Original Article

Resistance Pattern and Detection of Metallo-beta-lactamase Genes in Clinical Isolates of *Pseudomonas aeruginosa* in a Central Nigeria Tertiary Hospital

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Abstract

Background: Acquired metallo-β-lactamases (MBLs) pose serious problem both in terms of treatment and infection control in the hospitals and report across the world showed an increase in their prevalence. However, there is a paucity of data from Africa, and their report is rare in Nigeria. **Aim:** This study aimed to determine the prevalence of acquired MBL-resistant genes in carbapenem-resistant *Pseudomonas aeruginosa* in Abuja, North Central Nigeria. **Materials and Methods:** Two hundred nonduplicate, consecutive isolates of *P. aeruginosa* from clinical samples submitted to the Medical Microbiology Laboratory of National Hospital, Abuja were screened for carbapenem resistance using imipenem and meropenem. Phenotypic detection of MBL-producing strains was determined using Total MBL confirm kits and E-test strips on isolates that were resistant to both Imipenem and meropenem. The MBL genes were detected using multiplex polymerase chain reaction, while the gene variant was determined by sequencing. **Results:** Twenty-two MBL-producing strains were detected phenotypically, but only 5 harbored the blaVIM-1 gene, giving a prevalence of 2.5%. These 5 strains were resistant to all the antipseudomonal antibiotics tested except Aztreonam and Colistin. Other common MBL-genes were not detected. **Conclusion:** The prevalence of MBL-producing strains of *P. aeruginosa* which poses serious challenge for therapeutics and infection control is currently low in Abuja, North Central, Nigeria. Therefore, rational use of the carbapenems and other antipseudomonal antibiotics, regular surveillance and adequate infection control measures should be instituted to limit further spread.

Keywords: *Abuja, carbapenem-resistance, metallo-β-lactamases, Pseudomonas aeruginosa*

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Introduction

Pseudomonas aeruginosa, an organism widespread in nature, is an important cause of healthcare-associated infection, especially in patients with compromised host defense.[1-4] Its infections are associated with relatively high treatment failures and mortality due to its intrinsic and acquired resistance to commonly available antibiotics, particularly in patients hospitalized with burns, malignancies, and those with cystic fibrosis, as well as in those with fulminant infections such as sepsis and pneumonia, most of which are fatal with high mortality rate, often >50%.[5,6]

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With increasing inappropriate use of antimicrobials both in human and veterinary medicine, microorganisms are rapidly evolving from susceptible into resistant strains to various antibiotics with consequent therapeutic failures[7-9] At present, a number of pathogenic bacteria are being described as multidrug-resistant (MDR), extensively-drug-resistant, or pan-drug-resistant

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pathogens because they have developed or acquired resistance to a number of clinically useful antibiotics.[7,10,11] *P. aeruginosa* has been identified in all the three categories.

The carbapenems used to be the empirical agent of last resort when dealing with MDR Gram-negative bacteria, but there are increasing reports of resistance to them, especially in *P. aeruginosa*. In Nigeria, different researchers in Zaria, Lagos, and Kano have reported *P. aeruginosa* carbapenem resistance of between 20% and 60% using phenotypic methods of Detection.[12-14] 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.[15,16] Of all these, the most worrisome is the emergence of strains of *P. aeruginosa* with acquired metallo-β-lactamases (MBLs) which present the most clinically challenging situation both in terms of treatment and infection control.[17]

MBLs are carbapenemases with capability of hydrolyzing the penicillins, cephalosporins, cephamycins, and the carbapenems but not monobactams.[18] In addition, pathogens that harbor MBL genes tend to carry co-resistance genes for other classes of antibiotics. MBL genes are usually borne on mobile genetic elements such as integrons, transposons, plasmids, or associated with insertion sequences which confer it with the propensity to spread not just within a species but also between different species. MBLs have been reported in more than 28 species of Gram-negative bacteria from more than forty countries.[19] The mortality attributable to infections caused by MBL-producing *P. aeruginosa* is estimated to range from 70% to 90%.[5,6]

Since resistance is driven by antibiotics use,[20,21] there is increasing report of these strains, and the possibility of rising prevalence exist with escalating use of the carbapenems as they become more available for clinical use, even as better methods of detection of MBL producing strain are becoming available in clinical microbiology laboratories. A number of studies in Nigeria have reported antibiotics resistance in *P. aeruginosa*, but, there is a paucity of data on MBL-induced resistance.[1,3,12,19,22-25] In Abuja, the federal capital territory of Nigeria, neither the prevalence rate of MBL-producing strains of *P. aeruginosa* nor the MBL gene type harbored by any of such strains has been reported.

This study was carried out to determine the prevalence of MBL-producing strains of *P. aeruginosa* in a tertiary health-care setting in Abuja as well as characterize the common resistance gene-types harbored by the strains as a way of generating local data for planning and advocacy with respect to empiric therapy, antibiotic stewardship, and infection control.

Materials and Methods

This was a cross-sectional study carried out over a 34-month period ranging from February 2013 to November 2015 at the National Hospital Abuja (NHA), a 400-bed tertiary health-care institution located in Abuja, North Central Region of Nigeria. Two hundred consecutive, nonduplicate, isolates of *P. aeruginosa* from all samples except stool submitted to the Medical Microbiology Laboratory of NHA were identified using standard techniques,[26] and further subjected to a temperature of 42ºC to eliminate the other members of the fluorescence group of *Pseudomonads*. Antibiotics Susceptibility Testing was performed using Modified Kirby–Bauer disc diffusion method, in accordance with the CLSI standards. Antibiotics tested were piperacillin/tazobactam, ceftazidime, cefepime, aztreonam, imipenem, meropenem, gentamicin, amikacin, ciprofloxacin, and colistin. *P. aeruginosa* that showed resistance to imipenem and or meropenem were subjected to phenotypic MBL confirmation using Total MBL Confirm Kit from ROSCO Diagnostica (ROSCO Diagnostica A/S, Taastrupgaardsvej, Denmark) in accordance with the manufacturer's instructions. The sensitivity and specificity of total MBL confirm kit from ROSCO Diagnositica is 97.7% and 100%, respectively.[27] Further confirmation of MBLs was done with E-test strip from Liofilchem (Liofilchem, Roseto degli Abruzzi, Italy). A positive MBL test is indicated by the ratio of the MIC of IMP: IMD or MRP: MRD ≥8 (IMP/IMD ≥8 or MRP/MRD ≥8) or the presence of a phantom zone. *P. aeruginosa* ATCC 27853 was used as control strain for these procedures. Phenotypically confirmed MBL producers were stored at 20°C in 50% glycerol stock until they were further analyzed at the DNA Labs, Kaduna, for molecular confirmation. Using the following primers: bla_{IMP}-types, bla_{VIM}-types, bla_{GIM-1}, bla_{SPM-1}, bla_{SIM-1} and bla_{NDM-1}, multiplex polymerase chain reaction (PCR) was used to detect the specific type of MBL gene harbored by the MBL-producing strains of *P. aeruginosa*. In addition, one pair of class 1 integron primer was included to detect the presence of class 1 integron. DNA sequencing using Bechmann coulter CEQ-8000 (Beckman Coulter, Kraemer Boulevard, Brea, CA, USA) was carried out on sample No. 22. The resulting nucleotide sequence was analyzed using the BLAST program on NCBI website (http://blast.ncbi.nlm.nih.gov/Blast.cgi) which

shows the nucleotide sequence as 99% identical to bla_{VIM-1} gene with E-value of 6×10^{-180} and accession numbers KF975369 in the GenBank. This resulting nucleotide sequence was used as the positive control for the multiplex PCR while all the PCR reaction mixture without a primer was used as negative control as shown in Figure 1 in the result section.

Ethical approval was given by the Ethical Committee of NHA while informed consent was obtained from patient using standard consent form.

Results

Distribution of clinical samples/isolates

The 200 isolates of *P. aeruginosa* from various clinical samples were distributed as follows: wound swab/biopsy 103 (51.5%); ear swab 32 (16%); urine 27 (13.5%); and blood culture 20 (10%) [Table 1].

Antibiotics susceptibility profile of *Pseudomonas aeruginosa*

Of the 200 isolates 176 (88%), 169 (84.5%), 168 (84%), and 146 (73%) were susceptible to imipenem, piperacillin/tazobactam, colistin, and cefepime, respectively [Table 2]. In addition, 139 (69.5%) isolates were susceptible to amikacin, 133 (66.5%) to gentamicin, 135 (67.5%) to ciprofloxacin, and 128 out of the 178 tested (71.9%) susceptible to meropenem. One hundred and two isolates (51%) were resistant to ceftazidime.

Multiplex polymerase chain reaction result of metallo-β-lactamases-*Pseudomonas aeruginosa* and resistant pattern

Out of the 22 phenotypically detected MBL-*P. aeruginosa*, the bla_{VIM-1} gene was detected in only five[5] [Table 3]. The agarose gel electrophoresis result for the bla_{VIM-1} gene is shown in Figure 1.

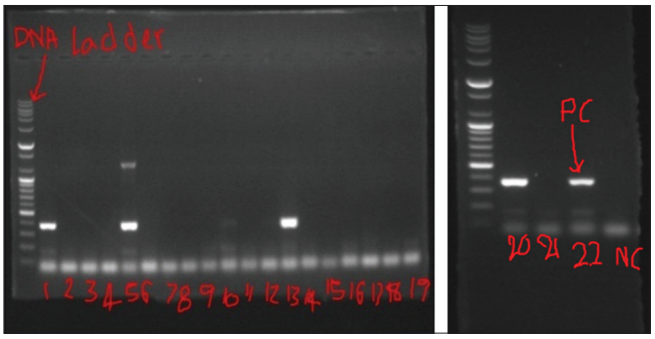

Figure 1: Agarose gel electrophoresis result for multiplex polymerase chain reaction product for the detection of metallo-β-lactamase genes. Lane 1 = Strain no: 10, 5 = 54, 13 = 112, 20 = 152, 22 = 185. PC: Positive Control, NC: Negative Control. NB: The bands in lane 1, 5, 13, 20, and 22 are the specific (positive) bands while the second band in lane 5 is a nonspecific band which should be ignored

Table 1: Distribution of isolates of *P. aeruginosa* in various clinical samples

Specimen Type	No of Isolates	Percentage (%)
Wound Swab/biopsy	103	51.5
Ear Swab	32	16
Urine	27	13.5
Blood Culture	20	10
Eye swab	10	5
Sputum	5	2.5
Nasal Swab	2	1
Bone Tissue	1	0.5
Total	200	100

Table 2: Susceptibility of *P. aeruginosa* isolates to antipseudomonal antibiotics

Antibiotics	No. tested	Result (%)		
		Susceptible	Intermediate	Resistance
Meropenem	178*	128 (71.9)	6 (3.4)	44 (24.7)
Imipenem	200	176 (88)	7 (3.5)	17 (8.5)
Colistin	200	168 (84)	-	32 (16)
Ceftazidime	200	94 (47)	4 (2)	102 (51)
Cefepime	200	146 (73)	23 (11.5)	31 (15.5)
Amikacin	200	139 (69.5)	31 (15.5)	30 (15)
Gentamicin	200	133 (66.5)	7 (3.5)	60 (30)
Ciprofloxacin	200	135 (67.5)	3 (1.5)	62 (31)
Pip/Tazo	200	169 (84.5)	21 (10.5)	10 (5)
Aztreonam	200	131 (65.5)	35 (17.5)	34 (17)

*Due to incomplete number of Meropenem disc

Table 3: Microbiological and molecular characteristics of Carbapenem-Resistance *P. aeruginosa* isolates

Strain no	Ward	Sample	MEM	IPM	CT	CAZ	CEF	AK	CN	CIP	TZP	ATM	KIT	E-test	Gene
10	SCBU	BC	R	R	S	R	R	R	S	R	R	R	+ve	+ve	bla_{VIM-1}
29	ICU	Urn	R	R	S	R	R	R	S	R	R	R	+ve	+ve	
34	IPFS	WS	R	R	S	R	S	R	I	R	S	S	+ve	-VE	
41	SCBU	BC	R	I	R	I	S	R	R	S	S	R	+ve	+VE	
49	ICU	BC	R	R	S	R	R	R	R	R	R	R	+VE	-VE	
54	GOPD	WS	R	I	S	R	I	I	R	R	I	I	+ve	+ve	bla_{VIM-1}
61	ICU	WS	R	R	S	R	R	S	R	R	R	R	+ve	+ve	
78	EPU	Conj	R	R	S	R	R	R	R	R	I	R	+ve	+VE	
88	ICU	WS	R	I	S	R	R	S	R	R	R	R	+ve	-VE	
89	IPMM	WS	R	R	S	R	R	R	R	R	I	R	+ve	+VE	
112	ICU	WS	R	I	S	R	I	I	R	R	I	I	+ve	-VE	bla_{VIM-1}
126	ICU	WS	R	R	R	R	R	R	R	R	R	R	+ve	+VE	
146	EPU	WS	I	R	S	R	I	S	R	R	I	I	+ve	-VE	
152	SCBU	WS	R	R	S	I	I	R	R	R	S	S	+ve	+VE	bla_{VIM-1}
154	ICU	Urn	I	R	S	R	I	I	R	R	I	I	+ve	+VE	
157	IPFS	WS	R	R	S	I	S	R	R	R	S	S	+ve	+VE	
161	POPD	WS	R	R	R	R	S	R	R	R	I	R	+ve	-VE	
167	IPFS	WS	R	R	R	R	R	R	R	R	I	R	+ve	-VE	
182	ICU	WS	R	R	S	R	S	S	R	R	I	S	+ve	+VE	
185	IPFS	WS	R	R	S	R	R	R	R	R	I	R	+ve	+VE	bla_{VIM-1}
189	ICU	WS	R	I	S	R	I	S	S	S	S	S	+ve	+VE	
190	IPMM	WS	R	R	S	R	R	I	R	R	R	I	+ve	+VE	

MEM=Meropenem; IPM=Imipenem; CT=Colistin; CAZ=Ceftazidime; CEF=Cefepime; AK=Amikacin; CN=Gentamicin; CIP=Ciprofloxacin; TZP=Piperacillin/Tazobactam; ATM=Aztreonam; KIT=Total MBL confirm kit; E-test=Epsilometer strip; SCBU=Special Care Baby Unit; ICU=Intensive Care Unit; EPU=Emergency Paediatrics Unit; IPFS=In-patient Female Surgical ward; POPD=Paediatrics Out-Patient Department; GOPD=General Out-Patient Department; WS=Wound Swab; Urn=Urine; BC=Blood Culture; Conj=Conjunctiva swab; S=Sensitive; I=Intermediately susceptible; R=Resistant; +VE=Positive; -VE=Negative; bla_{VIM-1}=Beta-lactamase gene for Verona Integron-borne metallo-β-lactamase variant No. 1

Discussion

Antibiotics susceptibility profile

Results of antibiotics susceptibility testing revealed that isolates of *P. aeruginosa* were most susceptible to imipenem, piperacillin-tazobactam, colistin, and cefepime but were largely resistant to ceftazidime. The previous retrospective studies conducted in this center also showed that imipenem had the highest susceptibility against *P. aeruginosa* in the range of 80%–90%, but with reduced susceptibility to ceftazidime, one-third-generation cephalosporin that was heavily relied on for its antipseudomonal activity.[28-31] The concordance of the retrospective studies with this prospective one implies that there has been little or no change in the antibiotics susceptibility pattern of *P. aeruginosa* between 2010 and 2015 in this locality.

The studies, however, revealed that ceftazidime could no longer be suitable as an agent for empiric therapy in serious infections suspected to be caused by *P. aeruginosa*. Ceftazidime has been the "workhorse" for the treatment of severe infections caused by *P. aeruginosa* in many tertiary healthcare institutions in Nigeria for over two decades, and this may account for the high resistance profile. This reduced susceptibility to ceftazidime and other commonly prescribed antipseudomonal antibiotics had previously been reported in studies from other tertiary health-care institutions in other parts of Nigeria.[23,24,26,32] Similar reason stated above may be adduced for these susceptibility patterns. However, a well-designed multicenter research may provide more representative data.

Imipenem and the carbapenems generally, are not commonly prescribed in our center, implying less exposure and pressure on this pathogen to these antibiotics; hence, the relatively high susceptibility profile. Similarly, piperacillin-tazobactam, cefepime, and colistin are not commonly prescribed, were not usually included in routine antibiotics susceptibility testing as reflected in the retrospective studies. Therefore, on the basis of this susceptibility profile, imipenem and piperacillin/tazobactam, would appear to be the most appropriate choices for empiric therapy in the treatment of patients with serious infections in which *P. aeruginosa* is suspected while colistin and cefepime should be reserved as last drugs of choice. Notwithstanding, the commonly prescribed antipseudomonal agents are still

clinically useful, provided their selection is based on an appropriate antibiogram and regular surveillance.

Prevalence of VIM-1 metallo-β-lactamases-*Pseudo monas aeruginosa*

Although the phenotypic method used in this study detected 22 (11%) MBL-producing *P. aeruginosa* strains, only five were found to harbor the gene for MBLs when subjected to molecular analyses, and they were all bla_{VIM-1} sub-types. This gave an overall prevalence of 2.5%. This, to the best of our knowledge, is the first report of the detection of bla_{VIM-1} in clinical isolate of carbapenem resistance *P. aeruginosa* in Nigeria. The multiplex PCR did not detect the MBL gene for IMP, NDM, SPM, SIM, and GIM that were assayed for. Most published studies on the detection of MBLs in *P. aeruginosa* in Nigeria were limited to phenotypic methods only.[14,33-35] Studies in Kaduna/Kano, Lagos, and Calabar using the ethylenediaminetetraacetic acid (EDTA) disc synergy method gave prevalence of 21.2%,[14] 8.8%[34] and 4.1%,[36] respectively; whereas an Enugu study gave a prevalence of 10%,[33] similar to the 11% in this study. The differences in the methods used partly accounted for the wide differences in prevalence as the EDTA disc synergy method is of lower specificity than the disc potentiating method used in this and the Enugu studies.

The phenotypic resistance to carbapenems in the absence of relevant genes for their enzyme elaboration, most probably suggests that resistant mechanisms other than MBLs could be at play in the isolates. Efflux pumps, impermeability of the outer membranes, modification of target site and carbapenemases other than MBLs may also mediate carbapenem resistance in *P. aeruginosa*.[15,16]

Interestingly, the findings in this study are a marked contrast from a similar study in Kenya[37] where all phenotypically detected MBL-producing, carbapenem-resistant *P. aeruginosa* (CRPA) harbored the bla_{VIM-2} gene, which is the much more common one while our study detected the much rarer bla_{VIM-1} gene, suggesting that this is the dominant strain circulating in Abuja, Nigeria. It would appear that bla_{VIM-2} does not presently exist in Abuja and probably Nigeria as a whole; an issue for further studies for conclusive determination.

The bla_{VIM-1} was first reported in Verona University Hospital, Italy, in 1999 by Laura Lauretti *et al*. in an isolate of CRPA.[38] Subsequently, it was frequently reported around the Mediterranean region and has largely remained confined there.[39,40] Its detection in Abuja may be a case of imported antibiotic resistance strains because many Nigerians frequently visit this region for pilgrimage (Israel, Saudi Arabia, Rome), medical tourism, and work. Two cases of imported MBL-producing *P. aeruginosa* from Ghana to Norway[41] and from Egypt to Hungary[42] have previously been reported and serve to illustrate this phenomenon. These two cases represented the first report of MBL-producing *P. aeruginosa* harboring bla_{VIM-2} gene in Norway and Hungary, a scenario which highlighted the significant role of international travel on the spread of antimicrobial resistance across the globe.

bla_{NDM-1} which has been linked with international travel, especially to the Indian Subcontinent, where it is commonly found, surprisingly was not detected in this study despite the fact that many Nigerians, particularly the inhabitants of Abuja, travel to India for medical care. Although bla_{NDM-1} gene had been more commonly reported among *Enterobacteriaceae* than *Pseudomonas*, the result of this study runs contrary to the common assertion that bla_{NDM-1} has great propensity for international dissemination as a consequence of international travel, especially to India. It is also possible that the rare bla_{VIM-1} detected in this study developed *de novo*.

The strains of *P. aeruginosa* isolated in this study that harbored bla_{VIM-1} gene was resistant to all the antipseudomonal antibiotics tested except aztreonam and colistin, but two (strains No. 10 and 185) were also resistant to aztreonam which may be due to additional resistant mechanisms. This reduced susceptibility to a broad array of antipseudomonal antibiotics has been reported by the previous studies and has a grave clinical implication for health care.[38,40]

Although it has been reported that bla_{VIM} genes are usually carried on gene cassettes inserted on a Class 1 integron,[40,43] it was not found in this study because the Class 1 integron primers used during the multiplex PCR reaction did not detect it.

Conclusion

Acquired MBL-producing strains of *P. aeruginosa* poses serious challenge for therapy as well infection control. However, the prevalence rate is currently low in Abuja, North Central Nigeria as reported in this study. With some harbouring the rare blaVIM-1-gene. Hence, rational use of antipseudomonal antibiotics, good infection control practice should be instituted to curb further spread of these strains.

Limitation of study

The limitation of this study was a result of limited resources and time constraint. Other genes responsible for MBL resistance and other mechanisms of resistance in these strains of *P. aeruginosa* could have been

detected using whole gene sequencing. Similarly, gene localization, gene cassette array, and genetic relatedness are information that could be derived from these strains where resources are available.

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Conflicts of interest

There are no conflicts of interest.

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