

Research Article

Molecular Detection of Carbapenemase-Encoding Genes in Multidrug-Resistant *Acinetobacter baumannii* Clinical Isolates in South Africa

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Introduction. Carbapenem-resistant *Acinetobacter baumannii* has been responsible for an increasing number of hospital-acquired infections globally. The study investigated the prevalence of carbapenemase-encoding genes in clinical multidrug-resistant *A. baumannii* strains. **Materials and Methods.** A total of 100 nonduplicate multidrug-resistant *A. baumannii* strains were cultured from clinical samples obtained from healthcare facilities in the O. R. Tambo district. The strains were confirmed by detecting the intrinsic *bla*_{OXA-51-like} gene. Antimicrobial susceptibility testing was performed by VITEK[®] 2 and autoSCAN-4 systems. The MIC of imipenem and meropenem was rechecked by E-test. Colistin MIC was confirmed by the broth microdilution method. Real-time PCR was performed to investigate the presence of carbapenemase-encoding genes. **Results.** Most strains showed high resistance rates (>80%) to the antibiotics tested. Resistance to amikacin, tetracycline, and tigecycline were 50%, 64%, and 48%, respectively. All strains were fully susceptible to colistin. The *bla*_{OXA-51-like} was detected in all strains whilst *bla*_{OXA-23-like}, *bla*_{OXA-58-like}, *bla*_{OXA-24-like}, *bla*_{IMP-1}, *bla*_{VIM}, and *bla*_{NDM-1} were found in 70%, 8%, 5%, 4%, 3%, and 2% of strains, respectively. None of the tested strains harboured the genes *bla*_{SIM} and *bla*_{AmpC}. The coexistence of *bla*_{OXA-23-like} and *bla*_{IMP-1} or *bla*_{OXA-58-like} was detected in 1% and 2% strains, respectively. A distinct feature of our findings was the coharbouring of the genes *bla*_{OXA-23-like}, *bla*_{OXA-58-like}, and *bla*_{IMP-1} in 2% strains, and this is the first report in the Eastern Cape Province, South Africa. The *intI1* was carried in 80% of tested strains whilst *ISAbal1/bla*_{OXA-51-like} and *ISAbal1/bla*_{OXA-23-like} were detected in 15% and 40% of the strains, respectively. The detection of *bla*_{OXA-23-like}, *ISAbal1/bla*_{OXA-51-like}, *ISAbal1/bla*_{OXA-23-like}, and *bla*_{OXA-23-like}, *bla*_{OXA-58-like}, and *bla*_{IMP-1} carbapenemases in strains had a significant effect on both imipenem and meropenem MICs. **Conclusions.** Results showed a high level of oxacillinases producing *A. baumannii* circulating in our study setting, highlighting the need for local molecular surveillance to inform appropriate management and prevention strategies.

1. Introduction

Acinetobacter baumannii, an opportunistic Gram-negative bacterium, has become a predominant cause of healthcare-associated infections worldwide [1]. This microorganism has emerged to become a major concern for clinicians worldwide causing infections such as septicaemia, meningitis, endocarditis, ventilator associated pneumonia, burns, urinary tract infections, surgical site infections, and wound infections in hospitals due to its intrinsic as well as its remarkable propensity to rapidly acquire resistance determinants to a wide range of antibacterial agents [2, 3]. The World Health Organization (WHO) lists *A. baumannii* among critical antibiotic-resistant “priority pathogens,” highlighting its serious threats to public health [4]. Risk factors for *A. baumannii* infections include immune suppression, burns, trauma, mechanical ventilation, catheters, invasive medical procedures, previous antibiotic treatment, an extended hospital stay (>90 days), and underlying diseases such as diabetes [5].

Carbapenem has been the drug of choice used to treat MDR *A. baumannii* infections over the past decade because they are highly efficacious and have low toxicity [6]. However, the resistance rate against carbapenem has increased dramatically due to its frequent application in recent years and is mainly mediated by the production of carbapenem-hydrolysing enzymes [7]. The most common mechanism of carbapenem resistance in *A. baumannii* is the production of β -lactamases, including enzymes of Ambler Classes D OXA-type carbapenemases consisting of *bla*_{OXA-23-like} (OXA-23, OXA-27 and OXA-49), *bla*_{OXA-40-like} (OXA-24/40, OXA-25, OXA-26 and OXA-72), *bla*_{OXA-58-like} (OXA-58 and OXA-96), *bla*_{OXA-143-like} and *bla*_{OXA-51-like} enzymes, and B Metallo- β -lactamases (MBLs) mainly *bla*_{VIM}, *bla*_{IMP-1}, *bla*_{NDM-1}, *bla*_{SPM}, *bla*_{GIM}, and *bla*_{SIM} types [8]. Among the multiple types of MBL genes described throughout the world, the *bla*_{IMP-1}, *bla*_{VIM}, and *bla*_{SIM} types are the most common [9]. The upstream of OXA-type class D carbapenemases in *Acinetobacter* is often associated with insertion sequence (IS), of which *ISAbal* is the most commonly detected. *ISAbal* and other IS may modulate the expression and transfer of OXA-type carbapenemase genes [10]. Integrons have been demonstrated to play a major role in the dissemination of multidrug-resistance among Gram-negative bacteria through the capture and expression of resistant genes embedded within cassettes [11].

Studies in Africa and the Gulf Cooperation Council (e.g., Saudi Arabia) have reported increasing carbapenem resistance among *A. baumannii* isolates [12–14]. Since nosocomial *A. baumannii* infection has generated an abundance of therapeutic obstacles for the treatment of hospitalized patients in South Africa, as well as other countries, the knowledge regarding the prevalence level of antibiotic resistance genes and the resistance pattern of this bacterium to the initial antibiotics is required to control, prevent, and cure *A. baumannii* driven infections. Therefore, the aim of the current study was to investigate the rates of carbapenem resistance as well as to detect the frequency of carbapenemase-encoding genes in MDR *A. baumannii* isolates

obtained from hospitalized patients receiving treatment in healthcare facilities in O.R. Tambo district in the rural Eastern Cape Province of South Africa.

2. Materials and Methods

2.1. Study Design and Settings. This was a prospective cross-sectional study conducted at healthcare facilities in the OR Tambo district municipality, Eastern Cape, South Africa. The municipality is formed by five local municipalities (King Sabata Dalindyebo, Nyandeni, Mhlontlo, Port St Johns, and Ingquza Hill) with an estimated total population of 1,760,389. Healthcare services are delivered by 1 Academic Central Hospital, 1 Regional Hospital, 12 District Hospitals; 11 Community Health Centers, 49 clinics, 52 Health Posts, and 15 mobiles. Patients’ clinical samples collected from all these facilities are sent for culture and susceptibility testing in the Department of Medical Microbiology at the National Health Laboratory Services (NHLS), located in the Nelson Mandela Central Hospital in Mthatha, Eastern Cape. Clinical samples from those various hospitals and clinics were sent as part of the patients’ routine standard of care.

2.2. Data Collection, Bacterial Identification, and Storage of *A. baumannii* Isolates. From August 2016 to July 2017, a total of 100 nonduplicate nonconsecutive MDR *A. baumannii* strains were collected arbitrarily from blood, pus, urine, sputa, catheter tips, tracheal aspirates, and cerebrospinal fluid of patients from the Microbiology Laboratory at the National Health Laboratory Services (NHLS) at NMAH, Mthatha (see Supplementary Materials). The strains were preliminarily speciated phenotypically using standard microbiological procedures: the VITEK® 2 automated machine with Gram-negative identification (GNI) cards (BioMérieux, France) and the MicroScan autoSCAN-4 System (Dade Behring Inc., Deerfield, IL) with Gram-negative ID type 2 panel. A high percentage ($\leq 95\%$) was utilized as the acceptance criterion for identification by the MicroScan autoSCAN system. Suspected colonies were also further verified using the *Acinetobacter* specific primer set Ac436F and Ac676r to amplify the 16S rRNA gene. Confirmation of *A. baumannii* strains was carried out by polymerase chain reaction analysis of the presence of inherent *bla*_{OXA-51-like} genes. All of the strains were stored at -80°C in skim milk with 15% glycerol until further use.

2.3. Antimicrobial Susceptibility Testing. A total of 18 clinically relevant antibiotics were tested using MicroScan autoSCAN-4 Gram-negative MIC panel, and results were analyzed and interpreted according to the recommended clinical breakpoints given in the clinical and laboratory standard institutes (CLSI) guidelines v27 [15]. These antibiotics were amikacin (AMK), ampicillin/sulbactam (AMS), cefepime (FEP), cefotaxime (CTX), ceftazidime (CAZ), ceftriaxone (CRO), ciprofloxacin (CIP), gentamicin (GEN), imipenem (IMP), meropenem (MEM), levofloxacin (LVX), tetracycline (TEC), tobramycin (TOB), trimethoprim/sulfamethoxazole (SXT), piperacillin/tazobactam (TZP),

TABLE 1: Quality control (QC) organisms used for the detection of resistant genes in this study.

Organism	ATCC or NCTC number	Resistant gene(s)
<i>A. baumannii</i>	NCTC 13302	OXA-26 (OXA-24-like; OXA-51-like)
<i>A. baumannii</i>	NCTC 13305	OXA-58
<i>Klebsiella pneumoniae</i>	ATCC-BAA 2146	NDM
<i>A. baumannii</i>	NCTC 13301	OXA-23
<i>A. baumannii</i>	NCTC 13303	OXA-26 (OXA-51-like)
<i>A. baumannii</i>	NCTC 13304	OXA-27 (OXA-51-like)
<i>Enterobacter cloacae</i>	NCTC 13405	AmpC
<i>Pseudomonas aeruginosa</i>	NSCC stock	VIM
<i>A. baumannii</i>	ATCC 19606	OXA-51
<i>Escherichia coli</i>	NCTC 13476	IMP-1
<i>Escherichia coli</i>	Se 131 (accession number: AJ238350)	<i>intI1</i>
<i>Pseudomonas aeruginosa</i>	ATCC 27853	OXA-24-like; OXA-51-like
<i>Klebsiella pneumoniae</i>	ATCC 8303	SIM-1
<i>A. baumannii</i>	AYE strain	AmpC, ISAb1, OXA-51-like

piperacillin (PRL), colistin (CST), and tigecycline (TGC), all from Beckman Coulter, South Africa. Determination of MICs to imipenem and meropenem rechecked by E-test minimum inhibitory concentration method using E-test strips as per the manufacturer's guidelines (BioMérieux, France) on Mueller-Hinton agar plates and CLSI breakpoints (strains displaying MICs ≥ 8 $\mu\text{g}/\text{mL}$ for imipenem and meropenem were considered resistant). Quality control strains used in antimicrobial susceptibility testing are *Escherichia coli* ATCC #25922, *Staphylococcus aureus* ATCC 29213, and *Pseudomonas aeruginosa* ATCC #27853 [16]. Colistin susceptibility testing was confirmed using the broth microdilution method according to the CLSI and by ComASP™ Colistin, Liofilchem® (REF. 75001), according to the manufacturer's recommendations. *Escherichia coli* NCTC 13846 (*mcr-1* positive) was used as a positive control for ComASP™ Colistin test. The United States Food and Drug Administration (US-FDA) approved breakpoints for members of the family *Enterobacteriaceae* were used as interpretative criteria for tigecycline (<http://www.fda.org.uk/sitemap.aspx>). Nonsusceptibility was defined as a combination of resistance and intermediate resistance. MDR *A. baumannii* isolates were defined as acquired nonsusceptibility to at least one agent in three or more antimicrobial categories [17]. All results were within quality control ranges.

2.4. Detection of Carbapenem-Resistant Genes. Genomic DNA from an overnight culture on tryptic soy broth of *A. baumannii* was extracted using the MagNaPure Compact® nucleic acid isolation kit I (ref. no. 03730964001, Roche Diagnostics, Mannheim, Germany) according to the manufacturer's protocol. A final elution volume of 200 μL of pure *A. baumannii* DNA was used. 2 μL of the DNA extract was used for each PCR analysis. The extracted DNA was stored at -80°C until required for further analysis. All MDR isolates were systematically screened by LightCycler 2.0 real-time PCR (Roche Diagnostics) instrument targeting some of the epidemiologically most relevant resistant genes. The presence of the genes encoding Amber class D Serine-Carbapenemase genes (*bla*_{OXA-51-like}, *bla*_{OXA-23-like}, *bla*_{OXA-24-like},

and *bla*_{OXA-58-like}), Ambler class B Metallo- β -lactamases (MBLs) (*bla*_{IMP-1}, *bla*_{VIM}, *bla*_{SIM}, and *bla*_{NDM-1}), and Ambler class C *bla*_{AmpC} were assessed. MDR *A. baumannii* strains were subsequently screened for the presence or absence of IS element ISAb1 [18] and class 1 integrons by real-time PCR [19]. To determine the overexpression of *bla*_{OXA-23-like} and *bla*_{OXA-51-like} families due to the upstream adjacent ISAb1, PCR was firstly performed for ISAb1, and in cases where *bla*_{OXA-like} is present, a combination of forward primer of ISAb1 and reverse primer of *bla*_{OXA-like} family was further used. Appropriate positive and negative controls were run simultaneously (Table 1). The primers used for PCR amplification of the carbapenemase genes are listed in Table 2.

2.5. Statistical Analyses. Data analyses were performed using the Statistical Package for Social Sciences Statistics for Windows software package version 23.0 (IBM, Armonk, NY). Results were expressed using frequency and percentages for qualitative variables and as mean (standard deviation) or median (interquartile range: IQR) for quantitative variables. The association between *A. baumannii* and drug resistance was analyzed by the chi-square test. All *P* values were two-sided. A *P*-value <0.05 was considered statistically significant.

2.6. Ethical Considerations. Ethical approval for this project was obtained from the Walter Sisulu University Research Ethics and Biosafety Committee (Reference number: 019/2016). No written informed consent was required because each MDR *A. baumannii* isolate obtained was delinked with the patient's personal information. Also authors had no direct interactions with patients from whom these isolates were isolated.

3. Results

3.1. Demographic Characteristics of the Study Population. The demographic characteristics of the patients with *A. baumannii* infection analyzed in our study showed a slight female preponderance of 47% males versus 53% females). The age of the overall patients ranged between 1 day and 91

TABLE 2: List of primers used for PCR amplification of resistant genes.

Gene	Oligonucleotide sequence	Fragment size (bp)	Location	References
16SrRNA	F: 5'TTT AAG CGA GGA GGA GG3' R: 5'ATT CTA CCA TCC TCT CCC3'	240	16SrRNA	[20]
OXA-23-like	F: 5'GATCGGATTGGAGAACCAGA3' R: 5'ATTTCTGACCGCATTTCAT3'	501	<i>bla</i> _{OXA-23}	[21]
OXA-24-like	F: 5'GGTTAGTTG GCC CCC TTA AA3' R: 5'AGTTGAGCGAAAAGGGGATT3'	246	<i>bla</i> _{OXA-24}	[21]
OXA-51-like	F: 5'TAATGCTTT GATCGG CCT TG3' R: 5'TGGATTGCACTT CAT CTT GG3'	353	<i>bla</i> _{OXA-51}	[21]
OXA-58-like	F: 5'AAGTATTGGGGCTTGTGCTG-3' R: 5'CCCCTCTGCGCTCTACATAC3'	599	<i>bla</i> _{OXA-58}	[21]
IMP-1	F: 5'GATGGTATGGTGGCTCTTGT3' R: 5'TTAATTTGCCGACTTAGGC3'	448	<i>Bla</i> _{IMP}	[22]
NDM-1	F: 5'ATTAGCCGCTGCATTGAT3' R: 5'CATGTCGAGATAGGAAGTG3'	154	<i>bla</i> _{NDM}	[23]
VIM-like	F: 5'ACTCACCCCATGGAGTTT3' R: 5'ACGACTGAGCGATTTGTGTG3'	815	<i>bla</i> _{VIM}	[24]
SIM-1-like	F: 5'TAATGCTTT GATCGG CCT TG3' R: 5'TGGATTGCACTT CAT CTT GG3'	353	<i>bla</i> _{SIM}	[21]
AmpC	F: ACAGAGGAGCTAATCATGCC R: GTTCTTTTAAACCATATACC	1243	<i>bla</i> _{AmpC}	[25]
<i>ISAbal</i>	F: 5-CACGAATGCAGAAGTTG-3 R: 5-CGACGAATACTATGACAC-3	599	<i>ISAbal</i>	[18]
<i>intI1</i>	F: 5'CAG TGG ACA TAA GCC TGT TC3' R: 5'CCC GAC GCA TAG ACT GTA3'	160	Integrase gene	[19]

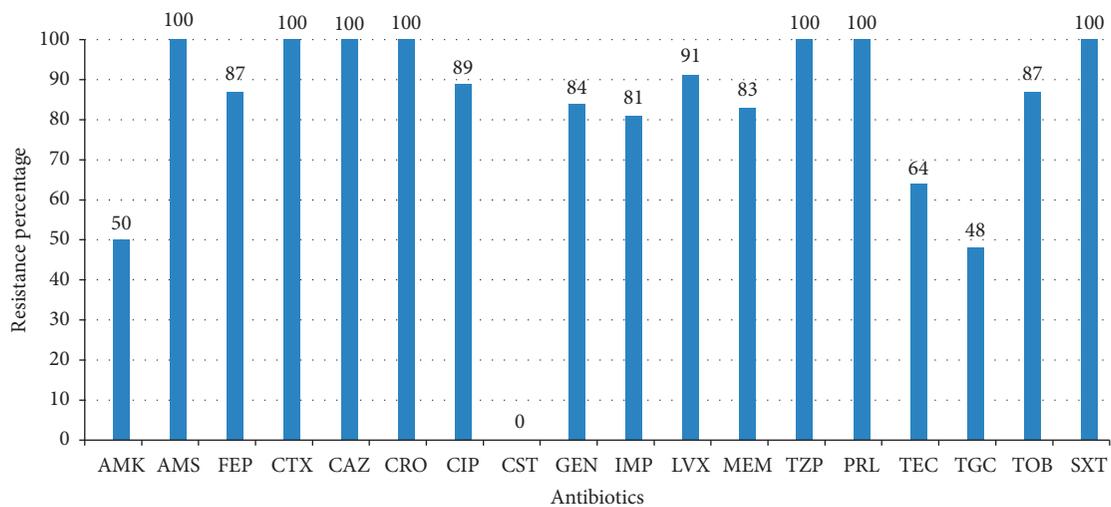


FIGURE 1: Antimicrobial resistance profile of 100 *A. baumannii* isolates. IMP: imipenem; MEM: meropenem; FEP: cefepime; CAZ: ceftazidime; CTX: cefotaxime; CRO: ceftriaxone; AMS: ampicillin/sulbactam; PRL: piperacillin; TZP: piperacillin/tazobactam; AMK: amikacin; TOB: tobramycin; GEN: gentamicin; TEC: tetracycline; CIP: ciprofloxacin; LVX: levofloxacin; SXT: trimethoprim-sulfamethoxazole; TGC: tigecycline; CST: colistin.

years (mean 44.4 years, SD \pm 36.3 years). *A. baumannii* were isolated highest from tracheal aspirate (32%) and lowest from cerebrospinal fluid (2%). *A. baumannii* was most prevalent in the ICU wards 41% followed by neonatal ward 22%, surgical ward 15%, medical ward 13%, and general ward 9%. *A. baumannii* was isolated from various age groups but was the most prevalent in age group 0–9 years (Supplementary Materials).

3.2. Antimicrobial Susceptibility Testing. Generally, the results for antimicrobial susceptibility testing for the 100 MDR *A. baumannii* revealed that *A. baumannii* strains showed a high resistance rate to most of the commonly used antibiotics tested. Overall, \geq 81% of all the strains were resistant to extended-spectrum cephalosporins, fluoroquinolones, and carbapenems antibiotics. Resistance to aminoglycosides ranges from 50% to 87% whilst 48% of strains were resistant

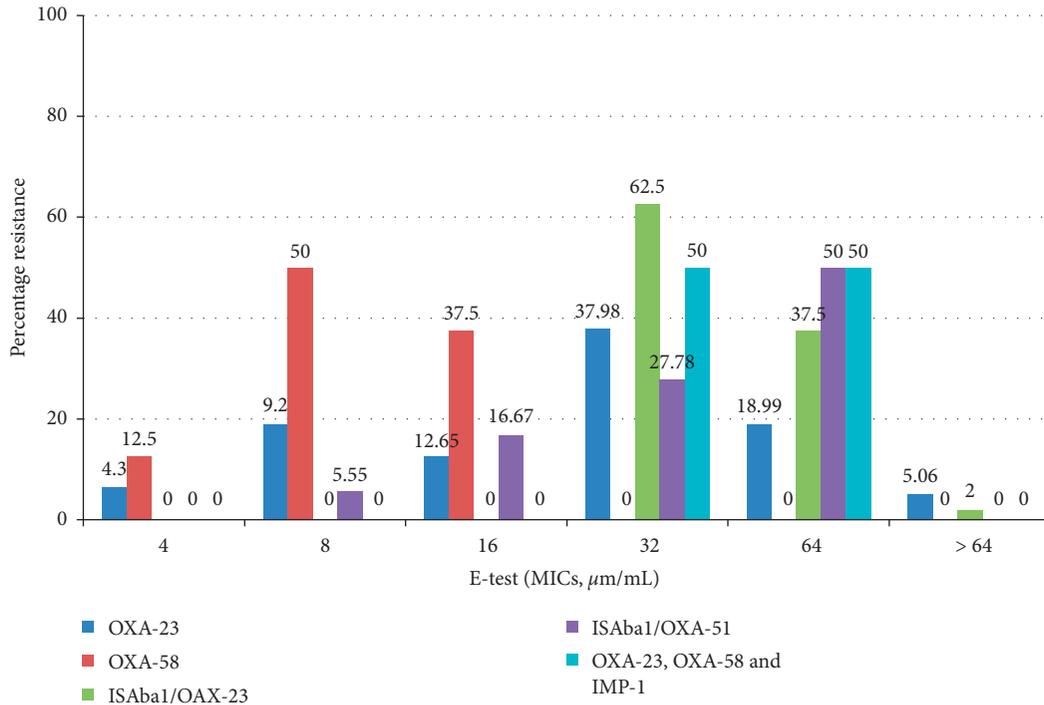


FIGURE 2: Distribution of imipenem MICs in 100 CRAB isolates with the *bla*_{OXA-23-like}, *bla*_{OXA-58-like}, *ISAbal/bla*_{OXA-23-like}, *ISAbal/bla*_{OXA-51-like} and *bla*_{OXA-23-like}, *bla*_{OXA-58-like} and *bla*_{IMP-1} genes.

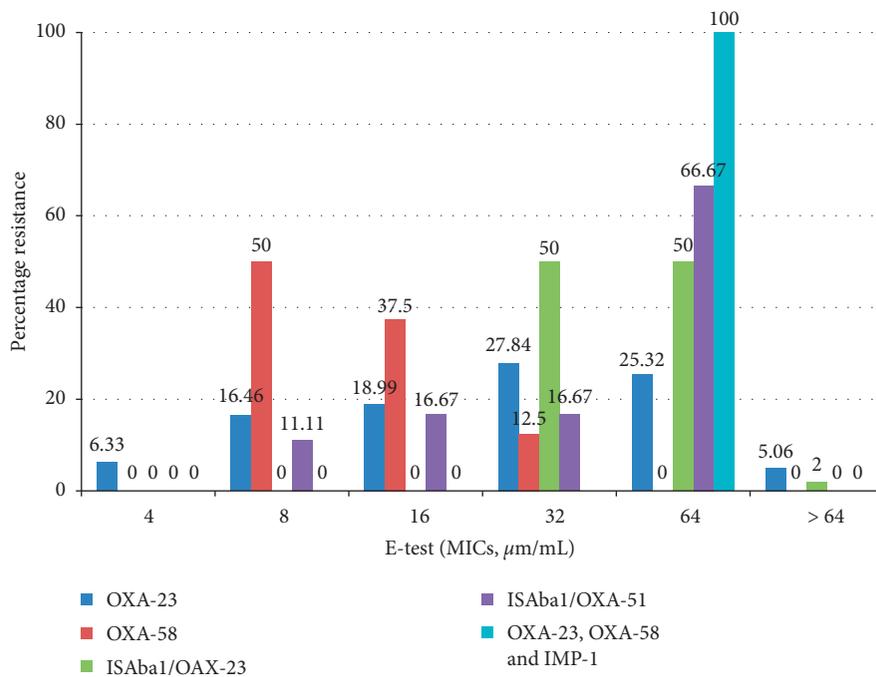


FIGURE 3: Distribution of meropenem MICs in 100 CRAB isolates with the *bla*_{OXA-23-like}, *bla*_{OXA-58-like}, *ISAbal/bla*_{OXA-23-like}, *ISAbal/bla*_{OXA-51-like} and *bla*_{OXA-23-like}, *bla*_{OXA-58-like} and *bla*_{IMP-1} genes.

to tigecycline. All strains were susceptible to colistin. Findings of the antimicrobial susceptibility patterns of all *A. baumannii* strains in this study are shown in Figure 1.

3.3. PCR Results of Carbapenemase-Encoding Genes. Results showed that all of the strains investigated in this study had the intrinsic *bla*_{OXA-51-like} gene. Among them, 70% were positive for the *bla*_{OXA-23-like}, 8% were positive for

TABLE 3: Percentage distribution of carbapenemases-encoding genes in 100 CRAB strains.

Carbapenemases-encoding genes	Number (%)
<i>bla</i> _{OXA-51-like} only	0
<i>bla</i> _{OXA-51-like} and <i>bla</i> _{OXA-23-like}	23
<i>bla</i> _{OXA-51-like} and <i>bla</i> _{OXA-58-like}	4
<i>bla</i> _{OXA-51-like} and <i>bla</i> _{OXA-24-like}	5
<i>bla</i> _{OXA-51-like} and <i>bla</i> _{IMP-1}	1
<i>bla</i> _{OXA-51-like} and <i>bla</i> _{VIM}	3
<i>bla</i> _{OXA-51-like} and <i>bla</i> _{NDM-1}	2
IS <i>Aba1</i> / <i>bla</i> _{OXA-51-like}	15
<i>bla</i> _{OXA-51-like} and IS <i>Aba1</i> / <i>bla</i> _{OXA23-like}	40
<i>bla</i> _{OXA-51-like} and <i>bla</i> _{OXA23-like} and <i>bla</i> _{IMP-1}	1
<i>bla</i> _{OXA-51-like} and <i>bla</i> _{OXA-23-like} and <i>bla</i> _{OXA-58-like}	2
<i>bla</i> _{OXA-51-like} and <i>bla</i> _{OXA-23-like} and <i>bla</i> _{OXA-58-like} and <i>bla</i> _{IMP-1}	2
IS <i>Aba1</i> / <i>bla</i> _{OXA-51-like} and IS <i>Aba1</i> / <i>bla</i> _{OXA-23-like}	2
<i>intI1</i> only	0

*bla*_{OXA-58-like}, and 5% positive for *bla*_{OXA-24-like}. For the MBLs, 4% strains were positive for *bla*_{IMP-1}, 3% carried *bla*_{VIM}, 2% carried *bla*_{NDM-1}, and none of the tested isolates harboured the *bla*_{SIM}. Alleles encoding *bla*_{AmpC} enzymes were not detected in any strain. The IS*Aba1* was located upstream the *bla*_{OXA-51-like} and the *bla*_{OXA-23-like} carrying strains 15% and 40%, respectively. Some of the strains coharboured different carbapenemase genes mostly yielding very broad-spectrum antibiotic resistance profiles to imipenem and meropenem. Both strains possessing the IS*Aba1*/*bla*_{OXA-23-like} and *bla*_{OXA-23-like}, *bla*_{OXA-58-like}, and *bla*_{IMP-1} genes had the highest carbapenem resistance with these strains having imipenem MICs of 32 µg/mL or more, and having meropenem MICs of 32 µg/mL or more. This was followed by the strains carrying the *bla*_{OXA-23-like} gene with 62% having imipenem MICs of 32 µg/mL or more, and 58% having meropenem MICs of 32 µg/mL or more (Figures 2 and 3). Screening for the presence of integron integrase genes showed the presence of the class 1 integrase gene in 80% MDR *A. baumannii* strains (Table 3).

4. Discussion

Understanding the fundamental mechanism that underlines *A. baumannii* infections, including original source of infecting strains, resistance patterns, and genes responsible for the development of resistance is critical for the development of appropriate infection control measures and more efficient treatment strategies [5]. Data from countries that lack routine surveillance, including South Africa, are necessary in order to understand and prevent the dissemination of carbapenem-resistant strains of species that are in epidemiological expansion, like *A. baumannii*.

Results from the present study revealed a 100% resistant rate of MDR *A. baumannii* to third-generation cephalosporins (ceftazidime, cefotaxime, and ceftriaxone). Resistance to cefepime was 87%. All strains were resistant to piperacillin, piperacillin/tazobactam, trimethoprim/sulfamethoxazole, and ampicillin/sulbactam. The percentage rates of resistance to remaining antibiotics range from 48% to 89% with the exception of colistin which had no resistant strain detected.

Carbapenem resistance of *A. baumannii* is a major concern since it is the drug of choice in the treatment of *A. baumannii* infections [26]. The results showed that the majority of *A. baumannii* strains tested were resistant to both imipenem 81% and meropenem 83%. Lowings et al. found all MDR *A. baumannii* isolates showed high level resistance (cefotaxime 100%; cefotaxime 100%; ceftazidime 89%; cefepime 90%; imipenem 86%; and meropenem 86%) in a study conducted at the Department of Medical Microbiology, Prins Hof Campus, University of Pretoria/National Health Laboratory Service (NHLS) in 2013 [27]. A previous study by Kock et al. in the same setting showed that the overall percentage of resistance to the cephalosporin antibiotics was cefepime (62%) and ceftazidime (45%) [28]. A report on antimicrobial resistance surveillance from sentinel public hospitals in South Africa in 2013 showed a resistance rate of 80% to cefepime and 73% to ceftazidime [29]. Comparing the results of the present study with these earlier studies indicates that the resistant rate of MDR *A. baumannii* to various antibiotics has not decreased. High levels of resistance to third and fourth generation cephalosporins in this study indicated that cephalosporins are no longer efficacious in the treatment of MDR *A. baumannii* isolated in South Africa. The full susceptibility of all MDR *A. baumannii* to colistin in this study indicated that colistin is still a better option of the drug for the treatment of infections caused by *A. baumannii* in South African hospitals. However, the issue of nephrotoxicity, neurotoxicity, colistin-resistance, and heteroresistance shown by colistin monotherapy is a challenge in the management of this infection [30]. The high resistance rate to other antibiotic categories may probably be due to the heavy selection pressure from overuse of these antibiotics.

Although *A. baumannii* infections are observed in all body parts, they are mostly observed in the respiratory system. Our study showed that *A. baumannii* isolates were predominantly found in the respiratory samples (tracheal aspirate) and the most frequent setting for MDR *A. baumannii* was found in the ICUs (41%). ICUs (Neonatal and general intensive care) are settings where most patients have indwelling devices and invasive procedures. Guckan et al. and Lowings et al. isolated 85.2% and 12% *A. baumannii* isolates, respectively, from ICUs [27, 31].

Molecular detection of carbapenemase-encoding genes has shown that the production of OXA-type carbapenemases is predominant in *A. baumannii* [30, 32]. The chromosomally encoded *bla*_{OXA-51-like} gene was detected in all the strains supporting those of other studies demonstrating that the detection of the *bla*_{OXA-51-like} gene can be used as a supplementary tool to identify *A. baumannii* at the species level together with additional methods [33]. It was observed that the expression of *bla*_{OXA-23-like} was the second dominant carbapenem resistance mechanism (70%) in MDR *A. baumannii* among the strains investigated aside the intrinsic *bla*_{OXA-51-like} gene. Its prevalence among isolates was higher than this in previous reports in South Africa. Its prevalence among strains was higher in this study than in the previous report (59%) by Kock et al., whilst Lowings et al. reported a prevalence of 77%. The *bla*_{OXA-23-like} gene is considered a virulence biomarker and a significant cause of carbapenem resistance worldwide [34]. This gene was first reported in 1985 in Scotland and since then outbreaks of OXA-23 carbapenemase-producing *A. baumannii* have been reported all over the world and is a dominant genetic determinant in the Asia subcontinent [35]. In Latin America, Brazil was the first country to report OXA-23-like-producing isolates in 2003, followed by Colombia, Argentina, and Mexico some years later [36].

This study also detected *bla*_{OXA-24-like} and *bla*_{OXA-58-like} genes in 5% and 8% isolates, respectively. Although the acquired *bla*_{OXA-23-like} is the dominant genetic determinant reported in most studies in South Africa, the detection of *bla*_{OXA-58-like} and *bla*_{OXA-24-like} genes in our study shows that these genes are becoming significant in carbapenem resistance in *A. baumannii* and may be responsible for the increase in carbapenem resistance in *A. baumannii*. It also suggested that these oxacillinases may locally spread in Mthatha, South Africa. These findings concur with a study by Ji et al. in China, who reported that *bla*_{OXA-23-like} are the most frequent carbapenem-resistant *A. baumannii*, whereas *bla*_{OXA-24-like} and *bla*_{OXA-58-like} genes pose potential threats of hospital outbreaks of multidrug-resistant *A. baumannii* [37]. For class B metallo- β -lactamases, we found that 4%, 3%, and 2% of the isolates had *bla*_{IMP-1}, *bla*_{VIM}, and *bla*_{NDM-1}, respectively, and no isolate was found to produce *bla*_{SIM} gene. None of the clinical *A. baumannii* isolates harboured *bla*_{AmpC}. IMP (primarily detected in South Korea) and NDM-1 (recently reported in many countries, such as India, Israel, Egypt, Germany, Spain, Switzerland, the United Arab Emirates, and China) are the two MBL genes most frequently detected in *A. baumannii* isolates [7, 33]. The *bla*_{NDM-1} producing bacteria are mainly reported in Enterobacteriaceae [38]. This gene was first identified from *Klebsiella pneumoniae* and *E. coli* strains from a patient previously hospitalized in India and has recently emerged in *A. baumannii* isolates [32]. Taking into consideration the diversity of the South African community (prominence of Asian and European communities where *bla*_{NDM-1} have been reported) and the frequency of travels made by individuals between these countries, it is believed that the spread of *bla*_{NDM-1} was likely to occur mostly through *A. baumannii* rather than Enterobacteriaceae. However, these findings need further investigation.

Interestingly, this study showed that some isolates were found to coharbour carbapenemase genes. Among these isolates include the coharbouring of carbapenemase genes *bla*_{OXA-23-like} and *bla*_{IMP-1} in 1% isolate, *bla*_{OXA-23-like} and *bla*_{OXA-58-like} in 2% isolates, and *bla*_{OXA-23-like}, *bla*_{OXA-58-like}, and *bla*_{IMP-1} in 2% isolates. To the best of our knowledge, this is the first report of the coharbouring of *bla*_{OXA-23-like}, *bla*_{OXA-58-like}, and *bla*_{IMP-1} in the Eastern Cape province of South Africa. We believe that coharbouring of these resistance genes is due to the presence of different genes in the same strain. This warrants the use of molecular typing methods to determine the clonal types of the isolates. The coharbouring of these major resistance mechanisms seriously limits therapeutic options, raising concerns regarding their transmission to other organisms. For example, we found that all isolates that coharboured *bla*_{OXA-23-like}, *bla*_{OXA-58-like}, and *bla*_{IMP-1} had imipenem MICs of 32 μ g/mL or more and meropenem MICs of 64 μ g/mL.

The study showed that carbapenem susceptibility phenotypes of our *A. baumannii* isolates clearly correlated with the presence or absence, in the genomes, of IS*Aba1*-linked *bla*_{OXA-23-like} or *bla*_{OXA-51-like} genes (Figures 2 and 3). For instance, the 100% MDR isolates with IS*Aba1* elements upstream of the *bla*_{OXA-23-like} had imipenem and meropenem MICs 32 μ g/mL or more, whereas 83.34 and 77.78% of MDR isolates with IS*Aba1* elements upstream of the *bla*_{OXA-51-like} had imipenem and meropenem MICs 32 μ g/mL, respectively. Presumably, the IS*Aba1* element likely provides exogenous promoter functions to overexpress the linked genes. Evidence showed that carbapenem resistance mediated by overexpression of *bla*_{OXA-like} families due to upstream IS elements is more prevalent [39–42]. Our data are consistent with the findings of previous studies in that acquisition IS*Aba1* are the main mechanism of carbapenem resistance among *A. baumannii* by acting as a promoter to increase oxacillinase expression [9, 39, 43]. However, there are limited studies in South Africa as most of the studies evaluated the prevalence of OXA-like families in detail. There is, therefore, the need for further investigations to find the contribution of other IS elements (IS*Aba2*, IS*Aba3*) upstream to OXA-like families in different parts of the country. The results suggested that class 1 integrons did not contribute resistance to carbapenems since its presence did not have an effect on the MICs of carbapenems.

4.1. Limitations. The study has some limitations. Firstly, the study was conducted in a single centre, NMAH in the Tambo district municipality, so our findings may not be generalized to other settings. Secondly, the study did not include molecular typing epidemiological study reference methods such as PFGE, MLST, REP-PCR, etc. which would have better evaluated the dissemination of a carbapenemase or some clones to understand CRAB isolates in the clinical setting. These studies are necessary to help highlight the need for area-specific surveillance to inform appropriate management.

5. Conclusion

In conclusion, the results of this study provide direct evidence of the spread of carbapenemase-encoding genes

especially *bla*_{OXA-23-like} in our setting. High presence of the *ISAbal* element upstream of the *bla*_{OXA-51-like} and *bla*_{OXA-23-like} increases expression of these carbapenemases and presents an emerging threat in this study setting. The results also provided clinically relevant insights into antimicrobial susceptibility and knowledge of the prevalent resistance mechanism among CRAB. This information will help in preventing the development of MDR *A. baumannii* in hospitals and also helps clinicians in the empirical treatment of patients. To prevent the development of Carbapenem-resistant *A. baumannii*, routine implementation of simple and cost-effective screening methods to detect carbapenemase production in hospitals is crucial in enhancing infection control practices and finally establishing antimicrobial stewardship programs. Contribution and interplay of different mechanisms to the rapid increase in antibiotic resistance of *A. baumannii* in South Africa warrant further investigation.

Data Availability

All data generated or analyzed during this study are included in this published article (and its supplementary information file).

Ethical Approval

Ethical approval for this project was obtained from the Walter Sisulu University Research Ethics Committee (Human) (reference number: 019/2016).

Conflicts of Interest

The authors declare that there are no conflicts of interest regarding the publication of this paper.

Authors' Contributions

Songca S. P., Apalata T., Vasaikar S. D., and Anane Y. A. conceived and designed the experiments. Vasaikar S. D. and Anane Y. A. performed the experiments. Songca S. P., Apalata T., Vasaikar S. D., Anane Y. A., and Okuthe G. E. analysed the data. Songca S. P., Okuthe G. E., and Vasaikar S. D. contributed reagents/materials/analysis tools. Songca S. P., Apalata T., Vasaikar S. D., and Anane Y. A. analysed data and wrote the manuscript. All authors read and approved the final manuscript.

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Supplementary Materials

Details of the genotypic features of *Acinetobacter baumannii* isolates consisting of genes encoding Amber class D serine-carbapenemase genes (*bla*_{OXA-51-like}, *bla*_{OXA-23-like}, *bla*_{OXA-24-like}, and *bla*_{OXA-58-like}), Amber class B metallo- β -lactamases (*bla*_{IMP-1}, *bla*_{VIM}, *bla*_{SIM}, and *bla*_{NDM-1}) and Amber class C *bla*_{AmpC}. Age range, gender, ward, and isolate source are also indicated. (*Supplementary Materials*)

References

- [1] A. Y. Peleg, H. Seifert, and D. L. Paterson, "Acinetobacter baumannii: emergence of a successful pathogen," *Clinical Microbiology Reviews*, vol. 21, no. 3, pp. 538–582, 2008.
- [2] G. Huang, X. Shen, Y. Gong et al., "Antibacterial properties of acinetobacter baumannii phage Abp1 endolysin (PlyAB1)," *BMC Infectious Diseases*, vol. 14, no. 1, p. 681, 2014.
- [3] W. A. Warner, S. N. Kuang, R. Hernandez et al., "Molecular characterization and antimicrobial susceptibility of *Acinetobacter baumannii* isolates obtained from two hospital outbreaks in Los Angeles County, California, USA," *BMC Infectious Diseases*, vol. 16, no. 1, p. 194, 2016.
- [4] Organization WHO, *WHO Publishes List of Bacteria for Which New Antibiotics Are Urgently Needed*, WHO, Geneva, Switzerland, 2017, <http://www.who.int/mediacentre/news/releases/2017/bacteria-antibiotics-needed/en/>.
- [5] S. El-Shazly, A. Dashti, L. Vali, M. Bolaris, and A. S. Ibrahim, "Molecular epidemiology and characterization of multiple drug-resistant (MDR) clinical isolates of *Acinetobacter baumannii*," *International Journal of Infectious Diseases*, vol. 41, pp. 42–49, 2015.
- [6] N. Z. Ning, X. Liu, C. M. Bao et al., "Molecular epidemiology of *bla*_{OXA-23}-producing carbapenem resistant *Acinetobacter baumannii* in a single institution over a 65-month period in north China," *BMC Infectious Diseases*, vol. 17, no. 1, 2017.
- [7] N. Karah, C. K. Dwibedi, K. Sjöström et al., "Novel aminoglycoside resistance transposons and transposon-derived circular forms detected in carbapenem-resistant *Acinetobacter baumannii* clinical isolates," *Antimicrobial Agents and Chemotherapy*, vol. 60, no. 3, pp. 1801–1818, 2016.
- [8] D. M. Ghaith, M. M. Zafer, M. H. Al-Agamy, E. J. Alyamani, R. Y. Booq, and O. Almoazzamy, "The emergence of a novel sequence type of MDR *Acinetobacter baumannii* from the intensive care unit of an Egyptian tertiary care hospital," *Annals of Clinical Microbiology and Antimicrobials*, vol. 16, no. 1, p. 34, 2017.
- [9] T.-H. Wang, Y.-S. Leu, N.-Y. Wang, C.-P. Liu, and T.-R. Yan, "Prevalence of different carbapenemase genes among carbapenem-resistant *Acinetobacter baumannii* blood isolates in Taiwan," *Antimicrobial Resistance & Infection Control*, vol. 7, no. 1, p. 123, 2018.
- [10] J. F. Turton, M. E. Ward, N. Woodford et al., "The role of *ISAbal* in expression of OXA carbapenemase genes in *Acinetobacter baumannii*," *FEMS Microbiology Letters*, vol. 258, no. 1, pp. 72–77, 2006.
- [11] E. Couvé-Deacon, T. Jové, P. Afouda et al., "Class 1 integrons in *Acinetobacter baumannii*: a weak expression of gene cassettes to counterbalance the lack of LexA-driven integrase repression," *International Journal of Antimicrobial Agents*, vol. 53, no. 4, pp. 491–499, 2019.

- [12] L. Al-Hassan, H. El Mehallawy, and S. G. B. Amyes, "Diversity in *Acinetobacter baumannii* isolates from paediatric cancer patients in Egypt," *Clinical Microbiology and Infection*, vol. 19, no. 11, pp. 1082–1088, 2013.
- [13] A. A. Alsultan, B. A. Evans, E. A. Elsayed et al., "High frequency of carbapenem-resistant *Acinetobacter baumannii* in patients with diabetes mellitus in Saudi Arabia," *Journal of Medical Microbiology*, vol. 62, no. 6, pp. 885–888, 2013.
- [14] M. E. Ibrahim, "Prevalence of *Acinetobacter baumannii* in Saudi Arabia: risk factors, antimicrobial resistance patterns and mechanisms of carbapenem resistance," *Annals of Clinical Microbiology and Antimicrobials*, vol. 18, no. 1, p. 1, 2019.
- [15] Clinical and Laboratory Standards Institute (CLSI), *Performance Standards for Antimicrobial Susceptibility Testing – 27th Edition. CLSI Document M100-27*, CLSI, Wayne, PA, USA, 2017.
- [16] R. N. Jones, T. R. Anderreg, J. M. Swenson, and Quality Control Working Group, "Quality control guidelines for testing gram-negative control strains with polymyxin B and colistin (polymyxin E) by standardized methods," *Journal of Clinical Microbiology*, vol. 43, no. 2, pp. 925–927, 2005.
- [17] A. -P. Magiorakos, A. Srinivasan, R. B. Carey et al., "Multi-drug-resistant, extensively drug-resistant and pandrug-resistant bacteria: an international expert proposal for interim standard definitions for acquired resistance," *Clinical Microbiology and Infection*, vol. 18, no. 3, pp. 268–281, 2012.
- [18] N. Woodford, M. Ellington, J. Coelho et al., "Multiplex PCR for genes encoding prevalent OXA carbapenemases in *Acinetobacter* spp," *International Journal of Antimicrobial Agents*, vol. 27, no. 4, pp. 351–353, 2006.
- [19] J. G. M. Koeleman, J. Stoof, M. W. van der Bijl, C. M. J. E. Vandenbroucke-Grauls, and P. H. M. Savelkoul, "Identification of epidemic strains of *Acinetobacter baumannii* by integrase gene PCR," *Journal of Clinical Microbiology*, vol. 39, no. 1, pp. 8–13, 2001.
- [20] K. Vanbroekhoven, A. Ryngaert, P. Wattiau, R. Mot, and D. Springael, "Acinetobacter diversity in environmental samples assessed by 16S rRNA gene PCR-DGGE fingerprinting," *FEMS Microbiology Ecology*, vol. 50, no. 1, pp. 37–50, 2004.
- [21] A. Cherkaoui, S. Emonet, G. Renzi, and J. Schrenzel, "Characteristics of multidrug-resistant *Acinetobacter baumannii* strains isolated in Geneva during colonization or infection," *Annals of Clinical Microbiology and Antimicrobials*, vol. 14, p. 1, 2015.
- [22] G. J. Silva, M. r. Correia, C. Vital et al., "Molecular characterization of blaIMP-5, a new integron-borne metallo- β -lactamase gene from an *Acinetobacter baumannii* nosocomial isolate in Portugal," *FEMS Microbiology Letters*, vol. 215, no. 1, pp. 33–39, 2002.
- [23] T. Naas, A. Ergani, A. Carrère, and P. Nordmann, "Real-time PCR for detection of NDM-1 carbapenemase genes from spiked stool samples," *Antimicrobial Agents and Chemotherapy*, vol. 55, no. 9, pp. 4038–4043, 2011.
- [24] O. Cardoso, R. Leitão, A. Figueiredo, J. C. Sousa, A. Duarte, and L. V. Peixe, "Metallo- β -Lactamase VIM-2 in clinical isolates of *Pseudomonas aeruginosa* from Portugal," *Microbial Drug Resistance*, vol. 8, no. 2, pp. 93–97, 2002.
- [25] C. Héritier, L. Poirel, and P. Nordmann, "Cephalosporinase over-expression resulting from insertion of ISAbal in *Acinetobacter baumannii*," *Clinical Microbiology and Infection*, vol. 12, no. 2, pp. 123–130, 2006.
- [26] O. Oikonomou, S. Sarrou, C. C. Papagiannitsis et al., "Rapid dissemination of colistin and carbapenem resistant *Acinetobacter baumannii* in central Greece: mechanisms of resistance, molecular identification and epidemiological data," *BMC Infectious Diseases*, vol. 15, p. 559, 2015.
- [27] M. Lowings, M. M. Ehlers, A. W. Dreyer, and M. M. Kock, "High prevalence of oxacillinases in clinical multidrug-resistant *Acinetobacter baumannii* isolates from the Tshwane region, South Africa - an update," *BMC Infectious Diseases*, vol. 14, p. 521, 2015.
- [28] M. M. Kock, A. N. Bellomo, N. Storm, and M. M. Ehlers, "Prevalence of carbapenem resistance genes in *Acinetobacter baumannii* isolated from clinical specimens obtained from an academic hospital in South Africa," *Southern African Journal of Epidemiology and Infection*, vol. 28, no. 1, pp. 28–32, 2013.
- [29] O. Perovic and V. Chetty, "Antimicrobial Resistance Surveillance from sentinel public hospitals, South Africa," *Commun Dis Surveill Bull*, vol. 14, pp. 56–67, 2015.
- [30] W. Tunyapanit, P. Pruekprasert, K. Laoprasopwattana, and S. Chelae, "Antimicrobial susceptibility of *Acinetobacter baumannii* isolated from hospital patients," *ScienceAsia*, vol. 40, no. 1, pp. 28–34, 2014.
- [31] R. Guckan, C. Kilinc, A. D. Demir, A. Capraz, and K. Yanik, "Antimicrobial susceptibility of *Acinetobacter baumannii* complex isolated from different clinical samples in A tertiary care hospital," *Journal of Antibiotics Research*, vol. 1, no. 1, p. 103, 2015.
- [32] P. R. Joshi, M. Acharya, T. Kakshapati, U. Leungtongkam, R. Thummeepak, and S. Sitthisak, "Co-existence of blaOXA-23 and blaNDM-1 genes of *Acinetobacter baumannii* isolated from Nepal: antimicrobial resistance and clinical significance," *Antimicrobial Resistance & Infection Control*, vol. 6, p. 21, 2017.
- [33] A. Howard, M. O'Donoghue, A. Feeney, and R. D. Sleator, "Acinetobacter baumannii," *Virulence*, vol. 3, no. 3, pp. 243–250, 2012.
- [34] T. L. Luo, A. H. Rickard, U. Srinivasan, K. S. Kaye, and B. Foxman, "Association of blaOXA-23 and bap with the persistence of *Acinetobacter baumannii* within a major healthcare system," *Frontiers in Microbiology*, vol. 6, p. 182, 2015.
- [35] B. A. Evans and S. G. B. Amyes, "OXA -Lactamases," *Clinical Microbiology Reviews*, vol. 27, no. 2, pp. 241–263, 2014.
- [36] T. P. G. Chagas, K. R. Carvalho, I. C. de Oliveira Santos, A. P. D. A. Carvalho-Assef, and M. D. Asensi, "Characterization of carbapenem-resistant *Acinetobacter baumannii* in Brazil (2008–2011): countrywide spread of OXA-23-producing clones (CC15 and CC79)," *Diagnostic Microbiology and Infectious Disease*, vol. 79, no. 4, pp. 468–472, 2014.
- [37] S. Ji, Y. Chen, Z. Ruan et al., "Prevalence of carbapenem-hydrolyzing class D β -lactamase genes in *Acinetobacter* spp. isolates in China," *European Journal of Clinical Microbiology & Infectious Diseases*, vol. 33, no. 6, pp. 989–997, 2014.
- [38] G. Revathi, L. K. Siu, P.-L. Lu, and L.-Y. Huang, "First report of NDM-1-producing acinetobacter baumannii in east Africa," *International Journal of Infectious Diseases*, vol. 17, no. 12, pp. e1255–e1258, 2013.
- [39] H. Salimizand, N. Noori, Z. Meshkat, K. Ghazvini, and S. J. Amel, "Prevalence of *Acinetobacter baumannii* harboring ISAbal/blaOXA-23-like family in a burn center," *Burns*, vol. 41, no. 5, pp. 1100–1106, 2015.
- [40] I. Goic-Barisic, B. Bedenic, M. Tonkic et al., "Occurrence of OXA-107 and ISAbal in carbapenem-resistant isolates of

- Acinetobacter baumannii* from Croatia,” *Journal of Clinical Microbiology*, vol. 47, no. 10, pp. 3348-3349, 2009.
- [41] P. Martínez and S. Mattar, “Imipenem-resistant *Acinetobacter baumannii* carrying the ISAbal-bla OXA-23, 51 and ISAbal-bla ADC-7 genes in Monteria, Colombia,” *Brazilian Journal of Microbiology*, vol. 43, no. 4, pp. 1274–1280, 2012.
- [42] S. Vijayakumar, S. Anandan, D. Prabaa et al., “Insertion sequences and sequence types profile of clinical isolates of carbapenem-resistant *A. baumannii* collected across India over four year period,” *Journal of Infection and Public Health*, no. 19, pp. 30361-30362, 2019, In press.
- [43] F. M. Elabd, M. S. Z. Al-Ayed, A. M. Asaad et al., “Molecular characterization of oxacillinases among carbapenem-resistant *Acinetobacter baumannii* nosocomial isolates in a Saudi hospital,” *Journal of Infection and Public Health*, vol. 8, no. 3, pp. 242–247, 2015.