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Antimicrobial susceptibility profile and detection of extended spectrum β-lactamase resistance genes in pseudomonas aeruginosa isolated from post-operative wounds

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ABSTRACT

The spread of *Pseudomonas aeruginosa* is difficult to control with disinfectants and antibiotics due to the resistance found in *P. aeruginosa* isolates. The study investigated the susceptibility profile and detect extended spectrum β-lactamase (ESBL) resistance genes in the *Pseudomonas aeruginosa* isolated from post-operative wound infection. Wound swabs were cultured on MacConkey and sub-cultured on Cetrimide agar. The isolates were identified base on their macroscopic and microscopic characteristics as well as 16SrRNA. Disc diffusion technique was used to check the susceptibility profile of the isolates and the extended spectrum beta-lactam (ESBL) producers were determined by growing the isolates on Mueller Hinton agar plate in the presence of amoxyclav. 40 positive isolates were recovered in the study with 21 isolates been multi-drug resistance (MDR). Before plasmid curing, Streptomycin, ceftazidime, Augmentin, and Gentamicin has the highest resistance rate of 80% (n=32), 60% (n=24), 60% (n=24) and 60% (n=24) respectively. However, after plasmid curing, the result showed reduction in resistance rates. Streptomycin and ceftazidime showed high reduction in resistance from 80% to 25% and from 60 to 12.7% respectively indicating that resistance was plasmid-mediated. *Pseudomonas aeruginosa* F23 and *Pseudomonas aeruginosa* S15 isolates shown to possess both the *plcH* and *bla* PER genes with bands amplicons size of 307bp and 520bp respectively. Antibiotic susceptibility pattern after plasmid curing showed that 45% of the isolates produces beta-lactamase enzymes i.e., 18 out of 40 isolates were inactive to all beta-lactam group of antibiotics.

Contribution/Originality: This study documents the antimicrobial resistance profile of *Pseudomonas aeruginosa* isolated from post-operative wound infection and also detect ESBL resistance genes in those isolates.

1. INTRODUCTION

Bacterial wound infections constitute a clinical disease state in which the multiplication of microorganisms in wounds provokes local and systemic reactions from the host [1]. Post-operative wound infections may be endogenous or exogenous, Endogenous infections (auto-infection's) are caused by organisms that have been a commensal in the patient's body [2], while exogenous infections [2] are spread from person to person which may happens due to accident and can causes skin trauma [3]. *Pseudomonas aeruginosa* is a common isolate from surgical wounds, and its frequency is related to the site and extent of the surgery and the underlying clinical state of the patient. *Pseudomonas aeruginosa* is a leading cause of health care associated infections and contributes substantially to wound-related morbidity and mortality worldwide [4]. Despite the resistant of *P. aeruginosa* to many antibiotics,

it is also susceptible to many semisynthetic penicillins. Increase in the resistance to most commonly antibiotics by several strains of *P. aeruginosa* is due to its ability to carry plasmids containing genes that regulate antimicrobial resistance [4-6]. The study was designed to evaluate the antimicrobial susceptibility profiles and detect the ESBL resistant gene in *P. aeruginosa* isolated from post-operative.

2. MATERIALS AND METHODS

2.1. Study Area

The study was conducted in Yola, Adamawa state Nigeria. Sampling was done in the two major health care facilities; Specialist Hospital located in Yola north local government Yola-Yola, and Federal Medical Center located at Yola south local government. Yola north local government is located on latitude 9^0, 13' and 9^0 17'N and longitude 12^0 24' and 12^0 28' E., [7], and Yola south local government has a geographical coordinate of 9^0 12' 0" North and East 12^0 29'0" East. Both hospitals are government-owned and cater for a wide variety of patients ranging from high to low-income patients [7].

2.2. Ethical Approval

The ethical approval was obtained before the commencement of the study. It was obtained from the managements of participating hospitals with a reference number of ADS/SHY/SUB/301/VOL.1 and FMCY/SUB/96N/T/X for specialist Yola and federal medical center Yola respectively. However, only patients who agreed to participate in the study were enrolled.

2.3. Sample Collection

Post-operative wound swabs of different anatomical sites, from the inpatients ward of surgery departments were used in this work. The samples were obtained irrespective of sex, age, status or race. 150 samples were obtained; 80 samples were collected from Specialist Hospital Yola, while 70 samples were collected from Federal Medical Center Yola respectively between the months of January and June, 2018. Seventy-two (72) samples were collected from male and seventy-eight (78) from female patients between the ages of 0 -10, 11-20, 21-30, 31-40, and 41 years above. The wounds were cleaned with sterile normal saline and samples were collected using sterile swabs in such a way that the surrounding skins were avoided.

2.4. Isolation of Pseudomonas aeruginosa

As described by Anupurba, et al. [8], primary isolation was done on MacConkey agar for 24hours at 37°C. The colonies were then transferred to a Cetrimide agar for 24hours at 37°C. A blue green pigmentation surrounding the growth on the agar plate was considered a confirmatory for *P. aeruginosa* [9].

2.5. Identification of Isolates

The bacteria isolated were identified using gram's staining techniques and certain biochemical test such as oxidase test, citrate test and triple sugar iron test as described by CLSI [10].

2.6. Antibiotic Susceptibility Testing before Plasmid Curing

The disc diffusion (Kirby Bauer) technique as described by CLSI [10] was employed. Small inoculums were added to 3ml of phosphate buffered saline and mixed gentle until it forms a suspension, and was adjusted to 0.5 McFarland standards which is equivalent to $1.5x10^8$cfu/mL. Twenty microlitres (20µl) inoculums spread on the surface of Mueller-Hinton (MH) agar plate, the plates were and allowed to dry for 3-5 minutes. Antibiotic discs used are gentamicin (10µg), streptomycin (10µg), cefrtriazone (30µg), ceftazidime (30µg), amoxicillin (10µg),

augmentin (30µg), cefotaxime (25µg), ofloxacin (30µg), ciprofloxacin (5µg) and amikacin (30µg) [11]. The zones of inhibition were measured using a meter rule to nearest millimeter (mm) after 24hours incubation at 37°C.

2.7. Susceptibility Test after Plasmid Curing

Overnight cultures of *Pseudomonas aeruginosa* were inoculated on nutrient broth containing sodium deodecyl sulphate (SDS) [12]. After inoculation the cultures were incubated at 37°C for 24hr. Cultures were sub-cultured onto Muller Hinton Agar after which susceptibility test was carried on the plasmid cured isolate using the method and antibiotics (with same concentration) as described by Kotb, et al. [12].

2.8. Identification of ESBL Producing P. aeruginosa

For the primary ESBL identification test, the test isolates with a turbidity equivalent to that of 0.5 McFarland standard was spread over a Mueller Hinton agar plate. The amoxyclav (amoxicillin-clavulanic acid 20µg/10µg) (augmentin) was place at the center while 30µg disc of third generation cephalosporin test antibiotic namely ceftazidime, cefrtriazone and cefotaxime were place at a distance of 20mm from center to center against the augmentin on the test isolate on Mueller Hinton Agar plate by Kirby Bauer disc diffusion method [2, 10]. The isolates were considered to be ESBL positive strains when the third-generation cephalosporin antibiotic zone of inhibition extended towards the Augmentin with diameter of ≤22mm for ceftazidime, cefrtriazone and ≤27mm for cefotaxime [2].

2.9. Determination of Multiple Antibiotic Resistance (MAR) Index

The following formula was used for the determination of Multiple Antibiotic Resistance (MAR) index for *P. aeruginosa* isolated in the study. The MAR was obtained by dividing the number of antibiotics the organism was resistant to by the total number of antibiotics used [13, 14].

$$\text{MAR index} = \frac{\text{Number of antibiotics isolate is resistant to}}{\text{Total number of antibiotics used}}$$

2.10. Molecular Detection of Chromosomal and Plasmid Encoded Genes in Pseudomonas aeruginosa

The molecular detection of the chromosomal and plasmid encoded genes was done using PCR in three stages which include DNA extraction, DNA amplification and sequencing. The isolates were first confirmed by PCR amplification of the 16SrRNA gene [15].

2.10.1. DNA Extraction

A single colony of an isolate was transferred to 1.5mL of nutrient broth which was grown in a water bath with shaker for 48 hours at 28°C, after which it was centrifuged at 4600g for 5 min. The pellets were then re-suspended in 520µL of TE buffer (10mMTris-HCl, 1mM EDTA, pH 8.0). 15µL of 20% SDS and 3µL of Proteinase K (20mg/mL) was then added. The mixture was incubated for 1 hour at 37 °C, then 100µL of 5M NaCl and 80µL of a 10% cetyltri-methyl ammonium bromide (CTAB) solution in 0.7M NaCl was added and vortexed.

The suspension was incubated for 10min at 65°C and kept on ice for 15min. An equal volume of chloroform: isoamyl alcohol (24:1) was added, followed by incubation on ice for 5min and centrifugation at 7200g for 20min. The aqueous phase was then transferred to a new tube and in the isopropanol (1: 0.6) was added and DNA precipitated at –20 °C for 16 h. DNA was collected by centrifugation at 13000/g for 10min, washed with 5000µL of 70% ethanol, air-dried at room temperature for approximately 3h and finally dissolved in 50µL of TE buffer [15, 16].

2.10.2. DNA Amplification of the 16SrRNA

The amplification of the 16SrRNA was done using PCR at the Central Laboratory University of Ibadan Nigeria. 10µL of 5x GoTaq colorless reaction, 3µL of 25mM $MgCl_2$, 1µL of 10mM of dNTPs mix, 1µL of 10pmol each 27F 5'- AGA GTT TGA TCM TGG CTC AG-3' and – 1525R, 5′-AAGGAGGTGATCCAGCC-3′ primers and 0.3units of Taq DNA polymerase (Promega, USA) made up to 42µL with sterile distilled water 8µL DNA template. PCR was carried out in a GeneAmp 9700 PCR System Thermalcycler (Applied Biosystem Inc., USA) with a PCR profile consisting of an initial denaturation at 94°C for 5min; followed by 30 cycles consisting of 94°C for 30s, 50°C for 60s and 72°C for 1minute, 30sec; and a final termination at 72°C for 10mins and chill at 4°C [16, 17].

Agarose gel was used for the to confirm amplification. 1g of agarose powder was dissolved in 100ml of 1X TAE buffer and boiled for 5 minutes; then allowed to cool to 60°C. The gel was stained with 3µL of 0.5g/mL ethidium bromide. The gel was casted on a tray with comb slotted in the tray; and was allowed to solidify. 2µL of 10X blue gel loading dye was added and loaded into the wells after the 100bp DNA ladder was loaded into well 1. The gel was visualized using ultraviolet trans-illumination and photographed. The estimation of the PCR products was done by comparing the mobility of the molecular ladder that was ran and that of the experimental samples in the gel [16].

2.11. Detection of a Chromosomal and Plasmid Encoded Resistant Gene (plc H, bla PEF gene) in Pseudomonas aeruginosa

Molecular detection of *plc H* and *bla PER* genes in two isolates of *Pseudomonas aeruginosa* (F23 and S15) which were found to be both multiple drug resistant and phenotypically ESBL producers was done by PCR on the extracted DNA using *Plc H* and *bla* PER specific primers unique to *Plc H* and *bla PER* coding regions. Primer sequences for plc H gene are F 5'-AAGCCATGGGCTACTTCAA-3' and R 3'- AGAGTGACGAGGAGCGGTAG-5' and *bla* PER F5'-GCTCCGATAATGAAAG-3' and R 3'-TTCGGCTTGACTCGGCTGACGT-5'. Buffer control was also added to eliminate any probability of false amplification. The amplified product was checked on a 1% Agarose gel. The gel was visualised using ultraviolet trans-illumination and photographed and the sizes of the PCR products was compared with the mobility of a 100bp molecular weight ladder that was ran alongside experimental samples in the gel [18].

3. RESULTS

3.1. Antibiotic Susceptibility Pattern of P. aeruginosa Isolates before Plasmid Curing

The susceptibility pattern of the antibiotic tested showed 21 out of 40 positive isolates recovered were multi-drug resistance (MDR). An isolate was considered MDR when it is resistant to at least two or four drugs' groups including Penicillin, Cephalosporin, Aminoglycoside and Quinolones.

Before plasmid curing, Streptomycin, ceftazidime, Augmentin, and Gentamicin has the highest resistance rate of 80% (n=32), 60% (n=24), 60% (n=24) and 60% (n=24) respectively. Followed by Amoxicillin, Cefotaxime, Cefrtriazone, with a moderate resistance of 57.5% (n=23), 45% (n=22) and 45% (n=18). Furthermore, the study has shown that ciprofloxacin and Amikacin are most effective on isolates as most isolates are more susceptible to antibiotic with a resistance rate of 10% (n=4) and 30% (n=12) only Table 1.

3.2. Antibiotic Susceptibility Pattern of Plasmid Cured P. aeruginosa Isolates

The resistance rate of *P. aeruginosa* reduced after plasmid curing. Streptomycin and ceftazidime showed high reduction in resistance from 80% to 25% and from 60 to 12.7% respectively indicating that resistance was plasmid-mediated. While reduction was not significant in Ciprofloxacin belonging to the class of Quinolone and Amikacin to the class of aminoglycoside as result shows 10% before plasmid curing and 7.5% after plasmid curing indicating that resistance was chromosome mediated. Most isolate showed that their resistance to the antibiotic was plasmid mediated than chromosomal mediated. Result showed that Ciprofloxacin is very effective in the treatment of. *P.*

aeruginosa and be used as the drug of choice Table 2. Antibiotic susceptibility pattern after plasmid curing showed that 18 out of 40 isolates were inactive to all beta-Lactam group of antibiotics (Cephalosporin), indicating that 45% of the isolates are able to produce the enzymes beta-lactamase that inhibit the activity of most antibiotic Table 2.

Table 1. Antibiotic susceptibility pattern before plasmid curing.

S/N	Isolate	CAZ	S	AK	CTX	CPX	AM	AU	CN	CRO	OFX
1.	*P. aeruginosa*F_2	R	R	S	S	S	R	R	S	S	S
2.	*P. aeruginosa* F3	S	R	R	R	S	R	R	S	S	S
3.	*P. aeruginosa* F9	R	R	S	R	S	S	R	S	S	S
4.	*P. aeruginosa* F11	R	R	S	S	R	S	R	R	R	S
5.	*P. aeruginosa* F14	S	R	R	R	S	S	S	S	R	S
6.	*P. aeruginosa* F18	S	R	S	R	S	R	S	S	R	S
7.	*P. aeruginosa* F20	R	S	S	R	S	S	R	R	R	S
8.	*P. aeruginosa* F23	R	R	S	R	S	S	R	R	R	R
9.	*P. aeruginosa* F24	S	R	R	R	R	R	S	R	S	S
10.	*P. aeruginosa* F25	S	R	S	R	S	R	R	S	S	R
11.	*P. aeruginosa* F35	R	S	S	R	S	R	R	R	S	S
12.	*P. aeruginosa* F37	S	S	R	R	S	R	S	R	S	R
13.	*P. aeruginosa* F40	R	S	S	R	S	R	S	R	R	S
14.	*P. aeruginosa* F53	S	R	R	R	S	R	S	R	S	S
15.	*P. aeruginosa* F60	R	R	S	R	S	R	S	R	S	S
16.	*P. aeruginosa* S5	S	R	R	S	S	R	R	R	S	S
17.	*P. aeruginosa* S7	S	S	R	S	S	R	R	R	S	R
18.	*P. aeruginosa* S8	R	S	S	R	S	R	S	S	S	S
19.	*P. aeruginosa* S11	R	R	S	S	S	R	R	S	R	S
20.	*P. aeruginosa* S13	R	R	S	R	S	R	S	R	S	S
21.	*P. aeruginosa* S14	R	R	S	S	S	S	R	R	S	R
22.	*P. aeruginosa* S15	R	R	R	R	S	S	R	R	R	S
23.	*P. aeruginosa* S20	S	R	S	R	S	S	S	R	S	R
24.	*P. aeruginosa* S22	S	R	S	R	R	S	S	S	R	S
25.	*P. aeruginosa* S27	R	R	S	S	S	R	R	R	R	S
26.	*P. aeruginosa* S30	S	S	S	S	S	R	S	R	R	R
27.	*P. aeruginosa* S31	R	R	S	S	S	R	R	S	R	R
28.	*P. aeruginosa* S32	R	R	S	R	S	R	R	S	S	S
29.	*P. aeruginosa* S37	R	R	R	S	S	S	R	S	S	S
30.	*P. aeruginosa* S38	R	R	S	S	S	S	R	S	R	R
31.	*P. aeruginosa* S40	R	R	S	S	S	R	R	R	S	S
32.	*P. aeruginosa* S50	R	R	R	S	S	S	R	R	S	R
33.	*P. aeruginosa* S53	R	R	S	R	S	S	S	R	R	R
34.	*P. aeruginosa* S56	S	R	R	S	S	R	R	R	R	S
35.	*P. aeruginosa* S64	R	S	S	S	S	S	S	R	R	R
36.	*P. aeruginosa* S68	S	R	S	S	S	S	S	R	S	R
37.	*P. aeruginosa* S70	S	R	R	S	S	S	R	R	R	R
38.	*P. aeruginosa* S71	R	R	S	R	S	S	S	S	S	R
39.	*P. aeruginosa* S72	S	R	S	R	R	R	R	S	S	R
40.	*P. aeruginosa* S75	R	R	S	S	S	R	R	R	R	S

Note: **CAZ:** Ceftazidime, S: Streptomycin, CN: Gentamicin, AU: Augmentin, AM: Amoxicillin, OFX: Ofloxacin, CPX: Ciprofloxacin, AK: Amikacin, CRO: Cefrtriazone, CTX: Cefotaxime

The data generated in the study was analysed using Chi-square statistical analysis. The results show that, there is significance difference in the resistance of *P. aeruginosa* (p= 0.002) before and after plasmid curing.

3.3. Antibiotic Resistant P. aeruginosa Before and after Plasmid Curing

The antibiotic resistant *P. aeruginosa* after plasmid curing showed a significant decrease in the resistant pattern of the organism. Highest reduction was recorded in streptomycin with a reduction in resistant from 32(80.0%) to 10(25.0%) after plasmid curing, followed by ceftazidime with reduction from 24(60.0%) to 7(17.5%) after curing.

This result indicated that the resistant exhibited by the organisms was plasmid mediated resistant. However, less reduction in resistant was observed after plasmid curing among ciprofloxacin and amikacin with reduction in resistant from 04(10.0%) to 03(7.5%), and 12(30.0%) to 10(25.0%) respectively as described in Table 3. This small decrease in resistant after plasmid curing indicated that the resistant was not plasmid mediated resistant, it could be a chromosome mediated resistant.

Table 2. Antibiotic susceptibility pattern after plasmid curing.

S/N	Sample	CAZ	S	AK	CTX	CPX	AM	AU	CN	CRO	OFX
1.	*P. aeuroginosa*F_2	S	S	S	S	S	S	S	S	S	S
2.	*P. aeruginosa* F3	S	S	R	R	S	S	S	S	S	S
3.	*P. aeruginosa* F9	S	S	S	S	S	S	S	R	S	S
4.	*P. aeruginosa* F11	S	S	S	R	R	S	R	S	S	S
5.	*P. aeruginosa* F14	S	S	R	S	S	S	S	S	R	S
6.	*P. aeruginosa* F18	S	R	R	R	S	R	S	R	S	S
7.	*P. aeruginosa* F20	S	S	S	R	S	S	R	S	S	S
8.	*P. aeruginosa* F23	R	S	S	R	S	S	R	S	R	S
9.	*P. aeruginosa* F24	S	S	S	R	R	R	S	S	S	S
10.	*P. aeruginosa* F25	S	R	S	S	S	R	S	S	S	R
11.	*P. aeruginosa* F35	S	S	S	S	S	R	R	R	S	S
12.	*P. aeruginosa* F37	S	S	S	S	S	R	S	S	S	S
13.	*P. aeruginosa* F40	S	S	S	S	S	S	S	R	R	S
14.	*P. aeruginosa* F53	S	S	R	S	S	S	S	R	S	S
15.	*P. aeruginosa* F60	R	R	S	S	S	S	R	S	S	S
16.	*P. aeruginosa* S5	S	S	R	S	S	S	S	R	S	S
17.	*P. aeruginosa* S7	S	S	S	S	S	R	R	S	S	S
18.	*P. aeruginosa* S8	S	S	S	R	S	R	S	S	S	S
19.	*P. aeruginosa* S11	S	S	S	S	S	S	R	S	S	S
20.	*P. aeruginosa* S13	S	R	S	R	S	S	S	S	S	S
21.	*P. aeruginosa* S14	R	S	S	S	S	S	S	R	S	R
22.	*P. aeruginosa* S15	S	S	R	S	S	R	S	S	S	S
23.	*P. aeruginosa* S20	S	S	S	R	S	S	S	S	S	S
24.	*P. aeruginosa* S22	S	R	S	R	R	S	S	S	S	S
25.	*P. aeruginosa* S27	S	S	R	S	S	R	R	R	S	S
26.	*P. aeruginosa* S30	S	S	S	S	S	R	S	S	R	S
27.	*P. aeruginosa* S31	S	R	S	S	S	S	R	S	S	S
28.	*P. aeruginosa* S32	S	R	S	R	S	S	R	S	S	S
29.	*P. aeruginosa* S37	R	S	S	S	S	S	S	S	S	S
30.	*P. aeruginosa* S38	S	S	S	S	S	S	R	S	R	R
31.	*P. aeruginosa* S40	R	S	S	S	S	R	S	R	S	S
32.	*P. aeruginosa* S50	S	R	R	S	S	S	S	S	S	S
33.	*P. aeruginosa* S53	S	S	S	S	S	S	S	R	R	R
34	*P. aeruginosa* S56	S	S	S	S	S	R	S	S	R	S
35.	*P. aeruginosa* S64	R	S	S	S	S	S	S	R	S	R
36.	*P. aeruginosa* S68	S	S	S	S	S	S	S	R	S	R
37.	*P. aeruginosa* S70	S	S	R	S	S	S	R	R	S	S
38.	*P. aeruginosa* S71	R	R	R	S	S	S	S	S	S	S
39	*P. aeruginosa* S72	S	R	S	R	S	R	R	S	S	S
40.	*P. aeruginosa* S75	S	S	S	S	S	R	R	S	R	S
	%Resistance	12.5	25	20	37.5	7.5	37.5	32.5	30	20	15

Note: CAZ: Ceftazidime, S: Streptomycin, CN: Gentamicin, AU: Augmentin, AM: Amoxicillin, OFX: Ofloxacine, CPX: Ciprofloxacin, AK: Amikacin, CRO: Cefrtriazone, CTX: Cefotaxime.

3.4. Identification of ESBL Producers

The phenotypic detection of ESBL production in *Pseudomonas aeruginosa* shown that *Pseudomonas aeruginosa* isolates were ESBL producers based on double disc synergy test as described by CLSI [10]. Positive Isolate exhibited zone of inhibition ≤ 22mm for ceftazidime and ≤27mm for cefotaxime indicating ESBL producers. While the remaining 55% show zone of inhibitions ≥22 for both ceftazidime and cefotaxime.

Table 3. Antibiotic resistant pattern of *P. aeruginosa* before and after plasmid curing.

S/N	Before curing			After curing	
	Antibiotics	Resistant	Susceptible	Resistant	Susceptible
1	Ceftazidime	24(60.0)	16(40.0)	07(17.5)	33(82.5)
2	Cefotaxime	22(55.0)	18(45.0)	12(30.0)	28(70.0)
3	Ceftriazone	18(45.0)	22(55.0)	08(20.0)	32(80.0)
4	Streptomycin	32(80.0)	08(20.0)	10(25.0)	30(75.0)
5	Amikacin	12(30.0)	28(70.0)	10(25.0)	30(75.0)
6	Ciprofloxacin	04(10.0)	36(90.0)	03(7.5)	37(92.5)
7	Amoxicillin	23(57.5)	17(42.5)	14(35.0)	26(65.0)
8	Augmentin	24(60.0)	16(40.0)	14(35.0)	26(65.0)
9	Gentamicin	25(62.5)	15(37.5)	13(32.5)	27(67.5)
10	Ofloxacine	16(40.0)	24(60.0)	06(15.0)	34(85.0)

3.5. Molecular Analysis of ESBL-Coding Genes via PCR

Pseudomonas aeruginosa F23 and *Pseudomonas aeruginosa* S15 isolates have been shown to possess both the *plcH* and *bla* PER genes with bands amplicons size of 307 bp Figure 1 and 520 bp Figure 1 respectively. Gel image indicates a positive amplification in the two samples. Figure 2 showed the gel image indicating a positive amplification with band size of approximately 520bp in all the samples. MK (100bp marker), S1 (F23), S2 (S15), B (buffer control), C (positive control) as indicated in Figure 2.

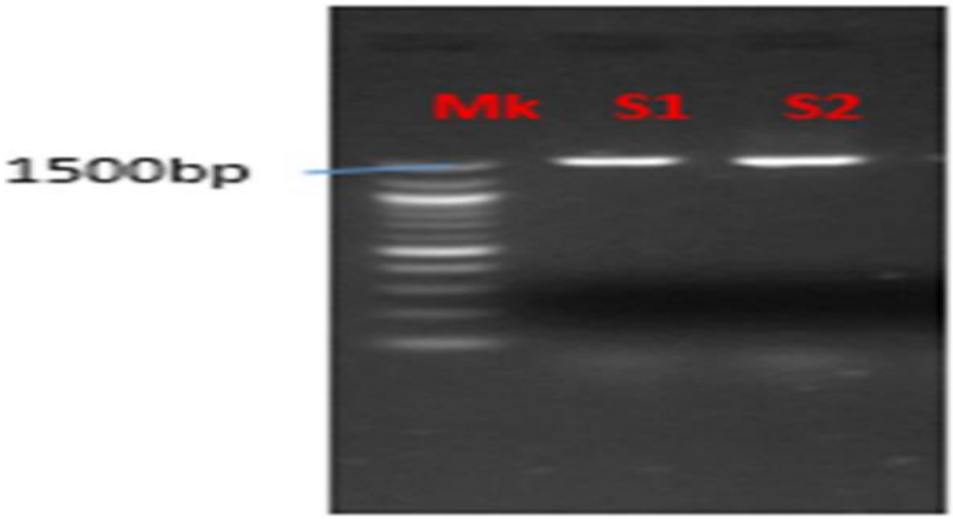

Figure 1. 1% Agarose gel depicting 1500bp fragment of 16S RNA of *P. aeruginosa* F23 and *P. aeruginosa* S15.

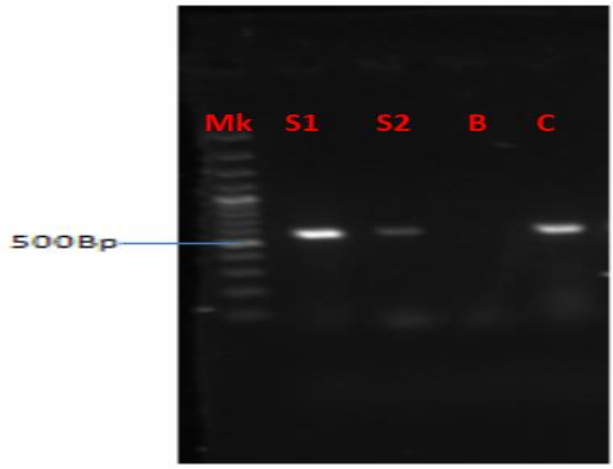

Figure 2. Gel image indicating positive amplification with band size approximately 320bp in all samples. MK (100bp marker), S1 (F23), S2 (S15), B (buffer control), C (positive control).

4. DISCUSSION

Antimicrobial resistance profile of *P. aeruginosa* isolates suggested that ceftazidime and streptomycin exhibited high resistance rate with percentage of 60%and 80%resistance rate respectively, hence ceftazidime and streptomycin are not the drugs of choice for the for treatment of *P. aeruginosa* in a hospital setting because of high resistance rate observed in this study. After plasmid curing the result shown a huge difference in the susceptibility of ceftazidime increasing to 12.5.5%sensitivity and streptomycin to 25%.Augmentin and gentamycin belonging to the class of aminoglycoside showed similar resistance rate of 60% each before plasmid curing with a reduction of 32.5% and 30% respectively after plasmid curing, the decreased in resistance was not much when compared with that of ceftazidime and streptomycin which increase in susceptibility from 60% and 80% to 12.5% and 25% respectively. This change in susceptibility pattern for ceftazidime and streptomycin is likely to be more of plasmid than chromosomal mediated.

Multidrug resistance index showed that 21 out of 40 (55.2%) isolate exhibited multidrug resistance before plasmid curing while multidrug resistance index after plasmid curing showed an increase in susceptibility from 55.2% to 32.5%. The increase in resistance to different class of antibiotic before plasmid curing could be as a result of multiple virulence factors associated with *P. aeruginosa.*

Although the quinolone ciprofloxacin and ofloxacin showed relative resistance of 10%, 40% respectively before plasmid cures and increased in susceptibility to 15% and 7.5% after plasmid curing, the class that showed the highest effectiveness of the antibiotic with susceptibility of 7.5% is ciprofloxacin. This result agrees with that of Pong, et al. [19]. However, antimicrobial susceptibility pattern showed that amikacin and ciprofloxacin were very effective against *Pseudomonas* species. Amikacin, and aminoglycoside antibiotic is known to have much effectiveness in *P. aeruginosa* better than others of the same group of antibiotics, this susceptibility may result from the fact that they are new synthesize antibiotics, and they are expensive and have not been exposed to extensive abuse in our community [13, 20]. The resistance of *P. aeruginosa* to ceftazidime may be due to its ability to produce beta-lactamase enzymes after (ESBL) test carried out.

The resistance of gentamicin, augmentin and ofloxacine was more of plasmid mediated resistance because of susceptibility increase with about 10mm after plasmid curing. ESBL also showed that *Pseudomonas aeruginosa* acquired resistant to penicillin and cephalosporin by hydrolyzing the antibiotics which are inhibited by beta-lactamase inhibitors such as clavulanic acid [21]. They have the ability to produce enzymes known as beta-lactamase that assists them confers resistance to antibiotics. Thus, not all beta antibiotics confers resistance because cefotaxime was susceptible with 20%. The DNA amplification of 16S RNA of isolates F23 and S15 has confirmed that the isolates were *P. aeruginosa* and isolates also possess both the *plcH* and *bla* PER genes.

5. CONCLUSION

Antibiotic susceptibility profile showed that ciprofloxacin belonging to the class of Quinolones was the most suitable drug in the treatment of post operational wound caused by *Pseudomonas aeruginosa.* Moreover, the resistances against streptomycin, ceftazidime and gentamicin were found to be very high. The high resistance of *P. aeruginosa* to the multiple antibiotics administered was plasmid mediated then chromosomal.

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