ORIGINAL ARTICLE

First report of colistin resistance among carbapenem-resistant *Acinetobacter baumannii* isolates recovered from hospitalized patients in Egypt

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Abstract

Acinetobacter baumannii is an opportunistic pathogen that poses an increasing threat in the health-care community. Colistin is one of the promising options for treatment of multidrug-resistant *A. baumannii*. The current study investigated the emergence of colistin resistance among carbapenem-resistant strains of *A. baumannii* in Egypt. It involved identification of clinically recovered *A. baumannii* isolates using the VITEK-2 system, and screening of their antimicrobial susceptibilities using broth microdilution techniques. Characterizations of carbapenemase and 16S rRNA methyltransferase genes were performed using PCR. Colistin-resistance determinants were characterized by sequencing. Carbapenem-resistant *A. baumannii* isolates (n = 40) showed resistance to amoxicillin-clavulanic acid, cefotaxime, gentamicin and amikacin. Most isolates revealed resistance to ciprofloxacin (95%; n = 38) and co-trimoxazole (92.5%; n = 37). Resistance to tobramycin and doxycycline was 80% (n = 32) and 62.5% (n = 25), respectively. Only two *A. baumannii* isolates demonstrated colistin resistance. Carbapenemase activity was tested by modified Hodge test and 78% of isolates were positive. All isolates carried $bla_{\text{OXA-51}}$-like genes whereas $bla_{\text{-OXA-23}}$ was detected in 80% (n = 32) of isolates. Among 16S rRNA methylase genes, *armA* was detected in 22.5% (n = 9) of the isolates. Analyses of *lpxA*, *lpxC*, *lpxD* and *pmrCAB* genetic sequences suggest that colistin resistance could be attributed to mutations in *pmrCAB* genes. Alarmingly, colistin resistance was associated with high levels of resistance to other antimicrobials. The current findings represent a serious health-care problem capable of restraining future therapeutic options.

Keywords: *Acinetobacter baumannii*, *armA*, $bla_{\text{OXA-23}}$, colistin resistance, *pmrABC*genes
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Introduction

Infections with multiple drug-resistant *Acinetobacter baumannii* are of increasing concern [22]. *Acinetobacter* is the main source of various infections including pneumonia, septicaemia, wound sepsis, urinary tract infection and meningitis [22]. Multidrug-resistant (MDR) *A. baumannii* strains are shown to be responsible for several worldwide outbreaks. *Acinetobacter baumannii* has the ability to survive harsh conditions, also their resistance to disinfectants allows them to persist in the hospital environment [9,20]. Carbapenems have been the most appropriate option for treatment of *A. baumannii* infections, but the development of carbapenem-resistant *A. baumannii* (CRAB) significantly limits their use. Several mechanisms are involved in carbapenem resistance, including production of carbapenem-hydrolysing β-lactamas (carbapenemase), reduced permeability and active efflux. Carbapenemase is the most common resistance mechanism, including the intrinsic $bla_{\text{OXA-51}}$-like and the acquired $bla_{\text{OXA-23}}$-like, $bla_{\text{OXA-24}}$-like, and $bla_{\text{OXA-58}}$-like forms [34].

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Nowadays, colistin (polymyxin E), is one of the last therapeutic options for CRAB strains. Colistin was recently reintroduced as the last resort for treatment of MDR *A. baumannii* infections [26,15] and colistin therapy with a new dosing regimen has lowered its toxic effects [15]. Nevertheless, some strains are now reported to be colistin resistant, as well as extensively drug resistant. MDR *A. baumannii* isolates have been recovered from intensive care units in Mediterranean hospitals and various countries [17,9].

A prominent mechanism involved in *A. baumannii* resistance to colistin is the mutation within the lipid A biosynthetic pathway that consequently causes a loss of outer lipopolysaccharide (LPS) and elimination of colistin target site [6]. Another potential mechanism is the alteration of lipid A components of LPS through mutations in the *pmrA* and *pmrB* genes of the regulatory system and *pmrC* that encodes a lipid A phosphoethanolamine transferase enzyme [30,32,4,29]. To the best of our knowledge, this is the first report addressing the emergence of colistin resistance, as well as its potential underlying mechanisms, among clinical isolates of CRAB in Egypt.

Materials and methods

Clinical isolates

Forty non-duplicated carbapenem-resistant *Acinetobacter baumannii* isolates were recovered from different clinical specimens (pus, sputum and urine) of inpatients admitted to El-Kasr El-Aini hospital (Cairo, Egypt) from January 2015 to July 2015.

Identification and susceptibility testing

Identification and antimicrobial susceptibility testing of clinical isolates were carried out using the VITEK-2 system (bioMérieux, Marcy l'Étoile, France). MICs were determined according to The CLSI guidelines using the broth microdilution method. *Escherichia coli* ATCC 25922 and *A. baumannii* ATCC17978 were used as control strains [8].

β-Lactamase assays

Clinical isolates of *A. baumannii* were screened for carbapenemase and metallo-β-lactamase (MBL) production using the modified Hodge test (MHT) and imipenem-EDTA double-disc synergy test, respectively. The two methods were performed as previously described [40,19].

Molecular characterization of carbapenemase and 16S rRNA methyltransferase genes

DNA extraction was performed using DNeasy Blood & Tissue Kits (Qiagen, Hilden, Germany). Carbapenemase-encoding genes ($bla_{OXA\text{-}23\text{-}like}$, $bla_{OXA\text{-}24\text{-}like}$, $bla_{OXA\text{-}51\text{-}like}$ and $bla_{OXA\text{-}58\text{-}like}$) were detected by PCR for the tested isolates. The amplification conditions involved, initial denaturation at 94°C for 5 min, 30 cycles of 94°C for 25 s, 52°C for 40 s, and 72°C for 50 s, and a final elongation at 72°C for 7 min, as described previously [39].

Furthermore, all *A. baumannii* isolates were subjected to multiplex-PCR for amplification of 16S rRNA methyltransferase genes (*armA*, *rmtB* and *rmtC* genes) using the primers and PCR conditions that were previously described by Doi et al. [11]. Specific primers used in this study are described in Table 1.

Investigation of colistin-resistant determinants

The colistin-resistant strains were screened for the presence of mutations in the *lpxA*, *lpxC*, *lpxD* and *pmrCAB* genes. The genes were amplified by PCR as described previously [4,30,23]. The PCR amplicons were sequenced on both DNA strands using ABI 310 Genetic analyser sequencing (Applied Biosystems, Foster City, CA, USA). Nucleotide sequences of *lpxA*, *lpxC*, *lpxD* and *pmrCAB* genes from colistin-resistant isolates were compared with the respective sequences obtained from colistin-susceptible and *A. baumannii* ATCC17978 strains.

Ethical approval

All experiments were carried out in accordance with the ethical standards of the institutional and national research committee

TABLE 1. List of primers used in this study

Gene	Primer sequences		Product size	References
$bla_{OXA\text{-}23}$	Forward	5′-GATCGGATTGGAGAACCAGA-3′	501 bp	16
	Reverse	5′-ATTTCTGACCGCATTTCCAT-3′		
$bla_{OXA\text{-}24}$	Forward	5′-TTCCCCTAACATGAATTTGT-3′	1024 bp	16
	Reverse	5′-GTACTAATCAAAGTTGTGAA-3′		
$bla_{OXA\text{-}51}$	Forward	5′-TAATGCTTTGATCGGCCTTG-3′	353 bp	16
	Reverse	5′-TGGATTGCACTTCATCTTGG-3′		
$bla_{OXA\text{-}58}$	Forward	5′-TGGCACGCATTTAGACCG-3′	507 bp	16
	Reverse	5′-AAACCCACATACCAACCC-3′		
armA	Forward	5′-ATT CTG CCT ATC CTA ATT GG-3′	315 bp	17
	Reverse	5′-ACC TAT ACT TTA TCG TCG TC-3′		
rmtB	Forward	5′-GCT TTCTGCGGG CGA TGTAA-3′	173 bp	17
	Reverse	5′-ATG CAA TGC CGC GCT CGT AT-3′		
rmtC	Forward	5′-CGA AGA AGT AAC AGC CAA AG-3′	711 bp	17
	Reverse	5′-ATC CCA ACA TCT CTC CCA CT-3′		

TABLE 2. Phenotypic and genotypic characterization of *Acinetobacter baumannii* isolates

	MIC (mg/L) (antibiotic susceptibility patterns)[a]														
Sample	**AMC**	**CTX**	**IPM**	**MEM**	**CIP**	**CN**	**AK**	**Tob**	**DO**	**SXT**	**CO**	**Type**	**Gender**	**MHT**	**Genes**
A101	512(R)	256(R)	64(R)	64(R)	128(R)	256(R)	512(R)	256(R)	32(R)	4/76(R)	0.25(S)	Sputum	Male	+	bla_{OXA-51}, bla_{OXA-23}, *armA*
A102	512(R)	128(R)	64(R)	64(R)	64(R)	256(R)	256(R)	1(S)	4(S)	16/304(R)	0.25(S)	Sputum	Female	+	bla_{OXA-51}, bla_{OXA-2}3
A103	256(R)	256(R)	64(R)	32(R)	64(R)	256(R)	256(R)	512(R)	2(S)	32/608(R)	0.25(S)	Sputum	Male	–	bla_{OXA-51}, *armA*
A104	512(R)	256(R)	128(R)	64(R)	64(R)	256(R)	128(R)	128(R)	2(S)	64/1216(R)	0.5(S)	Sputum	Female	–	bla_{OXA-51}
A105	512(R)	256(R)	64(R)	64(R)	32(R)	256(R)	32(R)	1(S)	1(S)	2/38(S)	0.25(S)	Sputum	Female	+	bla_{OXA-51}, bla_{OXA-23}
A106	512(R)	256(R)	64(R)	32(R)	128(R)	128(R)	256(R)	512(R)	64(R)	32/608 (R)	0.25(S)	Urine	Male	+	bla_{OXA-51}, bla_{OXA-23}, *armA*
A107	256(R)	256(R)	128(R)	64(R)	32(R)	128(R)	256(R)	256(R)	32(R)	16/304(R)	0.5(S)	Urine	Male	+	bla_{OXA-51}, bla_{OXA-23}, *armA*
A108	512(R)	256(R)	64(R)	32(R)	32(R)	256(R)	256(R)	1(S)	64(R)	32/608(R)	0.25(S)	Sputum	Female	+	bla_{OXA-51}, bla_{OXA-23}
A109	512(R)	256(R)	64(R)	64(R)	64(R)	512(R)	256(R)	512(R)	128(R)	16/304(R)	0.25(S)	Sputum	Male	+	bla_{OXA-51}, bla_{OXA-23}, *armA*
A110	512(R)	256(R)	64(R)	64(R)	32(R)	256(R)	256(R)	64(R)	2(S)	32/608(R)	0.5(S)	Urine	Male	+	bla_{OXA-51}, bla_{OXA-23}
A111	256(R)	256(R)	32(R)	32(R)	16(R)	128(R)	256(R)	512(R)	1(S)	16/304 (R)	0.25(S)	Urine	Male	–	bla_{OXA-51}, *armA*
A112	512(R)	256(R)	128(R)	64(R)	64(R)	256(R)	512(R)	512(R)	1(S)	32/608(R)	0.5(S)	Urine	Female	+	bla_{OXA-51}, bla_{OXA-23}, *armA*
A113	512(R)	256(R)	32(R)	64(R)	32(R)	128(R)	128(R)	128(R)	128(R)	64/1216(R)	0.5(S)	Pus	Male	+	bla_{OXA-51}, bla_{OXA-23}
A114	512(R)	256(R)	64(R)	64(R)	128(R)	256(R)	512(R)	512(R)	2(S)	16/304 (R)	0.25(S)	Sputum	Male	+	bla_{OXA-51}, bla_{OXA-23}, *armA*
A115	512(R)	256(R)	32(R)	32(R)	64(R)	256(R)	128(R)	1(S)	64(R)	1/19(S)	0.25(S)	Sputum	Female	+	bla_{OXA-51}, bla_{OXA-23}
A116	512(R)	512(R)	64(R)	64(R)	16(R)	256(R)	256(R)	1(S)	128(R)	32/608 (R)	0.5(S)	Urine	Female	+	bla_{OXA-51}, bla_{OXA-23}
A117	512(R)	128(R)	64(R)	32(R)	32(R)	128(R)	256(R)	64(R)	32(R)	32/608 (R)	0.25(S)	Urine	Male	+	bla_{OXA-51}, bla_{OXA-23}
A118	512(R)	256(R)	32(R)	32(R)	16(R)	128(R)	128(R)	128(R)	2(S)	64/1216 (R)	0.25(S)	Urine	Male	+	bla_{OXA-51}
A119	256(R)	128(R)	32(R)	64(R)	16(R)	64(R)	256(R)	64(R)	64(R)	32/608(R)	32(R)	Urine	Male	+	bla_{OXA-51}, bla_{OXA-23}
A120	512(R)	256(R)	64(R)	128(R)	64(R)	128(R)	256(R)	1(R)	32(R)	32/608(R)	0.25(S)	Sputum	Female	+	bla_{OXA-51}, bla_{OXA-23}
A121	512(R)	256(R)	32(R)	64(R)	64(R)	256(R)	256(R)	1(S)	64(R)	4/76(R)	1(S)	Sputum	Male	+	bla_{OXA-51}, bla_{OXA-23}
A122	512(R)	256(R)	32(R)	64(R)	32(R)	128(R)	128(R)	1(S)	128(R)	32/608 (R)	0.25(S)	Urine	Female	+	bla_{OXA-51}, bla_{OXA-23}
A123	512(R)	256(R)	32(R)	32(R)	32(R)	128(R)	256(R)	64(R)	32(R)	32/608 (R)	0.25(S)	Pus	Male	–	bla_{OXA-51}
A124	512(R)	128(R)	64(R)	64(R)	1(S)	256(R)	256(R)	128, R	64(R)	32/608 (R)	1(S)	Sputum	Male	+	bla_{OXA-51}, bla_{OXA-23}
A125	512(R)	512(R)	128(R)	64(R)	32(R)	512(R)	256(R)	64(R)	2(S)	64/1216 (R)	0.25(S)	Urine	Male	+	bla_{OXA-51}, bla_{OXA-23}
A126	512(R)	256(R)	32(R)	128(R)	64(R)	512(R)	256(R)	64(R)	16(R)	16/304(R)	1(S)	Pus	Male	+	bla_{OXA-51}, bla_{OXA-23}
A127	512(R)	512(R)	32(R)	32(R)	128(R)	512(R)	512(R)	64(R)	4(S)	64/1216 (R)	0.25(S)	Sputum	Male	–	bla_{OXA-51}, bla_{OXA-23}
A128	512(R)	512(R)	64(R)	64(R)	128(R)	256(R)	256(R)	128(R)	32(R)	32/608(R)	0.25(S)	Sputum	Male	–	bla_{OXA-51}
A129	512(R)	256(R)	32(R)	64(R)	1(S)	128(R)	512(R)	64(R)	1(S)	32/608(R)	0.25(S)	Sputum	Male	+	bla_{OXA-51}, bla_{OXA-23}
A130	512(R)	512(R)	32(R)	32(R)	32(R)	256(R)	512(R)	128(R)	32(R)	64/1216(R)	0.25(S)	Sputum	Female	–	bla_{OXA-51}, bla_{OXA-23}
A131	512(R)	128(R)	32(R)	32(R)	64(R)	512(R)	512(R)	64(R)	1(S)	16/304(R)	0.25(S)	Sputum	Male	+	bla_{OXA-51}, bla_{OXA-23}
A132	512(R)	512(R)	128(R)	64(R)	32(R)	256(R)	256(R)	128(R)	64(R)	32/608(R)	2(S)	Pus	Male	–	bla_{OXA-51}
A133	512(R)	256(R)	64(R)	64(R)	32(R)	256(R)	256(R)	64(R)	16(R)	32/608 (R)	2(S)	Sputum	Male	+	bla_{OXA-51}, bla_{OXA-23}
A134	512(R)	256(R)	64(R)	64(R)	16(R)	256(R)	128(R)	64(R)	32(R)	4/76(R)	1(S)	Pus	Male	+	bla_{OXA-51}, bla_{OXA-23}
A135	512(R)	256(R)	32(R)	64(R)	128(R)	256(R)	512(R)	128(R)	64(R)	4/76(R)	2(S)	Urine	Female	+	bla_{OXA-51}, bla_{OXA-23}
A136	512(R)	128(R)	64(R)	32(R)	128(R)	512(R)	256(R)	512(R)	128(R)	64/1216(R)	2(S)	pus	Female	+	bla_{OXA-51}, bla_{OXA-23}, *armA*
A137	512(R)	128(R)	32(R)	64(R)	128(R)	128(R)	128(R)	64(R)	16(R)	32/608 (R)	0.5(S)	sputum	Male	+	bla_{OXA-51}, bla_{OXA-23}
A138	512(R)	512(R)	128(R)	64(R)	32(R)	256(R)	256(R)	64(R)	64(R)	(R)	2(S)	pus	Male	–	bla_{OXA-51}
A139	512(R)	128(R)	32(R)	128(R)	64(R)	256(R)	256(R)	128(R)	2(S)	2/38(S)	0.5(S)	pus	Male	+	bla_{OXA-51}, bla_{OXA-23}
A140	512(R)	128(R)	64(R)	64(R)	128(R)	128(R)	64(R)	64(R)	1(S)	32/608 (R)	32(R)	Urine	Male	+	bla_{OXA-51}, bla_{OXA-23}

AK, amikacin; AMC, co-amoxiclav; CIP, ciprofloxacin; CN, gentamicin; CO, colistin; CTX, cefotaxime; Do, doxycycline; IPM, imipenem; MEM, meropenem; SXT, co-trimoxazole; TOB, tobramycin; +, positive; –, negative; MHT, modified Hodge test.
[a]Interpretive breakpoints of antibiotic susceptibility are based on the CLSI criteria.

and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards.

Results

Throughout the study period, 40 CRAB isolates were recovered from male (*n* = 28; 70%) and female (*n* = 12; 30%) patients in the El-Kasr El-Aini hospital (Cairo, Egypt). Almost half of the *A. baumannii* isolates were recovered from sputum samples (*n* = 19; 47.5%) followed by urine (*n* = 13; 32.5%) and pus (*n* = 8; 20%). All isolates (*n* = 40; 100%) were resistant to amoxicillin-clavulanic acid, cefotaxime, imipenem, meropenem, gentamicin and amikacin. Most isolates showed resistance to ciprofloxacin (95%; *n* = 38) and co-trimoxazole (92.5%; *n* = 37). Resistance to tobramycin and doxycycline was found in 80% (*n* = 32) and 62.5% (*n* = 25) of the tested clinical isolates, respectively. Only two *A. baumannii* isolates (A119 and A140) demonstrated colistin resistance and they were selected for further study (Table 2).

All *A. baumannii* isolates were negative for MBLs using an EDTA double-disc synergy test and 78% of the tested isolates showed positive carbapenemases activity by MHT (Table 2). In contrast, all isolates (100%; *n* = 40) harboured bla_{OXA-51}-like genes while *bla*-$_{OXA-23}$ was detected in 80% (*n* = 32) of isolates (Fig. 1). None of the tested isolates harboured bla_{OXA-58}-like or bla_{OXA-24}-like genes. Among 16S rRNA methyltransferase genes, *armA* was detected in 22.5% (*n* = 9) of the isolates. While *rmtB* and *rmtC* genes were not detected among the studied isolates.

Colistin-resistant isolates (5%; *n* = 2) were genetically screened for the existence of mutations in the *lpxA*, *lpxC*, *lpxD* and *pmrCAB* genes. The obtained sequences of the tested genes were analysed using BLAST at the National Center of Biotechnology Information website (http://www.ncbi.nlm.nih.gov/BLAST). Analyses of sequences were also carried out through the EXPASY translate tool at the Swiss Institute of Bioinformatics website (http://web/expasy.org/translate/) as well as the CLUSTALW2 multiple sequence alignment program (http://www.ebi.ac.uk/Tools/msa/clustalw2/).

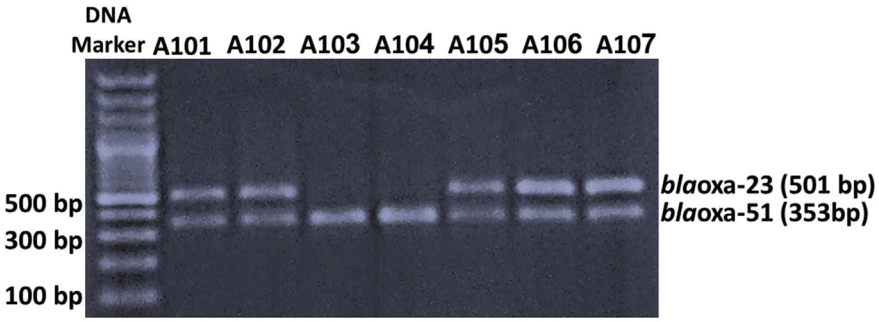

FIG. 1. Multiplex PCRs for detection of *blaOXA* carbapenemase genes. First lane represents DNA marker (100-bp DNA ladder). The agarose gel illustrates that *Acinetobacter baumannii* strains A101, A102, A105, A106 and A107 are positive for both bla_{OXA-23} and bla_{OXA-51}; *A. baumannii* A103 and A104 are bla_{OXA-23} gene negative.

Sequencing of *lpxA*, *lpxC* and *lpxD* genes revealed the presence of five amino acid substitutions (three in the *lpxA* gene (A82E, H135Y and H257Q), two in the *lpxC* gene (D296N and V324I), and none in the *lpxD* gene) in sequences of both colistin-resistant and colistin-susceptible *A. baumannii* isolates, compared with that of *A. baumannii* ATCC 17978. Consequently, the aforementioned amino acid substitutions may not play a role in colistin resistance in the tested *A. baumannii* isolates.

Regarding *pmrCAB* genes, unique mutation patterns of *pmrCAB* genes were detected in colistin-resistant isolates as follows: *pmrA* (L46F), *pmrB* (Y125F, A140T, P174L and A456V) and *pmrC* (A81K, I238G, V254A and K553T) in comparison with their sequences in *A. baumannii* ATCC 17978. Our GenBank accession numbers are LC371058 and LC371059 for *pmrC* and *pmrB*, respectively.

Discussion

Prevalence of *A. baumannii* has become a critical problem that threatens health care in Egypt. The current study involved 40 carbapenem-resistant isolates. Demographic data illustrated that most of these isolates were collected from male patients. Most of the *A. baumannii* isolates were associated with respiratory tract infections. None of amoxicillin-clavulanic acid, cefotaxime, imipenem, meropenem, gentamicin or amikacin was effective in treatment of the recovered isolates. Out of 40 isolates, 80% and 62.5% of isolates showed resistance to tobramycin and doxycycline, respectively. The highest susceptibility rate (95%) was observed with colistin, as only two isolates (5%) were colistin resistant. Accordingly, colistin remains the most effective agent for treatment *A. baumannii* infection in comparison with other tested antibiotics.

During the last decade, carbapenems were the treatment of choice for management of MDR *A. baumannii* [3]. Nevertheless, carbapenem misuse and over-use for management of *Acinetobacter* infections is responsible for the emergence of CRAB [5]. The importance of colistin as one of the last therapeutic options has been noted as a result of the escalation in CRAB. Carbapenemase is considered one of the main resistance mechanisms of *A. baumannii* to carbapenem. We screened the production of carbapenemase and MBL phenotypically using MHT and EDTA double-disc synergy, respectively. MHT revealed that 78% of isolates showed positive carbapenemase phenotype. On the other hand, all isolates were MBL negative according to an EDTA double-disc synergy test, indicating that CRAB isolates do not produce MBLs. This finding agrees with that of Mathlouthi et al. [28].

Worldwide dissemination of MDR *A. baumannii* harbouring OXA-type carbapenemase has been progressively reported [33]. The OXA-type carbapenemase has a relatively lower catalytic efficiency to hydrolyse carbapenems compared with MBLs, but it is essential to consider its presence as a crucial factor because the expression of OXA-type carbapenemase can be fundamentally unregulated by the upstream existence of insertion sequence elements such as insertion sequence Aba1 [37,35]. The impact of OXA-type carbapenemase on the resistance profile can be intensified when other mechanisms of resistance are present, such as increased expression of efflux pumps and/or loss of some porins [38,27,31].

In the current work, a bla_{OXA-51}-like gene was detected in all isolates whereas bla_{OXA-23} was harboured by 80% of the isolates. The bla_{OXA-51}-like genes are intrinsic genes in *A. baumannii* species. In this study, the results revealed the presence of *A. baumannii* isolates (20%) carrying only *bla*oxa$_{-51}$ carbapenemase, and showing a high level of resistance to carbapenem. This may be attributed to the existence of other carbapenem-hydrolysing enzymes. Furthermore, carbapenem resistance can possibly be mediated by a combination of *bla*oxa$_{-51}$ and efflux, as reported previously [18].

The noticeable prevalence of bla_{OXA-23}-carrying *Acinetobacter* in Egypt is a crucial health-care concern that necessitates strict interventions to eliminate such infections [13,12]. Neither bla_{OXA-24} nor bla_{OXA-58} was found among the tested isolates. This finding is consistent with that of Ghaith et al. [16].

The 16S rRNA methylation mechanism has been found to confer high aminoglycoside resistance levels on *A. baumannii*

[11]. The *armA* gene was reported in *A. baumannii* isolates recovered from Korea [24], China [41] and North America [11]. In the current study, the *armA* gene of 16S rRNA methyltransferase could be detected in 22.5% of the tested isolates. All the *armA*-positive isolates showed high aminoglycosides resistance rates with amikacin, gentamicin and tobramycin MICs of >256 mg/L. Many reports have addressed the coexistence of bla_{OXA-23} and *armA* among *A. baumannii* in China [36,42] and India [42,21]. In the current work, bla_{OXA-23} and *armA* coexisted in 17.5% of *A. baumannii* isolates. Some investigators have documented the emergence of colistin resistance among *A. baumannii* in China after colistin was reintroduced to treat infections with CRAB [7]. In this study, two *A. baumannii* isolates (5%) revealed colistin resistance, with a MIC value of 32 mg/L. This is the first report indicating the emergence of colistin resistance among clinical isolates of *A. baumannii* in Egypt. Many factors have been implicated in *A. baumannii* resistance to colistin. Alterations of the lipid A portion of LPS [2] or LPS biosynthetic alterations [29] were defined as the primary resistance mechanisms. Also, reduction of negative charges on the outer membrane reduces its affinity for positively charged molecules and may lead to colistin insensitivity [2,4].

It is worth noting that the emergence of colistin resistance was not linked with higher susceptibility to other antimicrobial agents. In accordance with previous reports, colistin resistance due to *pmrCAB* mutations is not associated with increased sensitivity to other antimicrobials, in contrast to *Lpx* variations [30,25].

Surveillance reports in US hospitals revealed that colistin resistance was significantly higher among imipenem non-susceptible strains compared with imipenem-sensitive strains [14]. Other reports from Bulgaria and Spain mentioned higher colistin resistance rates of 16.7% and 19.1%, respectively [7,9,10,1], compared with that in the current study.

Our findings suggest that colistin resistance was mostly attributed to mutations in *pmrCAB* genes rather than *lpx* genes. To the best of our knowledge, this is the first report addressing the emergence of colistin resistance as well as its potential underlying mechanisms among clinical isolates of CRAB in Egypt. More rigorous regulation of antibiotic prescription and administration, as well as antibiotic stewardship programmes are required in Egyptian hospitals to hinder the dissemination of CRAB and colistin-resistant *A. baumannii*.

Conclusions

Emergence of colistin-resistant/carbapenem-resistant *A. baumannii* in our health-care setting is an alarming issue. Colistin resistance was associated with mutations in *pmrABC* genes and OXA-23-like carbapenem-hydrolysing class was the most predominant carbapenemase. In addition, *armA* was the main methyltransferase gene among the clinical isolates of *A. baumannii*. Our findings revealed that colistin resistance was associated with a high resistance level to other antimicrobials. The current findings represent a serious health-care problem capable of restraining future therapeutic options. Strict regulation of antibiotic usage is needed in Egyptian hospitals to prohibit the spread of CRAB and colistin-resistant *A. baumannii* in clinical settings.

Transparency declaration

The authors declare that they have no competing interests.

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Conflict of interest

The authors declare no conflict of interest.

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