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Molecular Detection of Carbapenem Resistance in Multi-Drug Resistant *Pseudomonas aeruginosa* from Patients and Fomites in Federal Medical Centre Keffi, Nigeria

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Abstract

This study is the aimed at the molecular detection of carbapenem resistance in multidrug resistant *Pseudomonas aeruginosa* from patients and fomites in Federal Medical Centre, Keffi, Nigeria. The study was carried out in Federal Medical Centre, Keffi (FMCK), Nigeria, between the period of February- August, 2020. **Material and Methods:** A total of 365 samples comprising 265 clinical (urine, wound swab, eye swab, ear swab, high vigal swab (HVS) and endocervical swab (ECS)) and 100 fomites samples were collected from Federal Medical Centre, Keffi, Nigeria. **Results:** Out of 365 clinical and formitesscreened, FMCK had overall occurrence 42 (11.5%) both in clinical andfomite samples examined. The isolates from both formites and clinical samples were more resistant to nalidixic acid (85.7%) and ampicilin (71.4%) but less resistant to imipenem (33.3%), ofloxacin (42.8%) and streptomycin (45.2%).The occurrence of carbapenemase producing imipenem resistant isolates was 28.5%.The occurrence of bla_{KPC} was 100%.The occurrences of bla_{VIM} and bla_{OXA} genes were 75% each. The occurrence of isolates with co-carriage *of* $bla_{VIM/KPC/OXA}$ (75%) was higher than $bla_{VIM/KPC}$, $bla_{KPC/OXA}$and $bla_{VIM/OXA}$ (50%). Conclusions: The occurrence of the isolates from fomites and clinical

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samples was high and most antibiotics tested were not effective against the *P. aeruginosa* isolates.

Keywords: Carbapenem; Resistance, *Pseudomonas aeruginosa*, Fomites

INTRODUCTION

Pseudomonas aeruginosa (*P. aeruginosa*) is an opportunistic, gram-negative bacillus widely spread in nature which is associated with community and hospital-acquired infections such as respiratory tract infections, urinary tract infections, burns, wounds and otitis media, especially in patients with compromised host defence (Lister *et al.,* 2009).

This microorganism possesses intrinsic resistance mechanisms to different antibiotics including β-lactams, tetracyclines, trimethoprim/sulfamethoxazole and chloramphenicol (Potron, 2015; Alvarez-Ortega *et al.,* 2011). Besides, *P. aeruginosa* may acquire other resistance mechanism, through mobile genetic elements, which further complicate the treatment of the infections it causes (Lister *et al.,* 2009). The frequency of infections caused by this organism is increasing and multidrug-resistant (MDR) isolates are emerging especially in hospitalized patients (Hirsch *et al.,* 2010).

Carbapenems are a group of broad-spectrum β-lactams for the treatment of infections caused by multi-drug resistant bacteria due to their high capacity of entry, low toxicity, high affinity for penicillin-binding proteins (PBP´s) and stability against β-lactamases (Alvarez-Ortega *et al.,* 2011). Carbapenems have been kept as a last resort therapy for the control of MDR *P. aeruginosa* infection. However, in recent years the emergence of *P. aeruginosa* strains resistant to these antibiotics has emerged as a serious threat causing higher hospital stay costs and mortality rates (Lister *et al.,* 2009; Nordmann*et al.,*2010).

Carbapenem-resistant *P. aeruginosa* resistant to other classes of antimicrobial agents are associated with limited therapeutic options and high rates of mortality and morbidity especially in hospitalized and immunocompromised patients (Papp-Wallace *et al.,* 2011; Hong *et al.,* 2015). This organism employs different mechanisms such as reduced outer membrane permeability, target site modification, efflux pumps over-expression, expression of chromosomal AmpC β-lactamases, and the acquisition of β-lactamases to become resistant to this group of antibiotics (Livermore, 2002; Hancock *et al.,* 2000).

A wide range of metallo-β-lactamases (MBLs) (class B β-lactamases) such as *IMP, VIM, NDM-1*, *OXA* and *GIM-1* have been reported in *P. aeruginosa*. These enzymes play a major role in the resistance of *P. aeruginosa* to carbapenems (Pollini*et al.,* 2013). In addition, class A β-lactamase*Klebsiellapneumoniae*carbapenemases (*KPC*) carries the extended spectrum *KPC*-1 enzyme, which was first detected in an outbreak in North Carolina (Yigit*et al.,* 2001) has subsequently been identified in *P. aeruginosa* isolates (Nordmann, 2009).

This srudy was aimed at determining thecarbapenem resistance genes (bla_{IMP}, bla_{VIM}, bla_{NDM}, bla_{KPC} *and* bla_{OXA}) among different MDR *P aeruginosa* isolates from patient and formites in Federal Medical Centre, Keffi, Nasarawa State, Nigeria. *P aeruginosa* resistant to three or more classes of antibiotics will be regarded as Multi drug resistant.

MATERIALS AND METHODS

Study area

The study was carried out in Keffi. Keffi lies between longitude 8-5ºS and latitude 7ºN and above the sea level of latitude 630m. Keffi is approximately 53km away from the federal capital territory, Abuja and 133km away from the state capital lafia (Akwa*et al.,* 2007).

Ethical Clearance

The ethical approval for this study was obtained from the ethical committees of Federal Medical Centre Keffi, Nigeria..

Sample Size

The sample size for this study was determined using the formula below as earlier described by Fisher (1998).

$N=Z^2pq/d^2$

Where N is is the minimum sample size, P is population proportion or prevalence, Q is 1-p, and D is degree of freedom (precision), Z is normal deviant at the portion of 95% confidence interval

Umoro*et al.* (2018) = 27.5%

Z=1.96

P=27.5%=0.275 Q=1-p=1-0.275=0.725

D=5%=0.05

$N= (1.96)^2 *0.275* (0.725)/0.05 =365$ samples

Sample Collection

Clinical samples (urine, wound swab, eye swab, ear swab, high vigal swab (HVS) and endocervical swab (ECS) and fomites samples were collected from Federal Medical Centre, Keffi, Nigeria. Urine samples were collected in sterile universal bottles from patients suffering from urinary tract infection. Specimens of ear discharge, HVS, ECS, wound swab, eye and fomites were collected using sterile cotton swab sticks with the assistance of medical officer.

The samples were collected in clean, dry and sterile containers and stored in iced pack and transported to the laboratory for microbiological analysis within one hour or refrigerated at 4°C till further analysis were carried out.

Isolation of *Pseudomonas aeruginosa*

The *P. aeruginosa* was isolated from urine by streaking a loopful of urine sample on cetrimide agar (CA) (Oxoid, UK) plates. From other sample types (high vaginal, endocervical, ear, eye, wound, throat and fomites) swab samples were inoculated into 5 ml of nutrient broth and loopfuls were streaked on CA. All inoculated CA plates were incubated at 37°C for 24 h. The blue-green colonies on CA were presumptively selected as *Pseudomonas* spp.

Identification of *Pseudomonas aeruginosa*

Presumptive *Pseudomonas*spp were identified by Gram staining andCommercial Biochemical Kit KB005 H125™.

1. Gram Staining

The Gram staining technique was carried out as described by Cheesbrough (2006). A small portion of cultural organism was transferred onto a clean grease-free glass slide, and emulsified in a drop of distilled water until a thin homogeneous film is obtained, then the wire loop was be re-sterilized and the thin homogeneous film was allowed to air-dry, and heat-fixed by passing through the flame. The slide was then flooded with crystal violet for 1 min, and then rinsed with distilled water. The stain will be again flooded with Lugol's iodine for 1 min, and rinsed with distilled water and then decolorized, rapidly with acetone alcohol until no more colour appeared to flow from preparation and rinsed appropriately with distilled water. The stain was counter-stained with Safranin for 1 min, and rinsed with

distilled water and allowed to air dry and viewed microscopically using x100 oil immersion objective. A Pink colour inferred by the absorption of the counterstain (safranin) by the bacterial cell wall; will be taken as Gram negative reaction, *Pseudomonas* species are Gram negative bacteria.

2. Use of Commercial Biochemical Kit, KB005 H125™

Presumptive *P. aeruginosa*isolates that were Gram negative, rod shape were confirmed using KB005 H125™ Kit following the manufacturer's instruction as follows. Following purification, 3 pure colonies of suspected isolates from NA plate were transferred to 5 ml of sterile normal saline in a tube to prepare a suspension and the turbidity of the suspension was adjusted to the turbidity equivalent to the turbidity of 0.5 McFarland standards.The kit was aseptically open by sealing off the sealing foil and 50 µl of the adjusted suspension was inoculated into each wells of the kit and was sealed back using the sealing foil and incubated at 37ºC for 24 h. After incubation, 3 drops of reagent R036 and 1 drop of reagent R015 were added to well No 5; 2 drops of reagent R009 were added to well No. 6; 3 drops of reagent R029 and 1 drop of reagent R030 were added to well No. 9; 2 drops of reagent 1007 were added to well No. 10 and finally 2 drops of reagent R008 were added to well No. 11. The results were read and interpreted as per the standard given in the identification index.

Antibiotic Susceptibility Testing

The antimicrobial susceptibility testing of the bacterial isolates was carried out as earlier described by Clinical and Laboratory Standards Institute (CLSI, 2007). Briefly, three (3) pure colonies of the isolates were inoculated into 5 ml sterile 0.85% (w/v) NaCl (normal saline) and the turbidity of the bacteria suspension was adjusted to the turbidity equivalent to 0.5 McFarland's standard. The McFarland's standard was prepared as follows: 0.5 ml of 1.172% (w/v) $BaCl_2.2H_2O$ was a dded into 99.5 ml of 1% (w/v) H_2SO_4.

A sterile swab stick was soaked in standardized bacteria suspension and streaked on Mueller-Hinton agar plates and the antibiotic discs were aseptically placed at the center of the plates and allowed to stand for 1 h for pre-diffusion. The plates were incubated at 37ºC for 24 h. The diameter zone of inhibition in millimeter was measured and the result was interpreted in accordance with the susceptibility break point earlier described by Clinical and Laboratory Standards Institute (CLSI, 2007).

Determination of Multiple Antibiotic Resistance (MAR) Index

The MAR index values of the isolates were determined using the formula (Ngwai*et al.*, 2014):

$$\text{MAR Index} = \frac{\text{No. antibiotics isolate is resistant to}}{\text{No. of antibiotics tested}}$$

Classification of Antibiotic Resistance

Antibiotic resistance in the isolates were classified into: multidrug resistance (MDR: non-susceptible to at least one agent in at least three antimicrobial categories); extensive drug resistance (XDR: non-susceptible to at least one agent in all but two and below antimicrobial categories); pan drug resistance (PDR: non-susceptible to all antimicrobial listed) (Magiorakos*et al.*, 2012).

Phenotypic detection of Carbapenem resistant genes (Carbapenemases)

The presence of carbapenemases in Multi drug *P. aeruginosa* isolates were detected phenotypically using Modified Hodge test as describe by (Mona *et al.,* 2017). A diluted culture of *Escherichia coli* ATCC 25922 (0.5 McFarland standard) as recommend by (CLSI, 2015) was swabbed on the surface of Mueller-Hinton agar. Imipenem disk (10 µg) (Oxoid, Basingstoke, UK) was placed at the center of each plate. The tested isolates were streaked as thin line from the edge of the meropenem disk to the edge of the plate. Interpretation of result was done after 16-24 hours of incubation. Positive Modified Hodge test showed a clover leaf-like indentation of the *E. coli* 25922 strain growing along the test organism growth streak within the disk diffusion zone indicating production of carbapenemase and a negative test showed no growth of the *E. coli* ATCC 25922 along the test organism growth streak within the disk diffusion.

Molecular (PCR) Detection of Carbapenem Resistant Genes

1. DNA Extraction by Boiling Method

The DNA was extracted from the carbapenem resistant isolates using boiling method as described by Porteous*et al.* (1994). Briefly, one pure colony of carbapenem resistant isolate was inoculated into 2 ml of LB broth and incubate at 37ºC for 8 h and 200µl of LB culture was transfer into Eppendorf tube and centrifuge in micro centrifuge as 3200 rpm for 2min at room temperature and the supernatant was discarded, living the cells. The cells were

wash twice with washing buffer. About 0.5 ml of sterile phosphate buffer was added to the pellet and vortex for 5 sec after which it was heated at 90ºC for 10 min and rapid coiling was done by transferring the tubes into freeze for 10min and thereafter it will be centrifuge at 3200 rpm for 1 min to separate the DNA and the cell containing the DNA debris and 300 µl of supernatant was then transferred into 2 ml Eppendorf tube and stored at -10ºC until use**.**

2. Amplification of Target Genes

The DNA amplification of target carbapenem resistance genes incarbapenem resistant *P. aeruginosa* isolates was carried out using single plex method by modification of the method earlier described by Shams *et al.* (2015). Briefly the reactions were carried out in 25 µl reaction volume in a reaction tube made up of 5 µl of master mix (Qias), 2.4 µl of primers (0.4 µl each of forward and reverse primers), 0.5 µl of $MgCl_2$, 1.5 µl of DNA template and 15.6 µl of Nuclease free water. The reaction tubes were placed in the holes of the thermal cycler and the door of the thermal cycler was closed.bla_{IMP}, bla_{VIN}, bla_{NDM}, bla_{KPC} and bla_{OXA} genes were amplified under the following conditions, initial denaturation at 94ºC for 5 min, followed by 32 cycles of amplification at 94ºC for 45 sec, 53ºC for 45 sec, with final extension at 72ºC for 5 min.

3. Agarose Gel Electrophoresis

The amplified DNA samples were separated using 1.5% Agarose gel in Agarose gel electrophoresis to determine the base pair of the target genes and 100bp DNA ladder was used as a standard for 40 min at 120V followed by sequencing.

RESULTS

The occurrence of *P. aeruginosa* isolates from clinical and formite samplesin FMCK is as shown in Table 1. Out of 365 clinical and formites samples obtained, the occurrence of the isolates was 42 (11.5%). The occurrence of the isolates in relation to the sample was high in ear swab (50%), Endocervical swab (32.1%) and High Vaginal swab (29.6%) but was low in formites (2%). Throat swab (9.1%), urine (10.1%) as shown in Table 2. the differences in the occurrences of the isolates in relation to sample was statistically insignificant ($p>0.05$).

The antimicrobial resistance of the isolates in both formites and clinical samples in FMCK is as shown in Table 2. The isolates from both formites and clinical samples were more

resistant to nalidixic acid (85.7%) and ampicilin (71.4%) but less resistant to imipenem (33.3%), ofloxacin (42.8%) and streptomycin (45.2%) respectively.

The antimicrobial resistance phenotypes of *P. aeruginosa*isolates is as shown in Table 3. The isolates were distributed into different phenotypes and the commonest phenotype is IMP-CN-AU-CIP-SXT-S-AMP-CEP-OFX-NA, with frequency of 14.3%.

The MAR index of *P. aeruginosa* isolates is as shown in table 4. Most of the isolates were MAR isolates with MAR index of >0.2 and the most common MAR index in urine were 0.6 (22.2%) and 1.0 (22.2%), ECS were 0.1 (22.2%), 0.5 (22.2%) and 0.8 (22.2%) and HVS were 0.5 (25.0%) and 0.7 (25.0%) respectively.

The categories of antibiotic resistance in multi drug resistant *P. aeruginosa*isolates namely: NMDR MDR, XDR, and PDR is as shown in Table 5. Out of 42 antibiotic resistant isolates, the occurrence of MDR isolates (66.7%) was high while the occurrence of XDR and PDR were less with percentage occurrences of 4.8% and 14.3% respectively.

The occurrence of carbapenem producing imipenem resistant *P. aeruginosa*isolates is as shown in Table 6. Out of 12 imipenem resistant isolates, 4(28.5%) isolates were carbapenemase producers.

The occurrence of Carbapenem resistant genes in phenotypic carbapenemaseproducers is as shown in Table 7. The amplified DNA bands for bla_{VIM}, bla_{KPC} and bla_{OXA}genes are as shown in Plate 1, 2 and 3.

Table 1: Occurrence of *Pseudomonas aeruginosa*from patients and fomites in Federal Medical Centre, Keffi, Nigeria

Samples	No. of Samples	No. (%)*P. aeruginosa*isolated
Formites	100	02 (02.0)
Throat	11	01 (09.1)
Urine	179	18 (10.1)
Wound	15	02 (13.3)
Eye	4	01 (25.0)
HVS	27	08 (29.6)
ECS	27	09 (32.1)
Ear	2	01 (50.0)
Total	365	42(11.5)

Table 2: Antibiotic Resistance profile of*Pseudomonas aeruginosa*isolate from patients and fomites in Federal Medical Centre, Keffi, Nigeria

Antibiotics	**Code**	**No. (%) Resistance (n=42)**
Imipenem	IMP	13(33.3)
Ofloxacin	OFX	18(42.9)
Streptomycin	S	19(45.2)
Cefuroxim	CEP	21(50.0)
Gentamycin	CN	21(50.0)
Ciprofloxacin	CIP	26(61.9)
Trimethoprim/sulfomethoxazole	SXT	26(61.9)
Augmentin	AU	27(64.3)
Ampicillin	AMP	30(71.4)
Nalidixic acid	NA	36(85.7)

Table 3: Antibiotics Resistance Phenotypes of *Pseudomonas aeruginosa*from patients and fomites in Federal Medical Centre, Keffi, Nigeria

Phenotypic Resistance Pattern	No. of Samples	No. (%) Isolates
AMP	1	1(2.4)
NA	1	5(11.9)
SXT, NA	2	1(2.4)
AMP, NA	3	1(2.4)
CN,AU,CIP	3	1(2.4)
CEP, OFX, NA	3	1(2.4)
AU, AMP, NA	3	1(2.4)
AU,SXT, AMP, NA	4	1(2.4)
CN, AU, SXT, CIP	4	2(4.8)
CN, CIP, SXT, AMP, NA	5	1(2.4)
AU, CIP, S, AMP, NA	5	1(2.4)
IMP, AU, AMP, CEP, NA	5	1(2.4)
AMP, CIP, AMP, CEP, NA	5	1(2.4)
CN,AU,CIP,SXT,S,AMP	6	2(4.8)
IMP,AU,CIP,SXT,AMP,NA	6	1(2.4)
IMP, CN, CIP, AMP, CEP, NA	6	1(2.4)
CN,SXT,S, AMP, CEP, NA	6	1(2.4)

CIP, S, AMP, CEP, OFX, NA	6	1(2.4)
IMP, CN, AU, CPX, SXT, S, NA	7	1(2.4)
CN. SXT, S, AMP, CEP, OFX, NA	7	1(2.4)
AU, CIP, S, AMP, CEP, OFX, NA	7	1(2.4)
AU, CIP, SXT, AMP, CEP, OFX, NA	7	1(2.4)
CN, AU, CIP, SXT, AMP, CEP, OFX, NA	8	1(2.4)
CN, AU, SXT, S, AMP, CEP, OFX, NA	8	1(2.4)
AU, CIP, SXT, S, AMP, CEP, OFX, NA	8	1(2.4)
CN,AU, CIP, SXT, AMP, CEP, OFX, NA	8	1(2.4)
IMP, CN, CIP, SXT, S, AMP, CEP, NA	8	1(2.4)
AU, CIP, SXT, S, AMP, CEP, OFX, NA	8	1(2.4)
IMP, AU, CPX, SXT, AMP, CEP, OFX, NA	8	1(2.4)
IMP, CN, AU, CIP, SXT, S, AMP, OFX, NA	9	1(2.4)
IMP, CN, AU, CIP, SXT, S, AMP, CEP, OFX, NA	10	6(14.3)

IMP=Imipenem**;** CEP=Cefuroxim;AUG=Augmentin;NA=Nalidixic acid**;** AMP=Ampicillin;
S=Streptomycin;CIP=Ciprofloxacin**;**SXT=Sulfomethoxazole/trimethoprim;
CN=Gentamicin;OFX**=**Ofloxacin.

Table 4: Multiple Antibiotics Resistance (MAR) Index of *Pseudomonas aeruginosa* from patients and fomites in Federal Medical Centre, Keffi, Nigeria

No of Antibiotics Resistance to (a)	No of Antibiotics tested (b)	MAR Index ($^{a}/_{b}$)	Frequency (%) (n = 42)
10	10	1.0	6(14.3)
9	10	0.9	1(02.4)
8	10	0.8	6(14.3)
7	10	0.7	5(11.9)
6	10	0.6	6(14.3)
5	10	0.5	4(09.5)
4	10	0.4	3(07.1)
3	10	0.3	3(07.1)
2	10	0.2	2(04.8)
1	10	0.1	6(14.3)

Table 5: Categories of Antibiotic Resistance of *Pseudomonasaeruginosa* frompatients and fomites in Federal Medical Centre, Keffi, Nigeria

Categories of Antibiotic resistance	Total Frequency (%) n=42
XDR	2(4.8)
NMDR	6(14.3)
PDR	6(14.3)
MDR	28(66.7)

Table 6: Phenotypic detection of carbapenemproduction in imipenem resistant *Pseudomonasaeruginosa*from patients and fomites in Federal Medical Centre, Keffi, Nigeria

Samples	Number of imipenem resistant *P. aeruginosa*	No. of carbapenemase producers (n=4)
Formites	1	0(0.0)
HVS	1	0(0.0)
ECS	2	0(0.0)
EYS	1	1(25.0)
Urine	7	3(75.0)
Total	12	4(28.5)

Table 7: Occurrence of Carbapenem resistance genes in phenotypic Carbapenemase producing *Pseudomsonasaeroginosa* frompatients and fomites in Federal Medical Centre, Keffi, Nigeria

Carbapenemase genes	No. (%) *P. aeruginosa* isolates (n=4)
bla_{VIM} / bla_{KPC}	2 (50.0)
bla_{KPC} / bla_{OXA}	2 (50.0)
bla_{VIM} / bla_{OXA}	2 (50.0)
bla_{VIM} / bla_{KPC} / bla_{OXA}	3 (70.0)
bla_{OXA}	3 (75.0)
bla_{VIM}	3 (75.0)
bla_{KPC}	4 (100)

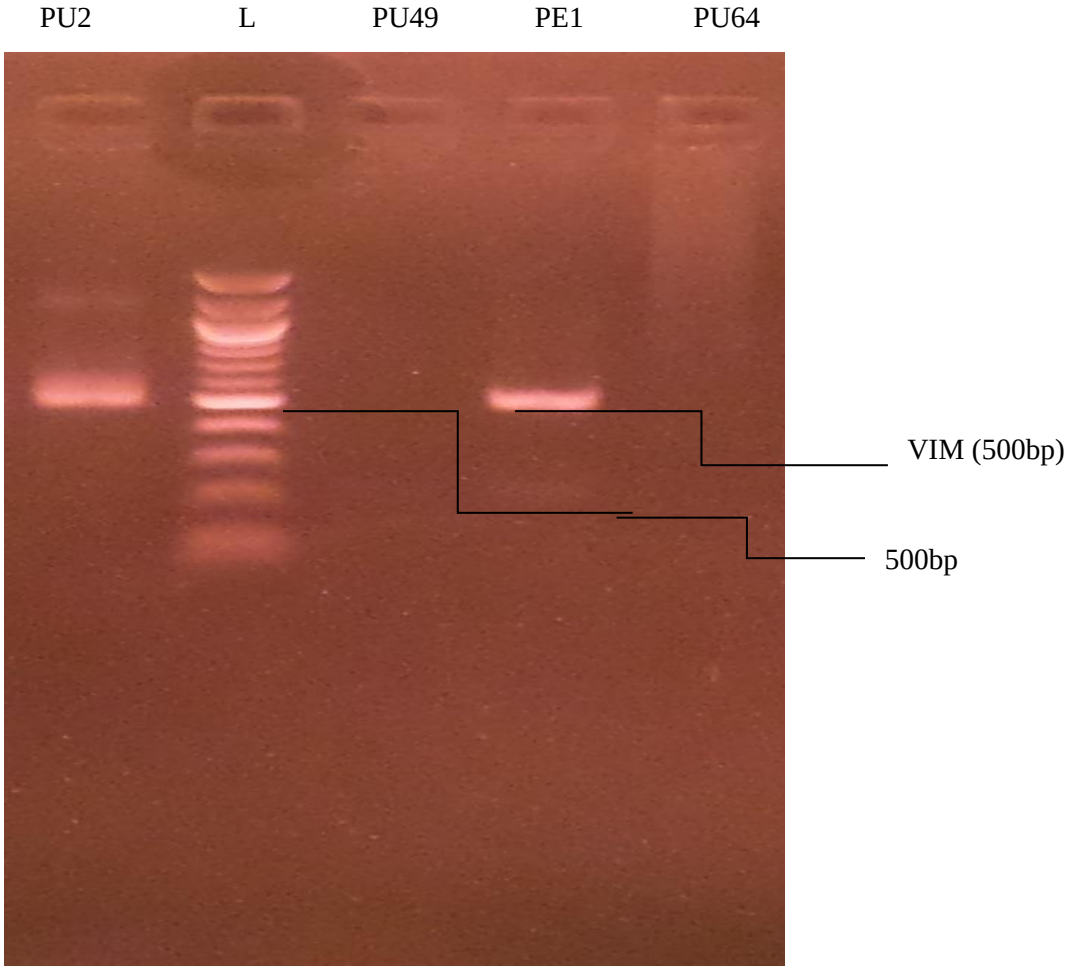


Plate 1: Agarose gel electrophoresis showing the amplified VIM gene.

- Lane PU2 and PE1 represent the VIM gene bands at 500bp
- lane L represents the 100bp molecular ladder

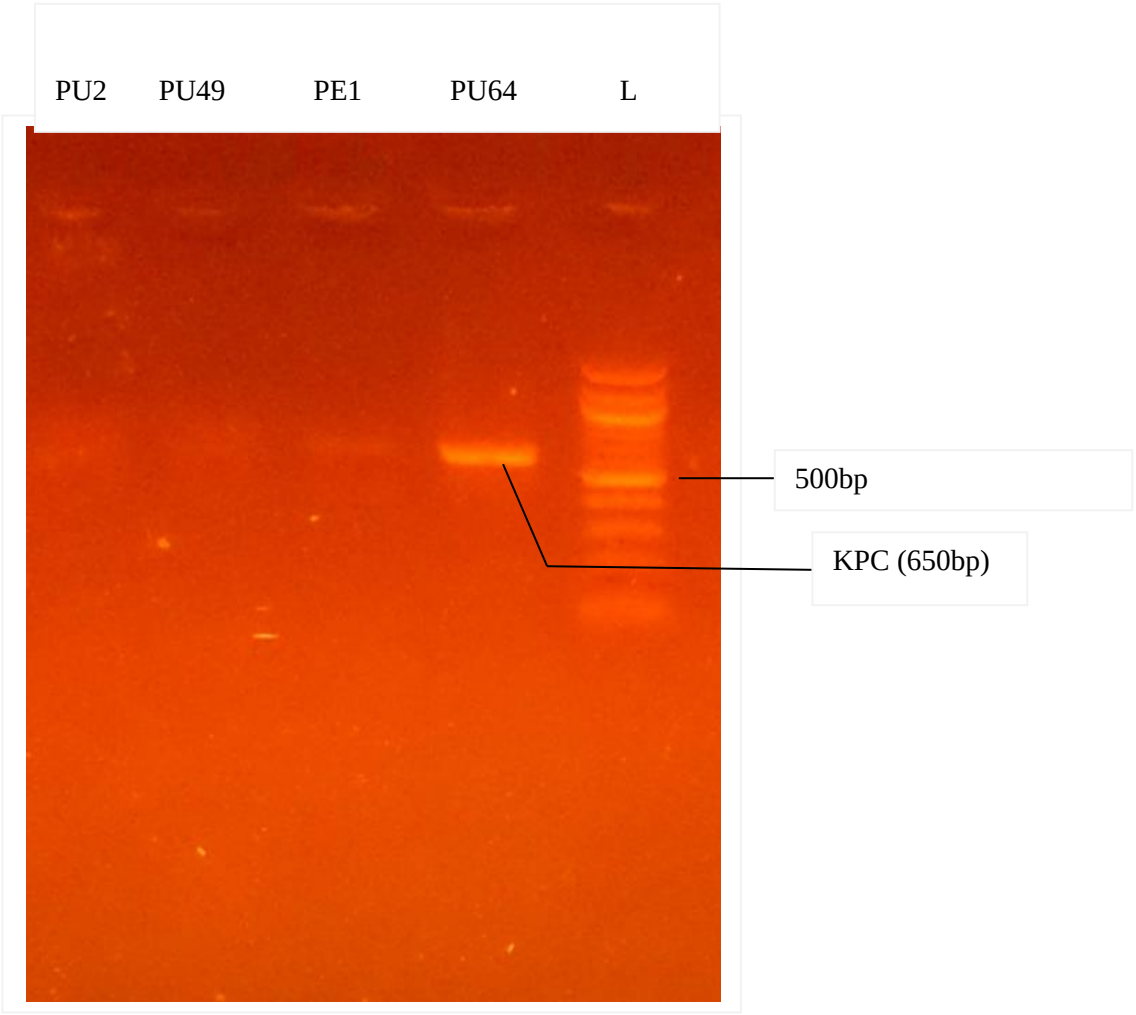


Plate 2: Agarose gel electrophoresis showing the amplified *KPC* gene.

- Lane PU2, PU49, PE1 and PU64 represent the *KPC* gene band at 650bp
- Lane L represents the 100bp molecular ladder

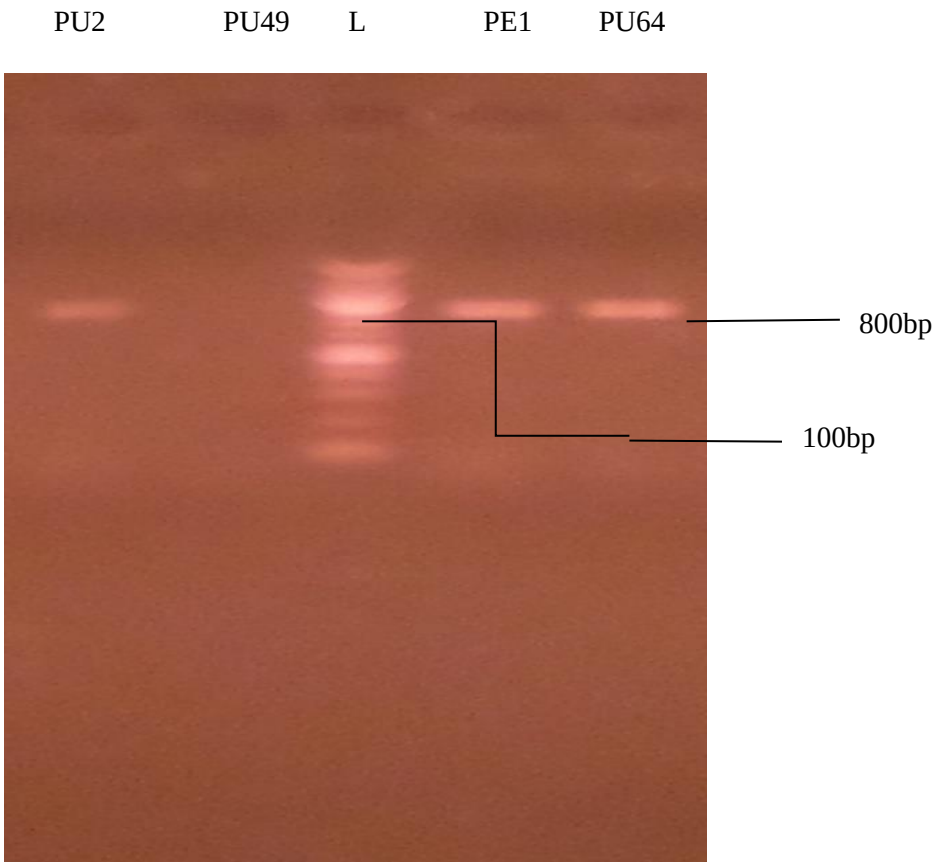


Plate 3: Agarose gel electrophoresis showing the amplified *OXA* gene.

- Lane PU2, PE1, PE1 and PU64 represent the OXA gene bands at 800 bp
- Lane L represents the 100bp molecular ladder.

DISCUSSION

Pseudomonas aeruginosa is one of the most common nosocomial pathogen found in hospital environement and may be responsible for infections such as wound infection, sepsis, urinary tract infection and ear infection ((Lister *et al.*, 2009).

The isolation of *P. aeruginosa* from clinical samples such as urine, HVS, ECS, throat swab, ear swab, eye swab, wound and fomites was expected and this is not different from the study earlier reported by Mona *et al.* (2017) and Gamal*et al.* (2007). The overall percentage of *P. aeruginosa* from clinical and fomiteswas 11.5%. This study was higher than 4.15% in a study conducted by Amrullah*et al.* (2019) but less than 27.5% reported by Umoro*et al.* (2018). The isolation of *P. aeruginosa* from clinical samples observed in this study was an

indication that this organinsm may be responsible for urinary tract infection, High Vaginal infection, eye infection andotitis media. The percentage occurrence of *P. aeruginosa* in ear isolates is higher than 2.4% and 12.5% reported by Amrullah*et al.* (2019) and Hayder (2011) respectively. The percentage occurrence of *P. aeruginosa*in urine 10.1% is relatively similar to 9.87% reported by Hayder, 2011. The high percentage occurrence of *P. aeruginosa* in HVS and ECS is quite surprising and alarming and this is not in agreement with the study earlier conducted by Amrullah*et al.* (2019) who reported 0% occurrence of *P. aeruginosa* from HVS and ECS samples. The differences may be due to geographical factor, drug misuse and water used in toileting. The occurrence of *P. aeruginosa*in throat is 9.1%, which is in contrast to 0% earlier reported by Amrullah*et al.* (2019). The occurrence of *P. aeruginosa* in wound (13.3%) is not in agreement with the study conducted by Ndip*et al.* (2005) and Amrullah*etal.* (2019) who reported 28% and 45.78% respectively but similar to 14.47% reported by Hayder (2011). The occurrence of *P. aeruginosa*in fomite is quite low which is not in agreement with 68.49% and 22.0% percentage occurrence as reported by the studies earlier reported by Hayder (2011).

The *P. aeruginosa* isolates from clinical and formites samples in this study were more resistant to antibiotics such as nalidixic acid, ampicicllin, sulfomethoxazole/trimethoprim, ciprofloxacin, ampicillin and gentamicin. The resistance of the isolates to the antibiotics mention is in agreement with the study earlier described by Amurullah*et al.* (2019) who reported high percentage resistance of *P. aeruginosa*isolates to ampicillin (96%), ciprofloxacin (61.9%) and gentamicin (48.27%). The resistance of the isolates to the antibiotics may be due to their inappropriate use for treatment of bacterial infections in hospital settings. The percentage resistance of the isolates to ciprofloxacin (61.9%) observed in this study was higher than 31.25% resistance to ciprofloxacin reported by Hayder (2011). The percentage resistance of the isolates to gentamicin as observed in this study was higher than 30% and 48.27% reported by Zubair and Iregbu (2018) and Amrulaah (2019). The 71.4% resistance to ampicilin observed in this study was less than 96% reported by Amrullah*et al.* (2019). Our findings in this study showed that the *P. aeruginosa*isolates were less resistant to streptomycin,imipenem and ofloxacin and these finding suggest that the antibiotics mentioned may not have been misusued or abused. The susceptibility of the isolates to antibiotics mentioned also suggests that the antibiotics are very effective against the *P.aeruginosa* in the study location. The percentage resistance of the

isolates to imipenem observed in this study is similar to 8.5% reported by Zubair and Iregbu (2018) but less than 27.6% reported by Amrullah*et al.* (2019).

Our findings also showed that most of the isolates were Multi drug resistant isolates and the percentage occurrence of the Multidrug resistant isolates was 66.7%. The occurrence of MDR isolates from clinical and fomites sample in the study area may have public health implication since MDR isolates have been known to cause infection that are difficult to treat using antibiotics.

The percentage occurrence of bla_{OXA} (75%), bla_{KPC} (100%) and bla_{VIM} (75%) observed in this study differs from the study earlier describe by Azar*et al.* (2019) who reported absence of bla_{KPC} (0.0%) and 18% of bla_{OXA}-48. The carbapenemasebla_{KPC} (100%) was the most frequent gene detected from imipenem resistant isolates observed in this study and this finding is not in agreement with the study earlier described by Azar*et al.* (2019) who reported the absence of bla_{KPC} and Moosavian&Rahimzadeh(2015) who reported that bla_{IMP} and bla_{VIM} were the most common genes conferring resistance to carbapenems. The detection of bla_{OXA}, bla_{KPC} and bla_{VIM} genes in imipenem resistant isolates observed in this study suggest that the gene may be responsible for the production of carbapenemase known to inactivate carbapenem antibiotic and this may have public implications since carbapenem antibiotics are the last resort of β-lactam antibiotics use for the treatment of other β-lactamase resistant isolates. The presence of these resistant isolates may be alarming because they may likely spread in the hospital settings where there is high level of unhygienic practices especially in third world countries.

CONCLUSION

The occurrence of the isolates from fomites and clinical samples was high and most antibiotics tested were not effective against the *P. aeruginosa* isolates. In addition, most of the isolates were multi-drug resistance and carbapenemase producers and bla_{KPC} gene was more frequently detected in carbapenemase producers than other carbapenemase resistance genes.

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