Nigerian Journal of Pharmaceutical and Applied Science Research, 10(1):61-69; March, 2021 (ISSN 1485-8059). Available at www.nijophasr.net

Class 1 Integrons and Associated Gene Cassettes in Multidrug Resistant *Acinetobacter baumannii* from Southwest Nigeria: Tertiary Hospitals Study

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ABSTRACT

Background: Acinetobacter baumannii is an opportunistic pathogen which has been implicated in numerous nosocomial infections worldwide. This study was conducted to investigate the correlation of class 1 integron and gene cassettes in multidrug resistant Acinetobacter baumannii (MDRAB) strains from clinical sources in tertiary hospitals in Southwest, Nigeria.

Methods: Identity of isolates was confirmed by using Oxoid MicrobactTM 2009GN system, and tracking of the presence of intrinsic blaOXA-51 gene by polymerase chain reaction (PCR). Antimicrobial susceptibility test was by Kirby-Bauer disc diffusion method and minimum inhibitory concentrations were according to Clinical and Laboratory Standards Institute guideline (CLSI). Interrogation of class 1 integron was by PCR while cassette content of the integrons was by sequencing.

Results: Seventy-two MDRAB were recovered with highest resistance rate against ampicillin, ceftazidime, ceftriaxone, and gentamicin (100%), while the highest susceptibility rate was against polymyxin B and amikacin (58%) at similar MICs -50 and -90 (2µg/mL). Sixty eight (95%) of the 72 MDRAB harbored class 1 integron, while the variable regions revealed arrays of gene cassettes.

Conclusion: To our best knowledge, this is the first report of MDRAB harboring class 1 integron gene cassettes in Southwest Nigeria. These findings call for strict surveillance to monitor the emergence and spread of MDRAB carrying integrons in Nigeria.

***Key words*— Acinetobacter baumannii, Multidrug resistant, Class 1 integron, Gene cassettes.**

1. INTRODUCTION

Multidrug-resistant *Acinetobacter baumannii* (MDRAB) has become a prominent opportunistic pathogen of public health concern having been responsible for severe hospital and community acquired infections, particularly among patients confined to the intensive care unit (ICU) [1]. Due to its propensity to survive harsh environments and multiple classes of antibiotics, it has thrived and spread as a nosocomial pathogen in many health care facilities, contributing to rising cases of morbidity and mortality [2]. In ventilator-associated pneumonia and bacteremia infections for instance, MDRAB remains a high leading cause of mortality rate (30% to 75%) and requires prolonged hospital stay especially among the critically ill patients [3]. In ICUs, the mortality rate linked to infections caused by *A. baumannii* has risen to an alarming 54% in the recent past [1]. The emergence of extensively drug-resistant *A. baumannii* (XDRAB) across the globe has narrowed the treatment options and caused considerable public health concern, especially to hospital-acquired infections (HAIs). While controlling MDRAB and XDRAB infections remains an important issue for the clinicians, therapeutic options have become difficult due to resistant to a wide spectrum of antimicrobial agents [2]. One of the important factors that contribute to the development of multidrug resistant phenotype is acquisition of mobile genetic elements (MGEs). In Acinetobacter species, antibiotic resistant determinants are commonly confined on MGEs including, the R-plasmids, transposons, genomic islands, and integrons [4]. Integrons are conserved sequences (3′-CS and 5′-CS), transposon-like DNA elements that are able to capture and mobilize gene cassettes, which can carry antibiotic resistance genes, by site-specific recombination [5]. Basically integrons are composed of an integrase gene (intI) encoding the IntI integrase, a recombination site gene (attI) encoding the cassette integration site, and one or more

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promoters regulating the expression of gene cassettes when present. There are five main classes of integrons based on sequence update. Of these classes, classes 1 and 2 are predominantly harbored among clinical isolates [6]. Reports on the other classes of integrons are not only scarce but also rudimentary. According to literature, class I integrons harbor numerous antimicrobial resistance gene cassettes encoding broad-spectrum β-lactamase, dfr (dihydroflavonol-4-reductase/trimethoprim), qacEΔ1 (disinfectants and tetravalent ammonium compounds), sul1 (sulfonamide), and aminoglycoside-modifying enzymes (AMEs) [1, 7]. The goals of this study were to identify the antimicrobial susceptibility profile, and the presence of class 1 integron in relation to antibiotic resistance gene cassettes in *A. baumannii* isolates obtained from eight different hospitals in Southwest Nigeria. This is first nationwide investigation of the class 1integron among *A. baumannii* isolates in Southwest Nigeria.

2. MATERIALS AND METHODS

2.1 Materials

2.1.1 *Equipment*

The following equipment were used in this study including Microscope, Autoclave machine, Incubator, Weighing balance, Centrifuge, Heating block, Thermocycler, Gel documentation machine, Gel electrophoresis machine, Test tubes, MacCathney bottles, Wire loop, Beaker, Round and flat bottom flasks, Automated sequence machine, Test tube racks, Petri dishes, Microbact 12E Gram-negative identification kit.

2.1.2 Biological Materials

Biological assay utilized in the course of this study include but not limited to the following, Acinetobacter baumannii isolates, Leeds Acinetobacter agar medium, Luria–Bertani broth *medium*, Antibiotics, 2% agarose gel, Gram staining reagents, ethidium bromide, Taq polymerase enzyme, DNase-free and RNase-free H_2O, PCR buffer solution, dNTP, Master mix, DNA template, Mg_2Cl.

2.2 Methods

2.2.1 Bacterial isolates

A total of 107 non-duplicated suspected isolates of *A. baumannii* were collected from the microbiology department of the tertiary health institutions including urine (39, 36%), wound (36, 34%), and blood (32, 30%) of male and female patients attending the University College Hospital (UCH), Ibadan (21, 20%), Lagos University Teaching Hospital (LUTH), Lagos (17, 16%), Obafemi Awolowo University Teaching Hospital (OAUTH), Ile-Ife (11, 10%), Olabisi Onabanjo University Teaching Hospital (OOUTH), Sagamu (12, 11%), Catholic Specialist Hospital (CSH), Oluyoro (13, 12%), Lagos State General Hospital (LSGH), Surulere (15, 14%), Federal Medical Center (FMC), Ido-Ekiti (11, 10%), and Federal Medical Center (FMC), Akure (07, 07%). The isolates were collected during a period of 14 months between January 2012 and May 2013 and identified using Oxoid Microbact^{TM}2009GN system (Basingstoke, UK). The species identification was confirmed by detection of intrinsic $bla_{OXA\text{-}51\text{-}like}$ gene as described by [8].

2.2.2 Antimicrobial susceptibility testing

Antimicrobial susceptibility testing was performed on Mueller-Hinton agar (MHA) (Oxoid, Basingstoke, UK) by agar disk diffusion (ADD) method as recommended by the Clinical and Laboratory Standards Institute (CLSI document M100-S29) [9]. The tested antibiotics were as follows: ampicillin (25µg), ampicillin-sulbactam (30µg), piperacillin (30µg), ceftazidime (30µg), cefepime (30µg), amikacin (30µg), gentamicin (10µg), tobramycin (30µg), ciprofloxacin (5µg), cefotaxime (30µg), levofloxacin (5µg), ceftriaxone (30µg), cefotetan (30µg), cephazolin (30µg), aztreonam (30µg), piperacillin/tazobactam (110µg), imipenem (10µg), and meropenem (10µg), colistin (10µg), polymyxin B (300 units) (Oxoid, Basingstoke, UK). In this study, MDRAB was defined as resistance to at least two classes of antibiotics among those antibiotic categories, include, aminoglycosides, anti-pseudomonal penicillins, carbapenems, third or fourth generation cephalosporins and quinolones. *A. baumannii* ATCC 17978 was used as a positive quality control (PQC) and *A. baylyi* ADP1 was used as a negative quality control (NQC).

2.2.3 Minimum inhibitory concentration (MIC)

The MIC titer for the aforementioned 20 antibiotics were tested using E-test strips according to the manufacturer's instructions (BioMérieux, Inc., USA) and CLSI guidelines. Suspension of each isolate in Mueller-Hinton broth, adjusted to the density of a 0.5 McFarland standard, was swabbed in all direction onto Mueller-Hinton agar plates to ensure uniform growth and incubated at 37°C for 18 to 24 hr. The interpretative values ≤2 µg/ml as susceptible (S) and ≥4 µg/ml as resistant (R) were used to interpret results as MICs were considered at point of complete inhibition of growth according to CLSI guideline.

2.2.4 Genomic DNA extraction

Genomic DNA used as a template for PCR assays was extracted by boiling method. The overnight cultures of *A. baumannii* strains in LB broth were suspended in 250 µL of sterile deionized water and incubated at 100 °C for 10 min. After centrifugation at 10,000 g for 5 min, the supernatant were used as a template DNA and stored at − 20 °C until use [7].

2.2.5 Amplification of class 1 integron

The presence of class 1 integron was investigated by amplification of integrase gene (*intI1*) using specific primers (Table 1). The primers were designed as described previously (14). The PCR reactions were prepared in total volume of 25µL and amplification was performed using the T100 Thermal Cycler (Bio-Rad Laboratories, Inc.) as follows: 5 min at 94 °C; 35 cycles of 1 min at 94 °C, 1 min at 55 °C, 30 s at 72 °C; 10 min at 72 °C for detection of *intI1* gene [10]. Reaction mixtures with a DNA template of *A. baylyi* ADP1 a non-pathogenic soil-dwelling *Acinetobacter* species which does not harbor resistance determinants [11] and *A. baumannii* ATCC 17978 harboring class 1 integron gene were used as the NQC and PQC respectively and were kindly provided by Peters Laboratory, Cornell University, Ithaca. The amplified products were electrophoresed on 1.2% agarose gel and after staining with ethidium bromide (EtBr) (0.5 mg/ml), visualized in gel document system (Bio-Rad Laboratories, Inc.). The amplicon sizes were determined by comparison with a DNA size marker (100-bp DNA ladder, Fermentas).

2.2.6 Amplification of internal gene cassette regions

All integron-positive MDRAB were tested for the presence of internal cassette genes of *IntI1* using specific primers for 5' and 3' conserved segments (5'CS and 3'CS) in the following combination 5'CS with VEB-R/TEM-R/PER-R/VIM-R/IMP-R and 3'CS with VEB-F/TEM-F/PER-F/VIM-F/IMP-F (Table 2). The following conditions was used for PCR reaction: initial denaturation at 94 °C for 5 min, followed by 35 cycles of denaturation at 94 °C for 1 min, annealing at 60 °C (VEB-F, TEM-F, PER-F, VIM-F, IMP-F/3'CS), 57 °C (VEB-R, TEM-R, PER-R, VIM-R, IMP-R/5'CS), for 1 min, and extension at 72 °C for 2 min, with final extension at 72 °C for 10 min [12]. After electrophoresis on 1.2% agarose gel and staining, the PCR products were visualized.

2.2.7 DNA sequencing and integron gene cassettes analysis

The amplified PCR products were sequenced after purification by an automated DNA sequencer (ABI3730XL DNA analyzer: Forster, USA) using 10 pmol of specific primers. The sequences were analyzed using Chromas Pro version 1.7.5 Technelysium and compared using online BLAST software (http://blast.ncbi.nlm.nih.gov/Blast.cgi). Upon sequencing of the CS-PCR products, the contents of the integrons cassettes were revealed.

2.3 Statistical analysis

In this study, all statistical analyses were carried out using Statistical Package for the Social Sciences (SPSS) for Windows (version 20.0), and all parametric variables were evaluated using the chi-square test, as appropriate. A difference was considered statistically significant if the $P \leq 0.05$.

3. RESULTS

Of the 107 suspected A. baumannii isolates evaluated, 72 isolates encoded intrinsic blaOXA-51-like a carbapenamase gene associated with A. baumannii (Figure 1). While the mean ages of patients was 54 years (range, 5 to 98 years), the female and male population in this study was (32, 44%) and (40, 56%) respectively.

3.1 Antimicrobial resistance profile in association with class 1 integron

All 72 isolates were MDRAB with highest resistance rate against ampicillin, ceftazidime, ceftriaxone, and gentamicin (100%), while the highest susceptibility rate was against polymyxin B and amikacin (58%) at similar MIC50 and MIC90 (2µg/mL). Similarly, resistance to ampicillin, aztreonam, ceftazidime, ceftriaxone, and gentamicin (100%) were observed as the common resistance pattern in all integron positive isolates followed by ampicillin/sulbactam, cefotaxime, meropenem, and levofloxacin (76%) (Table 2). The rates of antibiotic resistance of class 1 integron positive strains to the class of penicillins, cephems, meropenem of class carbapenems, gentamicin and tobramycin of class aminoglycosides, levofloxacin of class fluoroquinolones, and colistin belonging to class lipopeptides were expressively high compared with the integron negative strains, suggesting that the mechanism essential for the expression of antibiotic resistance resides with the class 1 integrons. Nonetheless, the rate of resistance of integron negative strains to some antibiotics was comparatively high, signifying that the multiplicity of drug resistance in A. baumannii strains is not exclusively associated with class 1 integrons but also with other drug resistance mechanisms, which calls for further investigation.

3.2 Detection of Class1 integron and characterization of gene cassettes in A. baumannii

The presence of class 1 integron was confirmed in (68, 95%) of the isolates (Figure. 2) which upon sequencing revealed 17 different arrays of gene cassettes within the variable regions (Table 3), including five genes encoding resistance to β-lactam antibiotics (blaTEM-1, blaVEB-1, blaPER-1, blaVIM-1, and blaIMP-1) and three genes for encoding resistance to aminoglycosides (aacA7, accC1, and aacC3). The correlation of integron-positive and antibiotic resistance profile are shown in Table 2. Similarly, the frequency of class 1 integron to gene cassette is demonstrated in Table 3.

Table 1: Primer sequences used in this study

Targets	Amplicon size (bp)	Sequence (5' to 3')	Reference
bla$_{OXA-51, -69, -71, -75, -78}$	353	`TAATGCTTTGATCGGCCTTG TGGATTGCACTTCATCTTGG	This study
IntI 1	493	GGTCAAGGATCTGGATTTCG ACATGCGTGTAAATCATCGTC	This study
variable region of class 1 integron	variable	GGCATCCAAGCAGCAAG AAGCACTTGACCTGA	This study
bla$_{TEM-1}$	891	ATGAGTATTCAACATTTC TTGCACAGTGCCGTAAACTT	This study
bla$_{VEB-1}$	900	ATGAAAATCGTAAAAAGGATATT TTATTTATTCAAATAGTAATTCC	This study
bla$_{PER-1}$	920	ATGAATGTCATTATAAAAG TTGGGCTTAGGGCAG	This study
bla$_{VIM-1}$	500	TTTGGTCGCATATCGCAACG CCATTCAGCCAGATCGGCAT	This study
bla$_{IMP-1}$	188	GTTTATGTTCATACWTCG GGTTTAAYAAAACAACCAC	This study

Table 2 : Correlation of resistogram and integron frequency in *A. baumannii* isolates from Southwest, Nigeria

Antibiotics (N= 20)	Antibiotic Susceptibility (N= 72)		MIC (µg/mL)		Integron-positive isolates		Integron-negative isolates	
	R (%)	S (%)	50	90	R (%)	S (%)	R (%)	S (%)
PENICILLINS								
AMP	72 (100)	0 (0)						
342 456	68 (100)	0 (0)	4 (100)		0 (0)			
PIP	68 (95)	4 (5)	128	128	66 (97)	2 (3)	2 (50)	2 (50)
CEPHEMS								
KZN	68 (95)	4 (5)	32	164	67 (99)	1 (1)	1 (25)	3 (75)
FEP	51 (71)	21 (29)	16	32	50 (74)	18 (26)	1 (25)	3 (75)
CTX	55 (76)	17 (24)	32	64	52 (76)	16 (24)	3 (75)	1 (25)
CTR	72 (100)	0 (0)	64	64	68 (100)	0 (0)	4 (100)	0 (0)
CTT	45 (62)	27 (38)	32	152	45 (66)	23 (34)	0 (0)	4 (100)
CAZ	72 (100)	0 (0)	34	76	68 (100)	0 (0)	4 (100)	0 (0)
MONOBACTAMS								
ATM	68 (95)	4 (5)	64	128	30 (44)	7 (10)	1 (25)	3 (75)
CARBAPENEMS								
IMP	35 (48)	37 (52)	32	32	33 (49)	35 (51)	2 (50)	2 (50)
MEM	55 (76)	17 (24)	32	128	52 (76)	16 (24)	3 (75)	1 (25)
BETA-LACTAM/β-LACTAMASE INHIBITORS								
SAM	55 (76)	17 (24)	32	64	52 (76)	16 (24)	3 (75)	1 (25)
TZP	58 (81)	14 (19)	32	64	55 (81)	13 (19)	3 (75)	1 (25)
AMINOGLYCOSIDES								
GEN	72 (100)	0 (0)	128	128	68 (100)	0 (0)	4 (100)	0 (0)
TBR	62 (86)	10 (14)	64	128	61 (90)	7 (10)	1 (25)	3 (75)
AMK	30 (42)	42 (58)	2	2	30 (44)	41 (60)	4 (75)	0 (0)
FLUOROQUINOLONES								
CIP	35 (48)	37 (52)	4	8	33 (49)	35 (51)	2 (50)	2 (50)
LEV	55 (76)	17 (24)	16	32	52 (76)	16 (24)	3 (75)	1 (25)
LIPOPEPTIDES								
POL	30 (42)	42 (58)	2	2	30 (44)	38 (56)	0 (0)	4 (100)
COL	44 (61)	28 (39)	4	8	42 (62)	26 (38)	2 (50)	2 (50)

Footnote: R - Resistance; S - Sensitive, AMP - Ampicillin (25µg), SAM - Ampicillin-sulbactam (30µg), PIP - Piperacillin (30µg), CAZ - Ceftazidime (30µg), FEP - Cefepime (30µg), AMK - Amikacin (30µg), GEN - Gentamicin (10µg), TBR - Tobramycin (30µg), CIP - Ciprofloxacin (5µg), CTX - Cefotaxime (30µg), LEV - Levofloxacin (5µg), CTR - Ceftriaxone (30µg), CTT - Cefotetan (30µg), KZN - Cefazolin (30µg), ATM - Aztreonam (30µg), TZP - Piperacillin-tazobactam (110µg), POL - Polymyxin B (300 units), COL - Colistin (10µg), IMP - Imipenem (10µg), MEM - Meropenem (10µg), MIC - Minimum Inhibitory Concentration

Table 3: Origin and molecular characterization of 68 class 1 integron-positive *A. baumannii* from Southwest, Nigeria

Source	Isolate (N) (%)	Class 1 integrons (N=68)	Inserted Cassette(s)
Blood	1 (1.5%)	+	*accC1, aacC3, bla$_{IMP-1}$*
	3 (4.4%)	+	*aacA7, bla$_{TEM-1,}$ bla$_{IMP-1}$*
	1 (1.5%)	+	*accC1, aacA7, bla$_{TEM-1}$, bla$_{IMP-1}$*
	2 (2.9%)	+	*accC1, aacA7, bla$_{TEM-1}$, bla$_{VEB-1}$*
	2 (2.9%)	+	*accC1, aacA7, bla$_{TEM-1,}$ bla$_{VIM-1}$*
	7 (10.3%)	+	*accC1, aacC3, bla$_{TEM-1,}$ bla$_{VEB-1}$, bla$_{IMP-1}$*
	2 (2.9%)	+	*accC1, aacA7, bla$_{TEM-1}$, bla$_{PER-1}$, bla$_{IMP-1}$*
	1 (1.5%)	+	*accC1, aacA7, bla$_{TEM-1}$, bla$_{VIM-1}$, bla$_{IMP-1}$*
	2 (2.9%)	+	*accC1, aacC3, bla$_{TEM-1}$, bla$_{VIM-1}$, bla$_{IMP-1}$*
	1 (1.5%)	-	*No cassette*
Urine	3 (4.4%)	+	*accC1, aacC3, bla$_{IMP-1}$*
	1 (1.5%)	+	*aacA7, bla$_{TEM-1,}$ bla$_{IMP-1}$*
	3 (4.4%)	+	*bla$_{TEM-1}$, bla$_{VEB-1,}$ bla$_{IMP-1}$*
	1 (1.5%)	+	*accC1, aacA7, bla$_{TEM-1}$, bla$_{VEB-1}$*
	1 (1.5%)	+	*accC1, aacA7, bla$_{TEM-1,}$ bla$_{VIM-1}$*
	4 (5.9%)	+	*accC1, aacA7, bla$_{TEM-1,}$ bla$_{PER-1}$*
	2 (2.9%)	+	*accC1, aacC3, bla$_{TEM-1,}$ bla$_{VEB-1}$, bla$_{IMP-1}$*
	5 (7.4%)	+	*accC1, aacC3, bla$_{TEM-1}$, bla$_{VIM-1}$, bla$_{IMP-1}$*
	4 (5.9%)	+	*aacA7, bla$_{TEM-1,}$ bla$_{VEB-1,}$ bla$_{VIM-1}$, bla$_{IMP-1}$*

Figure 1: PCR-based interrogation of OXA-51-like carbapenemase gene intrinsic to *A. baumannii* species

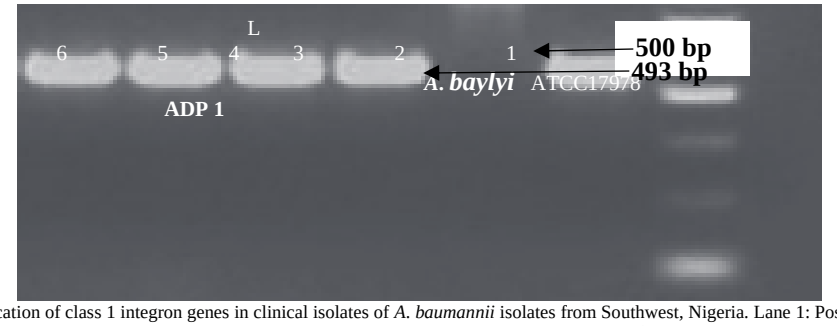

Figure 2: PCR amplification of class 1 integron genes in clinical isolates of *A. baumannii* isolates from Southwest, Nigeria. Lane 1: Positive control for class 1 integron (*A. baumannii* ATCC 17978), Lane 2: Negative control (*A. baylyi* ADP 1), Lane 3–6: Positive results for clinical *A. baumannii* harboring class 1 integron, Lane L: 100 bp molecular size marker.

4. DISCUSSION

Integrons are genetic elements that are capable of acquiring and rearranging open reading frames (ORFs) embedded in gene cassette units and converting them to functional genes by ensuring their correct expression [13]. As a MGE, it has increasingly become worrisome in clinical space on how much significance it has become in the propagation of diverse drug resistance gene cassettes from bacterial drug resistance genes in different strains resulting in multidrug resistance in bacteria [14]. Class 1 integrons are the most generic type in A. baumannii encoding genes that code for aminoglycoside resistance, Ambler A β-lactamases, oxacillinases, metallo-β-lactamases in addition to sulfonamides and antiseptics [5]. In this current study, blaOXA-51-like gene, which is intrinsic to *A. baumannii* was identified in 72 of the 107 suspected isolates collected and tested [8, 15, 16]. We also found these 72 A. baumannii strains to be MDR, resistance to multiple classes of antibiotics used in this study even though many of these drugs were not in use at a time in many of the health facilities in this region. This is not surprising as many studies in the past have demonstrated that acquisition of class 1 integrons carrying gene cassettes in *A. baumannii* can elicit MDR phenotype regardless of new therapeutic options [5, 6, 13, 14, 17]. This plausibly explains the MDR phenotype exhibited by many of our isolates against the antibiotics that have never been used in this region at a time but were tested in this study. While all the isolates (100%) were resistance to ampicillin, ceftazidime, gentamicin and ceftriaxone, high resistance profile (55, 76%) was also recorded against ampicillin-sulbactam, cefotaxime, levofloxacin, and meropenem as they appeared to be the most frequently observed drugs. This result is similar to those reported by [6, 17, 18]. Carbapenems are the drug of choice in the treatment of infection caused by *A. baumannii*, however, emergence of resistance against this class of antibiotic has become a gro wing public health concern among clinicians across the globe. In this study, while (35, 48%) of the isolates were resistance to imipenem, (55, 76%) were observed as resistance to meropenem. This observation and results corroborated the CHINET surveillance data which demonstrated that the resistance rate of *A. baumannii* to meropenem is higher than that of imipenem even though both drugs have continued to decline in their antimicrobial activity against bacterial infections [19, 20]. According to the data represented in Table 2, the high MIC50 and MIC90 values observed against carbapenems in this study, was a clear indication of reduction in the efficacy of these chemical agents due to abuse. Colistin and polymyxin B are said to be the last options for the treatment of infections caused by carbapenem-resistant *A. baumannii* [6]. In this study, it was alarming to observe a high rate of resistance for colistin (44, 61%) and polymyxin B (30, 42%), considering the fact that these agents were rarely used in this population. Although this phenomenon is rising worldwide for colistin [21], polymyxin B (42, 58%); however, in this experiment demonstrated excellent activity against many of the MDRAB tested (MIC=2µg/ml). This result is comparable with those of the other studies carried out in Iran [22, 23]. While the identification mechanism of polymyxins resistance in these isolates was beyond the scope of our study, the phenomenon could be best explained based on the modification of Lipopolysaccharide (LPS) at outer cell envelope. Again in this study, isolates resistant to aminoglycosides, such as gentamicin (72, 100%), and tobramycin (62, 86%), were high in their numbers except for amikacin (30, 42%), which overall demonstrated efficient activity (MIC=2µg/ml) just as polymyxin B. This result supports the recent finding by [24], where the lowest resistance rate was observed against amikacin and polymyxin E (colistin). On the other hand, our findings contradicts the reports by Nasr et al. and Zhu et al., where they found resistance rate to amikacin as 90% and 84.6% respectively [6]. Similar to other studies, our findings showed very low sensitivity to piperacillin, cephazolin and aztreonam (4%, 4%, 5%). In a recent study, while Gupta et al. and Nazmul et al. recorded 55% and 77.5% resistance respectively against piperacillin [25], Shakibaie et al. observed 100% resistance [25]. These results are in tandem with our finding in this current study. Aztreonam, a superior antibiotic to many of the third generation cephalosporins and more stable than carbapenemases was tested in this study and demonstrated a high prevalence of resistance (68, 95%). Even though the percentage value may seem much higher, it is on a par with a previous report from Iran, where *A. baumannii* was found to be 78.5% [23]. The result of the present study demonstrated a consistency in the trend of resistance pattern of *A. baumannii* species towards the piperacillin/tazobactam combination. Only (14, 19%) isolates were susceptible to piperacillin/tazobactam combination. In retrospect, previous studies from India and other countries have also shown low susceptibility rate of *A. baumannii* isolates to *piperacillin/tazobactam* combination [26, 27]. Again, two recent

studies from the USA [28, 29] reported 95.5% and 82.4% resistance rates respectively to *piperacillin/tazobactam* suggesting the elimination of this antibiotic from the list of therapeutic options for *A. baumannii* infections control. In this research we confirmed the presence of class1 integrons in (68, 95%) clinical isolates of MDRAB from tertiary hospitals in Southwest Nigeria. We hypothesized that there is an emergence of class 1 integron harboring gene cassettes among *A. baumannii* strains in the region as cases of multidrug resistance became evident in previous study [30]. As mentioned, integrons are commonly known for their role in the spread of antibiotic resistance, especially among Gram-negative bacteria. In addition, class 1 integrons have been confirmed globally as the most prevalent class among mobile integrons in MDRAB strains from clinical sources [6]. Other similar studies carried out in Korea (89.3%), Egypt (85%), and Taiwan (71.4%), high percentages of class 1 integrons were observed. These values are comparable to the finding in this current study as mentioned above (95%). The correlation in class 1 integrons with resistance to a number of antibiotics has been documented among multidrug-resistant pathogens [5]. In this study, class 1 integron was significantly associated with many of the antibiotics resistance, including aminoglycosides, fluoroquinolones, beta-lactam/beta-lactamase inhibitors, and lipopeptides. These results are summarized in Table 2 of this study. These relationships were not unexpected because class 1 integron carry many antibiotic resistance gene cassettes which encode resistance to a wide range of antibiotics in Acinetobacter spp. These findings was validated in [31] and comparable to [32] observation where significant association existed between class 1 integron and aminoglycoside resistance among pan-European *A. baumannii*. In another similar study, [33] showed a considerable association between carriage of integron and resistance to quinolones, new generation cephalosporins, aztreonam, and amikacin. On the contrary, our results disagree with the findings by Rasegar-Lari and coworkers as their result did not support any correlation between carriage of class1 integron and antibiotic resistance in MDR *A. baumannii*. In the same way, all the integron-negative isolates were resistance to ampicillin, ceftriaxone, ceftazidime, and gentamicin suggestive of the fact that the resistant determinant might be encoded on either the chromosome or other genetic mobile elements. According to the data in this report, many of the integron-positive strains were resistance to aminoglycosides (Table 2) corroborating the finding by [32] that many of the gene cassettes conferring resistance to aminoglycosides including aad and aac genes are part of class 1 integron architecture. This report is supported by our finding as many of the sequence results of the integron-positive isolates demonstrated the presence of aacA7, accC1, and aacC3 genes within the gene cassettes. This study also revealed significantly high resistance rate to beta-lactam antibiotics among the integron-positive isolates also suggesting the presence of beta-lactamase encoding genes (blaTEM-1, blaVEB-1, blaPER-1, blaVIM-1, and blaIMP-1) which was also detected within the gene cassettes upon sequencing (Table 3). Similar finding has also been reported in integron-positive P. aeruginosa carrying class 1 integrons from Malaysia [12]. To the best of our knowledge, this is the first study to report the detection of 17 different arrays of gene cassettes in class 1 integrons of *A. baumannii* from Nigeria. This study underlines the importance of IMP-, VIM-, VEB-, TEM-, and PER- genes carried within the gene cassettes of integron-positive *A. baumannii* to both β-lactams and β-lactam inhibitors resistance phenotypes. Since horizontal propagation of the class 1 integron-associated MBL and ESBL genes may contribute to the emergence of carbapenem-resistance in other Gram-negative bacteria, it is crucial for strict surveillance and control measures to be put in place to prevent the spread of MBL- and ESBL-producing organisms in health facilities. Routine studies similar to this should be carried out to better understand the impact of integrons on the spread of antimicrobial resistance in clinical practice.

5. CONCLUSION

The high prevalence of class 1 integron carrying carbapenemase, cephalosporinase and aminoglycoside encoding genes within the gene cassettes in these isolates is worrisome. This is because of the high potential for spreadability and the plausibility of further propagation of the highly antibiotic-resistant genes to other bacteria by horizontal gene transfer through this mobile genetic element. Hence, narrowing the therapeutic options and making treatment of cases very challenging, especially among the immunocompromised patient population. Overall, these findings are alarming and call for strict control mechanisms to prevent the spread of these genes in bacteria communities.

Acknowledgment

The authors wish to thank the staff of medical microbiology and parasitological departments of various medical facilities used in this study for their generous support in providing isolates. This work was supported in part by a grant from the NSF MCB 1244227 of the Peters Laboratory at department of microbiology, Cornell University, Ithaca, New York.

Conflict of Interest

The authors declare that the research was conducted in the absence of any potential conflict of interest.

Contribution of the Authors

WE, BA, and JP conceived and designed the experiments, WE, BA and JP performed the experiments
WE wrote the manuscript

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