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Molecular characterization of carbapenem-insensitive *Acinetobacter baumannii* in Egypt

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SUMMARY

Objectives: This study investigated the prevalence of diverse Ambler class β -lactamase-encoding genes in 40 carbapenem-insensitive *Acinetobacter baumannii* isolates collected from two hospitals in Egypt during the period January–March 2012.

Methods: The resistance levels to different groups of antimicrobial agents were determined. PCR was used to detect the different Ambler class β -lactamases encoding the following genes: *bla*_{TEM}, *bla*_{SHV}, *bla*_{CTX-M}, *bla*_{VEB}, *bla*_{PER}, *bla*_{GES}, *bla*_{VIM}, *bla*_{IMP}, *bla*_{SIM}, *bla*_{SPM}, *bla*_{GIM}, *bla*_{NDM}, *bla*_{ADC}, *bla*_{OXA-23}, *bla*_{OXA-24}, *bla*_{OXA-51}, and *bla*_{OXA-58}. *ISAbal* and *int1* were detected by PCR.

Results: The isolates were 100% resistant to amoxicillin–clavulanate, aztreonam, cefepime, cefotaxime, and ceftazidime. Of the isolates, 5% were resistant to colistin, 45% to amikacin, 70% to imipenem, and 85% to ciprofloxacin. The *bla*_{ADC} and *bla*_{OXA-51}-like genes were detected in the entire collection. The prevalences of *bla*_{OXA-23}, *bla*_{OXA-24}, and *bla*_{OXA-58} were 50%, 7.5%, and 5%, respectively. However, the prevalences of *bla*_{TEM}, *bla*_{PER}, and *bla*_{GES}-like genes were 87.5%, 55%, and 27.5%, respectively. SHV, CTX-M, VEB, KPC, and MBL encoding genes were not detected. The *ISAbal* was found upstream to *bla*_{OXA-51}, *bla*_{OXA-23}, and *bla*_{ADC} in 85%, 80%, and 50%, respectively. Of note, 45% (18/40) of the isolates co-produced extended-spectrum β -lactamases (PER and GES) and carbapenemases (OXA-23 and OXA-58).

Conclusions: The *bla*_{ADC}, *bla*_{TEM}, *bla*_{PER}, *bla*_{OXA-23}, and *bla*_{GES}-like genes were found to be the most prevalent types of β -lactamase-encoding gene in *A. baumannii* collected from Egypt. A high level of carbapenem resistance is mediated by *bla*_{OXA-23}, *bla*_{OXA-24}, and *bla*_{OXA-58} (minimum inhibitory concentration (MIC) 32 to >256 μ g/ml), and a low level of carbapenem resistance is mediated by *bla*_{GES} (MIC 4–16 μ g/ml) and by up-regulation of *ISAbal*–OXA-51 (MIC 1–4 μ g/ml). Class B MBL was not identified to play a role in carbapenem resistance in *A. baumannii* isolates from Egypt.

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1. Introduction

The genus *Acinetobacter* comprises Gram-negative, aerobic, glucose-non-fermenting, non-fastidious, non-motile, catalase-positive, and oxidase-negative bacteria. *Acinetobacter baumannii* is an opportunistic pathogen that is an important source of nosocomial infections, including pneumonia, urinary tract infections, and wound infections, with high mortality.¹ In addition, it is often

resistant to a wide variety of antimicrobial agents, including β -lactam antibiotics, fosfomycin, and trimethoprim. Therefore, infections caused by multidrug-resistant *A. baumannii* are currently among the most difficult to treat.^{1,2} A variety of molecular mechanisms conferring resistance to β -lactams have been reported in *A. baumannii*, such as the production of β -lactamases enzymes, alterations in the outer membrane protein, the production of penicillin-binding proteins, and increased activity of efflux pumps.¹ However, the most prevalent mechanism of extended-spectrum cephalosporin and carbapenem resistance in *A. baumannii* is enzymatic degradation by β -lactamases.^{1–3} The Ambler class A, B, C, and D β -lactamases confer various resistance phenotypes, such as extended-spectrum β -lactamases (ESBLs), metallo- β -lactamases

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(MBLs), carbapenem-hydrolyzing class D β -lactamases (CHDLs), and Acinetobacter-derived cephalosporinases (ADCs).^{1,2,4–7} Acquired resistance to carbapenem is mediated most often by the CHDLs (OXA-23, OXA-24/40, OXA-58, and OXA-143) and less frequently by MBLs (VIM, IMP, SPM, GIM, and NDM), which are responsible for high levels of carbapenem resistance.^{5,7–9} Recently, the Ambler class A carbapenemase GES has been described in *A. baumannii*, which is responsible for a low level of carbapenem resistance.^{2,3,10–13}

The resistance of *A. baumannii* to extended-spectrum cephalosporins is usually related to the over-expression of the resident Ambler class C *bla*_{ADC} gene,^{6,14,15} or infrequently to the acquisition of ESBL (TEM, SHV, CTX-M, VEB, PER, GES, and KPC) encoding genes.^{1–4,10–13,16,17} The PER-, VEB-, and GES-like types are the most common Ambler class A β -lactamases in *A. baumannii*.^{2–4,10–13,18}

There is little information on the frequency of occurrence, prevalence, and distribution of the Ambler class β -lactamases in Egypt. Therefore, this study was undertaken to determine the prevalences of the class A, B, C, and D β -lactamases that confer various β -lactamase resistance phenotypes and to determine the prevalences of *ISAbal* and class 1 integron among *A. baumannii* isolates collected from two Egyptian hospitals.

2. Materials and methods

2.1. Bacterial isolates

A total of 40 non-consecutive, unique, imipenem-insusceptible *A. baumannii* clinical isolates were collected from Kasr El Aini Hospital, Cairo and Dar Al Fouad Hospital, Sixth of October City, Egypt over a period of 3 months from January to March 2012. The isolates were identified using the API 20 NE system (bioMérieux, Marcy l'Etoile, France) and confirmed using PCR to detect the intrinsic *bla*_{OXA-51}.¹⁹

2.2. Determination of the minimum inhibitory concentration (MIC)

The MICs of amikacin, amoxicillin–clavulanate, aztreonam, cefepime, cefotaxime, ceftazidime, ciprofloxacin, imipenem, gentamicin, and colistin were determined for the 40 imipenem-insusceptible *A. baumannii* isolates using the British Society for Antimicrobial Chemotherapy (BSAC) agar dilution method, with BSAC/European Committee on Antimicrobial Susceptibility Testing (EUCAST) breakpoints.²⁰ *Escherichia coli* ATCC 25922 was used as the reference strain.

2.3. Phenotypic detection of ESBLs and MBLs

ESBL screening was performed using the disk diffusion method, in accordance with the Clinical and Laboratory Standards Institute (CLSI) recommended guidelines.²¹ ESBL production was confirmed

with Etest ESBL strips (AB Biodisk, Solna, Sweden). Imipenem/imipenem + ethylenediaminetetraacetic acid (EDTA) E-test MBL strips (AB Biodisk, Solna, Sweden) were used in accordance with the manufacturer's directions to investigate MBL production. A ratio of the MICs of imipenem to imipenem + EDTA of ≥ 8 or the presence of a phantom zone was taken as a positive result.

2.4. Molecular characterization of antimicrobial resistance determinants

A series of PCR reactions were performed to detect the different Ambler class *bla* genes and mobile genetic elements. Primers were designed to amplify the following *bla* gene groups: class A, *bla*_{TEM}, *bla*_{SHV}, *bla*_{CTX-M}, *bla*_{VEB}, *bla*_{PER}, *bla*_{KPC}, and *bla*_{GES}; class B, *bla*_{IMP}, *bla*_{VIM}, *bla*_{GIM}, *bla*_{SPM}, *bla*_{SIM}, and *bla*_{NDM}; class C, *bla*_{ADC}; and class D, *bla*_{OXA-23}-like, *bla*_{OXA-24}-like, *bla*_{OXA-51}-like, and *bla*_{OXA-58}-like.^{14,22–24} Integrase genes (*int1*) and IS elements (*ISAbal*) were amplified by PCR using previously described methods.^{25,26} PCR mapping experiments using combinations of the *ISAbal* primers and the OXA-51-like, OXA-23-like, OXA-24-like, OXA-58-like, and ADC-like reverse primers were carried out. All PCR assays were performed using Red Load Taq Master (Jena Bioscience, Jena, Germany) in a Techne thermocycler (Techne, UK). Positive and negative controls were included in all PCR assays.

3. Results

3.1. Antimicrobial resistance pattern

The resistance patterns of the 40 *A. baumannii* isolates and the MIC distributions of the tested antimicrobial agents are shown in Table 1. *A. baumannii* isolates were all resistant to amoxicillin–clavulanate, aztreonam, cefepime, ceftazidime, and cefotaxime. The resistance rates to ciprofloxacin, imipenem, and amikacin were 85% (34/40), 70% (28/40), and 45% (18/40), respectively. Colistin showed the highest activity against *A. baumannii* isolates; the resistance rate was 5% (2/40).

3.2. MICs of tested antibiotics against *A. baumannii* isolates

The MICs and MIC distributions of amoxicillin–clavulanate, cefotaxime, ceftazidime, cefepime, aztreonam, imipenem, amikacin, ciprofloxacin, and colistin are shown in Tables 1 and 2.

3.3. Prevalence of ESBLs and MBLs

Etest strips for ESBLs were applied to the 40 isolates, with 30 (75%) giving a positive result. The imipenem/imipenem + EDTA Etest gave negative MBL results for all isolates.

Table 1
Minimum inhibitory concentration (MIC) distributions of antimicrobial agents for 40 isolates of *Acinetobacter baumannii*

Antibiotic	Resistance pattern, n (%)			Distribution of MIC ($\mu\text{g/ml}$)										
	R	I	S	≤ 0.25	0.5	1	2	4	8	16	32	64	128	≥ 256
Amoxicillin–clavulanate	40 (100)	0	0	0	0	0	0	0	0	0	0	0	0	40
Cefotaxime	40 (100)	0	0	0	0	0	0	0	0	0	0	0	0	40
Ceftazidime	40 (100)	0	0	0	0	0	0	0	0	0	0	1	1	38
Cefepime	40 (100)	0	0	0	0	0	0	0	0	0	0	0	3	37
Aztreonam	40 (100)	0	0	0	0	0	0	0	0	0	7	7	4	22
Imipenem	28 (70)	7 (17.5)	5 (12.5)	0	0	2	3	4	3	4	1	3	8	12
Amikacin	18 (45)	9 (22.5)	13 (32.5)	0	0	1	2	3	7	9	4	3	6	5
Ciprofloxacin	34 (85)	0	6 (15)	1	2	3	2	1	4	4	2	7	8	6
Colistin	2 (5)	0	38 (95)	19	9	8	2	1	1	0	0	0	0	0

R, resistant; I, intermediate; S, susceptible.

Table 2
Clinical data, minimum inhibitory concentrations (MIC), and results of PCR for 40 *Acinetobacter baumannii* isolates

No.	Isolate	Clinical data				MIC (mg/l) ^a								PCR ^{b,c}									
		Specimen	Hospital	Date of collection	Gender	AMC	CT	TZ	FP	AT	IP	AK	CI	COL	TEM	PER	GES	IS/Aba1-ADC	ISAbal-1-OXA-51	OXA-23	ISAbal-1-OXA-23	OXA-24	OXA-58
1	AB1	Wound swab	Dar Al Fouad	3/1/2012	Male	≥256	≥256	≥256	≥256	≥256	>256	16	64	0.25	+	+	-	+	+	+	-	-	TEM + PER + ADC + OXA-51 + OXA-23 + ISAbal1 + int1
2	AB3	Blood	Dar Al Fouad	6/1/2012	Female	≥256	≥256	≥256	≥256	≥256	256	8	128	0.25	+	+	-	+	+	+	-	-	
3	AB5	Wound swab	Kasr El Aini	9/1/2012	Male	≥256	≥256	≥256	≥256	>256	128	16	64	0.25	+	+	-	+	+	+	-	-	
4	AB6	Drain	Dar Al Fouad	10/1/2012	Male	≥256	≥256	≥256	≥256	≥256	128	16	32	0.5	+	+	-	+	+	+	-	-	
5	AB9	ETT	Kasr El Aini	15/1/2012	Male	≥256	≥256	≥256	≥256	>256	16	>256	4	+	+	-	+	+	+	-	-		
6	AB13	Wound swab	Dar Al Fouad	27/1/2012	Male	≥256	≥256	≥256	≥256	≥256	>256	16	16	0.25	+	+	-	+	+	+	-	-	
7	AB18	ETT	Kasr El Aini	8/2/2012	Male	≥256	≥256	≥256	≥256	≥256	128	4	128	0.25	+	+	-	+	+	+	-	-	
8	AB19	CVP	Dar Al Fouad	9/2/2012	Female	≥256	≥256	≥256	≥256	≥256	64	8	64	0.25	+	+	-	+	+	+	-	-	
9	AB20	Sputum	Kasr El Aini	14/2/2012	Female	≥256	≥256	≥256	≥256	≥256	64	32	256	0.25	+	+	-	+	+	+	-	-	
10	AB23	Blood	Dar Al Fouad	23/2/2012	Female	≥256	≥256	≥256	≥256	≥256	128	16	32	0.5	+	+	-	+	+	+	-	-	
11	AB24	Sputum	Kasr El Aini	24/2/2012	Male	≥256	≥256	≥256	≥256	≥256	32	32	>256	1	+	+	-	+	+	+	-	-	
12	AB26	ETT	Kasr El Aini	27/2/2012	Male	≥256	≥256	≥256	≥256	≥256	256	64	128	0.25	+	+	-	+	+	+	-	-	
13	AB28	ETT	Kasr El Aini	2/3/2012	Male	≥256	≥256	≥256	≥256	≥256	64	16	128	0.5	+	+	-	+	+	+	-	-	
14	AB30	Sputum	Kasr El Aini	6/3/2012	Male	≥256	≥256	≥256	≥256	≥256	128	8	64	0.5	+	+	-	+	+	+	-	-	
15	AB33	Sputum	Kasr El Aini	10/3/2012	Male	≥256	≥256	≥256	≥256	128	128	32	128	1	+	+	-	+	+	+	-	-	
16	AB34	Urine	Kasr El Aini	13/3/2012	Female	≥256	≥256	≥256	≥256	≥256	>256	64	>256	1	+	+	-	+	+	+	-	-	
17	AB22	Urine	Kasr El Aini	18/2/2012	Female	≥256	≥256	≥256	≥256	≥256	>256	128	128	0.5	+	-	-	-	+	+	-	-	TEM + ADC + OXA-51 + OXA-23 + ISAbal1 + int1
18	AB40	Sputum	Dar Al Fouad	25/3/2012	Male	≥256	≥256	≥256	≥256	≥256	256	>256	16	8	+	-	-	-	+	+	-	-	
19	AB14	Blood	Kasr El Aini	31/1/2012	Female	≥256	≥256	≥256	≥256	128	256	>256	256	1	+	-	-	-	+	+	-	-	
20	AB7	Pus	Kasr El Aini	11/1/2012	Male	≥256	≥256	≥256	≥256	>256	>256	>256	256	0.25	+	-	+	+	+	+	-	+	TEM + GES + ADC + OXA-51 + OXA-23 + OXA-58 ISAbal1 + int1
21	AB4	Pus	Kasr El Aini	7/1/2012	Male	≥256	≥256	≥256	≥256	32	128	128	64	0.25	+	-	-	+	+	-	+	-	TEM + ADC + OXA-51 + OXA-24 + ISAbal1 + int1
22	AB8	Sputum	Kasr El Aini	13/1/2012	Male	≥256	≥256	≥256	≥256	≥256	128	256	128	0.25	+	-	-	+	+	-	+	-	
23	AB37	Unknown	Kasr El Aini	17/3/2012	Female	≥256	≥256	≥256	≥256	≥256	256	128	64	1	+	-	-	+	+	-	+	-	
24	AB38	Unknown	Kasr El Aini	19/3/2012	Male	≥256	≥256	≥256	≥256	128	>256	8	0.5	1	+	+	-	-	+	-	-	+	TEM + PER + ADC + OXA-51 + OXA-58 + ISAbal1 + int1
25	AB2	Pus	Kasr El Aini	5/1/2012	Female	≥256	≥256	≥256	128	64	8	16	1	2	+	-	+	-	+	-	-	“-	TEM + GES + ADC + OXA-51 + ISAbal1
26	AB10	Unknown	Dar Al Fouad	17/1/2012	Female	≥256	≥256	≥256	≥256	64	16	128	2	0.25	+	-	+	-	+	-	-	-	
27	AB15	Blood	Kasr El Aini	2/2/2012	Male	≥256	≥256	≥256	≥256	64	16	16	0.25	0.5	+	-	+	-	+	-	-	-	
28	AB16	Urine	Kasr El Aini	5/2/2012	Female	≥256	≥256	≥256	≥256	64	4	>256	8	0.25	+	-	+	-	+	-	-	-	
29	AB31	Blood	Kasr El Aini	8/3/2012	Male	≥256	≥256	≥256	≥256	128	4	128	4	1	+	-	+	-	+	-	-	-	
30	AB32	Pus	Kasr El Aini	8/3/2012	Male	≥256	≥256	≥256	≥256	64	16	8	8	2	+	-	+	-	+	-	-	-	
31	AB12	Pus	Kasr El Aini	23/1/2012	Male	≥256	≥256	≥256	≥256	≥256	8	2	8	0.25	+	-	+	-	+	-	-	-	
32	AB25	Blood	Kasr El Aini	26/2/2012	Male	≥256	≥256	≥256	≥256	64	8	128	1	0.5	+	-	+	-	+	-	-	-	
33	AB35	Tissue	Kasr El Aini	16/3/2012	Male	≥256	≥256	≥256	≥256	≥256	16	1	128	1	+	-	+	-	+	-	-	-	
34	AB21	CVP	Kasr El Aini	17/2/2013	Female	≥256	≥256	≥256	≥256	64	4	32	16	0.5	+	-	+	-	+	-	-	-	
35	AB11	Pus	Kasr El Aini	20/1/2012	Male	≥256	≥256	≥256	≥256	32	2	4	16	0.25	-	+	-	-	-	-	-	-	ADC + PER + OXA-51 + ISAbal1
36	AB17	Urine	Dar Al Fouad	6/2/2012	Male	≥256	≥256	64	128	32	4	8	0.5	0.25	-	+	-	-	-	-	-	-	
37	AB27	Blood	Kasr El Aini	1/3/2012	Male	≥256	≥256	≥256	≥256	32	1	8	64	0.25	-	+	-	-	-	-	-	-	
38	AB29	Blood	Kasr El Aini	5/3/2012	Female	≥256	≥256	≥256	≥256	32	2	64	8	0.5	-	+	-	-	-	-	-	-	
39	AB39	Sputum	Kasr El Aini	22/3/2012	Female	≥256	≥256	≥256	128	32	2	2	1	0.25	-	+	-	-	-	-	-	-	
40	AB36	Blood	Dar Al Fouad	16/3/2012	Male	≥256	≥256	128	≥256	32	1	4	2	0.25	+	-	-	-	-	-	-	-	TEM + ADC + OXA-51 + ISAbal1

ETT, Endotracheal tube; CVP, Central venous catheter.

^a AMC, amoxicillin-clavulanate; CT, cefotaxime; TZ, ceftazidime; FP, cefepime; AT, aztreonam; IP, imipenem; AK, amikacin; CI, ciprofloxacin; COL, colistin.

^b All isolates were negative to SHV, CTX-M, VEB, VIM, IMP, SIM, SPM, GIM, and NDM.

^c ADC, *int1*, OXA-51, *ISAbal1* were universal.

3.4. Prevalences of the Ambler class β -lactamase-encoding genes

The prevalences of four Ambler class β -lactamase-encoding genes among the 40 *A. baumannii* isolates are shown in Table 2. The intrinsic β -lactamase gene, *bla*_{OXA-51}-like, was amplified from all 40 *A. baumannii* isolates. The *bla*_{OXA-23} gene was amplified from 20 isolates (50%). The *bla*_{OXA-24/40}- and *bla*_{OXA-58}-like genes were detected in three isolates (7.5%) and two isolates (5%), respectively. Class B MBL genes were not detected in the 40 *A. baumannii* isolates.

The most prevalent Ambler class A β -lactamase-encoding gene was *bla*_{TEM}, which was identified in 35 (87.5%) isolates; the next most prevalent gene was *bla*_{PER}, which was identified in 22 (55%) of the isolates. Ambler class A carbapenemase-encoding gene *bla*_{GES} was detected in 11 (27.5%) of the 40 *A. baumannii* isolates. However, *bla*_{SHV}, *bla*_{CTX-M}, *bla*_{VEB}, and *bla*_{KPC} encoding genes were not detected. The prevalence of *bla*_{ADC}, an Ambler class C cephalosporinase, was 100% in the 40 *A. baumannii* isolates.

3.5. Upstream regulation of ISAbA1

All isolates were found to harbor class 1 integron and ISAbA1. The ISAbA1 element was found upstream to the corresponding genes *bla*_{OXA-51}, *bla*_{OXA-23}, and *bla*_{ADC} in 85% (34/40), 80% (16/20), and 50% (20/40), respectively. However, the ISAbA1 element was not found upstream of either *bla*_{OXA-24/40} or *bla*_{OXA-58} (Table 2).

4. Discussion

Antimicrobial resistance in *A. baumannii* has become a worldwide problem. The emergence of clinical *A. baumannii* isolates with diverse antibiotic resistance phenotypes causes difficulties in treating infections caused by this pathogen.³ In the present study, *A. baumannii* isolates were 100% resistant to amoxicillin–clavulanate, third- and fourth-generation cephalosporins, and monobactams; however 85% of the isolates were also found to be resistant to ciprofloxacin. Amikacin was found to be an effective drug in the treatment of *A. baumannii* isolates; 45% of the isolates were resistant. The present study is consistent to some extent with previous studies conducted on *A. baumannii* collected from Egypt.^{8,27,28} In the study of Mohamed and Raafat,⁸ 100% of *A. baumannii* isolates ($n = 23$) were found to be resistant to the third- and fourth-generation cephalosporins. Furthermore, high resistance rates to amikacin, tobramycin, and ciprofloxacin were found: 100%, 82.6%, and 69.6%, respectively. Ahmed et al.²⁷ reported that *A. baumannii* isolates ($n = 52$) were 100% resistant to amoxicillin–clavulanate, ceftazidime, ciprofloxacin, nalidixic acid, and chloramphenicol; however, the resistance rates to amikacin, cefepime, and cefradine were 76.9%, 80.8%, and 96.2%, respectively. Nasr and Attalah²⁸ found that all isolates ($n = 20$) were 100% resistant to ampicillin–sulbactam, ceftazidime, ceftriaxone, ciprofloxacin, and piperacillin–tazobactam. High resistance rates were also observed to amikacin (90%), gentamicin (85%), and doxycycline (75%).

Carbapenems have become the drugs of choice for the treatment of serious nosocomial infections caused by Acinetobacter; however, carbapenem-resistant strains of *A. baumannii* have been reported worldwide. In the present study, the majority of the isolates (70%) were resistant to imipenem (MIC >8 μ g/ml). Resistance to carbapenems in clinical *A. baumannii* isolates has been notable recently in Egypt. Few studies have determined the resistance rates for carbapenem in *A. baumannii* isolates from Egypt. High resistance rates to carbapenems have been observed in Egypt, ranging from 75% to 100% for imipenem and from 61% to 77% for meropenem.^{8,27–30} The resistance to imipenem reflects a problem that might be described as countrywide. In addition, in the present study, 50% of the isolates displayed unusually high

levels of resistance to imipenem, with MIC values ≥ 128 μ g/ml. In the Middle East and North Africa, the occurrence of imipenem-resistant *A. baumannii* is recognized with alarm. The resistance rate of *A. baumannii* to imipenem was found to be 95% in Turkey, 65% in Saudi Arabia, 47.9% in Algeria, 45% in Tunisia, and 19.14% in Kuwait.^{12,13,31–33} The emergence of *A. baumannii* strains with increased carbapenem resistance in this area of the world may be due to the extensive misuse of carbapenems.

Colistin and tigecycline are the last options for the treatment of carbapenem-resistant *A. baumannii*. Lately, *A. baumannii* isolates have frequently been found to be resistant to most antimicrobial agents, and evidence of pan-drug resistance among these isolates has been reported. *A. baumannii* isolates resistant to carbapenems, colistin, and tigecycline have been identified, making the treatment of these isolates particularly difficult. The rate of colistin resistance is relatively low, likely because of its infrequent use. In the present study, colistin retained its activity against most of the tested isolates, with a percentage of susceptibility of 95%. That is consistent with previous studies in Egypt, in which colistin was found to be active against 82.6%⁸ and 100%³⁰ of the tested isolates. In addition, in other studies, it was found that 100% of *A. baumannii* was sensitive to colistin in Algeria, 92.5% in Kuwait, and 70.9% in Saudi Arabia.^{12,31,33}

The most prevalent mechanism of carbapenem resistance in *A. baumannii* is the enzymatic degradation by carbapenem-hydrolyzing β -lactamases. The most widespread carbapenemases in *A. baumannii* are CHDLs and, to a lesser extent, MBL and class A carbapenemases.^{1,3,5,12,13,34} MBL, mostly VIM and IMP, has been reported sporadically in some parts of the world.¹ MBL NDM-1 and NDM-2 were first described in *A. baumannii* from Egypt,^{7,35} and then spread in the Middle East.³⁶ MBL VIM, SPM-1, and GIM-1 were detected previously in *A. baumannii* isolates from Egypt.^{8,30} Nevertheless, in the present study, none of the *A. baumannii* isolates harbored *bla*_{IMP}, *bla*_{VIM}, *bla*_{SPM}, *bla*_{SIM}, *bla*_{GIM}, or *bla*_{NDM} MBL-encoding genes.

CHDLs can be divided into four main subgroups: the intrinsic *bla*_{OXA-51}-like and the acquired carbapenemase genes *bla*_{OXA-23}-, *bla*_{OXA-24/40}-, and *bla*_{OXA-58}-like.¹ Numerous studies have recently reported that *bla*_{OXA-23} is the most frequent type of carbapenemase identified among carbapenem-resistant *A. baumannii*.^{1,12,13,29,30,37} In this study, the most prevalent CHDL-encoding gene in *A. baumannii* was *bla*_{OXA-23}, with a prevalence rate of 50% ($n = 20$), which is in agreement with previous studies.^{12,13,29} However, Fouad et al.³⁰ detected *bla*_{OXA-23} in their entire collection of *A. baumannii* isolates. *bla*_{OXA-24/40} has mostly been found in the Asian and Iberian peninsulas, but has also been detected in other areas.^{1,12,13,38,39} The OXA-58 gene has been reported in isolates of *A. baumannii* scattered throughout different parts of the world, including Algeria, Argentina, Italy, Kuwait, Turkey, the UK, and the USA.^{1,37,40–43} In this study, the prevalence of the OXA-58-encoding gene in the clinical isolates of *A. baumannii* was found to be 5% (2/40). In Egypt and Algeria, 9.1% and 14.7%, respectively, of carbapenem-resistant *A. baumannii* isolates were found to produce OXA-58.^{29,37} Additionally, in Italy and Turkey, carbapenem resistance of *A. baumannii* has consistently been related to the production of *bla*_{OXA-58}.^{41,42} Only a few studies on carbapenemases in *A. baumannii* in Egypt are available.^{7,8,29,35} In the present study, all three acquired class D carbapenemases OXA-23-, OXA-24/40-, and OXA-58-encoding genes were identified among the tested strains correlating with resistance to carbapenems, with prevalences of 50%, 7.5%, and 5%, respectively. In a recent study conducted in Egypt by Al-Hassan et al., the prevalences of OXA-23, OXA-40, and OXA-58 were 55.88%, 2.9%, and 14.7%, respectively.²⁹ The prevalence of OXA-23 in the present study is very similar to that found in the study of Al-Hassan et al.; however, the prevalence of OXA-24 in the present study is higher than that found in the

study of Al-Hassan et al. In addition, we found that the prevalences of *bla*_{OXA-23}, *bla*_{OXA-24}, and *bla*_{OXA-58} were lower. It is worth noting that the presence of the *bla*_{OXA-23} carbapenemase-encoding gene along with the coexistence of *bla*_{OXA-58} was detected in one strain in the present study. This finding is in agreement with other studies.^{29,37,38}

The *bla*_{OXA-51}-like gene is unique in that it occurs naturally in *A. baumannii*. Therefore, it is chromosomally located and is widely prevalent. Many studies have indicated that the identification of the *bla*_{OXA-51}-like gene is a reliable and rapid method to presumptively identify *A. baumannii*. In addition, the identification of this gene reveals that the rate of antibiotic resistance to various antibiotics is high in *A. baumannii* isolates.¹⁹ Insertion sequences (IS) may contribute to the over-production and dissemination of β -lactamase.^{25,43} The over-expression of CHDL-encoding genes, driven mostly by promoters provided by their upstream ISs, is one of the means by which *A. baumannii* acquires a high level of carbapenem resistance. *ISAbal* and *ISAbal825* upstream to the *bla*_{OXA-51}-like gene are associated with the over-expression of the *bla*_{OXA-51}-like and other CHDL-encoding genes along with carbapenem resistance in *A. baumannii*.^{12,13,43} However, some isolates harboring the *bla*_{OXA-51}-like gene with an upstream *ISAbal* are still susceptible to carbapenems (Pagano et al.⁴⁴). The present study revealed that all *A. baumannii* isolates had *bla*_{OXA-51} and *ISAbal*. The *ISAbal* element was found upstream to the corresponding genes *bla*_{OXA-51}, *bla*_{OXA-23}, and *bla*_{ADC} in 85% (34/40), 80% (16/20), and 50% (20/40), respectively. However, the *ISAbal* element was not found upstream of either *bla*_{OXA-24/40} or *bla*_{OXA-58}. The *ISAbal* element was not found upstream of *bla*_{OXA-51} in six (15%) isolates with imipenem MICs ranging from 1 to 4 μ g/ml. The up-regulation of *ISAbal* to *bla*_{OXA-51} was found in 34 isolates. Twenty-five out of 34 isolates were concomitant with other CHDLs that had markedly high MICs for imipenem (≥ 32 μ g/ml): *bla*_{OXA-23} ($n = 19$), *bla*_{OXA-24} ($n = 3$), *bla*_{OXA-58} ($n = 1$), and one isolate co-produced *bla*_{OXA-23} and *bla*_{OXA-58}. Nine of 34 isolates were not concomitant with other CHDLs, with lower MICs for carbapenems (2–8 μ g/ml). In the present study, up-regulation of *ISAbal* played an important role in the over-expression of *bla*_{OXA-51}, *bla*_{OXA-23}, and *bla*_{ADC}.

GES variants and KPC are Ambler class A carbapenemases that have been reported in the last 5 years in *A. baumannii*;^{3,12,13,17} our isolates were tested for their encoding genes. Our results revealed that *A. baumannii* were devoid of KPC, but GES was detected in 27.5% (11/40) of the isolates. In the present study, the prevalence of GES is in agreement with the findings of previous Turkish and Saudi studies,^{12,13} with prevalences of 23.8% and 34.5%, respectively. Several GES-1 mutants have been detected in *A. baumannii*, such as GES-11, GES-12, GES-14, and GES-22.^{2,3,10,11,13} Unfortunately, in the current study, GES-encoding genes were not sequenced; however from the MIC data it can be concluded that they may be GES-1 variants, which possess carbapenemase activity. Several studies detecting GES in *A. baumannii* have been published recently. GES-11 has been reported from Turkey, Egypt, Kuwait, Gaza, and France.^{3,10,11,13} GES-12 has been detected in Egypt, Belgium, and France, and in addition, GES-14 has been detected in Turkey and Kuwait.^{3,11} GES-22 has been detected in Turkey.¹³ GES was detected concomitant with OXA-51 ($n = 10$) or in combination with OXA-23 plus OXA-58 ($n = 1$). Ten GES-positive isolates, which had GES plus OXA-51, had imipenem MICs ranging from 4 to 16 μ g/ml. This result indicates that the GES-1 mutant is responsible for a high level of carbapenem resistance and/or up-regulation of *ISAbal* to OXA-51.

Several Ambler class A ESBLs have been identified in *A. baumannii*, such as CTX-15, PER-1, PER-2, PER-7, and VEB-1.^{4,16,18} PER and, to a lesser extent, VEB are the most common Ambler class A ESBLs in *A. baumannii*.^{4,12,18,45–48} Our isolates were tested for PER, VEB, TEM, SHV, and CTX-M genes. Our results

revealed that *A. baumannii* were devoid of VEB, SHV, and CTX-M, but TEM and PER were detected in 87.5% and 49.1% of *A. baumannii* isolates, respectively. PER has been documented in *Acinetobacter* isolates from France, Belgium, India, Iran, South Korea, Saudi Arabia, and Argentina.^{4,18,45–48} PER-1, PER-2, and PER-7 have been detected previously in *A. baumannii*.^{4,45–48} In Iran and Korea, 51% and 54%, respectively, of nosocomial isolates of *Acinetobacter spp* were found to produce PER-1,^{18,46} and our results agree with those studies.

The most common mechanism of resistance of *A. baumannii* to β -lactam antibiotics is attributed to the presence of a chromosomal cephalosporinase-encoding gene.^{14,15} Most AmpC-type β -lactamases naturally produced by Gram-negative bacteria hydrolyze amino- and ureidopenicillins, cephamycins, and, at a low level, oxyiminocephalosporins, such as ceftazidime, cefotaxime, ceftriaxone, and aztreonam. Several allelic variants of the *A. baumannii* AmpC enzyme have also been reported.¹⁴ Recently, a uniform designation for this family of cephalosporinases has been suggested: ADC, with AmpCs of *A. baumannii*.^{6,14} The enzyme is normally expressed at low levels and is not inducible, but over-expression occurs with the upstream insertion of *ISAbal* common in *A. baumannii*, which provides an efficient promoter for the *bla*_{AmpC} gene. In this study, the entire collection had ADC, 40% of which harbored the upstream *ISAbal*. The detection of *Int1* is considered a good indicator for the spread of epidemic *Acinetobacter* isolates and it can be responsible for the integration of resistance markers either on the chromosome or plasmid.²⁶ In the present study, the prevalence of *Int1* was universal; however in a recent study from Egypt, the prevalence of *Int1* was detected in 85% of *Acinetobacter* isolates.³⁰

Current knowledge of *A. baumannii* is presented in this paper. This report highlights the emergence of *bla*_{OXA-23}-like and *bla*_{GES}-like genes, especially those conferring carbapenem resistance in *A. baumannii*. We can conclude that these isolates were devoid of class B MBL. PER-1 is the dominant ESBL and ADC is the dominant extended-spectrum cephalosporinase. *ISAbal* plays an important role in the over-expression of *bla*_{OXA-51}. To our knowledge, PER-, GES- and ADC-like have not been reported in *A. baumannii* from Egypt. Heterogeneous groups of β -lactamases were identified in our isolates.

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