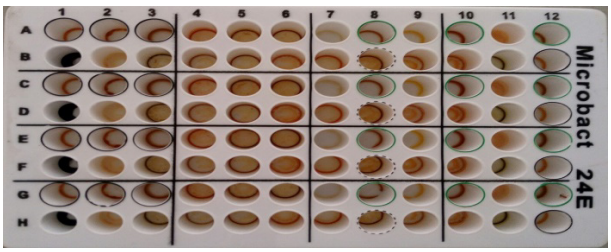

Fig. 1. Reaction well for Microbact assay (Oxoid, England).

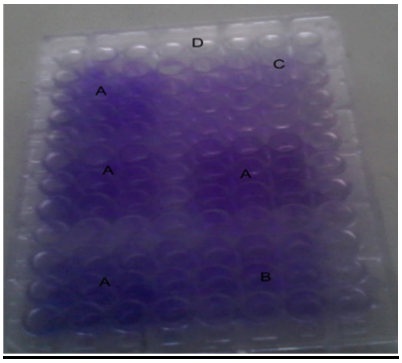

Fig. 2. Crystal violet assay indicating quantification of biofilms forming bacterial isolates. KEY: A= Strong Biofilms (OD-0.4± 0.03); B =Moderate Biofilms (0.2± 0.01); C= Weak Biofilms (0.1±0.001); D= No Biofilms (0.001 ± 0.004).

Table 4. Distribution of *Pseudomonas spp*. Isolates in Different Hospital Wards.

Wards	Total Sample collected	*P. aeruginosa*	*P. florescens*	*P. putida*	*P. chlorophis*	No of Pseudomonas spp. isolates
Accident &Emergency	314	21(60.00%)	7(20.00%)	4(11.43%)	3(8.57%)	35(11.75%)
Paediatrics	193	11(68.73%)	3(18.75%)	2(12.30%)	0(00%)	16(8.29%)
Intensive care unit	207	16(69.57%)	4(17.3%)	3(13.04%)	0(00%)	23(11.11%)
Obstr &Gynae	188	9(64.29%)	5(35.71%)	0(00%)	0(00%)	14(7.45%)
Surgical ward	213	11(40.74%)	6(22.22%)	7(25.93%)	3(11.11%)	27(12.68%)
Internal Medicine	207	15(62.50%)	5(20.83%)	4(16.67%)	0(00%)	24(11.59%)
Total	1314	83	30	20	6	139

Nucleotide sequence line of *Pseudomonas aeruginosa* (control strain)-ATCC49189
CAT CGT CCA GGC ACG CCC GGA AAC CGT GAA GAG CCG CGC CAG CGC CAC GGT CAT GGA GCG CTA CCT GCT GAA AGA GAA GGG GAC CGT CCT GGT GGA AGG GCG TGC CAT CGG CCA GCG CAT CGG TGC CGG TCC TGT CAA GGT GAT CAA CGA CGT GTC GGA AAT GGA CAA GGT CCA ACC GGG TGA CGT CCT GGT CTC CGA CAT GAC CGA CCC GGA CTG GGA GCC GGT GAT GAA GCG CGC CAG CGC CAT CGT CAC CAA CCG CGG CGG GCG TAC CTG CCA CGC GGC GAT CAT CGC TCG CGA ACT GGG CAT CCC GGC GGT GGT CGG TTG CGG CAA CGC CAC CCA GAT CCT GCA GGA TGG CCA GGG GGT GAC CGTT

Length 373

Nucleotide sequence line of biofilm-forming *Pseudomonas aeruginosa* (strain 1)
CCC GTA GGC CTC TCC GGC GGC GTG GAC TCC TCG GTG GTC GCC GCG CTG TTG CAC AAG GCC ATC GGC GAC CAA CTG ACCT GCG TGT TCG TCG ACA ACG GCC TGC TGC GCC TGC ACG AAG GCG ACC AG G TGA TGG CCA TGT TCG CCG AGA ACA TGG GCG TGA AGG TGA TCC GCG CCA ACG CCG AGG ACA AGT TCC yCG rCC GCC TGG CCG GCG TCG CCG ACC AGG AAG AGA AGC GCA AGA TCA TCG GCC GCA CCT TCA TCG AA G TCT TCG ACG AAG AAG CCC CAr GCT GCA GGA CGT GAA GTT CCT CGC CCA GGG CAC CAT CTA CCC CGA CGT GAT CGA GTC GGC CGG CGC CAA Gar GGC AAA GGC CCA CGT GAC

Length 373
Codon 63-66, 74, 78-80, 101- 103 are points of mutation and genetic polymorphism

Nucleotide sequence line of biofilm-forming *Pseudomonas aeruginosa* (strain 2)
GGG TTT CCA GGC ACG CCC GGA AAC CGT GAA GAG CCG CGC CAG CGC CAC GGT CAT GGA GCGC TAC CTG CTG AAA GAG AAG GGG ACC GTC CTG GTG GAA GGA CGT GCC ATT GGC CAG CGC ATC GGT GCC GGT CCG GTC AAG GTG ATC AAC GAC GTG TCG GAA ATG GAC AAG GTC CAA CCG GGT GAC GTC CTG GTC TCC GAC ATG ACC GAC CCG GAC TGG GAG CCG GTG ATG AAG CGC GCC AGC GCC ATC GTC AC C AAC CGC GG C GGG CGC ACC TGC CAC GCG GCG ATC ATC GCT CGC GAA CTG Gwr ATC CCG GCG rTG GTC GGT TGC GGC AAT GCC ACC CAG ATC CTG CAG GAT GGC CAG GGG ACC GTTT
Length 372
Note: Codon 99 – 105 contains mutations and point of genetic polymorphism

Fig. 3. Sequence lines of biofilm forming *Pseudomonas aeruginosa* isolates.

DISCUSSION

In this study, a high degree of reproducibility was achieved with conventional PCR for the confirmation of the identity of *Pseudomonas aeruginosa* isolates. The PCR showed a high degree of genomic similarity among the Biofilm forming *Pseudomonas aeruginosa* isolates in conformity with the research hypothesis that strain similarity exists among *Pseudomonas aeruginosa* isolates circulating within the hospital environment being studied. The similarity in the banding pattern of the isolates could be attributed to genetic convergence due to cross infections of the circulating biofilm forming *Pseudomonas aeruginosa* within this hospital Community.

The PCR results further showed that single oligonucleotide primers can be used to amplify genomic DNA segments and polymorphisms can be detected between the amplified products of isolates. This work clearly demonstrates the relevance of molecular typing techniques such as PCR in the analysis of relationships in an organism as well as providing reliable epidemiological data. The specific target sequence was determined by co-amplification of a highly similar sequence, an internal standard which was distinguished from the target sequence in the PCR product. A shortened version of the specific sequence was used as an internal standard [24]. It is possible to assert from this study that typing to evaluate the existence of genetically different strains of microorganisms like *Pseudomonas aeruginosa* is commonly advised in epidemiological investigations. However, the use of very high discriminating typing methods is recommended for differentiation between bacterial species [25].

The sequence of highly conserved gene region of Biofilm forming *Pseudomonas aeruginosa,* 16S rDNA helped in the final prediction of the correct identity of *Pseudomonas aeruginosa* isolates from this study. Complete knowledge of an organism's genetic makeup through sequencing allows for exhaustive identification of antimicrobial targets [26]. The sequence of **16S** rDNA was fully based on PCR amplification for identification and genetic level confirmation of biofilm-forming *Pseudomonas aeruginosa*.

Also observed in this study were common species of biofilm-forming *Pseudomonas aeruginosa* detected in the samples analyzed, thus indicating that there could be patient-to-patient spread in this Hospital or between the environments and the admitted patients. Complications due to biofilm forming *Pseudomonas aeruginosa* are usually associated with the opportunistic nature of the organism; therefore, rapid diagnosis of such infection in a reliable way becomes necessary. It is widely accepted that traditional phenotypic methods for typing *Pseudomonas aeruginosa* do not permit accurate investigation of infections because phenotypic markers are relatively unstable [24], thus molecular technique as applied in this study remains the gold standard.

CONCLUSION

Biofilm-forming bacteria especially *Pseudomonas spp.* seriously exist on noncritical surfaces of various hospital sections that could lead to nosocomial transmissions in the hospitals**.** In this study, the nucleotide sequence of the experimental isolates expressed genetic mutation which represents point of involvement to *Pel* B gene involved in biofilm formation. This was observed for (samples one and two) both of which represents the strong and weak biofilm forming *Pseudomonas aeruginosa* isolates. Previously published data showed that *Pel* B mutation resulted in a dramatic decrease in pellicle formation compared to the parent strain [27]. However, later biofilm formation stages are significantly influenced by *Pel* B gene. There may be other genes involved in biofilm formation in *Pseudomonas aeruginosa* which are mutants of the standard control isolate used in the study as most of the genes associated with biofilm formation are usually controlled by about 1% chromosomal genes. Although the gene responsible for biofilm formation is more or less mutant of the standard control isolates of *Pseudomonas aeruginosa*, there may be other factors like strain difference, culture conditions, rate of expression of these genes and presence of other genetic factors affecting biofilm formation of *Pseudomonas aeruginosa*. This study confirms the molecular character of biofilm-forming *Pseudomonas aeruginosa.* This calls for adequate healthcare infection control practices in hospitals [28] like Federal Medical Centre, Umuahia, Abia-Nigeria as nosocomial infection may pose serious health challenges and drug resistance due to mutation, among healthcare professionals, patients and patient relatives.

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CONTRIBUTION OF AUTHORS

The authors declare that this work was done by the authors named in this article and all liabilities pertaining to claims relating to the content of this article will be borne by them.

CONFLICT OF INTEREST

There is no conflict of interest associated with this work.

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