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Molecular Characterisation of Multidrug Resistant *Pseudomonas aeruginosa* from Clinical Specimen in University of Port Harcourt Teaching Hospital, Rivers State, Nigeria

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Abstract

Antibiotic resistance is an international communal wellbeing apprehension and occurs through several defined mechanisms. However, *Pseudomonas species* have been said to have genes that code for beta-lactamase production, some of such genes are SHV, CTX-M and AMPC. This study was aimed at detecting SHV, CTX-M and AMPC genes in multi-drug resistant *Pseudomonas aeruginosa*. The isolates were cultured on MacConkey agar and antibiotic sensitivity were done on Mueller-Hinton agar. Phenotypically identified was by Gram staining and biochemical testing. DNA extraction was carried out by boiling method and gene detection was done. Of the 135 *Pseudomonas* isolates studied, consequently, 81 (60%) of *Pseudomonas* spp isolates were multi-drug resistant. The distribution of the isolates by specimen revealed that, 32 (23.7%), 2 (1.5%), 37 (27.4%), 6 (4.4%), 2 (1.5%) and 2 (1.5%) were from urine, ear, wound, blood culture, abdominal fluid and pleural fluid respectively. Out of the six antibiotics used in this study, Ceftriaxone and Cefuroxime were the most resistant 75 (95.6%). The distribution of resistance genes showed that Molecular characterization with 16s rRNA markers revealed that some isolates that were phenotypically characterized as *Pseudomonas* spp whereas it was *Alcaligenes faecalis*. Conclusively, this study showed the presence of AmpC gene among *Pseudomonas* isolates from UPTH, thus patients can be treated effectively by the knowledge of the drug mechanism.

Keywords: *SHV; CTX-M; AMPC; Multidrug Resistant Pseudomonas*

Introduction

Background to the study

People with latent infection with *Pseudomonas aeruginosa* which is challenging to treat by antibiotic therapy, are a danger to public health. It is worth noting that *Pseudomonas aeruginosa* has two unique antibiotic resistance pathways. The first is the AmpC -lactamase,

which destroys -lactam antibiotics, and the second is the MexXYOprM, a three-protein efflux pump that expels aminoglycoside antibiotics from bacterial cells. Antibiotic resistance gene expression levels are expected to be a pivotal component in antibiotic resistance; however they have not been established throughout infection [5]. *Pseudomonas,* a Gram-negative bacterium with approximately 191 identified species [2], the effectively researched species usually involve *P. aeruginosa* in its contribution as an opportunism virulence factor, *P. syringae, P. putida, and P. fluorescens,* which seem to be herb and soil bacteria*, as well as P. syringae, P. putida*, and *P.* [3]. *Pseudomonas aeruginosa* rarely encountered as part of the living beings microbiota in normal subjects is a gram-negative rod that is not a glucose fermenter well with capability of making catalase and oxidase enzymes. It is an obligate aerobic, non-sporing organism, as well as the majority of them generate blue-green pigment (pyocyanin). *P. aeruginosa,* which is also found in the gut and skin, might well be separated via sinks, restrooms, pulmonary treatment equipment, and even disinfection products. *P. aeruginosa* is common in natural ecosystems and is an important pathogen for people, causing a wide range of diseases such as urinary tract infections, burns, lung infections, and septicemia [76]. It is the most common cause of ventilation-related pneumonia in the critical care unit [54]. There is no evidence of chromosomal AmpC b-lactamase synthesis above the basic threshold. The AmpC gene encodes AmpC b-lactamase [34]. In *P. aeruginosa*, similar processes control the enzyme's activity. Many genes play a role in ampC expression, which is closely related to peptidoglycan recycling [34]. This regulator is required for the induction of b-lactamase [34]. The transcriptional regulatory action of AmpR is associated with peptidoglycan processing [32,33].

AmpG encodes a transmembrane protein that serves as a permease for 1,6-anhydromurapeptides, which are thought to serve as the signal molecules implicated in ampC induction [23-25]. AmpD, the third gene, encodes a cytosolic N-acetyl-anhydromuramyl-L-alanine amidase that hydrolyzes 1,6-anhydromurapeptides and acts as an AmpC repressor [6,25]. AmpD point mutations knockdown in P. aeruginosa PAO1 results in largely depressed production of AmpC blactamase (Langaee., *et al*. 2000). *P. aeruginosa* hospital-acquired illnesses have recently been highlighted as a major issue in hospitals because to its intrinsic multi-drug resistant classes of antimicrobial drugs and its capacity to develop feasible resistance to all effective medications [13,22]. The bacterium possesses a number of virulence factors that contribute to microbial invasion and cytotoxicity [72-74]. *P. aeruginosa* is intrinsically resistant to many compounds with different antibacterial drugs due to the low conductivity of its outer membrane (1/100 of the wettability of *E. coli's* cellular surface) [15,43-45], the constitutive assertion of numerous efflux systems with broad substrate specificity [15] and the naturally occurring chromosomal AmpC b lactamase (also known as cephalosporinase) [16-18,27-30].

P. aeruginosa vulnerability (the so-called wild-type) would include sensitivity to carboxypenicillins (carbenicillin, ticarcillin), ureidopenicillins (azlocillin, piperacillin), a few really third generation cephalosporins (ceftazidime, cefsulodin, cefoperazone), all fourth generation cephalosporins, the monobactam aztreonam (Pechere and Kohler, 1999). There has recently been a widespread occurrence of *P. aeruginosa* resistance to routinely used antibiotics because to the existence of antibiotic-degrading enzymes such as extended-spectrum -lactamases such as PER-1, PER-2, VEB-1, and AmpC. As a result, there is an urgent need to determine and comprehend the operation of these enzymes in this environment. In individuals with substantial baseline medical disorders, *Pseudomonas aeruginosa* can cause a variety of devastating illnesses. It is commonly recognised that patients suffering from severe infections caused by MBL-producing organisms have a bad prognosis when given antibiotics to that they are entirely resistant. To acquire and track the degree of resistance, baseline data of SHV, CTX-M, and AmpC beta-lactamase genes in *Pseudomonas* sp. are required. This investigation is therefore justified. The goal of this research was to discover SHV, CTX-M, and AmpC genes in Multidrug Resistant *Pseudomonas aeruginosa* clinical specimens at the medical microbiology laboratory of the University of Port Harcourt Teaching Hospital (UPTH).

Materials and Methods

Area of study

The Medical Microbiology Laboratory of University of Port Harcourt Teaching Hospital (UPTH)was the study area. The hospital which is a major tertiary care teaching and research facility is located in east west road, Choba, Port Harcourt, Rivers State, Nigeria.

Collection of sample

One hundred and thirty-five (135) bacteria isolates were obtained from different clinical specimens (wound, urine and sputum) from the Medical Microbiology Laboratory of University of Port Harcourt Teaching Hospital (UPTH)for a period of three (3) months.

Sample size calculation

$N = Z^2 X\ p\ (1 - p)/\ d^2$

Where:

N = Minimum sample size

d = Desired level of significance 5% (0.05)

Z = Confidence interval 95% (1.96)

P = Prevalence rate 6.5% [4]

Therefore

$N = 1.962\ X\ 0.065\ (1 - 0.065)/0.05^2$

N = 94.

Sample processing

All the specimen were cultured on MacConkey agar in order to isolate *P. aeruginosa*. The plates were incubated overnight at 37°C. *P. aeruginosa* colonies were identified by standard bacteriological methods. Gram staining was used to confirm the gram negative status of the organism. Biochemical test was done like oxidase test.

Antibiotic susceptibility

Preparation of MacFarland Standard for Kirby Bauer method of susceptibility testing was done. The antibiotic susceptibility test was carried out for all the isolates on Mueller-Hinton plates and zones of inhibition were measured in accordance with the recommendations of Clinical and Laboratory Standard Institute (CLSI).

Molecular identification

Sixteen isolates were chosen at random from a pool of phenotypically tested ESBL producers for molecular studies.

DNA extraction (Boiling method)

5 ml of the bacterium isolate's overnight bacterial suspension in Luria Bertani (LB) were spun at 14000 rpm for 3 minutes. The cells were reconstituted in 500 ul of saline solution and warmed on the heating block for 20 minutes at 95°C. This heated bacterial suspension was quickly cooled for 10 minutes before being spun for 3 minutes at 14000 rpm. The DNA-containing supernatant was moved to a 1.5 ml microcentrifuge tube and kept at -20°C for further procedures.

DNA quantification

The Nanodrop 1000 spectrophotometer was used to measure the isolated genomic DNA. The equipment's software was launched by double tapping on the Nanodrop icon. The apparatus was cleared with DNA elution buffer after being initiated with 2 ul of sterile PCR water (nuclease free water). After loading 2 ul of extracted DNA onto the lower pedestal, the higher pedestal was lowered to make contact with the extracted DNA on the lower pedestal. By clicking on the "measure" button, the DNA concentration was determined.

Extended spectrum beta- lactamase detection

Amplification of SHV genes

SHV F: 5' CGCCTGTGTATTATCTCCCT-3' and SHV R: 5'-CGAGTAGTCCACCAGATCCT-3' primers were used to amplify SHV genes from the isolates using an ABI 9700 applied biosystems thermal cycler at a final volume of 30 ul for 35 cycles. The PCR mix includes the Inqaba, South Africa-supplied X2 Dream taq Master mix (taq polymerase, DNTPs, MgCl), the primers at a concentration of 0.4M, and 50 ng of the extracted DNA as template. The following PCR conditions were used: Initial denaturation was at 95°C for 5 minutes; denaturation was at 95°C for 30 seconds; annealing was at 56°C for 40 seconds; extension was at 72°C for 50 seconds for 35 cycles; and final extension was at 72°C for 5 minutes. The result was resolve on a 1% agarose gel at 120V for 25 minutes and visualized on a UV transilluminator for a 281 bp product size.

Amplification of CTX-M genes

CTX-M genes were amplified from the isolates using the CTX-MF: 5'-CGCTTTGCGATGTGCAG-3' and CTX-MR: 5'-ACCGCGATATCGTTG-GT-3' primers using an ABI 9700 Applied Biosystems thermal cycler for 35 cycles at a final volume of 30 ul. The PCR mix includes the Inqaba, South Africa-supplied X2 Dream Taq Master mix (taq polymerase, DNTPs, MgCl), the primers at 0.4M concentration, and 50 ng of the extracted DNA as template. The following were the PCR conditions: Denaturation at 95°C for 5 minutes; denaturation at 95°C for 30 seconds; annealing at 52°C for 30 seconds; extension at 72°C for 30 seconds for 35 cycles; and final extension at 72°C for 5 minutes. The product was resolved on a 1% agarose gel at 120V for 25 minutes before being examined with a UV transilluminator.

Amplification of AMPC genes

AMPC genes from the isolates were amplified using the primers AMPCF: 5'-ATGCAGCCAACGACAAAGG-3' and AMPCR: 5'-CGCCCTC-GCGCGCGCTTC-3' on an ABI 9700 Applied Biosystems thermal cycler at a final volume of 30 ul for 35 cycles. The PCR mix includes the X2 Dream Taq Master mix from Inqaba, South Africa (taq polymerase, DNTPs, MgCl), the primers at 0.4uM concentration, and 50 ng of the extracted DNA as template. The PCR conditions were as follows: Initial denaturation at 95°C for 5 minutes; denaturation at 95°C for 30 seconds; annealing at 58oC for 30 seconds; extension at 72°C for 30 seconds for 35 cycles; and final extension at 72°C for 5 minutes. The product was resolved on a 1% agarose gel at 130V for 25 minutes and visualized on a blue trans-illuminator for a 650 bp product size.

Sequencing

Sequencing was performed on a 3510 ABI sequencer by Inqaba Biotechnological in Pretoria, South Africa, using the BigDye Terminator kit. The sequence was carried out in a final volume of 10 ul, using 0.25 ul of BigDye® terminator v1.1/v3.1, 2.25 ul of 5 x BigDye sequencing buffer, 10 uM Primer PCR primer, and 2-10 ng PCR template per 100 bp. The conditions for sequence analysis were as continues to follow: 32 cycles of 96°C for 10s, 55°C for 5s, and 60°C for 4 minutes.

Phylogenetic analysis

Trace edit was used to alter the retrieved sequences, and BLASTN was used to retrieve comparable sequences from the National Center for Biotechnology Information (NCBI) repository. These sequences were matched using MAFFT. MEGA 6.0 was utilized to infer the evolutionary origins using the Neighbor-Joining approach [67]. The evolutionary history of the species studied is represented by the bootstrap consensus tree estimated from 500 replicates. The Jukes-Cantor technique was used to estimate the evolutionary distances.

Statistical analysis

Using SPSS version 21, the Chi-square statistical analysis test was conducted to establish the presence or absence of a significant difference.

Results

Of 135 specimens tested for *Pseudomonas* sp., 81 (60%) were multi-drug resistant, including 32 (23.7%), 2 (1.5%), and 37 (27.4%). 6 (4.4%), 2 (1.5%), and 2 (1.5%), respectively, were from urine, ear, wound, blood culture, abdominal fluid, and pleural fluid. *Pseudomonas* spin wound specimens were substantially more common than other specimens (P0.05) (Table 1).

Specimen	Number Examined (%)	Number of Positives (%)	X^2	P value
Urine	40 (29.6)	32 (23.7)	17.487	0.004
Ear	4 (3.0)	2 (1.5)		
Wound	63 (46.7)	37 (27.4)		
Blood Culture	20 (14.8)	6 (4.4)		
Abdominal Fluid	2 (1.5)	2 (1.5)		
Pleural Fluid	6 (4.4)	2 (1.5)		
Total	135	81 (60.0)		

Table 1: *Distribution of P. aeruginosa by source.*

P < 0.05 = Significant.

69 (51.1%) isolates from males and 67 (49.9%) colonies from females were recovered from the 135 specimens examined for *Pseudomonas* sp. According to the age distribution of the isolates, 21 (15.6%), 4 (3.0%), 14 (10.4%), 38 (28.1%), 20 (14.8%), 22 (16.3%), 10 (7.4%), and 6 (4.4%) were isolated from subjects aged 0 - 10, 11 - 20, 21 - 30, 31 - 40, 41 - 50, 51 - 60, 61 - 70, 71 - 80, respectively. The incidence of *Pseudomonas* sp was not statistically significant among age groups (P > 0.05) (Table 2).

Age	Number Examined (%)	Male (%)	Female (%)	X^2	P value
0 - 10	21 (15.6)	8 (5.9)	13 (9.6)	3.826	0.800
11 - 20	4 (3.0)	2 (1.5)	2 (1.5)		
21 - 30	14 (10.4)	8 (5.9)	6 (4.4)		
31 - 40	38 (28.1)	21 (15.6)	17 (12.6)		
41 - 50	20 (14.8)	9 (6.7)	11 (8.1)		
51 - 60	22 (16.3)	13 (9.6)	9 (6.7)		
61 - 70	10 (7.4)	4 (3.0)	6 (4.4)		
71 - 80	6 (4.4)	4 (3.0)	2 (1.5)		
Total	135 (100)	69 (51.1)	66 (48.9)		

Table 2: *Distribution of P. aeruginosa by age and gender.*

P > 0.05 = Not Significant.

Antimicrobial susceptibility patterns indicated that 81 isolates of *Pseudomonas* sp. were multi-drug resistant, with 59 (72.8%), 39 (48.1%), 75 (95.6%), 75 (95.6%), 73 (90.1%), 73 (90.1%) being meropenem, ceftriaxone, cefuroxime, gentamicin, and ciprofloxacin, respectively. The incidence of drug resistance in *Pseudomonas* spin was considerably greater for ceftriaxone and cefuroxime than with other antibiotics (P0.05) (Table 3). The distribution of 81 isolates of multi-drug resistant *Pseudomonas* sp revealed 32 (23.7%), 2 (1.5%), and 37

(27.4%), 6 (4.4%), 2 (1.5%), and 2 (1.5%) were from urine, ear, wound, blood culture, abdomen fluid, and pleural fluid, respectively. The frequency of *Pseudomonas* spin wound specimen was substantially greater than that of other specimens (P0.05) (Table 4).

Antibiotics	Susceptible (%)	Resistant (%)	Total	X^2	P value
Meropenem (MEM)	22 (27.2)	59 (72.8)	81	83.449	0.000
Ceftazidime (CAZ)	42 (51.9)	39 (48.1)	81		
Ceftriaxone (CRO)	6 (7.4)	75 (92.6)	81		
Cefuroxime (CXM)	6 (7.4)	75 (92.6)	81		
Gentamicin (CN)	8 (9.9)	73 (90.1)	81		
Ciprofloxacin (CIP)	8 (9.9)	73 (90.1)	81		

Table 3: *Antimicrobial susceptibility pattern of P. aeruginosa.*

P < 0.05 = Significant.

Specimen	Number Examined	Resistant (%)	Non resistant (%)	X^2	P value
Urine	40	32 (23.7)	8 (5.9)	17.487	0.004
Ear	4	2 (1.5)	2 (1.5)		
Wound	63	37 (27.4)	26 (19.3)		
Blood culture	20	6 (4.4)	14 (10.4)		
Abdominal fluid	2	2 (1.5)	0		
Pleural fluid	6	2 (1.5)	4 (3.0)		
Total	135	81 (60)	54 (40)		

Table 4: *Distribution of multidrug resistant drugs in P. aeruginosa by source.*

P < 0.05: Significant.

The distribution of multidrug resistance medications in *P. aeruginosa* by age and gender revealed 41 men (50.6%) and 40 females (49.4%). In the age range 31 - 40, 15 (18.5%) were separated from men, while 12 (14.8%) were isolated from females, but no organism was recovered from males or females in the age group 11 - 20. The incidence of *Pseudomonas* sp among age groups was not statistically significant (P > 0.05) (Table 5).

Age	Number of Positives (%)	Male (%)	Female (%)	X^2	P value
0 - 10	10 (12.3)	2 (2.5)	8 (9.9)	8.875	0.181
11 - 20	0	0	0		
21 - 30	8 (9.9)	4 (4.9)	4 (4.9)		
31 - 40	27 (33.3)	15 (18.5)	12 (14.8)		
41 - 50	12 (14.8)	6 (7.4)	6 (7.4)		
51 - 60	14 (17.3)	8 (9.9)	6 (7.4)		
61 - 70	6 (7.4)	2 (2.5)	4 (4.9)		
71 - 80	4 (4.9)	4 (4.9)	0		
Total	81 (100)	41 (50.6)	40 (49.4)		

Table 5: *Distribution of multidrug resistant drugs in P. aeruginosa by age and gender.*

P > 0.05: Not significant.

Ten (62.5%) of the 16 specimens chosen for gene extraction were positive for the AmpC gene. There were no specimens with SHV or CTX-M genes. AmpC gene distribution by source indicated 2 (12.5%), 1 (6.3%), and 7 (43.8%) for urine, ear, and wound specimens, respectively. *Pseudomonas* sp prevalence by source was not significant (P > 0.05) (Table 6).

Number examined (16 samples)					
Specimen	**AmpC (%)**	**Male (%)**	**Female (%)**	**X^2**	**P value**
Urine	2 (12.5)	1 (6.3)	1 (6.3)	1.143	0.565
Ear	1 (6.3)	0	1 (6.3)		
Wound	7 (43.8)	4 (25.0)	3 (18.8)		
Blood Culture	0	0	0		
Abdominal Fluid	0	0	0		
Pleural Fluid	0	0	0		
Total	10 (62.5)	5	5		

Table 6: *Distribution of AmpC gene in P. aeruginosa by source.*
P > 0.05: Not significant.

Ten (62.5%) of the 16 specimens chosen for gene extraction were positive for the AmpC gene. There were no specimens with SHV or CTX-M genes. AmpC gene distribution by age and gender was found to be 2 (12.5%), 1 (6.3%), and 7 (43.8%) for urine, ear, and lesion specimens, respectively. The incidence of *Pseudomonas* sp among age groups was not statistically significant (P > 0.05) (Table 7).

Number examined (16 samples)					
Age	**AmpC (%)**	**Male (%)**	**Female (%)**	**X^2**	**P value**
0 - 10	1 (6.3)	0	1 (6.3)	6.875	0.230
11 - 20	0	0	0		
21 - 30	1 (6.3)	0	1 (6.3)		
31 - 40	4 (25.0)	1 (6.3)	3 (18.8)		
41 - 50	2 (12.5)	2 (12.5)	0		
51 - 60	0	0	0		
61 - 70	1 (6.3)	0	1 (6.3)		
71 - 80	1 (6.3)	1 (6.3)	0		
Total	10 (62.5)	4	6		

Table 7: *Distribution of AmpC gene in P. aeruginosa by age and gender.*
P > 0.05: Not significant.

Some phenotypically defined specimens were also genotypically identified by sequencing. *P. aeruginosa* was found in P1, P3, and P4, while P5 was *Pseudomonas* sp. After sequencing, P2 revealed *P. aeruginosa* and *Alcaligenes faecalis* for phenotypic and genotypic characterisation, respectively (Figure 1).

The positive bands of the isolates are represented by the image of an agarose gel electrophoresis revealing positive bands for the 16S gene with 1500bp of DNA isolated from clinical specimens 1-2 and 3-6, while lane L shows the 1kp quick-load molecular ladder (Plate 1).

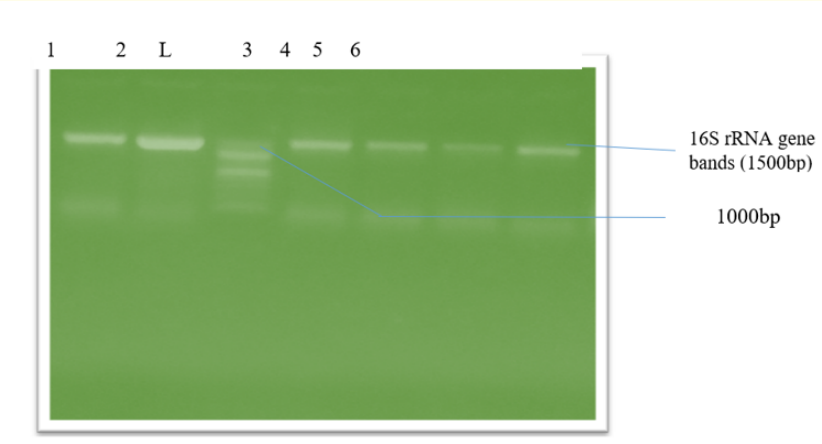

Plate 1: *Agarose gel pix showing the amplified 16S rRNA gene bands. Lanes 1-6 show the bands at 1500bp while Lane L represents the 100 bp molecular ladder.*

The image of Agarose gel electrophoresis displaying enhanced AmpC. Lanes 1-3, 5, 8, 12, and 13 depict the AmpC gene, whereas lane L depicts the 1kp quick-load molecular ladder (Plate 2).

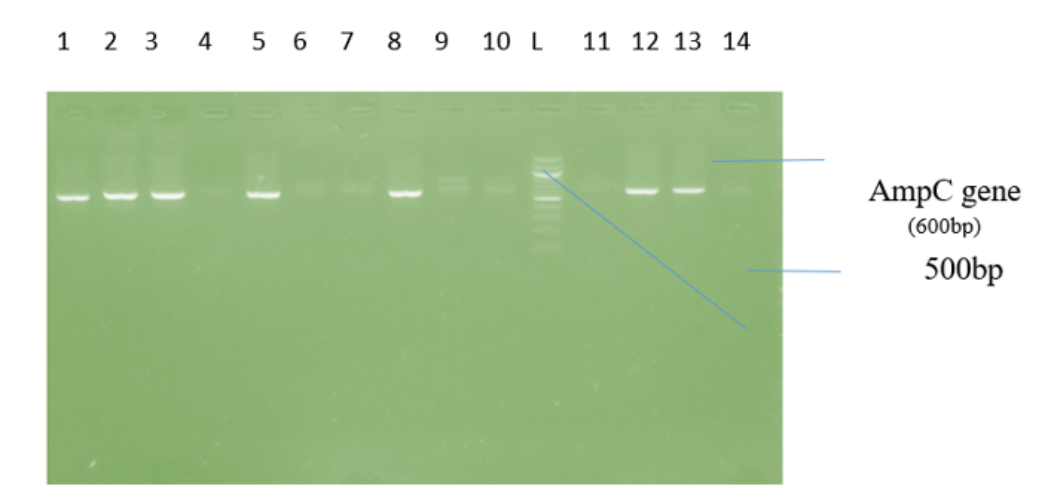

Plate 2: *Agarose gel electrophoresis showing the amplified AmpC. Lane 1-3, 5, 8, 12, 13 showing the AmpC gene while lane L represents the 100bp molecular ladder.*

Using a megablast search for extremely similar sequences from the NCBI non-redundant nucleotide (nr/nt) database, the isolate's 16s rRNA sequence returned a perfect match. The isolates P3, P4, P1, and P5 shared 100% of their 16S rRNA with other species (Plate 1 and 2). The evolutionary distances calculated with the Jukes-Cantor technique were in agreement with the phylogenetic placement of the 16S rRNA of the isolates within the *Pseudomonas* sp and revealed a closely relatedness to *Pseudomonas aeruginosa* than other *Pseudomonas* sp, P2 was closely related to Alcaligenes faecalis (Figure 1).

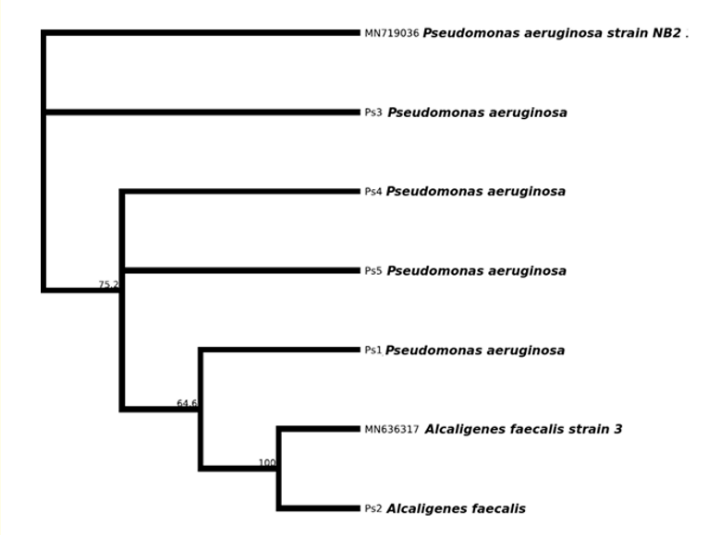

Figure 1: *Phylogenetic tree showing the evolutionary distance between the bacterial isolates.*

Discussion

Pseudomonas sp were recovered from 81 (60%) of the 135 specimens tested for *Pseudomonas* sp. This indicates that a greater proportion of the samples isolated for this bacterium were resistant. The wound sample was found to be the most frequent 37 (27.4%) of the time when the samples were distributed by source. This suggests that wound samples contain the majority of multidrug resistant *Pseudomonas* sp.

Pseudomonas sp. was found in 69 (51.1%) male isolates and 67 (49.9%) female isolates. There was no significant variation in the distribution of the isolates by gender. The frequency of *Pseudomonas* sp agrees with that observed by Niloufar., *et al*. (2017) and Sahar., *et al*. (2017); demonstrating that the most commonly diagnosed infection is caused by *Pseudomonas* sp.

Pseudomonas sp. were multi-drug resistant, with 59 (72.8%), 39 (48.1%), 75 (95.6%), 75 (95.6%), 73 (90.1%), 73 (90.1%) resistant to meropenem, ceftriaxone, cefuroxime, gentamicin, and ciprofloxacin, respectively Sahar., *et al*. (2017) found 78.7% resistance to a wide range of antibiotics, and Zongo., *et al*. (2015). This might be due to the widespread usage of broad-spectrum antibiotics, which could have resulted in the encoded antibiotic resistance genes. Alsterlund., *et al*. (2009) agreed, confirming the existence of ESBL resistance based on prior antibiotic usage.

In this investigation, the distribution of multi-drug resistant *Pseudomonas* sp was 32 (23.7%), 2 (1.5%), and 37 (27.4%). 6 (4.4%) 2 (1.5%) came from urine, 2 (1.5%) from ear, wound, blood culture, abdominal fluid, and 2 (1.5%) from pleural fluid, respectively. *Pseudomonas* spin wound specimens were substantially more common than other specimens (P 0.05). This conclusion was consistent with the findings of Niloufar., *et al*. (2017), who found over 70% resistance across wound swab samples, with 55 bacteria harboring multiple resistant genes.

The percentage of antibiotic resistance assessed across gender revealed that males had greater than females; 41 (50.6%) males and 40 (49.4%) females. The incidence of *Pseudomonas* sp across the age groups was not significant (P > 0.05), with the age group 41 - 50 years being the most resistant (12.5%), followed by the age group 31 - 40 years. Among the 16 specimens chosen for gene extraction, 10 (62.5%) were positive for the AmpC gene.

Most *Pseudomonas aeruginosa* strains, like nearly all members of the *Enterobacteriaceae* family, exhibit an inducible chromosomally encoded AmpC-lactamase (cephalosporinase), which is classified as class C in Ambler's classification and as group 1 in Bush's classification as found in Sanders., *et al*. (1992), Ambler, (1980) and Bush., *et al*. (1995). There were no specimens with SHV or CTX-M genes. AmpC gene distribution by source indicated 2 (12.5%), 1 (6.3%), and 7 (43.8%) for urine, ear and wound specimen respectively. The prevalence of *Pseudomonas* sp by source was not significant (P > 0.05).

Ten (62.5%) of the 16 specimens chosen for gene extraction confirmed positive for the AmpC gene. There were no specimens with SHV or CTX-M genes. AmpC gene distribution by age and gender was found to be 2 (12.5%), 1 (6.3%), and 7 (43.8%) for urine, ear, and wound specimens, respectively. The occurrence of *Pseudomonas* sp did not differ by age group (P > 0.05).

Sequencing demonstrated that a phenotypically described specimen was also genotypically recognized. *P. aeruginosa* was found in P1, P3, and P4, while P5 was *Pseudomonas* sp. After sequencing, P2 revealed *P. aeruginosa* and *Alcaligenes faecalis* for phenotypic and genotypic characterisation, respectively. Marchesi (1998) showed that phenotypically similar *P. aeruginosa* and *Alcaligenes faecalis* may be proven better genotypically.

In agreement with Gutell., *et al*. (1985), an agarose gel electrophoresis showed positive bands for the 16S gene with 1500bp of DNA extracted from the clinical samples with the 1kp quick-load molecular ladder which showed that the 16SrRNA region of the ribosome of the isolates were successfully amplified.

The amplified AmpC gene was visible on agarose gel electrophoresis, whereas lane L represented the 1kp quick-load molecular ladder.

Conclusion

In summary, using a molecular technique to identify bacteria and their ESBL genes responsible for multi-resistance is more trustworthy. The AmpC gene was found in *Pseudomonas* isolates from the University of Port Harcourt Teaching Hospital in this investigation. The SHV and CTX-M genes were not present. The limitations of phenotypic characterization and a shortage of reagents may result in incorrect results. This research work carried out revealed that Multi-Drug Resistance *Pseudomonas aeruginosa* isolated at UPTH harbors AmpC gene and that 16s rRNA as a genetic marker or signature for bacteria. Similarly, the multidrug resistance of *Pseudomonas aeruginosa* in this study, provides a clue to the clinical treatment and infection control measure for patients. From the findings of this study, we therefore recommend that further research should be carried out to know all the genes associated with multidrug resistance in *Pseudomonas* sp in this region. Also, appropriate measures should be taken to reduce or stop the recurrence of multidrug resistance and the use of PCR for identification of pathogenic organisms in hospitals should be encouraged.

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