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DISTRIBUTION OF AMPC, SHV AND CTX-M GENES IN MULTIDRUG RESISTANT *PSEUDOMONAS AERUGINOSA* FROM CLINICAL SPECIMENT IN UNIVERSITY OF PORTHARCOURT TEACHING HOSPITAL RIVERS STATE, NIGERIA

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ABSTRACT

Antibiotic resistance is a global public health concern 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 AMPC, SHV and CTX-M. This study was aimed at detecting AMPC, SHV and CTX-M genes in multi- drug resistant *Pseudomonas aeruginosa*. The isolates were cultured and phenotypically identified by Gram staining and biochemical testing. DMA extraction was carried Qutiby boiling method and gene detection was done. Two hundred and thirty five (235) clinical samples collected from University of Port Harcourt Teaching Hospital (UPTH) were cultured on blood agar and MacConkey agar and incubated at 37°C for 24hours. 135 organisms were isolated consisting 81(60%) of Pseudomonas aeniginosa. 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 sp whereas it was Alcaligenes faecalis. The result showed the presence of Amp C and the absence of SHV and CTX-M gene among Pseudomonas isolates from UPTH, thus patients can be treated effectively by the knowledge of the drug mechanism.

INTRODUCTION

Pseudomonas, a Gram-negative bacterium, with about 191 described species (Euzeby, 1997), the best studied species include P. aeniginosa in its role as an opportunistic human pathogen, P. syringae, P. putida, and P. fluorescent which are plant and soil bacteria(Akanji et al., 2011). Pseudomonas aeniginosa infrequently found as part of the human microflora in healthy individuals is a gram-negative rod that does not glucose fermenter with the potential of producing catalase and oxidase enzymes. It is an obligatory aerobic, non-sporing and most of them produce pigment which is blue-green (pyocyanin). P. aeruginosa can be isolated from sinks, toilets, respiratory therapy equipment and even disinfectant solutions, also present in intestine and skin. P. aeruginosa is widespread in natural environments and it is an opportunistic pathogen for humans lead to a broad spectrum of disease such as urinary, burn, respiratory infections, and septicemia (Yang el al., 2011). It is the primary cause of ventilation associated pneumonia in the intensive care unit (Nseir el al., 2011).

The susceptible P. aeruginosa phenotype (the so called wild-type) includes susceptibility to carboxypenicillins (carbenicillin, ticarcillin), ureidopenicillins (azlocillin, piperacillin), some third generation cephalosporins (ceftazidime, cefsulodine, cefoperazone), all the fourth generation cephalosporins, the monobactam aztreonam, and the carbapenems imipenem and meropenem (Pechere and Kohler, 1999). There are several basic resistance phenotypes. Often called "intrinsic resistance

to carbenicillin', this phenotype is characterized by a fourfold to eightfold increase of MIC for most of the b-lactams, including meropenem but not imipenem. No production of chromosomal AmpC b- lactamase above the basic level is found. This phenotype includes resistance to non-b- lactam antibiotics like quinolones, trimethoprim, tetracycline and chloramphenicol.

AmpC b-lactamase is encoded by the AmpC gene (Lodge etal. 1993). Mechanisms regulating AmpC expression have been studied in detail for Enterobacter cloacae. Similar mechanisms regulate the expression of the enzyme in P. aeruginosa. Of the genes involved, AmpR, is contiguous to AmpC but divergently transcribed, and it encodes a positive transcriptional regulator that is a member of the LysR family (AmpR). This regulator is necessary for the b-lactamase induction (Lodge el al., 1993). AmpR transcriptional regulatory activity is related to peptidoglycan processing (Jacobs el al., 1994). The second gene. AmpG, encodes a transmembrane protein that acts as a permease for 1, 6-anhydromurapeptides, which are considered to be the signal molecules involved in ampC induction (Dietz el a/., 1997). The third gene, AmpD, encodes a cytosolic N-acetyl-anhydromuramyl-L-alanine amidase, which hydrolyses 1,6- anhydromurapeptides, acting as a represser of AmpC expression (Ho'ltje et al., 1994).

In Gram negative microbes, the β-lactamase mediated resistance is either plcismid mediated or expressed chromosomally. Nevertheless, the spread of β-lactamases is frequently linked with plasmid mediated ESBLs, specifically the CTX-M family. CTX-M. Although a bit recently discovered, CTX-M enzymes are the most increasingly reported types of enzymes associated with resistance. CTX-M enzymes are plasmid based encoded cefotaximases that constitute the fast growing family of ESBLs. CTX-Ms are named after their extended activity against cefotaxime compared to ceftazidime and the origin of its first isolation (Munich, Germany) (Birbrair and Frenette, 2016). Among other ESBLs, CTX- M enzymes have been proven to be the most efficacious in terms of promiscuity and its predominance abundance in diverse epidemiological settings, where they have largely replaced and outnumbered other ESBL types such as TEM (Birbrair and Frenette, 2016).

CTX-M expression is quite often associated with co-resistance along with expression of other resistance elements critically reducing response to treatment. Unlike the TEM and SHV ESBLs, CTX-M type enzymes did not arise as a result of alterations of existing enzymes; they were acquired de novo by lateral gene transfer from Kluyvera sp. A phylogenetic tree can be drawn based on the amino acid sequence to determine the relatedness among the members of CTX-M /?-lactamases. CTX-M has been divided into six sublineages or groups (CTX-M-1, CTX-M-2, CTXM-8, CTX-M-9. CTX-M-25, and KLUC, entitled after the first member of the group that was described). Members within a group have >94% amino acid relatedness and <90% relatedness across the groups. Additionally, there are about four CTX-M variants that exhibit a hybrid structure, namely, CTX-M-45 (formerly Toho-2), which is a hybrid of CTXM-14 with a protein of unknown origin, and CTX- M-64, CTX-M-123, and CTX-M-132 that are hybrids of CTX-M-15 with different segment CTX-M-14 . While the main variants of CTX-Ms are biologically different, CTX-M-15 and CTX-M-14 are the most common variants detected globally in important microbes, followed by CTX-M-2, CTX-M-3, and CTX-M-1. In the early 1990s, reports from distant countries suggested the potential of spread of these enzymes and its ability to disseminate. During this time, diversification was also noticed, illustrated well by the CTX-M-3, closely related to CTX-M-1 differing in four amino acid positions (V77, D114, S140A, and N288D). In this context, CTX-M-10 was reported in the Mediterranean areas and CTX-M-15 in New Delhi. The CTX-M-10 differs in two amino acids (at positions A27A and R38Q) from CTX-M-3, while CTX-M-15 differs in a single amino acid at position (D240G); presumably, all these three might have been produced from a common ancestor (D'Andrea el al., 2013). SHV ESBLs: SHV types of enzymes are mostly found in Klebsiella species (especially K. pneumonias} most often housed by a plasmid. However, a number of species have been shown to carry SHV-1 gene within the chromosome. SHV denotes sulfhydryl variable as it was believed that the inhibition of SHV activity by chloromercuribenzoate was substrate-related and was found inconstant according to the substrate used for the assay (Zhao and Hu, 2013). SHV-2 was the 1st SHV-ESBL type detected in Klebsiella ozaenae isolated from Germany, in 1983. This enzyme originated from point mutation in SHV-1 which resulted in substitution of glycine by serine at the 238 positions and extension of its hydrolytic substrate profile to include cefotaxime and to a minor degree ceftazidime (Zhao and Hu. 2013).

The aim of this study was the Molecular detection of AmpC genes in Multidrug Resistant Pseudomonas aeruginosa from clinical specimen in medical microbiology laboratory of the University of Port Harcourt Teaching Hospital (UPTH).

Material and MethodsArea 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 East West Road, Port Harcourt, Rivers State, Nigeria.

Collection of Samples

One hundred and thirty-five (135) clinical samples comprising Urine (100), Ear (4), Wound (93), Blood (30) Abdominal Fluid (2) and Pleural Fluid (6) obtained from the Medical Microbiology Laboratory of University of Port Harcourt Teaching Hospital (UPTH) for a period of three (3) months were studied.

Sample processing

All the specimens were cultured on Blood agar and MacConkey agar, incubated at 37°C for 24hours. The organisms were isolated and identified using standard bacteriological methods as described by Cheesbrough (2000). The standard identification to isolate and identify P. aeruginosa such as Gram staining and some biochemical test were done.

Antibiotic susceptibility

Preparation of MacFarland Standard for KJrby 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 Clinicaland Laboratory Standard Institute (CLSI).

Molecular Identification

Among phenotypically tested ESBL producers, sixteen isolates were randomly selected for molecular characterization.

DNA extraction (Boiling method)

5ml of an overnight broth culture of the bacterial isolate in Luria Bertani (LB) was centrifuged at 14000rpm for 3mins. The cells were re-suspended in SOOul of normal saline and heated at 95°C for 20mins on the heating block. The heated bacterial suspension was fast cooled for lOmins and centrifuged for 3mins at 14000rpm. The supernatant containing the DNA was transferred to a 1 .5ml microcentrifuge tube and stored at -20°C for other downstream reactions.

DNA quantification

The extracted genomic DNA was quantified using the Nanodrop 1000 spectrophotometer. The software of the equipment was lunched by double clicking on the Nanodrop icon. The equipment was initialized with 2ul of sterile PCR water (nuclease free water) and blanked using DNA elution buffer. 2ul of the extracted DNA was loaded onto the lower pedestal, the upper pedestal was brought down to contact the extracted DNA on the lower pedestal. The DNA concentration was measured by clicking on the "measure'" button.

16SrRNA Amplification

The 16s rRNA region of the rRNA gene of the isolates were amplified using the 27F: 5'-AGAGTTTGATCMTGGCTCAG-3[1] and 1492R: 5'-CGGTTACCTTGTTACGACTT-3' primers on a ABI 9700 Applied Biosystems thermal cycler at a final volume of 40 til for 35 cycles. The PCR mix included: the X2 Dream taq Master mix supplied by Inqaba, South Africa (taqpolymerase, DNTPs, MgCl), the primers at a concentration of 0.5uM and the extracted DNA as template. The PCR conditions were as follows: Initial denaturation, 95°C for 5 minutes; denaturation, 95°C for 30 seconds; annealing, 52°C for 30 seconds; extension, 72°C for 30 seconds for 35 cycles and final extension, 72°C for 5 minutes. The product was resolved on a 1% agarose gel at 130V for 30 minutes and visualized on a blue light transilluminator for a 1500bp amplicons.

Amplification of AMPC genes

AMPC genes from the isolates were amplified using the AMPCF: 5'- ATGCAGCCAACGACAAAGG -3[5] and AMPCR: 5'~€GCCCTCGCGAGCGCGCTTC~3[!] Primers on a ABI 9700 Applied Biosystems thermal cycler at a final volume of 30 ul for 35 cycles. The PCR mix included: the X2 Dream taq Master mix supplied by Inqaba, South Africa (taq polymerase, DNTPs. MgCl), the primers at a concentration of 0.4uM and 50ng of the extracted DNA as template. The PCR conditions were as follows: Initial denaturation, 95°C for 5 minutes; denaturation, 95°C for 30 seconds; annealing, 58°C for 30 seconds; extension, 72°C for 30 seconds for 35 cycles and final extension, 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 1243bp product size.

Amplification of SHV genes

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

Amplification of CTX-M genes

CTX-M genes from the isolates were amplified using the CTX-MF: 5'- CGCTTTGCGATGTGCAG-3' and CTX-MR:5'-ACCGCGATATCGTTGGT-3' primers on a AB19700 Applied Biosystems thermal cycler at a final volume of 30 ul for 35 cycles. The PCR mix included: the X2 Dream taq Master mix supplied by Fnqaba. South Africa (taq polymerase, DNTPs, MgCl), the primers at a concentration of 0.4M and 50ng of theextracted DNA as template. The PCR conditions were as follows: Initial denaturation, 95°Cfor 5 minutes; denaturation, 95°C for 30 seconds; annealing, 52°C for 30 seconds; extension, 72°C for 30 seconds for 35 cycles and final extension, 72°C for 5 minutes. The product was resolved on a 1% agarose gel at 120V for 25 minutes and visualized on a UV transilluminator for a 550bp product size.

Sequencing

Sequencing was done using the BigDye Terminator kit on on a 3510 ABI sequencer by Inqaba Biotechnological, Pretoria South Africa. The sequencing was done at a final

volume of 1 Oul, the components included 0.25 ul BigDye® terminator v 1.1 /v3.1, 2.25ul of 5 x BigDye sequencing buffer, lOuM Primer PCR primer, and 2-1 Ong PCR template per lOObp.The sequencing condition were as follows 32 cycles of 96°C for 10s, 55°C for 5s and 60°C for 4min.

Phylogenetic analysis

Obtained sequences were edited using the bioinformatics algorithm Trace edit, similar sequences were downloaded from the National Center for Biotechnology Information (NCBI) data base using BLASTN. These sequences were aligned using MAFFT. The evolutionary history was inferred using the Neighbor-Joining method in MEGA 6.0 (Saitou and Nei, 1987). The bootstrap consensus tree inferred from 500 replicates (Felsenstein. 1985) is taken to represent the evolutionary history of the taxa analyzed. The evolutionary distances were computed using the Jukes-Cantor method (Jukes and Cantor 1969).

Statistical analysis

Chi-square statistical analysis test was used to determine whether or not there is any significant difference using SPSS version 21.

RESULTS

Out of 135 specimen examined for Pseudomonas sp. 81(60%) of Pseudomonas sp isolated were multi-drug resistant of which 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. The prevalence of Pseudomonas sp in wound specimen was significantly higher than that of other specimen (P<0.05) (Table 1).

Table 1: Distribution of P. aeruginosa by source.

Specimen	***Number Examined(%)***	***Number ofPositives (%)***	***X^2 P value***
Urine	*40(29.6)*	*32(23.7)*	*17.487*
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)***	

P<0.05 = Significant

of the 135 specimen analyzed for Pseudomonas sp. 69(51.1%) isolates were obtained from males and 67 (49.9%) from females. The distribution of the isolates by age revealed that, 21(15.6%), 4(3.0%), 14(10.4%), 38(28.1%), 20(14.8%). 22(16.3%), 10(7.4%), 6(4.4%) were isolated from subjects within age bracket, 0-10, 11-20, 21-30, 31-40, 41-50, 51-60, 61-70. 71-80 respectively. The prevalence of Pseudomonas sp was not significant across age bracket (P>0.05) (Table 2).

Table 2: Distribution of P. aeruginosa by Age and Gender.

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)***		

P>0.05=Not Significant

Antimicrobial susceptibility pattern revealed that 81 isolates of Pseudomonas sp, were multi-drug resistant of which 59(72.8%), 39(48.1%), 75(95.6%), 75(95.6%). 73(90.1%). 73(90.1%) were Meropenem, Ceftazidime, Ceftriaxone, Cefuroxime. Gentamicin and Ciprofloxacin respectively. The prevalence of drug resistant in Pseudomonas sp in Ceftriaxone and Cefuroxime were significantly higher than that of other antibiotics (P<0.05) (Tables 3).

Table 3: Antimicrobial Susceptibility Pattern of P. aerugmosa.

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*	

P<0.05 = Significant

The distribution of 81 isolates of multi-drug resistant Pseudomonas sp showed, 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. The prevalence of Pseudomonas sp in wound specimen was significantly higher than that of other specimen (P<0.05) (Table 4).

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

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)*		
ABDOMINALFLUID	*2*	*2(1.5)*	*0*		
PLEURALFLUID	*6*	*2(1.5)*	*4(3.0)*		
TOTAL	*135*	*57(60)*	*54(40)*		

P<0.05: Significant

The Distribution of multidrug resistant drugs in P. aeruginosa by age and gender showed, 41(50.6%) males and 40(49.4%) from females. The age group 31-40, 15(18.5%) were isolated from males, while 12(14.8%) in females as the highest, while age group 11-20, no organism was isolated from males and females. The prevalence of Pseudomonas sp across the age group was not significant (P>0.05) (Table 5).

Table 5: Distribution of multidrug resistant drugs in P. aerugmosa by Age and Gender.

Age		*Numberof 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)***		

P>0.05: Not significant

The distribution of AmpC gene by source revealed, 2(12.5%), 1(6.3%), 7(43.8%) for urine, ear and wound specimen respectively. The prevalence of Pseudomonas sp by source wasnot significant (P>0.05) (Table 6).

Table 6: Distribution of Amp C, SHV and CTX-M genes in P. aeruginosa by source.

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

P>0.05: Not significant

Of selected 16 specimen for gene extraction, 10(62.5%) specimen were positive for AmpC gene. The distribution of AmpC gene by age and gender was showed, 2(12.5%), 1(6.3%), 7(43.8%) for urine, ear and wound specimen respectively. The prevalence of Pseudomonas sp across the age group was not significant (P>0.05) (Table 7).

Table 7: Distribution of AmpC gene in P. aerugmosa by Age andGender.

Age	*AmpC*	*Male (%)*	*Female(%)*	X^2	*P value*
0 - 10	*1(6.3)*	*0(0)*	*1(6.3)*	*6.875*	*0.230*
11 -20	*0*	*0*	*0*		
21 -30	*1(6.3)*	*0(0)*	*1(6.3)*		
31 -40	*4(25.0)*	*1(6.3)*	*3(18.8)*		
41 -50	*2(12.5)*	*2(12.5)*	*0(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***		

P>0.05: Not significant

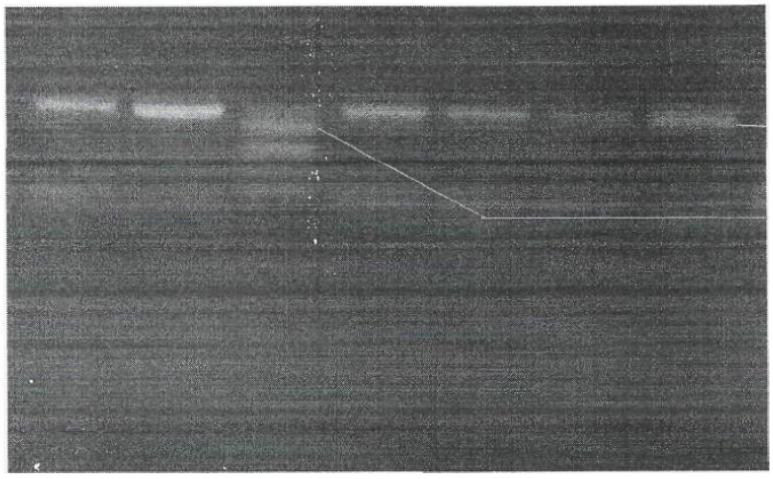

Plate 1: Agarose gel pix showing the amplified 16S rRNA gene bands. Lanes 1-6 show thebands at I500bp while Lane L represents the 100 bp molecular ladder.

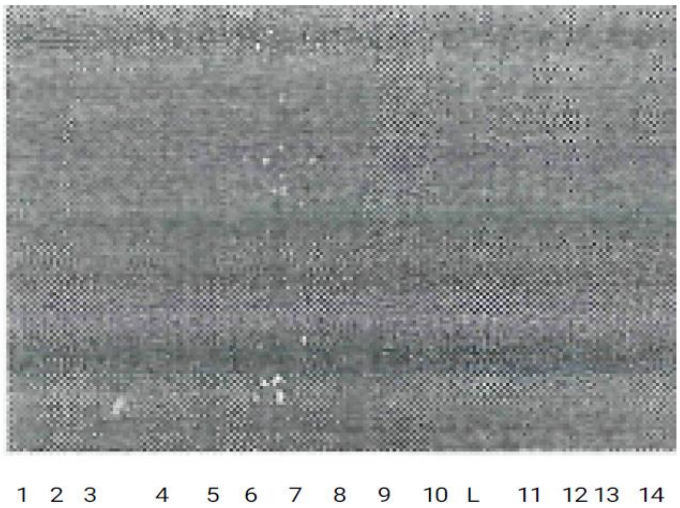

Plate 2: Agarose gel electrophoresis showing the amplified AmpC. Lane 1-3. 5, 8, 12, 13 showing the AmpC gene while lane L represents the l00bp molecular ladder.

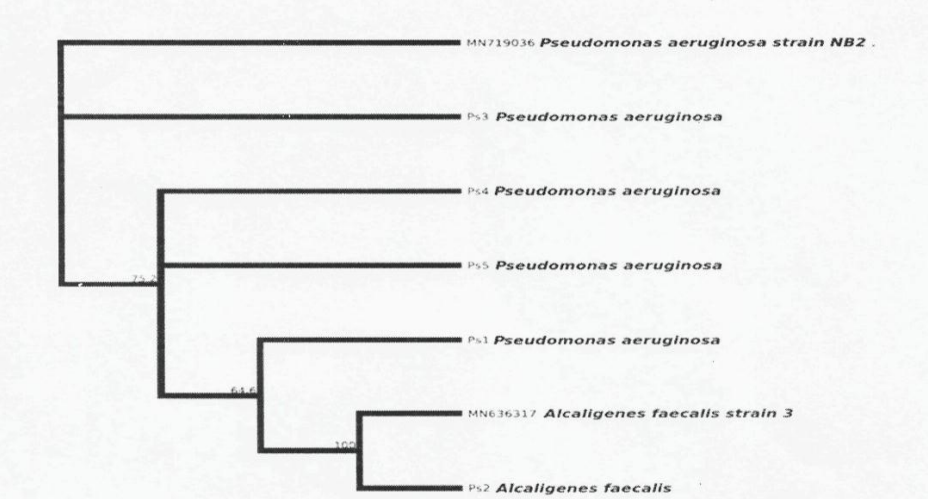

Fig 1: Phylogenetic tree showing the evolutionary distance between the bacterial isolates

DISCUSSION

From the 135 specimen examined for Pseudomonas sp, 81(60%) of Pseudomonas sp isolated were multi-drug resistant. This shows that more of samples isolated for this organism was resistant. The distribution of the sample by source revealed the wound sample was the most prevalent 37(27.4%). This indicates that most multidrug resistant Pseudomonas sp are found in wound samples.

The occurrence of Pseudomonas sp were 69(51.1%) isolates obtained from males and 67 (49.9%) from females. The distribution of the isolates by gender there was not much difference. The prevalence of Pseudomonas sp corresponds with that observed by Niloufar el al., (2017) and Sahar el al., (2017); revealing that Pseudomonas sp are the most common nosocomial infection.

Susceptibility pattern revealed that Pseudomonas sp, were multi-drug resistant of which 59(72.8%). 39(48.1%), 75(95.6%), 75(95.6%), 73(90.1%), 73(90.1%) were

Meropenem, Ceftazidime, Ceftriaxone, Cefuroxime, Gentamicin and Ciprofloxacin respectively. The high resistance to a wide variety of antibiotic was reported by Sahar el al., (2017) with 78.7% and Zongo el al 2015. This may be as a result of general useof broad spectrum antibiotics which may have resulted to the encoded antibiotic resistance genes. A view also held by Alsterlund el a].. 2009 reporting the presence of ESBL resistance based on previous use of antibiotics.

The distribution of multi-drug resistant Psendomonas sp in this study showed, 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. The prevalence of Pseudomonas sp in wound specimen was significantly higher than that of other specimen (P<0.05). This result was in agreement with the study conducted by Niloufar et al (2017) which had over 70% resistance among the wound sab samples while 55strains harboured multiple resistant genes.

The percentage resistance of antibiotics across gender showed that more were observed among males than females; 41(50.6%) males and 40(49.4%) from females. The prevalence of Pseudomonas sp across the age group was however not significant (P>0.05), with age group 41-50years being the most predominant resistant (12.5%) while age group 31-40years.

Selected 16 specimen for gene extraction, 10(62.5%) specimen were positive for AmpC gene. Most Pseudomonas aeruginosa strains, like almost all members of the family Enterobacteriaceae, express an inducible chromosomally encoded AmpC p-lactamase (cephalosporinase), which is placed in class C of Ambler's classification andwhich is in Bush's group 1. (Sanders et al., 1992, Ambler, 1980 and Bush et al., 1995). No specimen showed SHV or CTX-M genes. The distribution of AmpC gene by source revealed, 2(12.5%), 1(6.3%), 7(43.8%) for urine, ear and wound specimen respectively. The prevalence of Pseudomonas sp by source was not significant (P>0.05). The obtained 16s rRNA sequence from the isolate produced an exact match during the megablast search for highly similar sequences from the NCBI non-redundant nucleotide (nr/nt) database. The 16S rRNA of the isolates P3. P4, PI, P5 showed a percentage similarity to other species at 100%. The evolutionary distances computed using the Jukes-Cantor method 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 (Fig. 1).

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