GENOMIC INVESTIGATION OF *bla-PAO* AND *bla-OXA50* IN MULTIDRUG-RESISTANT *PSEUDOMONAS AERUGINOSA* FROM CLINICAL SAMPLES IN ABEOKUTA, OGUN STATE

ABSTRACT

Pseudomonas aeruginosa is one of the pathogens of human concern with high intrinsic multi-drug resistance capabilities. The genomic investigation of blaPAO and blaOXA-50 was done on multi drug-resistant *Pseudomonas aeruginosa* that were also resistant to carbapenem among the isolates collected from a total of 128 clinical samples in Abeokuta, Nigeria. *Pseudomonas aeruginosa* isolates were obtained from pure culture, and profiled for antibiogram by disc diffusion method. Genomic DNA from isolates were typed for blaPAO and blaOXA-50 with PCR. A total of 75 samples (58.6%) yielded the growth of bacterial isolates. Bacteria isolated were *Escherichia coli* (18.8%), *Klebsiella pneumoniae* (16.4%), *Staphylococcus aureus* (8.6%,), *Pseudomonas aeruginosa* (7.8%), *Streptococcus pnuemoniae* (3.1%), *Proteus mirabilis* (2.3%), and *Enterobacter aerogenes* (1.6%). Only ten (30%) isolates were confirmed to be *Pseudomonas aeruginosa*. All the *P. aeruginosa* isolates were resistant to ampicillin, cloxacillin, erythromycin, and tetracycline. Out of these ten multidrug *Pseudomonas aeruginosa* isolates, only three (30%) were resistant to carbapenems. Only two of these isolates expressed blaOXA-50 and blaPAO, while one possessed only blaPAO. Close Continuous monitoring of these antibiotic-resistant pathogens and hospital surveillance needs to be adopted to reduce their spread to other healthcare facilities.

Keywords: *Pseudomonas aeruginosa;* antibiotic resistance; blaPAO; blaOXA-50

INTRODUCTION

Pseudomonas aeruginosa is one of the most common pathogens isolated from patients who have been hospitalized longer than one week and it is a leading cause of nosocomial infections in hospital settings and death cases have also been reported (1). *Pseudomonas aeruginosa* are ubiquitous and are found in several environmental niche and can spread to patients and healthcare providers in clinical settings particularly when exposed to contaminated clinical instruments, surfaces, beddings, water or soil. There is high risk of infection with patients on ventilators or other medical devices such as intravenous catheters (2). Nosocomial *Pseudomonas* aeruginosa infection include bloodstream infection, pneumonia, urinary tract infections and surgical wound infection. These infections mostly affect hospitalized patients especially those with weak immune systems and those on long term treatments (3). *Pseudomonas aeruginosa* has been recognized to have survival and adaptation abilities in a wide range of environments such as soil, water, sewage and hospitals (4). Despite therapy the mortality due to nosocomial is approximately 70%. *Pseudomonas aeruginosa* develops resistance to most of antibiotics thereby preventing the selection of appropriate treatment (5). *P. aeruginosa* are particularly problematic because the organism is inherently resistant to many drug classes and is able to acquire resistance to all effective antimicrobial drugs (6). Multi Drug Resistant *Pseudomonas aeruginosa* (MDRPA) is a condition that bacteria resistant to three or more classes of antibiotics such as penicillins, cephalosporins, monobactam, carbapenem, aminoglycosides and fluoroquinolones. Inappropriate antibiotics administration can cause *P. aeruginosa* resistant to several classes of antibiotics (7). *P. aeruginosa* nosocomial infections is generally difficult to treat because of the possibility intrinsic resistance and its ability to obtain faster resistance mechanism against many groups of antimicrobials (8). *P. aeruginosa* is naturally resistant to beta-Lactams. Furthermore, they easily acquire resistance to new antibacterial agents by mutational changes or acquisition of genetic material. Most pseudomonads known to cause diseases in humans are associated with opportunistic infections. These include *Pseudomonas fluorescence*, *Pseudomonas putida*, *Pseudomonas cepacia*, *Pseudomonas stutzeri*, *Pseudomonas maltophila* and *Pseudomonas putrefaciens*. In Nigeria, a study from the South west showed species, *P. mallei* and *P. pseudomallei*, isolated from human disease glanders and meliodosis. *Pseudomonas aeruginosa* are both invasive and toxigenic bacteria and has become increasingly recognized as an emerging

opportunistic pathogen of clinical relevance (9). This organism has been incriminated in cases of meningitis, septicaemia, pneumonia, ocular and burn infections (10). Wound infections related to burn patients often leads to bacteraemia. Bacteraemia and septic shock are mostly observed in immuno-compromised patients that are associated with high mortality rates (from one-third to almost two-thirds of cases) (11). Different conditions such as severe neutropenia, mucosal ulcers, and malignancies lead to a risk for bacteraemia (12). *Pseudomonas aeruginosa* is most commonly found in cystic fibrosis patients. The abnormal airway epithelia of these patients allow long-term colonization by this bacterium and, once they get infected, they rarely fade away and lead to chronic lung diseases (12, 13). *P. aeruginosa* possess various *virulence* genes that contribute to its pathogenicity such as exotoxin The blaOXA -50 gene (formerly known as the PA5514 gene) is an oxacillinase gene identified in silici in the genome of Pseudomonas PAO1 (14). It has been reported that blaOXA – 50 naturally exists in all *P. aeruginosa* and does not appear to have been acquired based on the similar GC% content of the blaOXA – 50 gene to the overall *P. aeruginosa* genome (15). Carbapenems are β-lactam antibiotics that consist of a four-membered β-lactam ring fused with a secondary five-membered thiazolidine ring through the nitrogen and adjacent tetrahedral carbon atom. Unlike other β-lactams, carbapenems have two substitutions, at position one there is a substitution of sulfur for a carbon atom and at the fourth position of the thiazolidinic moiety, a carbon is substituted for a sulfone (16, 17). So far, four carbapenems, including ertapenem, meropenem, doripenem, and imipenem, have been approved for use in the US. These members differ in their side chains, influencing their antimicrobial activity. Carbapenems inhibit cell wall synthesis by preventing the formation of cross-linkages in peptidoglycan via binding to peptidoglycan binding protein (PBP), thus leading to cell lysis and death (18).

MATERIALS AND METHODS

Sampling, Isolation and Identification

One hundred and twenty-eight clinical samples from Federal Medical Centre, Abeokuta, in southwest Nigeria were analyzed between June 2023 and January 2024. *P. aeruginosa* isolates obtained from the clinical samples were preserved in semi-solid Brain Heart Infusion (BHI) (Oxoid, Basingstoke, UK) supplemented with glycerol and re-characterized for confirmation following standard biochemical methods previously described (19).

144

145 **Antimicrobial Susceptibility**

146 The susceptibility profile of antibiotics commonly prescribed for *Pseudomonas aeruginosa*
147 infections was determined using Kirby-Bauer disc diffusion in accordance with CLSI
148 recommendations and guidelines (20, 21). Overnight culture from cetrimide agar was sub-cultured
149 on BHI and further incubated for 24 h at 37 ◦C. Bacterial suspension of 0.5 MacFarland turbidity
150 was spread on BHI using sterile swab stick. Twelve different antibiotics with different disc
151 concentrations, such as Gentamycin (Gen) 10 µg/disc, Erythromycin (Ery) 15 µg/disc, Ceftriaxone
152 (Cef) 30 µg/disc, Imipenem (Imp) 10 µg/disc, Meropenem (Mem) 10 µg/disc, Tetracycline (Tet)
153 30 µg/disc, Cefuroxime (Str) 30 µg/disc, Cloxacillin 30 µg/disc (Cxc), Ampicillin (Amp), 30
154 µg/disc, Cefuroxime (Cxm) 30 µg/disc, Ceftazidime (Caz) 30 µg/disc, Cefepime (Cef) 30 µg/disc
155 and Ciprofloxacin (Cip) 5 µg/disc were used in this study. The antimicrobial sensitivity test of
156 each isolate was carried out as described by the Kirby-Bauer disc diffusion method. The turbidity
157 of the bacterial suspensions was compared with 0.5 Macfarland's barium sulfate standard solution.
158 The standardized bacterial suspension was inoculated on Muller Hinton Agar (Lab M Laboratories,
159 Mumbai, India) and left to dry for 10 minutes before placing the antimicrobial sensitivity discs.
160 Antibiotic-impregnated discs of 8 mm in diameter were used for the test. After incubation, the
161 diameter of the zone of inhibition was measured and compared with the zone diameter
162 interpretative chart (20, 21) to determine the sensitivity of the isolates to antibiotics. The standard
163 strain, *P. aeruginosa* ATCC 27853, was used as a control. Isolates showing resistance to at least
164 one agent in more than three classes of the antibiotic group were classified as multi-drug resistant
165 *P. aeruginosa* (MDR P. aeruginosa) according to Magiorakos *et al.* (22). Multi antibiotic resistance
166 index (MARI), was calculated for each isolated tested, using the formula below

167 MARI = No of resistant antibiotics ÷ Total number of antibiotics tested

168

169 **Chromosomal DNA Extraction**

170 Purified genomic bacterial DNA was extracted from overnight cultures of the three multidrug-
171 resistant Pseudomonas aeruginosa isolates after growth on TSA medium using a genomic DNA
172 mini kit (QIAGEN, QIAamp®, USA) according to the manufacturer's instructions. This serves as
173 the template DNA. The concentration of the eluted DNA was measured using a NanoDrop 2000
174 spectrophotometer.

175
176
177 **Agarose gel electrophoresis**
178 Powdered agarose (0.8% w/v) was boiled in tris-acetic EDTA (TAE) buffer intermittently until
179 the solution became a clear gel. The agarose solution was allowed to cool to 45^{o}C before 7 µl of
180 ethidium bromide was added. The clear gel solution was poured into the gel tray with the comb in
181 place and allowed to solidify. Thereafter, the gel tray and the comb were removed. The gel was
182 placed into the tank containing the gel buffer. Then, 2µl of the tracking dye (bromophenol blue)
183 was mixed with 1µl of the marker and loaded into the first well. Thereafter, 20µl of the
184 bromophenol blue with 20µl of the sample was mixed and loaded into other wells. The cover of
185 the tank was carefully placed on it and plugged into the power source to run from a negative to a
186 positive direction, making sure it did not run a distance of far more than ¾ of the gel for
187 approximately 30 minutes. Then, the gel was viewed via the UV transilluminator (23). A 1.5kb
188 standard DNA molecular weight marker (Gene Mate, UK) was used in the study.
189 **Polymerase Chain Reaction**
190 The pure DNA of each of the three different *Pseudomonas aeruginosa* was subjected to the
191 polymerase chain reaction (PCR), with the genes blaPAO and blaOXA-50 being targeted. The
192 PCR reaction was performed with primer sets blaPAO forward F-
193 TGCCTGGTAGTGGGGGATAA, reverse F-TGCCTGGTAGTGGGGGATAA, and blaOXA50
194 forward F-AATCCGGCGCTCATCCATC reverse R: GGTCGGCGACTGAGGCGG a total
195 volume of 50 µL, containing 25 ng of DNA template, 10 mM Tris-HCl, 50 nmol KCl, 1.5 mM
196 MgCl2, 200 µM dNTP (Fermentas), 12.5 pmol of each primer, 1 U Taq DNA polymerase
197 (Fermentas), and 5 µL PCR buffer 10X. Reactions were initiated at 1 cycle at 94° for 3 min,
198 followed by 30 cycles at 94°C for 30 s, 55 for 1 min, 72°C for 1.5 min, and a final elongation step
199 at 72°C for 5 min (24).
200
201 **Data analysis**
202 The results were analyzed using descriptive Statistical methods. Graphs were generated with the
203 libraries ggplot2; Rcolorbrewer and pheatmap in R-studio (www.rcoreteam). The dendogram was
204 generated un DendroUPGMA software using the MARI scores of the isolates to generate a

similarity matrix for the dendrogram construction. The subsequent tree was visualized in TVBOT (https://www.chiplot.online/tvbot.html).

RESULTS

From a total of 128 clinical samples were collected in this study; 75 (58.6) had the growth of bacteria (table 1). Bacteria isolated were *Escherichia coli* (24), *Klebsiella pneumoniae* (21), *Staphylococcus aureus* (11), *Pseudomonas aeruginosa* (10), *Streptococcus pnuemoniae* (4), *Proteus mirabilis* (2), and *Enterobacter aerogenes* (2), which constituted 18.8%, 16.4%, 8.6%, 7.8%, 3.1%, 2.3%, and 1.6%, respectively (table 2). The distribution of Pseudomonas aeruginosa isolates from the different clinical samples in relation to sex and age is shown in table 4. Age groups within 11–20 years, 51–60 years, and 61–70 years had 0.8% Pseudomonas aeruginosa occurrence, respectively, while age groups 11–20 years and 31–40 years had 1.6% Pseudomonas aeruginosa occurrence, respectively.

The resistance pattern of the *Pseudomonas aeruginosa* isolated from clinical samples to antibiotics showed that all the *Pseudomonas aeruginosa* isolates identified were resistant to 4 of the antibiotics used namely; ampicillin, cloxacillin, erythromycin and tetracycline respectively, and had 30% resistant to imipenem and meropenem respectively (Figure 1). The multi-drug-resistant *Pseudomonas aeruginosa* isolates (n=3, 30%) were resistant to carbapenem. The result revealed that one of the *Pseudomonas aeruginosa* isolates was resistant to seven classes of antibiotics, while three were resistant to six classes. Antibiotic resistance related was investigated using dendroUPGMA and the isolates clustered into 2 clades based on their MARI scores (Figure 2a), both clusters harbored positive PCR positive ESBL isolates. Figure 2b shows a heatmap containing antibiotic resistance profiles of the isolates according to the clases of antibiotics tested, including the bla-OXA50 and bla-PAO positivity rates of the isolates tested. From the heat map majority of the isolates were fully susceptible to carbapenem and quinolonce class of antibiotics while the chephalosporins class showed the highest resistance rates (Figure 2b). Figure 3 shows the Agarose gel pictures of the PCR reaactions, showing the bla-PAO bands seen in multi-drug resistant *Pseudomonas aeruginosa* while plate 2 showed the bla-OXA50 bands seen in the multi-drug resistant *Pseudomonas aeruginosa*.

Table 1. Distribution of bacteria growth among clinical samples

Samples	No. of samples (%)	No. of Yielded Growth (%)	No. of Non-Bacteria Growth (%
Urine	58(45.3)	36(28.1)	22(17.2)
Wound swab	22(17.2)	11(8.6)	11(8.6)
Sputum	20(15.6)	12(9.4)	8(6.3)
Pus	15(11.7)	7(5.5)	8(6.3)
Ear swabs	8(6.3)	6(4.7)	2(1.6)
Burns	5(3.9)	3(2.3)	2(1.6)
Total	128(100.0)	75(58.6)	53(41.4)

Table 2. Percentage of occurrence of bacterial isolates in the clinical samples

Isolates	n = 128	Number (%) of bacterial isolates
Escherichia coli		24(18.8)
Klebsiella pnuemoniae		21(16.4)
Pseudomonas aeruginosa		10(7.8)
Staphylococcus aureus		11(8.6)
Streptococcus pneumoniae		4(3.1)
Proteus mirabilis		3(2.3)
Enterobacter aerogenes		2(1.6)
Total		75(58.6)

Table 3. Distribution of *Pseudomonas aeruginosa* isolated from clinical samples in relation to sex and age.

Sex	Total examined n (%)	Number (%) yielded growth of *Pseudomonas aeruginosa*
Male	55(42.9)	4(3.2)
Female	73(57.1)	6(4.6)
	128(100)	10(7.8)
Age group		
≤10	12(9.4)	0(0.0)
11 – 20	6(4.6)	2(1.6)
21 – 30	34(26.6)	1(0.8)
31 – 40	28(21.9)	2(1.6)
41 – 50	22(17.2)	3(2.3)
51 – 60	18(14.1)	1(0.8)
61 – 70	14(10.9)	1(0.8)
≥ 71	4(3.1)	0(0.0)
Total	128(100)	10(7.8)

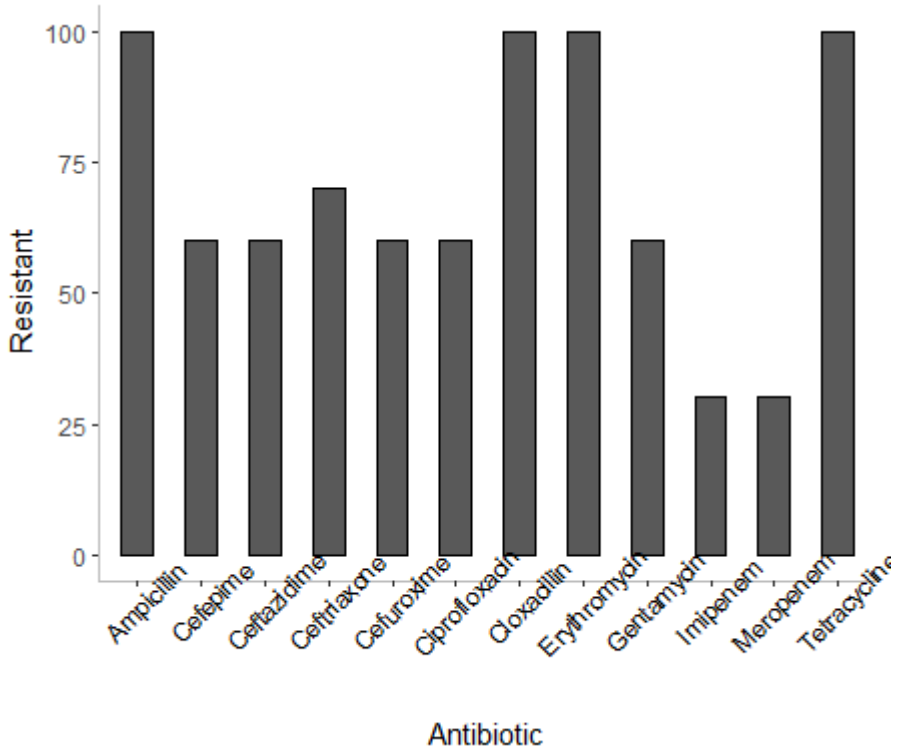
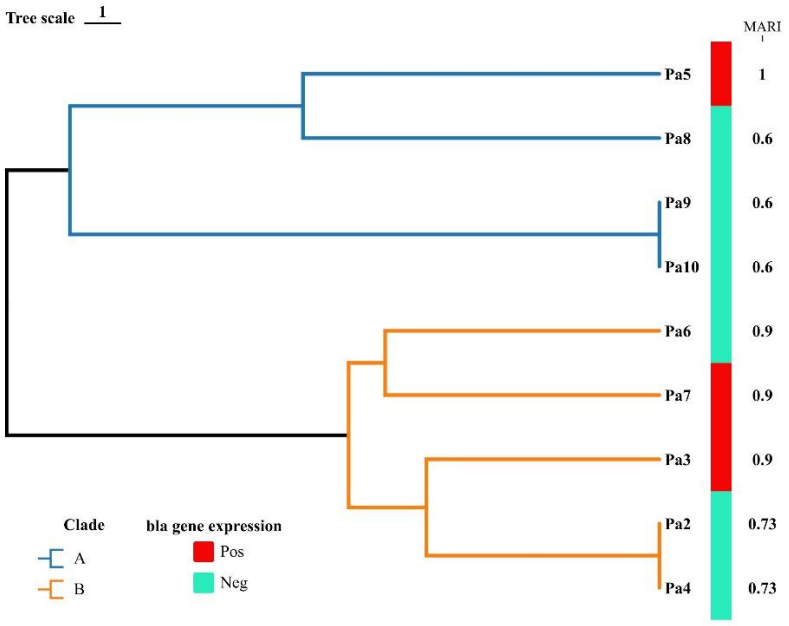
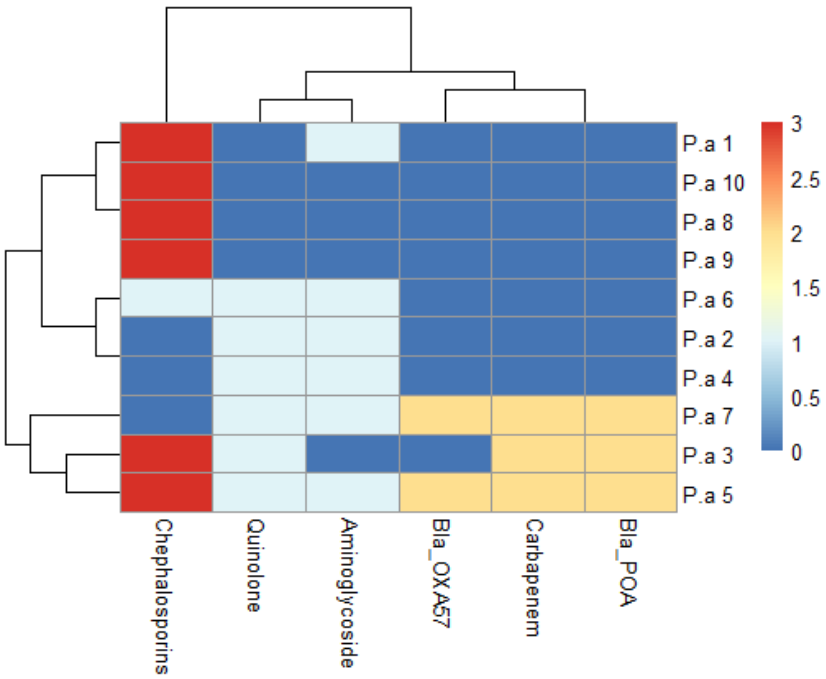


Figure 1. Bar chart showing antibiotic resistance distribution among the various antibiotics tested against *Pseudomonas aeruginosa* isolated from patients in Abeokuta, Nigeria.



283

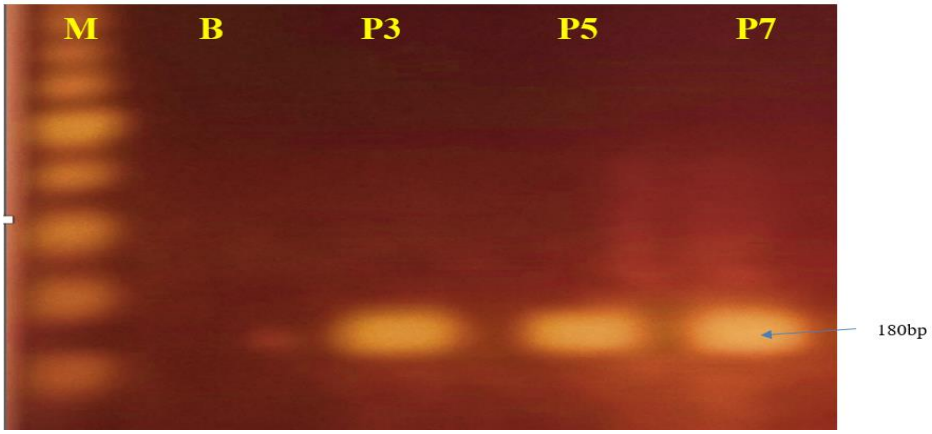
284 Figure 2a



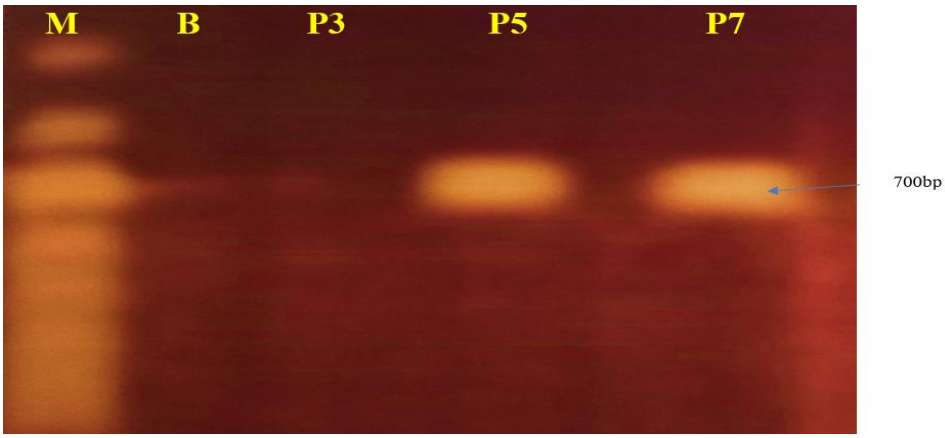
285

286 Figure 2b

287 Figure 2a. Dendogram showing the antimicrobial based clustering and relatedness, showing,
288 MARI scores and ESBL-PCR positivity. Figure 2b. Heatmap showing resistance profiles of the
289 *Pseudomonas aeruginosa* isolates against the different classes of antibiotics.



Bla-PAO positive



bla-OXA50 positive

Figure 3. Agarose gel pictures of PCR positive samples showing, DNA ladder on the first row, no template control along with PCR positive samples.

DISCUSSION

Pseudomonas aeruginosa is a versatile bacterium that causes a wide range of severe opportunistic infections in patients with serious underlying medical conditions. The prevalence of *Pseudomonas aeruginosa* in this study was 7.8%. *Pseudomonas aeruginosa* is currently one of the most frequent nosocomial pathogens, and infections due to this organism are often difficult to treat due to antibiotic resistance (25). All the *P. aeruginosa* isolates were completely resistant to ampicillin, cloxacillin, erythromycin, and tetracycline. This finding was in agreement with Akingbade *et al.* (26), who reported that *P. aeruginosa* was 90% resistant to ampicillin and cloxacillin in south-west Nigeria. According to Juan *et al.,* (27) *P. aeruginosa* is the third most prevalent bacterium identified from infections contracted in intensive care units and is the main cause of morbidity and death in people with cystic fibrosis (CF), chronic obstructive pulmonary disease (COPD), diabetes, and severe kidney and liver failure. Hospital and community Pseudomonas aeruginosa infection control could suffer major setbacks due to high-level resistance to cell wall biosynthesis inhibitors (particularly augmentin, ceftazidime and ampicillin) which are most prescribed antibiotics (28).

All ten *Pseudomonas aeruginosa* isolates were resistant to three or more classes of antibiotics, and their multidrug patterns cut across all the commonly used drugs prescribed in the clinical setting. This is also consistent with other findings from Egypt (29). The spread of these antibiotic-resistant *P. aeruginosa* strains is increasing within the hospital environment. This multiple resistance could be attributed to the misuse of antibiotics, which necessitates strict prescription policies to overcome this problem.

Antibiotic resistance is a major problem observed among most of the *P. aeruginosa* infections in the clinical setting. It can also be seen from these results that *P. aeruginosa* isolates were resistant to most of the commonly used antibiotics. *P. aeruginosa* screened had 60% resistant to gentamycin in this study, and this is similar to the low susceptibility rate reported in two different studies (32.2% and 33%, respectively) by Samad *et al*. 2017 (30) and Diggle *et al*. 2020 (31). The result is also in agreement with those from Spagnolo *et al*., 2021 (69%) (32), Langendonk *et al*., 2021 (77%) (33), and Shimaa *et al.*, 2023 (73%) (34), but differs from those of Tuon *et al.*, 2022 (31%)

(35). Aminoglycosides are an essential part of the antipseudomonal chemotherapy used to treat a number of illnesses caused by *P. aeruginosa* (36, 37). The result also showed that ciprofloxacin inhibited the growth of only 40% of *P. aeruginosa*, and this is in contrast to a 2012 report by Akingbade *et al.* (26) in Abeokuta, Ogun State. Imipenem, a member of the carbapenem class, is the most effective antibiotic (70%) against the strain of *P. aeruginosa* in this study. This is in line with research conducted by Shimaa *et al.* (2023) (34) that found 87.2% of the *P. aeruginosa* isolates susceptible to imipenem. This study revealed the presence of blaPAO and blaOXA-50 among the three strains of MDR-*P. aeruginosa* using the PCR technique. Many studies have reported the prevalence of blaPAO and blaOXA50 in the *P. aeruginosa* genome (38). Due to the ability of *P. aeruginosa* to develop resistance to a wide variety of antibiotics through diverse molecular pathways, the emergence of MDR-*P. aeruginosa* is, in fact, a worldwide health concern.

In the present study, MDR-*P. aeruginosa* showed resistance to different antibiotics, such as ampicillin, cloxacillin, erythromycin, tetracycline, cefuroxime, ceftazidime, ceftriaxone, and cefepime. It was also resistant to aminoglycosides (gentamycin) and fluoroquinolones (ciprofloxacin). Recent studies have provided detailed descriptions of each resistance mechanism and contribution to each class of antibiotics (5, 39). It is known that some strains of *P. aeruginosa* have highly developed and acquired resistance mechanisms that enable them to withstand the majority of antibiotics. The molecular analysis of three multidrug-resistant *P. aeruginosa* shows that two of the *P. aeruginosa* possessed the two types of genes screened for (blaPAO and blaOXA-50). Similar studies have also shown a high incidence of the blaPAO and blaOXA-50 genes among MDR-*P. aeruginosa* (40, 41). It was observed that the two *P. aeruginosa* isolates (p-5 and p-7) that possessed both blaPAO and blaOXA-50 genes were resistant to penicillin, aminoglycosides, tetracyclines macrolides and carbapenems classes respectively. One of this *Pseudomonas aeruginosa* isolates (P7), was also, sensitive to cephalosporin classes while the other (p-5) was resistant to cephalosporin classes. The *P. aeruginosa* isolate (p-3) that possess only blaPAO gene was resistant to penicillin, aminoglycosides, tetracyclines, cephalosporins, macrolides and carbapenems classes respectively and was sensitive to gentamycin, an aminoglycoside class. Hospital and community antibiotic stewardship need to be strengthened with proper information on combining these antibiotic classes and regulation of drug prescription, particularly in local

outlets (28). The decreased susceptibility of *P. aeruginosa* to commonly used antibiotics has also been shown in different studies (39, 5, 42).

conclusion :

, this study shows that the three MDR-Pseudomonas aeruginosa that have blaPAO and blaOXA-50 beta-lactamases are resistant to penicillin, macrolide, tetracycline, fluoroquinolones, tetracycline, and carbapenems. Newer clinical approaches are needed to curtail the increasing resistance by considering the innovative integrated system in prescription and therapeutic formulation, with combined synergistic mechanism of action (28).

ETHICS STATEMENT

The current study was approved by the ethical committee of Federal Medical Centre, Abeokuta, Nigeria. Informed consent was also given by all participants in the study.

DATA AVAILABILITY STATEMENT

The authors declare that all available data regarding the manuscript is already included within it. Any addition information regarding data of the work will be provided upon request by the corresponding author.

REFERENCES

1. Qin Shugang., Wen Xiao and Min Wu (2022). Pseudomonas aeruginosa; pathogenesis, virulence factors, antibiotic resistance, interaction with host, technology advances and emerging therapeutics. 7;199

2. Stephanie G. C., Martha V., Lasse K and AARON M. S (2019). The environmental occurrence of Pseudomonas aeruginosa. Apim 128 (3). 100-104

3. Preeti P., Ragini G. and Puneet Gandhi (2019). Emergence of antibiotic resistance Pseudomonas aeruginosa in intensive care unit; a critical review. Genes and Diseases 6:109-119

4. Ali Khursheed a, Bilal Ahmed a, Mohammad Saghir Khan a, Javed Musarrat a (2018). Differential surface contact killing of pristine and low EPS Pseudomonas aeruginosa with Aloe vera capped hematite (α-Fe2O3) nanoparticles. Journal of Photochemistry and Photobiology B: Biology Volume 188, November 2018, Pages 146-158

5. Obritsch MD, Fish DN, Maclaren R, Jung R (2004). The national surveillance of antimicrobial resistance in the Pseudomonas aeruginosa isolates obtained from intensive care unit patients from 1993 to 2002. Antimicrob Agents Chemother. 48:4606–4610.

6. Alnour Tang MS, Eltayib Hassan and Ahmed – Ahmed (2017). Multi drug *Pseudomonas aeruginosa*: Medical impact, Pathogenicity, resistance mechanisms and epidemiology JSM Microbiology Sci med Central

7. Japoni A, AlborziA, Kalani M, Nasiri J, Hayati H and Farshad S (2009). Susceptibility patterns and cross resistance of antibiotics against Pseudomonas aeruginosa isolated from burn patients in the Southof Iran. Burns 32, 343-347

8. Strateva T, Yordanov D. (2009). Pseudomonas aeruginosa — a phenomenon of bacterial resistance. Journal of Medical Microbiology. 58:1133–1148.

9. Stephen P. Diggle and Marvin Whiteley (2020). Microbe Profile: *Pseudomonas aeruginosa:* opportunistic pathogen and lab rat. Microbiology (Reading0 166(1): 30 - 33

10. Hernandez J. Ferus M.A., and Hernandez M., (1997) Fingerprinting and serotyping of clinical *Pseudomonas aeruginosa* strains. FEMS Immunology Med. Micribiol. 17:37-47

11. Collin BA, Leather HL., Wingard JR., Ramphal (2001). Evolution, incidence and susceptibility of bacterial bloodstream isolates from 519 bone marrow transplant patients. Clin. Infect. Dis 33(7);947-53

12. Pollack M (2000). Pseudomonas aeruginosa. In: Principles and practice of infectious diseases 5th ed.Mandell GL, Bennett JE and Dolin R (ed.), Edinburgh,Churchill Livingstone , Scotland , p.2310–2335

13. Davies JC (2002). Pseudomonas aeruginosa in cystic fibrosis: pathogenesis and persistence. Paediatr Respir Rev 3(2): 128 - 34

14. Delphine Girlich, Thierry Naas and Patrice Nordmann (2004). Biochemical Characterization of the Naturally occurring oxacillinase OXA-50 OF Pseudomonas aeruginosa. Antimicrob. Agents Chemother. 48 (6): 2043 – 2048

15. Girlich, D.; Naas, T.; Nordmann, P. (2004). Biochemical Characterization of the Naturally Occurring Oxacillinase Oxa-50 of Pseudomonas aeruginosa. Antimicrob. Agents Chemother. 48, 2043–2048

16. Bonfiglio, G.; Russo, G.; Nicoletti, G (2002). Recent Developments in Carbapenems. Expert Opin. Investig. Drug, 11, 529–544.

17. Nicolau, D.P. Carbapenems: A Potent Class of Antibiotics. Expert Opin. Pharmacother. 2008, 9, 23–37.

18. Zhanel, G.G.; Lawrence, C.K.; Adam, H.; Schweizer, F.; Zelenitsky, S.; Zhanel, M.; Lagacé-Wiens, P.R.S.; Walkty, A.; Denisuik, A.; Golden, A. (2018). Imipenem-Relebactam and Meropenem-Vaborbactam: Two Novel Carbapenem-B-Lactamase Inhibitor Combinations. Drugs 2018, 78, 65–98

19. Zahedani, S.S.; Tahmasebi, H.; Jahantigh, M. (2021). Coexistence of Virulence Factors and Efflux Pump Genes in Clinical Isolates of Pseudomonas aeruginosa: Analysis of Biofilm-Forming Strains from Iran. Int. J. Microbiol, 5557361.

20. Clinical and Laboratory Standards Institute. Performance Standards for Antimicrobial Susceptibility Testing, Sixteenth Informational Supplement; Document M100-S20; CLSI: Wayne, PA, USA, 2018

21. EUCAST Clinical Breakpoints and Dosing. Available online https://www.eucast.org/clinical_breakpoints

22. Magiorakos, A.P.; Srinivasan, A.; Carey, R.B.; Carmeli, Y.; Falagas, M.E.; Giske, C.G.; Paterson, D.L. (2012). Multidrug-resistant, extensively drug-resistant and pandrug-resistant bacteria: An international expert proposal for interim standard definitions for acquired resistance. Clin. Microbiol. Infect. 2012, 18, 268–281

23. Pulimamidi Rabindra R and Nomula,R. 2012. Gel electrophoresis and it application, Gel electrophoresis - Principles and basics. In Tech ISBSN: 978-953-51-0458-2

24. Liu, B.; Zheng, D.; Zhou, S.; Chen, L.; Yang, J. Vfdb 2022: A General Classification Scheme for Bacterial Virulence Factors. Nucleic Acids Res. 2022, 50, D912–D917.

25. Reynolds D and Kollef M. (2021). The Epidemiology and Pathogenesis and Treatment of Pseudomonas aeruginosa Infections: An Update. Drugs. (18):2117-2131.

26. Akingbade, O.A., Balogun, S.A., Ojo, D.A., Afolabi, R.O., Motayo, B.O., Okerentugba, P.O., and Okonko, I.O (2012). Plasmid profile analysis of multidrug resistant *P. aeruginosa* isolated from wound infections in South West, Nigeria. World Applied Sciences Journal 20 (6): 766-775.

27. Juan C, Torrens G, Gonzalez-Nicolau M, Oliver A (2017). Diversity and regulation of intrinsic β-lactamases from non-fermenting and other Gram-negative opportunistic pathogens. FEMS Microbiol Rev;41(6):781–815.)

28. Akinduti, Paul A. Onome W. George, Hannah U. Ohore, Olusegun E. Ariyo, Samuel T. Popoola, Adenike I. Adeleye, Kazeem S. Akinwande, Jacob O. Popoola, Solomon O. Rotimi, Fredrick O. Olufemi, Conrad A. Omonhinmin and Grace I. Olasehinde (2023). Evaluation of Efflux-Mediated Resistance and Biofilm Formation in Virulent Pseudomonas aeruginosa Associated with Healthcare Infections

29. Abbas Hisham A, Amira M El-Ganiny, and Hend A Kamel (2018). Phenotypic and genotypic detection of antibiotic resistance of Pseudomonas aeruginosa isolated from urinary tract infections. Afr Health Sci.

30. Samad Abdul, Tanveer Ahmed , Afaq Rahim, Abdul Khalil and Iftikhar A (2017). Antimicrobial susceptibility patternsof clinical isolates of *Pseudomonas aeruginosa* isolated from patients of respiratory tract infections in Tertiary Care Hospital. Peshawar. Pak J. Med. Sci. 33(3): 670 -674

31. Diggle S, P, Whiteley M. (2020). Microbe profile: *Pseudomonas aeruginosa*: opportunistic pathogen and lab rat. Erratum in: Microbiology 167 (8)

32. Spagnolo F, Trujillo M, Dennehy JJ. (2021). Why Do Antibiotics Exist? mBio Dec 21;12(6).

33. Langendonk R. Frèdi, Daniel R. Neill, and Joanne L. Fothergill (2021). The Building Blocks of Antimicrobial Resistance in Pseudomonas aeruginosa: Implications for Current Resistance-Breaking Therapies. Front Cell Infect Microbiol.

34. Shimaa M. Ghanem, Gamal, F. M and Rehab Mahmoud Abd El -Baky; Nancy G.F (2023). Association between resistance, Biofilm formation and LASB gene in Pseudomonas aeruginosa isolated from different clinical specimens. Bullentin of Pharmaceutical Science Assiut University 10. 21608

35. Tuon FF, Dantas LR, Suss PH, Tasca Ribeiro VS. (2022). Pathogenesis of the Pseudomonas aeruginosa Biofilm: A Review. Pathogens. Feb 27;11(3):300.

36. CDC (2016). Antibiotic resistance threats in the United States. Atlanta, GA: CDC; 2013.

37. Greipel L, Fischer S, Klockgether J, et al. Molecular epidemiology of mutations in antimicrobial resistance loci of Pseudomonas aeruginosa isolates from airways of cystic fibrosis patients. Antimicrob Agents Chemother. 2016;60(11):6726–6734.

38. Madaha Estelle Longla, Charlotte Mienie, Hortense Kamga Gonsu, Rhoda Nsen Bughe, Marie Christine Fonkoua, Wilfred Fon Mbacham, (2020). Whole-genome sequence of multi-drug resistant Pseudomonas aeruginosa strains UY1PSABAL and UY1PSABAL2 isolated from human broncho-alveolar lavage, Yaoundé, Cameroon

40. Arya M, Arya P, Biswas D, Prasad R (2005). The antimicrobial susceptibility pattern of the bacterial isolates from post-operative wound infections. Indian J Pathol Microbiol. 48(2):266–269.

41. Du SJ, Kuo HC, Cheng CH, Fei ACY, Wei HW, Chang SK (2010). Molecular mechanisms of ceftazidime resistance in Pseudomonas aeruginosa isolates from canine and human infections. Vet Med. 55(4):172–182.

42. McAulay K, Schuetz AN, Fauntleroy K, (2021). Multidrug-resistant *Pseudomonas aeruginosa* in healthcare facilities in Port-au-Prince, Haiti. J Glob Antimicrob Resist.;25:60–65.

43. Algun A, Arisoy GT, Ozbakkaloglu B (2004). The resistance of Pseudomonas aeruginosa strains to fuoroquinolones group of antibiotics. Ind J MedMicro. 22(2):112–114.