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Antimicrobial Susceptibility Pattern of Biofilm forming *Pseudomonas aeruginosa* Isolated from Noncritical Surfaces in a Tertiary Healthcare Facility in South Eastern Nigeria

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Abstract **Background:** The presence of biofilm forming *Pseudomonas* species on noncritical surfaces in various hospital areas are the basis of Healthcare Associated Infections. **Justification**: The Healthcare associated infections are on the increase, affecting both care givers and patients with many showing resistant to many antibiotics and therefore calls for study for better understanding of the susceptibility of *Pseudomonas aeruginosa* isolated from noncritical surfaces in the facility. **Aim and objectives:** The study was to assess the susceptibility of commonly prescribed antibiotics in the south eastern healthcare facility and to be able to educate the staff, students and patients. **Methodology:** The study used an experimental design carried out in 800 beds capacity Federal Medical Center, Umuahia, and South East Nigeria. These bacteria were isolated using the swab to collect samples for analysis. Samples were collected from different noncritical surfaces surrounding hospitalized patients and equipment in the tertiary healthcare facility. The 450 positive samples out of the 1314 samples collected were analyzed for bacterial isolation and identification using bacterial cultural and microscopic identification techniques, biochemical tests and the Microbact 24E assay. **Result:** Biofilm forming *Pseudomonas aeruginosa* were identified through crystal violet assay while Antimicrobial susceptibility test was done using agar well diffusion method which was carried out on the isolated biofilm forming *Pseudomonas aeruginosa*. **Conclusion:** The susceptibility showed that biofilm forming *Pseudomonas aeruginosa* isolates were resistant to Gentamicin and Augmentin, but sensitive to Vancomycin, Azithromycin and Meropenem. *Pseudomonas aeruginosa* has the highest potential to form biofilms and could be recognized as a major agent of nosocomial infections in healthcare facilities in South East. Its notable resistance to some major antibiotics used in those centres calls for an urgent need for caregivers to carry out susceptibility testing before antibiotic prescription.

Keywords: *antimicrobial susceptibility, biofilm forming pseudomonas aeruginosa, hospital, South East-Nigeria*

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1. Introduction

The potential for a contaminated environmental surfaces to contribute to transmission of healthcare-associated pathogens depends on a number of factors including the ability of the pathogen to remain viable on a variety of dry environmental surfaces, frequency with which the contaminated surfaces are regularly touched by patients and healthcare workers and whether or not such contaminations are sufficiently high to result in transmission to patients. Noncritical surfaces are at the center of such transmission cycle and thus serve as sources of contamination and spread of nosocomial pathogen because they are surfaces that are frequently exposed to sources of contaminations by both patients, their relatives and caregiver unlike critical surfaces that are usually handled by professionals that understand the principles of sterility and maintenance of hygiene when handling patients in preventing nosocomial infections. Transmission from healthcare worker`s hand or gloves has been documented in some studies [1]. There could be direct transmission from contaminated noncritical surfaces

to patients [2] when noncritical surfaces are contaminated, patients can directly acquire pathogenic organisms from these surfaces directly because of the proximity of some of the noncritical surfaces to patients during healthcare delivery e.g. electronic thermometer and sphygmomanometer. Elimination of the environmental source of contamination reduces transmission of several pathogens that otherwise would have resulted in outbreak [3].

Pseudomonas aeruginosa is known as a leading cause of nosocomial infections worldwide especially in hospital environments and Federal Medical Center, Umuahia may not be exempted. There is generally an increase resistance of biofilm forming *Pseudomonas aeruginosa* to most antibiotics [4]. Biofilm forming *Pseudomonas aeruginosa* shows multidrug resistance (MDR) pattern that are statistically significant in comparison to non-biofilm producers and a greater understanding of the nature, intercellular communications within the biofilm and their susceptibility or resistance shall help in development of new and more effective treatment pattern towards an improved patient management especially in healthcare associated infections [5]. Drug resistance and healthcare associated infections (HAIs) is now a global burden that needs world attention ranging from bacteria gotten from noncritical surfaces of hospital environment [6] to the ones gotten from food and environment. [7]

This study is aimed at assessing the susceptibility of commonly prescribed antibiotics in the healthcare facility, so as to be well informed, thus serving as a tool for massive education campaign to staff, students and patients.

2. Materials and Method

This study was conducted in Federal Medical Center, Umuahia in Abia State after obtaining management and ethical approvals from the institution. Samples were collected from noncritical surfaces like Sphygmomanometer cuff, Sink, Electronic thermometer, stethoscope, bed rail, hand gloves, delivery couch, ultra sound probe and toilet door handle. Samples were collected with sterile cotton swabs pre-moistened with sterile normal saline and rubbed (10cm radius) on the selected noncritical surfaces and inserted into bijou bottles containing same medium and transported to the laboratory within 30 minutes for immediate processing [8].

2-3 colonies of 20h growth of the *Pseudomonas aeruginosa* on Muller Hinton agar were suspended in 50 ml pre-warmed (37°C) Mueller Hinton broth. The suspension was incubated overnight at 37°C, diluted 1 in 250 in the same pre-warmed medium and incubated in water bath with agitation (50 rpm). The absorbance of the culture was monitored with a spectrophotometer (6405 Jenway, Barloworld Scientific Ltd. Dunmow, Essex CMB 3LB), using a wavelength 450 nm and 19 mm diameter spectrophotometer tubes until absorbance of 0.1 was reached [9].

Biofilm forming *Pseudomonas aeruginosa* isolates, identified through Crystal Violet Assay [10] were tested for their susceptibility to most commonly used antibiotics in the hospital by agar well diffusion method with slight modification. 2-3 colonies of the isolate were taken from a pure culture and transferred to a tube containing 5ml sterile brain heart infusion broth (BHIB) and mixed gently by shaking until a homogenous suspension was formed which was then diluted to 0.5 McFarlane standard ($1.5x10^8$) [10].

Using a micropipette, 100µL of bacteria suspension in BHIB was evenly inoculated over the entire surface of Mueller Hinton agar plate and made uniformed with the aid of a sterile cotton swab stick. Five (5) holes of 8mm was punched aseptically with a sterile cork borer in each of the culture plates. One of the holes was punched at the center of the plate where 10µL of sterile TSB was added as a negative control.

100µL volume of antibiotics was added per well in the other holes and all the plates were then incubated at 37°C for 24 h. The clear zones of inhibition around the antibiotics were measured and result interpreted as sensitive, intermediate and resistant in line with the National committee on clinical laboratory standards [11] chart. The experiment was done in triplicate. The concentration of antibiotics used for the susceptibility tests is shown in Table 1. The minimum inhibitory concentration of commonly used antibiotics was evaluated by tube dilution method [12] with slight modifications.

The minimum bactericidal concentration (MBC) of antibiotics was tested by determining their bactericidal activity through counting of the number of bacteria in the initial suspension using the surface plate method. After ascertaining the MIC, the number of bacteria was counted in each of the tubes of the broth that showed invisible turbidity after overnight incubation and was compared with the number of bacteria in the initial suspension. According to NCCLS [11], the lowest concentration of the antibiotics solution that allowed 0.1% of the original inoculum to survive was taken to be the minimum bactericidal concentration (MBC). The Minimum Biofilm Inhibitory Concentration (MBIC) of commonly used antibiotics in Federal Medical Center, Umuahia as shown in Table 1, against biofilm forming *Pseudomonas aeruginosa*, was determined according to Cernohorska and Votava [13], with slight modifications. The experiment was done on 96-wells polystyrene micro titer round bottomed plates.75µL of an overnight standard culture of $1.5x\ 10^8$cfu (i.e. 0.5 McFarlane standards) was added to wells of micro titer plates and the plates incubated for 24h at 37°C. These wells were washed three times with Phosphate buffer saline (PBS) under aseptic conditions to remove unattached bacteria and dried in an inverted position.

Volumes of 100µL of appropriate twofold dilutions of the respective antibiotics were transferred into the wells with established biofilms. The micro titer plates were incubated for 18h to 24h at 37°C. Following incubation, 40µl of 0.2% of INT (2-4-Iodophenyl-3-4-nitrophenyl-5-phenyl-2H-tetrazoliumchloride or Iodo Nitro Tetrazolium) was added in all the wells and incubated for further 30 min at 37°C and the MBIC determined, as the concentration which corresponds to the lowest concentration of the antibiotics that inhibited growth of biofilm cells of the *Pseudomonas aeruginosa* as indicated by the first clear well. The positive and Negative controls were vancomycin and Tryptone soy broth (TSB) respectively. The experiment was carried out in triplicate. The result was analyzed in percentages.

Table 1. Concentrations of Antibiotics used in the study.

Antibiotics	Abv.	Concentration
Ciprofloxacin	(CF)	5µg,
Meropenam	(MP)	25 µg,
Azithromycin	(AZ)	30µg,
Erythromycin	(E)	15 µg,
Streptomycin	(S)	10 µg,
Gentamicin	(G)	10 µg,
Nalidixic acid	(NA)	30 µg,
Colistin	(CL)	10 µg,
Oxacilln	(OX)	10 µg,
Augmentin	(AG)	20/10µg
Vancomycin	(VC)	20 µg

The standard reference strain of *Pseudomonas aeruginosa* (ATCC 27853) was used as a quality control.

3. Result

Pseudomonas aeruginosa colonized majority of the noncritical surfaces/equipment in the various wards when compared to other species of *Pseudomonads* identified. Sink, Toilet door handle, hand gloves, electronic thermometer and Stethoscope showed significant level of colonization ranging between 14.28% to 66.67%. Moderate colonizationwas observed on noncritical surfaces like bed sheet and bed rail at 9.09% colonization while delivery couch was not colonized by biofilm forming *Pseudomonas aeruginosa*. The distribution of biofilm forming *Pseudomonas aeruginosa* on noncritical surfaces located in the hospital wards is shown in Table 2.

The resistance profile of biofilm forming *Pseudomonas aeruginosa* isolates to some commonly used antibiotics in Federal Medical Centre, Umuahia at statistical significance, P value < 0.05 indicates that 73.43% and 65.05% of the isolates assayed, demonstrated resistance to Aminoglycosides (Gentamycin) and β-Lactamase (Augmentin) respectively, 45.78% and 42.16% were resistant to tetracycline and polypeptide (Colistin), 37.34% and 36.14% were resistant to Macrolides (Erythromycin) and Nitrofurantoin respectively, 25.30% and 21.68% demonstrated resistance to quinolone(Nalidixic acid) and fluoquinolone (Ciprofloxacin)respectively. The least resistant rates were demonstrated by 12.05%, 7.29% and 4.81% of the isolates to Macrolides (Azithromycin), Carbapenem (Meropenem) and Glycopeptides (Vancomycin) respectively. Significant correlation was found between the total percentages resistances to the antibiotics tested. Table 3 showed the resistance profile of biofilm forming *Pseudomonas aeruginosa* isolates.

Table 2. Distribution of biofilm forming *Pseudomonas aeruginosa* on noncritical surfaces in various wards

Hospital ward/unit	Total № of *Pseudomonas aeruginosa* isolated	Noncritical surfaces colonized	№ of biofilm forming *P. aeruginosa* isolated	% colonization
Accident and Emergency	21	Sphygmomanometer cuff	5	23.80
		Sink	7	33.33
		Indoor air space	3	14.28
		Elect. Thermometer	1	4.76
		Stethoscope	2	9.52
		Hand gloves	3	14.28
Pediatrics ward	11	Sink	5	45.45
		Bed sheet	1	9.09
		Elect. Thermometer	1	9.09
		bed rail	1	9.09
		Stethoscope	2	18.18
		Indoor air space	1	9.09
		Ultrasound probe	1	6.25
Intensive care unit	16	Sink	6	37.5
		Bed sheet	1	6.25
		Delivery couch	0	00.00
		Electronic thermometer	3	18.75
		Indoor air space	4	25.00
		Ultrasound probe	1	11.11
Obstetrics and gynecology	9	Sink	6	66.67
		Delivery couch	0	00.00
		Electronic thermometer	0	00.00
		Indoor air space	2	22.22
		Stethoscope	0	00.00
Surgical ward	11	Indoor air space	2	18.18
		Electronic Thermometer	1	9.09
		Sink	4	36.36
		Stethoscope	0	00.00
		Sphygmomanometer cuff	3	27.27
		Hand glove	1	9.09
Internal medicine ward	15	Stethoscope	3	20.00
		Sink	5	33.33
		Sphygmomanometer cuff	1	6.67
		Electronic thermometer	1	6.67
		Hand glove	2	13.33
		Indoor air space	3	20.00

Table 3. Antibiotics Resistant Profile of Biofilm forming Pseudomonas aeruginosa Isolates

Antibiotics Class	Antimicrobial Drugs	№ of *P. aerug.*	% Resistance	95% C.I.
B-Lactamase	Augmentin	54	65.06	62.10 -68.50
Aminoglycoside	Gentamicin	61	73.43	70.74 - 77.46
Quin/fluroquin.	Ciprofloxacilin	18	21.68	18.97 - 25.63
Glycopeptides	Vancomycin	4	4.81	1.50 -7.73
Macrolides	Azithromycin	10	12.05	9.20 - 15.76
Quinolone	Nalidixic acid	21	25.30	22.00-28.00
Nitro.Compd.	Nitrofurantion	30	36.14	33.18-39.02
Polypeptides	Colstin	35	42.16	38.98 - 44.22
Carbapenems	Meropenam	6	7.29	4.39-8.72
Macrolides	Erythromycin	31	37.4	34.54 - 40.46
Tetracycline	Tetracycline	38	45.78	42.07 - 48.53

Key: Nitro. – Nitrofurantoin, Compd - compound. Quin.-Quinolone, fluroquine – fluoroquinolone.

Pseudomonas aeruginosa colonized majority of the noncritical surfaces/equipment in the various wards when compared to other species of *Pseudomonads* identified. Sink, Toilet door handle, hand gloves, electronic thermometer and Stethoscope showed significant level of colonization ranging between 14.28% to 66.67%. Moderate colonizationwas observed on noncritical surfaces like bed sheet and bed rail at 9.09% colonization while delivery couch was not colonized by biofilm forming *Pseudomonas aeruginosa*. The distribution of biofilm *Pseudomonas aeruginosa* on noncritical surfaces located in the hospital wards is shown in Table 2.

Table 4. MIC, MBC and MBIC of commonly used antibiotics against biofilm forming *Pseudomonas aeruginosa* isolates

Antibiotics	MIC (µg/ml)	MBC	MBIC
TSB (Neg. control)	NA	NA	NA
Gentamicin	3.81	0.0561	4.721
Augmentin	3.77	0.0520	4.510
Tetracycline	2.50	0.4960	3.816
Colstin	2.48	0.0480	3.770
Erythromycin	2.33	0.3700	2.812
Nitrofurantoin	2.32	0.3618	2.706
Nalidixic acid	2.12	0.3423	2.620
Ciprofloxacilin	2.04	0.0341	2.600
Azithromycin	1.03	0.2104	1.288
Meropenem	0.93	0.0170	0.112
Vancomycin	0.62	0.0014	0.051

NB: Values are means of duplicate experiments.
KEY: MIC-Minimum Inhibitory Concentration, MBC-Minimum Bactericidal Concentration, MBIC-Minimum Biofilm Inhibitory Concentration, NA-Indicates lack of event /activity within the range of concentration tested.

Antibacterial activities of most commonly used antibiotics in Federal Medical Centre, Umuahia, shows Minimum Inhibitory Concentration (MIC), Minimum bactericidal concentration (MIC) and Minimum Biofilm Inhibitory Concentration (MBIC) as in Table 4.

4. Discussion

Resistance of biofilm forming *Pseudomonas aeruginosa* to commonly use antibacterial agents, is becoming an increasing clinical problem and a recognized Public health threat [14]. In this study, the highest resistance of *Pseudomonas aeruginosa* was observed with aminoglycosides (gentamicin) which was 43.88% (61/139). This resistance rate of *Pseudomonas aeruginosa* isolate is in contrast to studies done in India which was 63% [15] and Turkey which was 57.5% [16]. Higher resistance rates of 75% were also reported from Jordan [17], Bangladesh was 77.3% [18], Saudi Arabia was 85.3% [19] and Malaysia was 94.3% [20]. Bacterial resistance to gentamicin is mainly due to an enzymatic modification of the antibiotic as indicated by Poole [21].

The widely usage of gentamicin in this hospital setting may have also contributed to the high resistance rate seen in this study. The outcome of biofilm forming *Pseudomonas aeruginosa* resistance to gentamicin is also not in agreement with high resistance of 87.5% for trimethoprim-sulphamethoxazole observed in Addis Ababa [22], 93.5% in Bangledesh [18] and 100% in Iran [23], which are entirely a different antimicrobial agent (trimethoprime-sulphamethodazole).

As indicated by McDonnell and Russell [24], reduced susceptibility of *Pseudomonas aeruginosa* to any disinfectant can be associated with the ability of the bacterium to form biofilms. Growth within biofilms gives rise to extensive genetic diversity that, in turn, enhances the potential for resistance against disinfectants to underlying cell modulation of the microenvironment and genetic tolerance to disinfectants.

Contrary to the inhibition posed against planktonic cells by the antimicrobial agents, biofilms of *Pseudomonas aeruginosa* were less susceptible to these agents (antibiotics and disinfectants). Bacteria living as biofilm are often more difficult to eradicate compared to the planktonic mode of growth [25]. Contemporary testing of minimum inhibitory concentration (MIC) which measures only planktonic susceptibility may be the possible explanation for treatment failures and resistant development among bacterial biofilms. In the present study, the result of MBIC, MBC, MIC and MBEC as highlighted shows the interesting activity of disinfectants commonly used in Federal Medical Center, Umuahia as seen in Agbo *et al*. [6]

5. Conclusion

The study further indicated that *Pseudomonas aeruginosa* isolated from this health facility was less susceptible to commonly prescribed antibacterial drugs, an evidence of circulating drug resistant strain with biofilm phenotype in the hospital community enviroments. [26,27] Despite this observation, Vancomycin, Azithromycin and Meropenem showed very good activity, showing that these antibiotics seem to be a promising therapy for biofilm related *Pseudomonas aeruginosa* infections especially in emergency situation.

Regular antimicrobial susceptibility surveillance is essential. An effective national and state level area wise monitoring of the resistance patterns of antibiotics policy and draft guild lines should be produced to preserve the antibiotics for better patient managements.

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Conflict of Interest

None.

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