

RESEARCH ARTICLE

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Molecular Characterization of Multiple Antibiotic-Resistant *Acinetobacter baumannii* Isolated from Egyptian Patients

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Abstract

Acinetobacter baumannii is an opportunistic microorganism commonly found in intensive care units (ICUs), and it is responsible for a broad span of hospital-acquired infections. Persistence of nosocomial infection caused by multidrug-resistant (MDR) *A. baumannii* is an alarming health care issue in Egypt, and at present, colistin remains the treatment of choice for the management of MDR *A. baumannii* infections. *A. baumannii* possesses great capacity to develop and acquire resistance to a broad range of antibiotics. The acquisition and dissemination of antibiotic-resistant determinants in *A. baumannii* strains are mediated by integrons, especially class I integrons. This study focuses on the characterization of some genetic mechanisms underlying the multidrug-resistant phenotypes of *A. baumannii* isolates in Egypt. Forty-eight *A. baumannii* specimens were isolated from different hospitalized patients; least resistance was observed against amikacin and tigecycline, with 60% and 58.5% of the isolates resistant, respectively, whereas 62.5% of the isolates were resistant to imipenem and meropenem. The highest sensitivity was found for colistin. Genetic analysis revealed that *bla*_{oxa-51} was detected in all isolates, the *bla*_{oxa-23}-like gene was detected in 80% of the isolates, and *bla*_{oxa-24} and *bla*_{oxs-58} were not detected in any isolate. Finally, PCR analysis revealed that 6.6% of isolates carried the class I integron gene.

Keywords: *Acinetobacter baumannii*, Multidrug-resistant (MDR)

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INTRODUCTION

Acinetobacter baumannii is an opportunistic microorganism commonly found in intensive care units (ICUs). It is responsible for a broad range of infections such as urinary tract infections, surgical wound infections, ventilator-associated pneumonia, meningitis, bacteremia, and other life-threatening infections. The primary factor leading to the wide spread of this nosocomial microorganism is its capacity for possessing a broad spectrum of antibiotic resistance genes. Selective pressure due to environmental stress causes the prevalence of these antibiotic-resistant clones¹. Its broad assortment of antimicrobial resistance genes makes infections difficult to treat, thereby causing financial burdens that ultimately create problems in infection management in hospitals. The quickly increasing prevalence of multi-drug resistant *A. baumannii* isolates is becoming a significant concern in global public health. The spread of resistant genes in hospitals and in society is caused by horizontal gene transfer² and movable segments such as plasmids, transposons, and integrons are the primary genetic elements responsible for the spread and prevalence of these resistance genes³. Over the past decades, despite the discovery of new drugs and treatment choices, *A. baumannii* strains have been shown to possess a capability to rapidly evolve multidrug resistance (MDR). This rapid increase in MDR is not only caused by the inherent resistance genes carried by these strains, but also by their extraordinary capacity to obtain resistance elements from other microbes. The roles of efflux pumps, class B β -lactamase (metallo- β -lactamase), chromosomal class C β -lactamase, AmpC, class D β -lactamase (OXA-type carbapenemase), integrons, and associated insertion sequence elements in MDR occurrence have recently been well documented³. The acquisition and spread of antimicrobial resistance determinants in multidrug-resistant *A. baumannii* strains are frequently mediated by integrons, especially class I integrons. Integrons are a key element in MDR spread, particularly in gram-negative pathogens⁴. These are usually immobile, but they can be transported through mobile genetic elements such as plasmids and transposons. Integrons have the ability to merge into the microbial genome,

and they possess numerous gene cassettes that confer antibiotic resistance⁵. Integrons can be categorized into three segments based on sequence conservation: the 5'-conserved segment, the variable region, and the 3'-conserved segment. The 5'-conserved segment contains a promoter, P_C, and an *intI* gene encoding integrase; the variable region usually incorporates various gene cassettes, and the 3'-conserved segment comprises sequences acquired from transposons such as *qacEΔ*, *sul1*, and *orf5*, which are found in class I integrons⁶. Integrons carry divergent gene cassettes that are rearranged under antibiotic selective pressure⁷. In *A. baumannii*, these gene cassettes frequently comprise efflux pump genes, β -lactam resistance genes, and aminoglycoside resistance genes. Up to now, a few categories of integrons have been recognized based on the succession of the integrase gene. Among these categories, class I integrons are the most common class type, and these are fundamental in the development and spread of resistance genes, followed by class II. Reports on the different classes of integrons are scarce^{4,8}, though based on literature, class 1 integrons and the pool of associated gene cassettes are the significant causes of *A. baumannii* MDR and could thus become a valuable instrument for studying molecular epidemiology in possible cross-infection cases, particularly in critical wards of hospitals such as ICUs².

MATERIALS AND METHODS

Bacterial isolates

Our study was conducted from 2016 to 2019. Forty-eight non-repeated *A. baumannii* isolates were collected from various clinical samples of hospitalized patients in El-Fayoum General Hospital. Isolates were identified using Gram staining, followed by specific biochemical reactions to identify *Acinetobacter* up to the genus level⁹. The isolates were then confirmed using the VITEK 2 system, following the manufacturers' instructions¹⁰. Finally, isolates were confirmed by examination of the *bla*_{oxa_51_like} gene, which is intrinsic to *A. baumannii*¹¹.

Antimicrobial sensitivity testing

In vitro sensitivity tests were carried out using a panel of 11 antibiotics for all isolates using the disk diffusion method, in accordance

with the guidelines established by the Clinical and Laboratory Standards Institute (CLSI)¹². The following antibiotic disks were used: amoxicillin/clavulanate (AMC), piperacillin/tazobactam (TPZ), cefoxitin (FOX), cefotaxime (CTX), cefepime (FEP), imipenem (IPM), aztreonam (ATM), ciprofloxacin (CIP), tigecycline (TGC), amikacin (AK), and colistin (COL). The standard reference strain *Escherichia coli* ATCC 25922 was used as a quality control strain in every test run.

Determination of the minimum inhibitory concentration (MIC)

MIC was tested for each isolate using the broth dilution method, as described by Irith Wiegand¹³. Bacteria were cultured in Muller-Hinton broth with a serial dilution of antibiotics. Growth (turbidity) was then determined after incubation for a specific duration (16-20 h), and the MIC value was then determined.

Extraction of genomic DNA

A. baumannii isolates were refreshed by culturing in Luria-Bertani (LB) broth and incubating overnight at 37°C. Fresh culture was then used for DNA extraction by boiling 1 mL of each isolate in LB suspension in an Eppendorf tube at 100°C for 5 min in a water bath to lyse the bacterial cell wall and liberate the DNA into the medium. The tubes were then centrifuged at maximum speed for 5 min, and the supernatant was then utilized for PCR¹⁴.

Detection of integron genes in *A. baumannii* isolates

For mining integrons, the following primers were used: *int1*, *int2*, and *int3* (Table 1)¹⁵. PCR reactions were performed using 5 µL of DNA, 0.2 mM of each type of deoxynucleoside triphosphate (dNTP), and 2 µL of 10× PCR buffer. One unit of *Taq* polymerase, 1.5 mM MgCl₂, and 1.25 µM of each primer were then added to the reaction. PCR conditions used were as follows: 35 cycles of 30 s of denaturation at 94°C, 30 s of annealing at 55°C, 30 s of extension at 72°C, with a final extension at 72°C for 10 min¹⁶.

Detection of carbapenem resistance genes

PCR was carried out in a reaction mixture containing 1× PCR buffer with 1.5 mM MgCl₂, 0.2 mM dNTP, 1.5 U *Taq* polymerase, 3 µL DNA, and 50 pmol of forward and reverse primers per reaction. Primer sequences used to amplify genes encoding *bla*_{oxa-23} like, *bla*_{oxa-24} like, *bla*_{oxa-51} and *bla*_{oxa-58} like genes are summarized in Table 1. The PCR conditions used to amplify OXA-type carbapenemases were as follows: initial denaturation at 94°C for 5 min, followed by 30 cycles of 94°C for 25 s, 52°C for 40 s, and 72°C for 50 s, with a final elongation step at 72 °C for 6 min¹⁷.

Table 1. PCR primers for the detection of genes encoding OXA carbapenemase and class-I, class-II and class-III integron

Primer name	Primer sequence	Product size	Ref.
<i>bla</i> _{oxa-23like} -F	GAT CGG ATT GGA GAA CCA GA	501 bp	17
<i>bla</i> _{oxa-23like} -R	ATT TCT GAC CGC ATT TCC AT		
<i>bla</i> _{oxa-24like} -F	GGT TAG TTG GCC CCC TTA AA	246 bp	
<i>bla</i> _{oxa-24like} -R	AGT TGA GCG AAA AGG GGA TT		
<i>bla</i> _{oxa-51like} -F	TAA TGC TTT GAT CGG CCT TG	353 bp	32
<i>bla</i> _{oxa-51like} -R	TGG ATT GCA CTT CAT CTT GG		
<i>bla</i> _{oxa-58like} -F	AAG TAT TGG GGC TTG TGC TG	599 bp	17
<i>bla</i> _{oxa-58like} -R	CCC CTC TGC GCT CTA CAT AC		
<i>Int1F</i>	CAG TGG ACA TAA GCC TGT TC	160 bp	16
<i>Int1R</i>	CCC GAG GCA TAG ACT GTA		
<i>Int2F</i>	GTA GCA AAC GAG TGA CGA AAT G	788 bp	33
<i>Int2R</i>	CAC GGA TAT GCG ACA AAA AGG T		
<i>Int3.F</i>	GCC TCC GGC AGC GAC TTT CAG	979 bp	
<i>Int3.R</i>	ACG GAT CTG CCA AAC CTG ACT		

RESULTS

Bacterial isolates

All isolates showed gram-negative coccobacilli arranged in diploids. The *bla*_{oxa-51-like} gene was found in each isolate as a PCR product of 353 base pairs (bp).

Antibiotic sensitivity of bacterial isolates

All 48 isolates were examined. Isolates were obtained from patients with different types of infections: 29 (60%) had respiratory tract infection, 7 (15%) had blood and wound infections, 7 (15%) had urinary tract infection, and 5 (10%) had miscellaneous infections.

Antimicrobial sensitivity of *A. baumannii* isolates using the disk diffusion method with *E. coli* ATCC 25922 as the control strain showed resistance patterns as follows: (AMC): 89.6%, (TPZ): 81%, (FOX): 95.8%, (CTX): 97.9%, (FEP): 87.5%, (IPM): 62.5%, (ATM): 62.5%, (CIP): 64.5%, (TGC): 58.5%, (AK): 60.4%, and (COL): 100%.

The MIC values for carbapenems were above 32 µg/mL for (30) 62.5% of isolates, and above 128 µg/mL, 256 µg/mL and 128 µg/mL for (44) 91.6% of second-generation, (47) 97.9% of third generation, and (42) 87.5% of fourth-generation cephalosporin, respectively. Amoxicillin/clavulanate and piperacillin/tazobactam showed MIC values above 256 µg/mL for (36) 75% and (34) 70.8% of isolates,

respectively. On the other hand, monobactam had values above 256 µg/mL for (31) 64.5% of isolates and above 256 µg/mL for amikacin and ciprofloxacin. Finally, colistin gave values ≤ 1 µg/mL for all 48 isolates. MIC values for the tested antibiotics are summarized in Table 2.

Integron detection in *A. baumannii* isolates

Two isolates were found to possess the class I integron gene, as shown in Fig. 1. None of the class II or class III integrons were detected.

Detection of carbapenem resistance genes

All carbapenem-resistant *A. baumannii* isolates were subjected to PCR screening for the presence of CHDLs. The *bla*_{oxa-51-like} gene was detected in all isolates, *bla*_{oxa-23-like} gene was detected in 24 isolates, and the *bla*_{oxa-24-like} and *bla*_{oxa-58-like} genes were not detected (Fig 2).

DISCUSSION

A total of 48 *Acinetobacter* clinical samples were tested for antimicrobial susceptibility, and all were found to be greater than 58% resistant to all the selected antibiotics. Ninety percent of the samples were resistant to amoxicillin-clavulanate, 96% resistant to second-generation cephalosporin, 98% resistant to third-generation cephalosporin, 88% resistant to fourth-generation cephalosporin, 62.5% resistant to carbapenems and monobactam, 64.5% resistant to quinolone,

Table 2. Minimum inhibitory concentration (MIC) distributions of antimicrobial agents for 48 isolates of *A. baumannii*

	≤0.25	0.5	1	2	4	8	16	32	64	128	≥256
AMC	0	12	0	0	0	0	0	0	0	0	36
TPZ	0	0	0	0	14	0	0	0	0	0	34
CN	10	5	5	0	0	0	0	0	20	8	0
FOX	0	0	2	0	0	0	0	0	0	44	2
CTX	0	1	0	0	0	0	0	0	0	0	47
CAZ	5	0	0	0	0	0	0	0	3	35	5
FEP	0	0	5	0	0	0	0	0	1	42	0
IPM	0	0	0	12	1	3	2	30	0	0	0
MEM	0	0	0	10	3	3	2	30	0	0	0
ATM	0	0	0	0	0	0	5	5	4	3	31
CIP	0	7	10	0	0	0	0	0	0	0	31
TGC	0	7	8	0	0	0	0	4	20	9	0
AK	0	8	10	0	1	0	0	0	0	0	30
COL	3	5	40	0	0	0	0	0	0	0	0

AMA: Antimicrobial agent, SD: Antimicrobial serial dilution, AMC: Amoxicillin/Clavulanic acid, TPZ: piperacillin/tazobactam, FOX: Cefoxitin, CTX: ceftriaxone, CAZ: Ceftazidime, FEP: Cefepime, IPM: Imipenem, ATM: Aztreonam, CIP: Ciprofloxacin, TGC: Tigecycline, AK: Amikacin and COL: Colistin.

and 60.4% to resistant amikacin. In contrast, all isolates showed 0% resistance to colistin. These data indicate the extreme drug-resistant risk of nosocomial infections, consistent with data from the Middle East, published by Al-Sweih NA¹⁸ and with previous results obtained from Egypt¹⁹.

In our study, the respiratory tract was the primary site of infection, which is similar to data published by Joly-Guillou ML²⁰. Moreover, we found that colistin is the only treatment option left for managing *A. baumannii* infections.

MIC values for carbapenems were above 32 µg/mL for 62.5% of isolates, and above 128 µg/mL, 256 µg/mL and 128 µg/mL for 91.6% of second-generation, 97.9% for third-generation, and 87.5% for fourth-generation cephalosporins. Amoxicillin/clavulanate and piperacillin/tazobactam showed

MIC values above 256 µg/mL for 75% and 70.8% of isolates, respectively. On the other hand, monobactam revealed values above 256 µg/mL for 64.5% of isolates and above 256 µg/mL for amikacin and ciprofloxacin; these data are consistent with data from Egypt published by Fouad et al.²¹

Based on the obtained sensitivity and MIC data, all carbapenem-resistant *A. baumannii* (CRAB) isolates were subjected to further investigation.

The most common carbapenemases in *A. baumannii* are the carbapenem hydrolyzing class D β-lactamases (CHDLs) and metallo-β-lactamase (MBL), while Class A carbapenemases were found at a lesser extent²². CHDLs can be classified into four main subgroups: the intrinsic *bla*_{oxa-51-like}

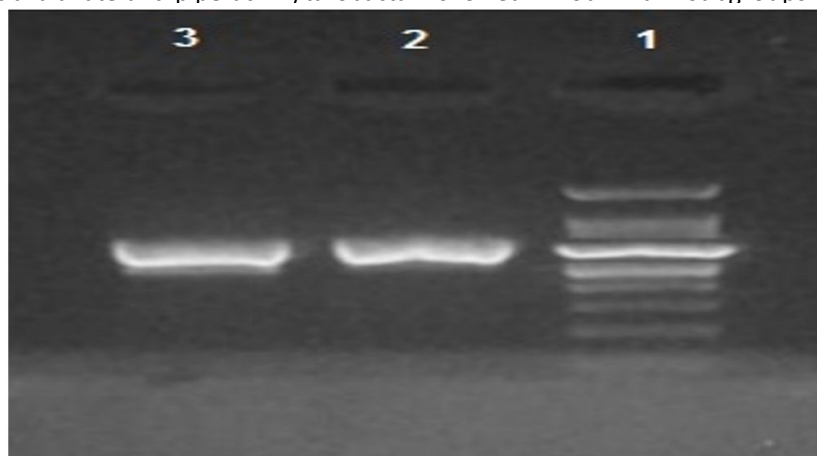


Fig. 1. Gel electrophoresis of PCR amplified class-I integron gene in *A. baumannii* isolates. Lane 1 was 1kb DNA ladder, lane 2 and 3 were (160 bp) amplicons of class-I integron gene positive *A. baumannii* isolates.

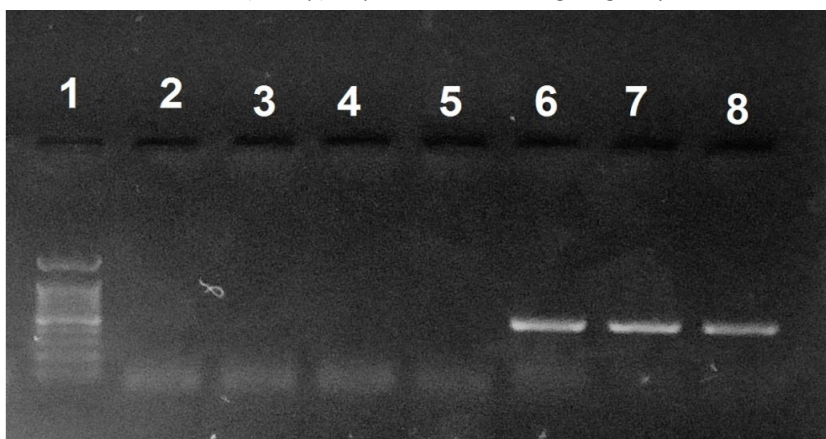


Fig. 2. Gel electrophoresis of PCR amplified *bla*_{oxa-23like} gene in *A. baumannii* isolates. Lane 1 was 1kb DNA ladder, lane 6, 7 and 8 were (501 bp) amplicons of *bla*_{oxa-23like} gene positive *A. baumannii* isolates while lanes 2, 3, 4 and 5 were *bla*_{oxa-23like} amplicon gene negative *A. baumannii*.

and the acquired carbapenemase genes *bla*_{oxa-23-like}, *bla*_{oxa-24/40-like} and *bla*_{oxa-58-like}¹⁷.

The *bla*_{oxa-51-like} gene is intrinsic to *A. baumannii*^{17,23}, and its discovery in all isolates of the present study confirmed their identity, as Feizabadi, et al. observed the *bla*_{oxa-51-like} gene in all 108 *A. baumannii* isolates in contrast to 20 negative non-*A. baumannii* isolates²⁴. Moreover, Sofy et al. used the *bla*_{oxa-51-like} gene for confirmation of *A. baumannii* isolates²⁵. These studies show that PCR analysis of the *bla*_{oxa-51-like} can be used as a basic and reliable technique for *A. baumannii* recognition.

In this study, multiplex PCR showed that 24/30 (80%) of CRAB isolates harbored *bla*_{oxa-23-like} genes, whereas *bla*_{oxa-24/40-like} and *bla*_{oxa-58-like} genes were not detected, consistent with numerous studies that reported *bla*_{oxa-23-like} as the most prevalent carbapenemase among carbapenem-resistant *A. baumannii*²⁶⁻²⁸. In Egypt, Fouad et al. confirmed the importance of the *bla*_{oxa23-like} gene among *A. baumannii* isolates²¹.

The class I integron has been documented in several studies of multi-drug resistant *A. baumannii* worldwide²⁹. In this study, 6.6% of CRAB isolates were positive for class I integrons, while class II and class III integrons were not detected; this is consistent with a study performed in Turkey, in which 6.4% CRAB isolates were positive for class I integrons³⁰. On the other hand, our results are not consistent with that of Mehdi et al., who reported a significant prevalence of class I and class II integrons³¹.

CONCLUSION

Acinetobacter baumannii poses a serious threat in Egyptian hospitals, and our current findings suggest a critical healthcare problem that can lead to the loss of effectivity of future therapeutic options. As such, stricter infection control measures are required to decrease intrahospital and interhospital spread of multi-drug resistant *A. baumannii*.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

AUTHORS' CONTRIBUTION

All authors listed have made a substantial, direct and intellectual contribution to the work, and approved it for publication.

FUNDING

None.

DATA AVAILABILITY

All datasets generated or analyzed during this study are included in the manuscript.

ETHICS STATEMENT

Not applicable.

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