



Co-Existence of Carbapenemase-Encoding Genes in *Acinetobacter baumannii* from Cancer Patients

Reham Wasfi · Fatma Rasslan · Safaa S. Hassan · Hossam M. Ashour ·

Ola A. Abd El-Rahman

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ABSTRACT

Introduction: *Acinetobacter baumannii* is an opportunistic pathogen, which can acquire new resistance genes. Infections by carbapenem-resistant *A. baumannii* (CRAB) in cancer patients cause high mortality.

Methods: CRAB isolates from cancer patients were screened for carbapenemase-encoding genes that belong to Ambler classes (A), (B), and (D), followed by genotypic characterization by enterobacterial-repetitive-Intergenic-

consensus-polymerase chain reaction (ERIC-PCR) and multilocus-sequence-typing (MLST).

Results: A total of 94.1% of CRAB isolates co-harbored more than one carbapenemase-encoding gene. The genes *bla*_{NDM}, *bla*_{OXA-23}-like, and *bla*_{KPC} showed the highest prevalence, with rates of 23 (67.7%), 19 (55.9%), and 17 (50%), respectively. ERIC-PCR revealed 19 patterns (grouped into 9 clusters). MLST analysis identified different sequence types (STs) (ST-268, ST-195, ST-1114, and ST-1632) that belong to the highly resistant easily spreadable International clone II (IC II). Genotype diversity indicated the dissemination of carbapenem-hydrolyzing, β -lactamase-encoding genes among genetically unrelated isolates. We observed a high prevalence of metallo- β -lactamase (MBL)-encoding genes (including the highly-resistant *bla*_{NDM} gene that is capable of horizontal gene transfer) and of isolates harboring multiple carbapenemase-encoding genes from different classes.

Conclusion: The findings are alarming and call for measures to prevent and control the spread of MBL-encoding genes among bacteria causing infections in cancer patients and other immunocompromised patient populations.

R. Wasfi
Department of Microbiology and Immunology,
Faculty of Pharmacy, October University for Modern
Sciences and Arts (MSA), Giza, Egypt

F. Rasslan · O. A. Abd El-Rahman
Department of Microbiology and Immunology,
Faculty of Pharmacy (Girls), Al-Azhar University,
Cairo, Egypt

S. S. Hassan
Department of Clinical Pathology, National Cancer
Institute, Cairo University, Cairo, Egypt

H. M. Ashour (✉)
Department of Integrative Biology, College of Arts
and Sciences, University of South Florida, St.
Petersburg, FL, USA
e-mail: hossamking@mailcity.com

H. M. Ashour
Department of Microbiology and Immunology,
Faculty of Pharmacy, Cairo University, Cairo, Egypt

Keywords: Cancer; Carbapenem-resistant *Acinetobacter baumannii* (CRAB); Metallo- β -lactamase (MBL); Multilocus sequence typing (MLST)

Key Summary Points

The majority (94.1%) of carbapenem-resistant *A. baumannii* (CRAB) isolates from cancer patients harbored more than one carbapenemase-encoding genes.

We observed a high prevalence of metallo- β -lactamase-encoding genes including blaNDM, blaOXA-23-like, and blaKPC.

MLST analysis identified different STs that belong to the highly resistant easily spreadable International clone II.

Measures should be implemented to control the spread of this clone.

DIGITAL FEATURES

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INTRODUCTION

Cancer patients are high-risk, immunocompromised, and may experience long hospital stays. Thus, they are more prone to infections with opportunistic bacteria such as *Acinetobacter baumannii* [1]. *A. baumannii* is a Gram-negative, aerobic, non-motile coccobacillus. Moreover, it is an opportunistic pathogen that can cause severe, life-threatening healthcare-associated infections such as bacteremia, pneumonia, meningitis, and endocarditis [2]. The fairly recent emergence and increased prevalence of multidrug-resistant (MDR) *A. baumannii* is worrisome. *A. baumannii* is now listed by the World Health Organization as one of the critical pathogens, which highlights the need for the development of new antimicrobials [5]. This can be attributed to the increased resistance to multiple antibiotics, including last resort

antibiotics, such as carbapenems, which are reserved for cases when all alternatives have been exhausted (typically used with MDR bacteria in hospitalized patients) [6]. The high resistance patterns of *A. baumannii* are due to the upregulation of intrinsic antimicrobial resistance genes in addition to their genomic plasticity, allowing the acquisition of new resistance genes through mobile genetic elements such as plasmids and transposons [7]. Many mechanisms can decrease susceptibility of *A. baumannii* to carbapenems, including the production of carbapenemase enzyme [10], loss of outer membrane proteins [11], overexpression of multidrug efflux pumps [12], and alterations in penicillin-binding proteins [2].

Among the previous mechanisms, the production of carbapenemase enzyme is considered to be the main mechanism of resistance to carbapenems [10]. Serine carbapenemases and metallo- β -lactamases are two carbapenemase groups that have been defined according to their active sites. Serine carbapenemases include class (A) penicillinases and class (D) oxacillinases, whereas class (B) carbapenemases belong to metallo- β -lactamases (MBL) that are inhibited by EDTA. Class (A) carbapenemases include the IMI/NMC, SME, KPC, and GES enzymes, whereby KPC and GES enzymes are plasmid-encoded and thus highly spreadable [13]. Class (B) MBL include IMP, VIM, GIM, SIM, and NDM enzymes, whose genes are mainly found in transferable plasmids. The NDM-encoding gene was first detected in a *Klebsiella pneumoniae* isolate [14]. NDM-1 has spread worldwide and is one of the most common carbapenemases in Enterobacteriaceae and *A. baumannii* [15]. Class (D) β -lactamases are also known as oxacillinases, for ‘oxacillin-hydrolyzing’, or OXA β -lactamases. Genes encoding OXA β -lactamases are present in plasmids and chromosomes. Worldwide, the most common OXA-encoding gene groups in *A. baumannii* are the OXA-23, OXA-24/40, and OXA-58 groups, whereas OXA-143 has only been detected from *A. baumannii* isolates in Brazil [16]. OXA-48 can typically be detected in *K. pneumoniae* [13]. The oxacillinase are relatively lower in activity than other types of carbapenemases, but overexpression of these genes has been observed in the presence of

insertion sequences (IS) upstream of these genes which can provide additional promoters [17]. There are three “worldwide” clonal lineages (International clones: ICs I, II, and III) for *A. baumannii* [18]. The international clone II shows worldwide spread in many hospitals which can be attributed to the ability of this lineage to incorporate new genes and their adaptation to hospital environment [2]. The rapid spread of multidrug-resistant *A. baumannii* clinical isolates among cancer patients in the last two decades is worrisome because infection by this bacteria is associated with a high rate of mortality among this vulnerable group [8].

The aim of the current study was to investigate the dissemination of carbapenemase-encoding genes among carbapenem-resistant *A. baumannii* (CRAB) isolates from cancer patients followed by the genotypic analysis of these isolates. This will help to tailor the antimicrobial protocols in healthcare settings and to improve infection control policies.

METHODS

Bacterial Isolates

A total of 520 isolates were recovered from blood samples in cases of blood infection of cancer patients at the National Cancer Institute (NCI), Giza, Egypt, from July 2017 to January 2018. Ethical approvals were obtained from the Ethics committees of the NCI and the Faculty of Pharmacy, October University for Modern Sciences and Arts. Consents from patients were obtained before the inception of the study. Samples were streaked on CHROMagar Acinetobacter supplemented with CR102 (CHROMagar, France) for isolation of multidrug-resistant *Acinetobacter* sp., then isolated colonies were identified using the VITEK2 automated system (BioMerieux, Marcy-l'Étoile, France). Identification was confirmed by polymerase chain reaction (PCR) amplification of the intrinsic *bla*_{OXA-51-like} [19], in addition to using matrix-assisted laser desorption ionization–time of flight mass spectrometry (MALDI-TOF/MS) (Microflex LT; Bruker Daltonics) [20]. All isolates were preserved in glycerol broth at –20 °C.

Antimicrobial Susceptibility Testing

Antimicrobial susceptibility patterns were determined by the VITEK2 system and the results were interpreted according to the Clinical and Laboratory Standards Institute (CLSI) guidelines [21]. The classes of antibiotics used in this test included β -lactams, aminoglycosides, quinolones, folate pathway inhibitors, glycol-cycline, and polymyxin. Minimum inhibitory concentrations (MICs) of meropenem and colistin, for all CRAB isolates, were determined by the agar dilution, and micro-broth dilution methods, respectively, according to the CLSI [21] guidelines.

Detection of the Carbapenemase-Encoding Genes

Pure colonies of carbapenem-resistant isolates were used for DNA extraction using the Thermo Scientific™ GeneJet™ genomic DNA purification kit (Thermo Scientific, MA, USA), according to the manufacturer's recommendations and kept at –20 °C. PCR was performed to screen for the presence of carbapenemase-encoding genes belonging to Ambler classes (A), (B), and (D), using the primers and annealing temperatures described in Table 1. Genes were amplified by initial denaturation at 94 °C for 5 min, followed by 35 cycles consisting of 3 phases: DNA denaturation at 94 °C for 0.5 min, annealing according to Table 1 for 0.5 min, and elongation at 72 °C for time (1 Kb/1 min); finally, elongation at 72 °C for 10 min. A combination of forward IS*Aba1* primer and reverse primers of class (D) encoding genes were used to detect the presence of the IS*Aba1* insertion element upstream carbapenemase-encoding genes [17]. Negative control was included in all PCR assays.

Molecular Typing of CRAB Isolates by Enterobacterial Repetitive Intergenic Consensus-PCR (ERIC-PCR) and Multilocus Sequence Typing (MLST)

Clonal relatedness of collected isolates was examined via enterobacterial repetitive

Table 1 Primers used for amplification, expected product lengths, and annealing conditions

Ambler class	Genes to be amplified	F/ R	Primer sequences (5'–3')	Annealing temp. (°C)	Expected product size (bp)	References
Class A	<i>bla_{KPC}</i>	F	GCC GTG CAA TAC AGT GAT AAC	54	276	This study
		R	GCC GGT CGT GTT TCC CTT			
	<i>bla_{GES}</i>	F	GAT ACA ACT ACG CCT ATT GCT	52	99	This study
		R	CAG CCA CCT CTC AAT GGT G			
Class B	<i>bla_{NDM}</i>	F	GGTTTGGCGATCTGGTTTTC	53	621	[61]
		R	CGGAATGGCTCATCACGATC			
	<i>bla_{VIM like}</i>	F	GATGGTGTTTGGTCGCATA	54	390	[61]
		R	CGAATGCGCAGCACCAG			
	<i>bla_{IMP like}</i>	F	TTT GTG GAG CGT GGC TAT AAA	54	117	This study
		R	TAA TTC AGA TGC ATA CGT GGG			
	<i>bla_{SPM}</i>	F	TGG CTA AGA CTA TGA AGC	46	272	This study
		R	AAA CGA GAA GAC CTT GCC			
	<i>bla_{GIM}</i>	F	TCG ACA CAC CTT GGT CTG AA	54	477	[37]
		R	AAC TTC CAA CTT TGC CAT GC			
	<i>bla_{SIM}</i>	F	TAC AAG GGA TTC GGC ATC G	54	571	[37]
		R	TAA TGG CCT GTT CCC ATG TG			
Class D	<i>bla_{oxa 51}</i>	F	TAA TGC TTT GAT CGG CCT TG	54	370	[61]
		R	TGG ATT GCA CTT CAT CTT GG			
	<i>bla_{OXA 23}</i>	F	GAT CGG ATT GGA GAA CCAGA	54	501	[61]
		R	ATT TCT GAC CGC ATT TCC AT			
	<i>bla_{OXA-24/40}</i>	F	GGT TAG TTG GCC CCC TTA AA	54	246	[61]
		R	AGT TGA GCG AAA AGG GGA TT			
	<i>bla_{OXa 58}</i>	F	AAG TAT TGG GGC TTG TGC TG	54	599	[61]
		R	CCC CTC TGC GCT CTA CAT AC			
Insertion element	<i>ISAbal</i>	F	CAC GAA TGC AGA AGT TG	46	549	[61]
		R	CGA CGA ATA CTA TGA CAC			
ERIC primers	ERIC2		AAGTAAGTGACTGGGGTGAGCG	49	–	[22]

intergenic consensus (ERIC)–PCR, which was carried out as described previously by Versalovic et al. [22] using ERIC2 primer (Table 1). Electrophoretic patterns were analyzed by using the Bionumerics software v.7.6 (Applied Maths, Sint-Martens-Latem, Belgium). The BioNumerics analysis was performed using the Dice coefficient and the unweighted pair group method of averages (UPGMA) with a 1% tolerance limit and 1% optimization. Isolates that clustered with $\geq 80\%$ similarity were grouped into one ERIC type. Representative isolates from ERIC clusters of 100% similarity were subjected to typing by multilocus sequence typing (MLST), which was performed according to the Oxford scheme protocol using primers listed in the *A. baumannii* MLST database website (<https://pubmlst.org/abaumannii/>). Amplification of the seven conserved housekeeping genes (*gltA*, *gryB*, *gdhB*, *recA*, *cpn60*, *rpoD*, and *gpi*) was performed according to the protocol proposed by Bartual et al. [23]. Sanger sequencing was carried out using the ABI 3730xl DNA Analyzer at the MacroGen sequencing facility (MacroGen®, South Korea). The allelic numbers and sequence types (STs) were defined by means of the *A. baumannii* MLST database. Clusters of related STs (defined as clonal complexes; CCs) were analyzed using the Global Optimal eBURST (goeBURST) by the Phyloviz 2.0 software (<https://www.phyloviz.net/goeburst/>). Analysis of clonal complex was carried out at the level of single locus variant (SLV) and double loci variant (DVL).

RESULTS

Antibiotic Susceptibility of Collected Isolates

Forty-eight *A. baumannii* non-duplicate isolates were recovered from 520 blood samples representing 15.1% of total isolates. The *A. baumannii* isolates were recovered from 43 hospitalized patients and 5 outpatients. The identification of the *A. baumannii* isolates up to the species level was confirmed by the detection of the intrinsic *bla*_{OXA-51-like} gene and the MALDI-TOF/MS. The antibiotic resistance of the isolates was

determined by VITEK2, and six resistance patterns were detected. A total of 34 out of the 48 isolates (70.8%) were found to be resistant to meropenem and ertapenem and were, hence, designated as CRAB. The 34 CRAB isolates were recovered from cancer patients (5 outpatients and 29 inpatients). Resistance to tigecycline and colistin (last-resort antibiotics) was detected in 7/34 (20.5%) and 1/34 (2.9%) of CRAB isolates, respectively (Table 2). The MICs of meropenem against tested isolates ranged from 8 to ≥ 128 $\mu\text{g/ml}$ (Table 3).

Detection of Carbapenemase-Encoding Genes

Co-existence of more than one carbapenemase-encoding gene was detected in 28/34 (82.3%) of CRAB isolates. The carbapenemase-encoding resistance genes *bla*_{NDM}, *bla*_{OXA-23-like}, and *bla*_{KPC} showed the highest prevalence of 67.7%, 55.9%, and 50% of CRAB isolates, respectively. The Ambler class (B) MBL genes were detected in 31/34 (91%) of isolates. Among the MBL genes detected, in addition to *bla*_{NDM}, were *bla*_{GIM}, *bla*_{SPM}, *bla*_{SIM}, and *bla*_{IMP} in 38.2%, 29.4%, 8.8%, and 5.8% of CRAB isolates, respectively, while *bla*_{VIM} was not detected in any of the collected isolates. The class (D) oxacillinase coding genes *bla*_{OXA24/40} and *bla*_{OXA58} were detected in 26.4% and 2.9% of CRAB isolates, respectively. Class (A) *GES* gene was detected in 9/34 (26.4%) of isolates. The insertion element was detected upstream of *bla*_{OXA 23-like} in two isolates (ID.27 and 41), *bla*_{OXA24/40-like} in two isolates (ID 38 and 41), while it was upstream of *bla*_{OXA 51-like} in one isolate (ID.7) (Table 3).

Molecular Typing of CRAB Isolates

ERIC-PCR typing revealed that the 34 CRAB isolates were grouped into 9 clusters and classified into 19 ERIC types according to 80% cut off. Eric type “3” was the most prevailing and represented by 7 isolates (Fig. 1). The predominant cluster was G which contains 7 isolates, followed by D(5), H (5), C (4), B (3), E (3), F (3), A (2), and I (2). Isolates with 100% ERIC typing

Table 2 Antimicrobial sensitivity patterns of carbapenem resistant *A. baumannii* (CRAB) isolates

Isolate ID	Antimicrobial resistance profile					
	Beta lactam	Aminoglycoside	Quinolone	Glycylcycline	Polymyxin	Folate pathway inhibitors
1-2-4-7-14-22-24-25-28-29-30-31-32-33-35-36-37-38-39-40-41-43	MER-ERT- CXT-CAZ- CTX-CPM- AMC-SAM	GEN-TOB- AMK	CIP-LEV	–	–	SXT
6-15	MER-ERT- CXT-CAZ- CTX-CPM- AMC-SAM	GEN-TOB- AMK	CIP-LEV	–	–	–
9	MER ^a -ERT ^a - CXT-CAZ- CTX-CPM- AMC-SAM	GEN-TOB- AMK	CIP-LEV	TGC	COL	SXT
12	MER-ERT- CXT-CAZ- CTX-CPM- AMC-SAM	GEN-TOB	CIP-LEV	–	–	SXT
21-44	MER-ERT- CXT-CAZ- CTX-CPM- AMC-SAM		CIP-LEV	–	–	SXT
23-26-27-45-46-47	MER-ERT- CXT-CAZ- CTX-CPM- AMC-SAM	GEN-TOB- AMK	CIP-LEV	TGC	–	SXT

MER Meropenem, *ERT* Ertapenem, *CXT* Cefoxitin, *CAZ* Ceftazidime, *CTX* Cefotaxime, *CPM* Cefepem, *AMC* Amoxicillin/Clavulanic acid, *SAM* Ampicillin/sulbactam, *Gen* Gentamicin, *TOB* Tobramycin, *AMK* Amikacin, *CIP* Ciprofloxacin, *LEV* Levofloxacin, *TGC* Tigecycline, *COL* Colistin, *SXT* Sulfamethoxazole/trimethoprim

(–) Indicates sensitivity to antimicrobial agent

^a Indicates moderate resistance

similarities were found to be collected within 0–3 months. MLST was carried out for six isolates as representative for each of the groups in Table 3, and belonging to ERIC clones showing 100% similarity. The most prevalent MLST type was ST 286 (3 isolates), followed by ST 195, ST 1114, and ST 1632, represented by one isolate

each (Table 4). Isolates typed as MLST ST 286 were found to be isolated from the same hospital floor but from different wards. ST relatedness to CCs was analyzed by the eBURST algorithm. eBURST analysis showed that all the identified STs belong to a founder ST208. ST 286 and ST 195 were SLV of *gpi* locus from founder,

Table 3 Minimum inhibitory concentration (*MIC*), carbapenemase-encoding genes, ERIC type of 34 carbapenem-resistant *A. baumannii* isolates

	ID	Inpatient/ outpatient	MIC µg/ ml	Carbapenem-hydrolyzing enzymes				ERIC type
				Mer	Class A	Class B	Class D	
One class of carbapenemase-encoding genes	2	Inpatient	32	–	–	OXA ₂₃ , OXA ₅₁	+	E2a
	4	Inpatient	32	–	–	OXA ₂₃ , OXA ₅₁	+	E2b
Two classes of carbapenemase-encoding genes	1	Outpatient	16	–	NDM, GIM	OXA ₂₃ , OXA-24/40, OXA ₅₁	+	E1a
	14	Inpatient	32	KPC	–	OXA ₂₃ , OXA ₅₁	–	E2c
	15	Inpatient	32	–	NDM, GIM	OXA ₂₃ , OXA ₅₁	–	E2d
	24	Inpatient	32	–	NDM	OXA ₂₃ , OXA ₅₁	–	E3b
	25	Inpatient	128	–	NDM	OXA ₅₁	+	E9
	30	Inpatient	> 128	–	NDM	OXA ₅₁	+	E11
	33	Inpatient	> 128	–	NDM, SPM, GIM	OXA ₂₃ , OXA ₅₁	+	E14
	40	Inpatient	32	–	SPM, GIM	OXA-24/40, OXA ₅₁	+	E3f
	44	Inpatient	32	KPC, GES	–	OXA ₅₁	+	E1b
	45	Inpatient	> 128	–	NDM, SPM, GIM	OXA ₂₃ , OXA ₅₁	+	E18a
Three classes of carbapenemase-encoding genes	6	Inpatient	> 128	KPC	GIM	OXA ₅₁	+	E3a
	7	Outpatient	32	KPC	NDM, GIM	OXA ₂₃ , OXA ₅₁ ^a	+	E4
	9	Inpatient	8	KPC	NDM, SPM, GIM	OXA ₅₁ , OXA ₂₃	+	E5
	12	Outpatient	64	KPC, GES	NDM, SPM	OXA ₅₁ , OXA-24/40	–	E6
	21	Inpatient	64	KPC	NDM, SPM	OXA ₅₁	–	E7
	22	Inpatient	64	KPC	NDM	OXA ₂₃ , OXA ₅₁	–	E8a
	23	Inpatient	> 128	KPC	NDM, GIM	OXA ₅₁ , OXA ₂₃	–	E2e
	26	Outpatient	> 128	GES	NDM	OXA ₅₁	+	E10
	27	Outpatient	32	KPC	NDM, SPM	OXA ₂₃ ^a , OXA ₅₁	+	E8b
	28	Inpatient	> 128	KPC	NDM	OXA ₂₃ , OXA ₅₁	–	E3c
	29	Inpatient	> 128	KPC	NDM, SIM	OXA ₅₁ , OXA ₂₃	+	E3d
	31	Inpatient	> 128	KPC	NDM	OXA ₂₃ , OXA ₅₁	+	E12
	32	Inpatient	32	KPC	NDM	OXA ₂₃ , OXA ₅₁	+	E13
	35	Inpatient	> 128	GES	NDM, GIM	OXA ₂₃ , OXA ₅₈ , OXA ₅₁	–	E15a
36	Inpatient	> 128	KPC	NDM, GIM	OXA ₅₁	+	E3e	
37	Inpatient	> 128	KPC	NDM, SIM	OXA-24/40, OXA ₅₁	–	E16	
38	Inpatient	> 128	GES	SIM	OXA ₂₃ , OXA-24/40 ^a , OXA ₅₁	+	E15b	
39	Inpatient	16	GES	SPM, GIM	OXA ₂₃ , OXA-24/40, OXA ₅₁	–	E17	
41	Inpatient	16	GES	IMP, SPM	OXA ₂₃ ^a , OXA-24/40 ^a , OXA ₅₁	+	E3g	
43	Inpatient	16	GES	SPM, GIM	OXA ₂₃ , OXA-24/40, OXA ₅₁	–	E8c	
46	Inpatient	32	GES	SIM	OXA ₅₁	–	E19	
47	Inpatient	> 128	KPC	NDM, IMP	OXA-24/40, OXA ₅₁	+	E18b	

Mer Meropenem, (+) detected gene, (–) no detected genes

^a *ISAbal* upstream Ambler Class D carbapenemase-encoding gene

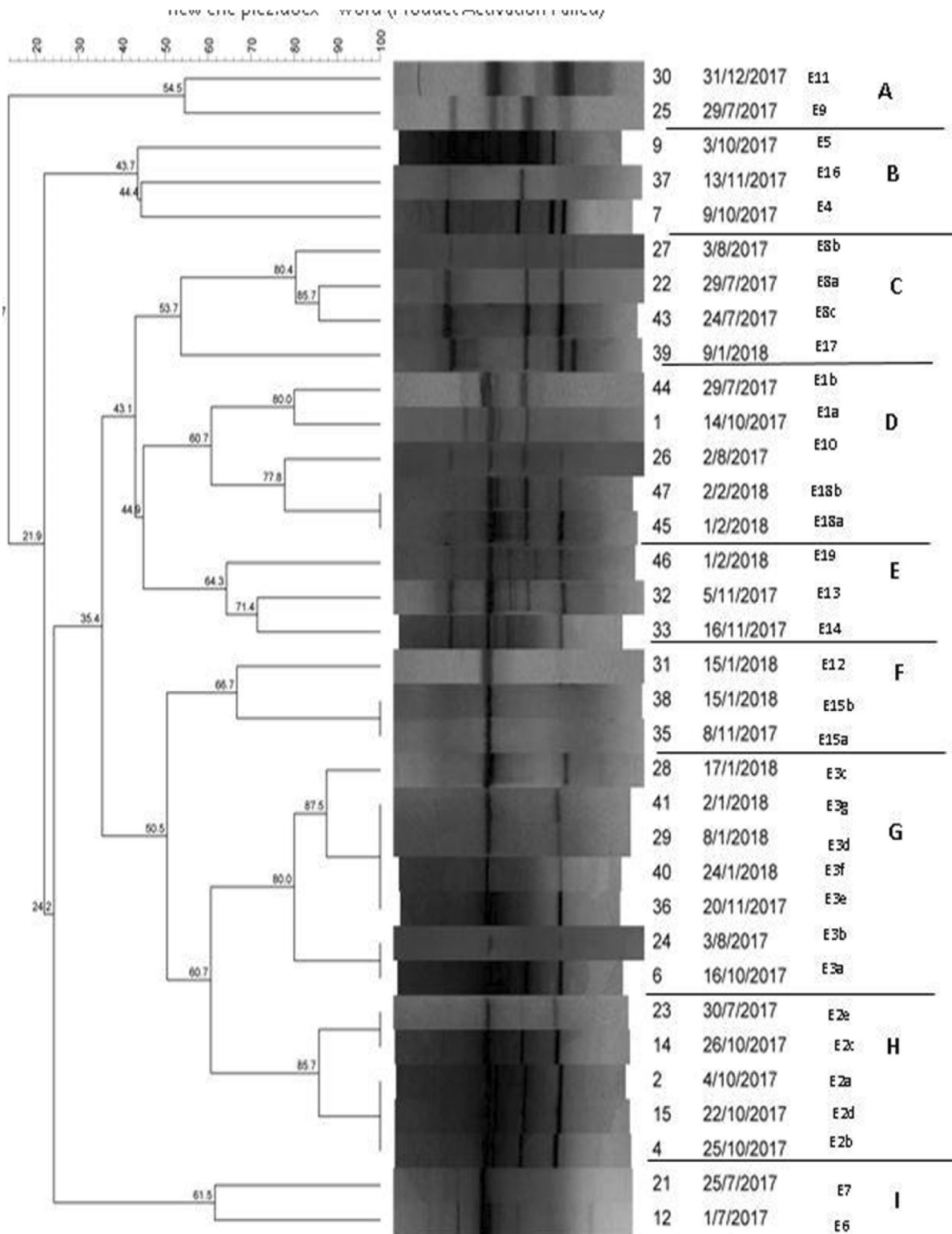


Fig. 1 Dendrogram generated by Dice coefficient and the UPGMA clustering method with 1% tolerance limit and 1% optimization, showing the genetic similarity among *A. baumannii* isolates by enterobacterial repetitive intergenic consensus (ERIC) genotyping

while ST 1114 and ST 1632 were SLV from ST437 and DLV from ST 208 founder in *gyrP* and *gpi* loci (Fig. 2). All identified STs were found to belong to CC92 (International clone II).

DISCUSSION

Cancer patients are at higher risk of acquiring *A. baumannii* infections due to several factors, including their immunocompromised state and lengthy hospital stays [10]. Infections by CRAB isolates pose a great threat for cancer patients because they are associated with a high mortality rate. The ability of *A. baumannii* to resist the reserved antibacterial agents including carbapenems is alarming, hence raising the importance of studying its prevalence and mechanism of resistance to control its spread.

Carbapenem insensitivity was detected in 70.8% (34/48) of collected isolates, which is similar to other studies carried in Egypt by Sultan and Seliem [24]. Others have shown a higher prevalence for CRAB reaching 90% [10, 25, 26], which suggests that Egypt is the topmost country in the region in CRAB

prevalence [27]. CRAB treatment typically relies on other last-resort antibiotics such as colistin and tigecycline and sometimes antibiotic combinations [28]. This becomes more challenging when isolates also exhibit resistance to last-resort antibiotics. About 21% of CRAB isolates were resistant to tigecycline; a similar prevalence of tigecycline resistance was detected by Kamel et al. [29].

Co-occurrence of a variety of intrinsic and acquired carbapenemase-encoding genes has been detected with increased prevalence for acquired carbapenemase-encoding genes known to be carried on mobile elements. Isolates co-harboring more than one acquired carbapenemase-encoding genes account for 32/34 (94.1%) of CRAB isolates. Clones carrying multiple carbapenemase-encoding genes have been detected in many studies carried out in the Middle East region [30–32], and in China [33].

Ambler class (A) *bla*_{KPC} and *bla*_{GES} genes were detected in 50% and 26.5% of CRAB isolates, respectively. The increased spread of the *bla*_{KPC} and *bla*_{GES} in *A. baumannii* clinical isolates in Egypt reached a prevalence of 56% and 48%, respectively, in Benmahmod et al.’s [26] study. The high spreading capacity of the *bla*_{KPC} and *bla*_{GES} genes could be attributed to their linkage to mobile elements such as *Tn4401* located on conjugative plasmids [34] and integrons [35], respectively, facilitating their horizontal transfer. The *bla*_{GES}-encoding gene is usually associated with a low level of carbapenem resistance (MIC 4–16 µg/ml) [9], while in the current study carbapenem MIC in isolates harboring *bla*_{GES}-

Table 4 Multilocus sequence types (MLSTs), allele profiles, and clonal complex of six carbapenem-resistant *A. baumannii* isolates

ID	Allele profile							ST	Clonal complex (CC)
	<i>gltA</i>	<i>gyrB</i>	<i>gdhB</i>	<i>recA</i>	<i>cpn60</i>	<i>gpi</i>	<i>rpoD</i>		
4	1	3	3	2	2	163	3	286	92
6	1	12	3	2	2	195	3	1632	92
23	1	3	3	2	2	96	3	195	92
35	1	3	3	2	2	163	3	286	92
41	1	3	3	2	2	163	3	286	92
45	1	12	3	2	2	79	3	1114	92

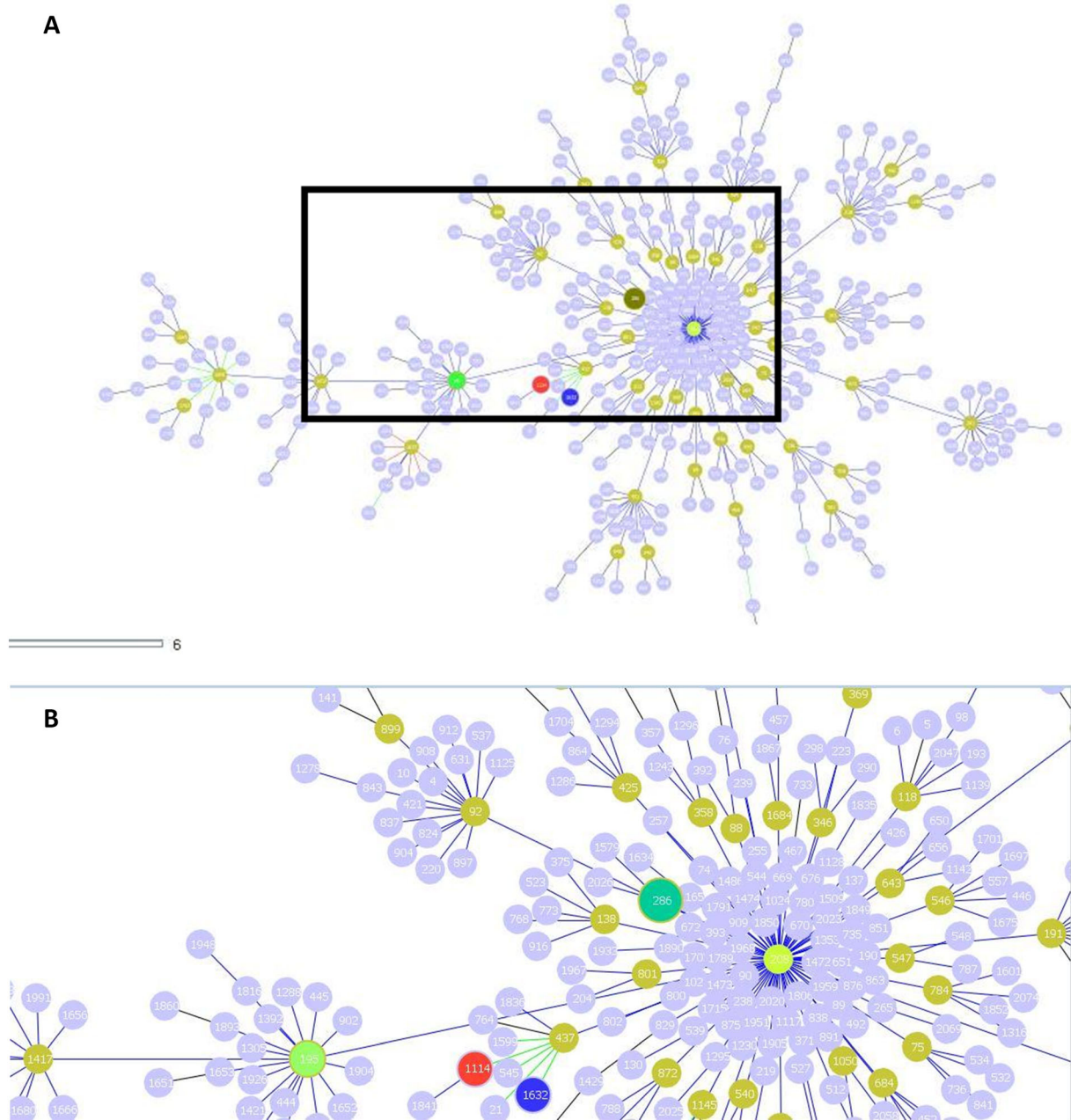


Fig. 2 eBURST analysis of *A. baumannii* strains using the Phyloviz 2.0 software. **a** Related and unrelated ST groups to the identified isolates were compared with 2087 sequences (in the MLST Oxford database). **b** STs 286 (dark green), 1114 (red), 1632 (blue), and 195 (light green)

are related to the founder ST 208 and belong to CC92. A circle represents an ST, and its size corresponds to the number of isolates

encoding gene ranged from 16 to ≥ 128 $\mu\text{g/ml}$ due to the co-existence of other carbapenemase-encoding genes. Infection with *A. baumannii*

carrying *bla*_{KPC} is usually associated with a high level of morbidity and mortality [36].

MBL-encoding genes were detected in 91% of CRAB isolates which is worrisome, because

these genes are usually correlated with high MIC [37], and they are characterized by rapid spread and high transferability between bacteria [38]. MBL-producing bacteria are a potentially great threat to modern intensive care treatment protocols [39]; hence, rapid detection and good infection control are required to reduce their impact. MICs in MBL-carrying isolates ranged from 16 to ≥ 128 $\mu\text{g/ml}$ except for one isolate (ID 9) which might indicate mutation in the MBL gene of this isolate. Class (B) carbapenemase-encoding genes including *bla*_{NDM}, *bla*_{GIM}, *bla*_{SPM}, *bla*_{SIM}, and *bla*_{IMP} were detected in 67.7%, 38.2%, 29.4%, 8.8%, and 5.8% of CRAB isolates, respectively.

High prevalence of *bla*_{NDM} gene (67.7% of CRAB isolates) was observed in our study compared to previous studies carried out in Egyptian hospitals which showed *bla*_{NDM} prevalence of 8% [40] and 39.3% [25] among CRAB isolates. The *bla*_{NDM-1} and *bla*_{NDM-2}-encoding genes were first described in *A. baumannii* isolated from Egyptian patients [41] and then a noteworthy spread of *bla*_{NDM}-positive *A. baumannii* was detected in the Middle East [42]. There are several proved mechanisms for horizontal transfer of *bla*_{NDM}-encoding gene. Jamal et al. [43] found that the transfer of MBL carbapenemase genes was associated with Tn125-type transposon and can be harbored by a plasmid or rarely integrated into the chromosome. The plasmid-harboring *bla*_{NDM-1} gene in *A. baumannii* was found to exhibit high transformation frequency via outer membrane vesicles [44]. Another mechanism for horizontal spread of *bla*_{NDM} gene among *A. baumannii* is by phage transduction [45]. A noteworthy observation in this study was the co-existence of MBL-encoding genes in some isolates which has been detected in many studies carried out in countries which suffer from uncontrolled antibiotic misuse [9, 31, 32, 46]. Lee and his colleagues found that strains harboring multiple plasmids that encode different carbapenemases showed increased fitness and virulence even in the absence of antibiotics which could increase the spread of this strain and emphasize the need for a strategy to combat this strain [47].

The increased prevalence of co-existing MBL could be attributed to the association of these

genes by class 1 (sometimes class 3) integrons, which, in turn, are embedded in transposons, resulting in a highly transmissible genetic apparatus [48]. Integrons facilitate movement of resistance genes between integrons in plasmids, and the plasmids allow transfer of genetic material to different bacteria.

Oxacillinase-encoding genes can be intrinsic (*bla*_{OXA-51-like}) or acquired (*bla*_{OXA-23-like}, *bla*_{OXA-24/40-like}, *bla*_{OXA-58-like}). Two isolates (ID 2, 4) were found to harbor solely the *bla*_{OXA-like} genes and they were found to be inhibited by relatively lower concentrations of meropenem compared to isolates harboring other types of carbapenemase genes. The oxacillinase enzymes only weakly hydrolyze carbapenems, but it was found that the insertion of a sequence such as *ISAbal* upstream of the *bla*_{OXA-like} genes may enhance the gene expression by conferring strong promoter activity. This insertion sequence could also explain the high capacity of the *bla*_{OXA-like} genes for horizontal transfer and increased clonal diversity [49]. Numerous studies in Egypt and the Mediterranean regions have classified *bla*_{OXA-23 like} gene as the most common carbapenemase-encoding gene [9, 40, 50–52]. In our study, the *bla*_{OXA-23 like} gene was detected in 64% of CRAB isolates, which is similar to the previously mentioned results. Our findings showed that clusters carrying *ISAbal* are widely distributed in our hospital, reaching 21/34 (61%), which might explain the high spread of acquired resistance genes among isolates. The *bla*_{OXA-58} gene prevalence reported here (2.9%) is lower than previously published rates from Tunisia (4%), Egypt (9.1%) [53], and Algeria (14.7%) [54].

The diversity of ERIC patterns obtained in our study suggests dissemination of carbapenem-hydrolyzing β -lactamase-encoding genes among genetically unrelated isolates of *A. baumannii*. This may be attributed to horizontal gene transfer of plasmids carrying resistance determinants. Isolates with ERIC typing similarity of 100% showed similarity in antibiotic resistance pattern, except for isolates (ID.6, 24), (ID 2, 5), and (ID14,23).

MLST was carried out for six isolates as representative for ERIC clones showing 100% similarity, and which was isolated from the same

hospital ward. The Oxford scheme of MLST typing was chosen due to its high discriminatory power, which is comparable to the resolution obtained with pulsed-field gel electrophoresis [55]. MLST analysis identified different sequence types: ST286 (3 isolates), ST195 (1 isolate), ST1114 (1 isolate), and ST1632 (1 isolate). ST195 was previously detected in Egyptian hospitals [10, 56], and it is also prevalent in the Gulf area [57] and many countries, including China [58]. The ST195 strain was previously isolated from environmental as well as clinical samples (<https://pubmlst.org/abaumannii/>). ST1114 was detected in an outbreak in a tertiary hospital in Egypt [59]. ST 1632 was not detected in previous Egyptian studies, but this type was SLV of ST437 which has been previously isolated from Mediterranean countries such as Italy and Greece (<https://pubmlst.org/abaumannii/>). All detected ST types were either SLV or DLV to ST 208 which is the common ST type detected in previous studies in Egypt [10], and also in Saudi Arabia [57]. All identified STs belong to international clone II (IC II), which is the most widely distributed highly resistant clone worldwide [40, 53, 56], and responsible for the majority of outbreaks reported around the world [55]. A correlation between the IC II and the prevalence of resistance genes and mobile elements has been detected which necessitates the need for adequate control for the spread of this clone.[60]. Some research have explained the spreadability of IC II due to prevalence of *bla*_{OXA-23-like} gene in this clone conferring high resistance for this strain [10], but, in contrast to our result, no *bla*_{OXA-23-like} gene was detected in the ST195 MLST typed isolate.

The main mechanism of carbapenem resistance is the production of carbapenemase enzyme. However, this does not eliminate the possibility of the presence of other mechanisms of carbapenem resistance. One limitation of the current study is that it does not cover these other possible mechanisms of resistance. Deciphering the role of efflux pumps in carbapenem resistance could be the scope of a future study.

CONCLUSION

The high prevalence of carbapenemase-encoding genes (including MBL-encoding genes such as *bla*_{NDM}) and their co-existence in CRAB isolates is worrisome. This is due to the potential for high spreadability and the possibility of further dissemination of the highly antibiotic-resistant genes to other bacteria. This can make treatment of these cases very challenging, especially in the immunocompromised cancer patient population. Overall, the findings are alarming and call for strict control measures to prevent the spread of these genes among bacteria causing infections in immunocompromised patient populations.

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Data Availability. The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

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