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The context of bla_{OXA-23} gene in Iraqi carbapenem-resistant *Acinetobacter baumannii* isolates belonging to global clone 1 and global clone 2

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Abstract

Background and objectives Of the genes conferring resistance to carbapenems in *Acinetobacter baumannii*, the bla_{OXA-23} gene is the most widely found across the world. The gene carrying bla_{OXA-23} transposons in *A. baumannii* isolates of global clones GC1 and GC2 is found worldwide. Here, we examined whether transposons play a role in the dissemination of the bla_{OXA-23} in globally distributed clones, GC1 and GC2 *A. baumannii* isolates from Iraq.

Materials and methods The 119 non-repetitive *A. baumannii* isolates including 94 recovered from clinical specimens and 25 isolates from hospital environment between September 2021 and April 2022 from different medical centers located at various regions in Baghdad, Iraq. The global clones (GC) and the genes encoding carbapenem resistance, including bla_{OXA-23}, bla_{OXA-24}, and bla_{OXA-58} were identified using multiplex PCR assays. Antibiotic susceptibility testing was performed by the Kirby-Bauer disk diffusion susceptibility method. The transposons carrying bla_{OXA-23} were examined using PCR mapping. In cases when carbapenem susceptible *A. baumannii* isolates were found, they were subjected to E test, full length sequencing of bla_{OXA-Ab} ($bla_{OXA-51-like}$) and Institut Pasteur multi-locus sequence typing scheme.

Results All but two isolates (92 clinical and 25 environmental) were identified carbapenem-resistant *A. baumannii* (CRAB). Of 117 CRAB isolates, 20 belong to GC1, 19 contained bla_{OXA-23}; of them, 17 isolates harbored the bla_{OXA-23} located on *Tn*2006. Among the 46 CRAB belonging to GC2, 39 contained bla_{OXA-23}; of them, 34 carried the bla_{OXA-23} located on *Tn*2006. The remaining GC1 and GC2 isolates, one GC1 as well as one GC2 isolate, were susceptible to imipenem, doripenem, and meropenem and considered carbapenem-susceptible *A. baumannii* (CSAB). Full-length sequencing of the bla_{OXA-Ab} and MLST for the two CSAB isolates belonging to GC1 and GC2 confirmed that the GC1 isolate belongs to ST 623 and contained an allele that encodes an bla_{OXA-69} variant of the bla_{OXA-Ab} while the GC2 belong to ST2 and carried an bla_{OXA-66} variant.

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Conclusion This study provides evidence for the dissemination of bla_{OXA-23} on the Tn*2006* in CRAB isolates in Baghdad, Iraq. It appears that this transposon is widespread in GC1 and 2 isolates as in the other parts of the world. Interestingly, one GC1 and one GC2 isolate from Iraq were found to be susceptible to carbapenem while the isolates belonging to GC1 and GC2 have so far rarely been found to be susceptible to carbapenem globally.

Keywords *Acinetobacter baumannii*, Carbapenem-resistance, Iraq, Tn*2006*, bla_{OXA-23}

Introduction

Acinetobacter baumannii is the World Health Organization's (WHO) number one critical priority pathogen due to its increased resistance to most antibiotics, especially carbapenems [1–4]. Carbapenems are used as last resort in the treatment of infections caused by multidrug-resistant *A. baumannii* [5–7]. In the recent period, the rise of carbapenem-resistant *A. baumannii* (CRAB) has been a global concern because it reduced the treatment choices for infections caused by this pathogen [7–9]. The antibiotic resistant strains of *A. baumannii* are members of the two main, globally distributed clones, known as global clone 1 (GC1) and global clone 2 (GC2) [10, 11]. Carbapenem resistance in *A. baumannii* is mostly related to the acquisition of carbapenem-hydrolyzing oxacillinases genes including bla_{OXA-23}, bla_{OXA-24}, and bla_{OXA-58} [12, 13]. The bla_{OXA-23} gene has been found within CRAB in various countries, nevertheless the bla_{OXA-24} and bla_{OXA-58} genes have indeed been recorded as endemic in some countries around the world [6, 14]. bla_{OXA-23} might be disseminated from one strain to another through transposons including Tn*2006*, Tn*2007*, and Tn*2008* [6, 15, 16]. The Tn*2006* has two ISAba1 insertion sequences with distinct orientations on both sides that surround the bla_{OXA-23} [17]. Because it carries ISAba1, this transposon could be incorporated into both chromosomes and plasmids, enabling the bla_{OXA-23} gene to be disseminated [16].

A. baumannii was identified as the most prevalent bacterium among US troops with wound infections returning from Iraq where particular clones of *A. baumannii* might be originated from [18]. However, knowledge on different aspects of CRAB from Iraq including the clonal diversity and the carbapenem resistance genes and their genetic context still limited. Here, we examined whether transposons play a role in the dissemination of the bla_{OXA-23} in globally distributed clones, GC1 and GC2 *A. baumannii* isolates from Iraq as one of the Middle East countries.

Materials and methods

Bacterial isolates

The study was approved by the local ethical committee of Tehran University of Medical Sciences (IR.TUMS.MEDICINE.REC.1400.1074). A total of 119 non-repetitive *A. baumannii* isolates including 94 isolates recovered from clinical specimens and 25 isolates from the intensive care unit (ICU) environment of hospitals; hereafter, called environmental isolates. The isolates were collected from six hospitals named Shahid Ghazi al Harery Surgical, Baghdad teaching hospital, Specialized Burn Hospital, Ibn Al balady Maternity & Children's Hospital, Fatmih alzahraa Maternity Hospital, Al-Imamian Al-Kadhimiyain Medical City, and a central laboratory in Baghdad, Iraq at different time periods of October 2021 to April 2022. The identification of *A. baumannii* was performed using a combination of biochemical and molecular tests; the biochemical identification was done using a variety of tests, such as the oxidase test reaction, the Triple Sugar Iron Agar (TSI), and the oxidation-fermentation test (OF) [19], and the molecular identification was done using PCR for the bla_{OXA-Ab} gene [20].

Antimicrobial susceptibility testing

The antibiotic susceptibility testing was performed using 27 antibiotic discs (Oxoid, Basingstoke, United Kingdom) including streptomycin (25 µg), spectinomycin (25 µg), sulfamethoxazole (300 µg), tetracycline (30 µg), kanamycin (50 µg), neomycin (30 µg), cefotaxime (30 µg), ceftazidime (30 µg), gentamicin (10 µg), ciprofloxacin (5 µg), amikacin (30 µg), nalidixic acid (30 µg), tobramycin (10 µg), netilmicin (30 µg), imipenem (10 µg), meropenem (10 µg), rifampicin (30 µg), ampicillin-sulbactam (20 µg), cefepime (30 µg), doripenem (10 µg), piperacillin-tazobactam (110 µg), ceftriaxone (30 µg), minocycline (30 µg), doxycycline (30 µg), levofloxacin (5 µg), timentin (ticarcillin-clavulanic acid) (85 µg), trimethoprim-sulphamethoxazole (25 µg), according to the standard Kirby-Bauer disk diffusion susceptibility method [21]. The results were interpreted according to the Clinical and Laboratory Standards Institute (CLSI) supplement M100, 33 rd ed. guideline for *Acinetobacter* spp [21] or Calibrated Dichotomous Sensitivity disk diffusion assay (CDS) (http://cdstest.net/). As demonstrated previously, the members of GC1 and GC2 are mainly responsible for the bulk of globally distributed multi-resistant *A. baumannii*, including CRAB [22]. However, two of isolates examined in the current study belonged to GC1 and GC2, while they showed susceptibility to carbapenem. To verify the susceptibility to carbapenem in these isolates of GC1 and GC2, disc diffusion was performed on two distinct single colonies from each isolate, followed by an E-test (bioMérieux, Marcy-l'Étoile, France) to determine

the minimum inhibitory concentration of imipenem, doripenem, and meropenem against the isolates.

PCR assays

Two multiplex PCR assays amplifying three alleles of *ompA*, *csuE* and intrinsic $bla_{\text{OXA-Ab}}$ ($bla_{\text{OXA-51-like}}$) were performed to determine the GC1 (group 2) and GC2 (group1) of the isolates [23]. Primers for these alleles and annealing temperature are shown in the Table 1 and reaction conditions as previously described [23]. One multiplex PCR assay was performed to identify the genes encoding the carbapenem resistance, including $bla_{\text{OXA-23}}$, $bla_{\text{OXA-24}}$, and $bla_{\text{OXA-58}}$ [20]. Primers targeting these genes and annealing temperature are shown in the Table 1 [20]. The PCR for amplifying full length $bla_{\text{OXA-Ab}}$ was performed to identify the variant of $bla_{\text{OXA-Ab}}$ family genes [24] using the primers for this gene as shown in the Table 1. The manufacturer of reagents is Ampliqon for Master mix, and Metabion for all the primers. The sequencing was done using Applied Biosystems ABI sequencer.

PCR mapping for identifying the genetic context of $bla_{\text{OXA-23}}$

All the isolates harbored $bla_{\text{OXA-23}}$ were screened for Tn*2006* and Tn*2008* using two overlapping PCRs linking the $bla_{\text{OXA-23}}$ gene to the upstream and downstream copies of ISAba1 [17, 25]. The primers used for PCR mapping are shown in Table 1.

Multilocus sequence typing

Multilocus sequence typing (MLST) was performed for the two isolates of carbapenem-susceptible *A. baumannii* (CSAB) belonging to GC1 and GC2. Sanger sequencing was used and the sequence type was analyzed according to the Institute Pasteur (IP) MLST scheme, which uses seven housekeeping genes (*cpn60*, *fusA*, *gltA*, *pyrG*, *recA*, *rplB*, and *rpoB*) [26]. The ST number was assigned by comparing the allele sequences to the known ones on the MLST website (http://pubmlst.org/abaumannii/).

Nucleotide accession numbers

The sequence of intrinsic $bla_{\text{OXA-Ab}}$ ($bla_{\text{OXA-51-like}}$) gene of *A. baumannii* isolates; Q26, Q30L, A98/1and A87S have been deposited in GenBank and are publicly available under the accession numbers OQ916423.1, OQ916422.1, OQ916421.1and OQ916420.1.

Statistical analysis

Data analysis was performed using SPSS version 22.0 (SPSS Inc., USA). Descriptive results were shown as frequencies. For comparison of the categorical variables, Chi-square and Fisher's exact tests of nonparametric data were used. *P* values of less than 0.05 were considered as significant.

Table 1 Primers used for PCR assays

PCR	Primer	Sequence (5′-3′)*	Annealing temperature (˚ C)	Amplicon length (bp)	Reference
Group1	*ompA*F306	F: GATGGCGTAAATCGTGGTA	57	355	[23]
	*ompA*R660	R: CAACTTTAGCGATTTCTGG		702	
	*csuE*F	F: CTTTAGCAAACATGACCTACC		559	
	*csuE*R	R: TACACCCGGGTTAATTCGT			
	*oxa66*F89	F: GCGCTTCAAAATCTGATGTA			
	*oxa66*R647	R: GCGTATATTTTGTTTCCATTC			
Group2	*ompA*F378	F: GACCTTTCTTATCACAACGA	57	343	
	*ompA*R660	R: CAACTTTAGCGATTTCTGG		580	
	*csuE*F	F: GGCGAACATGATCTATTT		162	
	*csuE*R	R: CTTCATGGCTCGTTGGTT			
	*oxa69*F169	F: CATCAAGGTCAAACTCAA			
	*oxa69*R330	R: TAGCCTTTTTTCCCATC			
$bla_{\text{OXA-23}}$	*oxa23*F-like	F: GATCGGATTGGAGAACCAGA	60	501	[20]
	*oxa23*R-like	R: ATTTCTGACCGCATTTCCAT			
$bla_{\text{OXA-24}}$	*oxa24*F-like	F: GGTTAGTTGGCCCCTTAAA	60	246	
	oxa24R-like	R: AGTTGAGCGAAAAGGGGATT			
$bla_{\text{OXA-58}}$	*oxa58*F-like	F: AAGTATTGGGGCTTGTGCTG	60	599	
	*oxa58*R-like	R: CCCCTCTGCGCTCTACATAC			
$bla_{\text{OXA-Ab}}$	OXA-69 A	F: CTAATAATTGATCTACTCAAG	57	975	[24]
	OXA-69B	R: CCAGTGGATGGATGGATAGATTATC			
ISAba1-*oxa23*	ISAba1B *oxa23*-F	R: CATGTAAACCAATCGTCACC	59	2725	[17]
		F: GATCGGATTGGAGAACCAGA			[25]
oxa23- ISAba1	*oxa23*-R	R: ATTTCTGACCGCATTTCCAT	59	1369	[25]
	ISAba1B	F: CATGTAAACCAATGCTCACC			[17]

*F and R indicate the forward and reverse primers, respectively

Results

Antibiotic resistance profiles

Of the 119 isolates examined, 117 showed resistance to carbapenems including imipenem, doripenem, and meropenem; Of them, 92 and 25 were clinical and environmental isolates, respectively. They were also resistant to different antibiotics including ampicillin, streptomycin, spectinomycin, kanamycin, sulfonamides, ceftazidime and cefotaxime, timentin, (ticarcillin-clavulanate), ceftriaxone, ciprofloxacin, nalidixic acid (Figure S1).

Identification of global clones

Multiplex allelic-specific PCR for the identification of GCs, revealed that 20 (16.80%) of *A. baumannii* isolates belonged to GC1 including 17 clinical and 3 environmental isolates. Of the 47 (39.49%) isolates belonging to GC2, 38 and 9 were clinical and environmental, respectively. One clinical isolate belonged to GC3 and the remaining isolates, 51 (42.85%) were of other clones.

bla$_{\text{OXA-like}}$ genes and the context of *bla*$_{\text{OXA-23}}$ gene

The *bla*$_{\text{OXA−23}}$ gene was identified in 76 (80.85%) of the clinical isolates, whereas the *bla*$_{\text{OXA−24}}$ gene was identified in 11 (11.70%). The *bla*$_{\text{OXA−23}}$ and *bla*$_{\text{OXA−24}}$ genes were identified in 18 (72%), 6 (24%) of the environmental isolates, respectively. Furthermore, no isolates carried the *bla*$_{\text{OXA−58}}$ gene. Tn*2006* were found in 68 (73.9%) and 17 (68%) of the clinical and environmental isolates of *A. baumannii* that carried the *bla*$_{\text{OXA−23}}$ gene, respectively (Figure S2). The structure analysis of the gene context is provided in Figure S3.

Of 117 CRAB, 19 belong to GC1 contained *bla*$_{\text{OXA−23}}$; of them 17 isolates harbored the *bla*$_{\text{OXA−23}}$ located on Tn*2006*. Of notable that of 20 GC1 isolates tested in this study, one (isolate 98/1) was susceptible to imipenem, doripenem, and meropenem. Among 46 CRAB belonging to GC2, 39 contained *bla*$_{\text{OXA−23}}$; of them 34 carried the *bla*$_{\text{OXA−23}}$ located on Tn*2006*. It is notable that of 47 GC2 isolates tested in this study, one (Q26) was susceptible to imipenem, doripenem, and meropenem (Table 2). Full-length sequencing of the *bla*$_{\text{OXA−Ab}}$ and MLST for the two CSAB isolates belonging to GC1 and GC2 confirmed that the GC1 isolate belongs to ST 623 (*cpn60-1*, *fusA-1*, *gltA-1*, *pyrG-1*, *recA-5*, *rplB-1* and *rpoB-1*) and contained an allele that encodes an *bla*$_{\text{OXA−69}}$ variant of the *bla*$_{\text{OXA−Ab}}$ while the GC2 belong to ST2 (*cpn60-2*, *fusA-2*, *gltA-2*, *pyrG-2*, *recA-2*, *rplB-2* and *rpoB-2*), and carried an *bla*$_{\text{OXA−66}}$ variant.

Discussion

Carbapenem-resistant *A. baumannii* (CRAB) is listed as the number one critical priority pathogen by the World Health Organization (WHO) amongst a published list of 12 antibiotic-resistant bacteria [1, 27]. The wars in

Table 2 Antibiotics susceptibility profile of carbapenem- susceptible *Acinetobacter baumannii* isolates

Strain	GC	MIC µg/mL			ST	Antibiotics susceptibility profile																							
		Ipm	Mem	DOR		Sm	Sp	Su	Tc	Km	Nm	CTX	CAZ	Gm	Cip	AK	Nx	Tm	Ne	Ipm	Mem	Rif	SAM	FEP	DOR	TZP	CRO	MIN	DOX	LVX	TS	TIM
A98	GC1	0.5	0.5	0.5	623	19	12	29	17	6	12	6	6	8	6	9	6	9	24	22	18	11	24	12	22	15	6	22	20	11	19	6
Q26	GC2	0.5	0.5	0.5	2	6	6	6	6	6	14	6	8	14	6	11	6	6	6	22	24	12	15	17	22	17	6	14	6	6	6	6

*: Sm: Streptomycin, Sp: Spectinomycin, Su: Sulfamethoxazole, Tc: Tetracycline, Km: Kanamycin, Nm: Neomycin, CTX: Cefotaxime, CAZ: Ceftazidime, Gm: Gentamicin, Cip: Ciprofloxacin, AK: Amikacin, Nx: Nalidixic Acid, Tm: Tobramycin, Ne: Netilmicin, Ipm: Imipenem, Mem: Meropenem, Rif: Rifampicin, SAM: Ampicillin-sulbactam, FEP: Cefepime, DOR: Doripenem, TZP: Piperacillin-Tazobactam, CRO: Ceftriaxone, MIN: Minocycline, DOX: Doxycycline, LVX: Levofloxacin, TS: Trimethoprim-sulphamethoxazole, TIM: Timentin (Ticarcillin-clavulanic acid). Inhibition zone diameters highlighted white, light gray and dark gray indicate susceptibility, intermediate susceptibility, and resistance, respectively

the last several decades brought focus on the infections caused by *A. baumannii* among US military returned from the Middle East, particularly Iraq and Afghanistan [18]. Furthermore, the multiply antibiotic resistant *A. baumannii* and its particular lineage are hypothesized to have originated from Middle East region. However, there are limited data from this geographical region. On the other hand, there is only one study have reported the presence of $bla_{\text{OXA–23}}$ in *A. baumannii* from Erbil city located in Kurdistan Region, Iraq [28]. Here, for the first time we analyzed the clonal diversity of CRAB Iraqi isolates that recovered in September 2021 to April 2022 from six hospitals in Baghdad, Iraq and determined the role of transposons in the dissemination of the most widespread carbapenem resistance gene, $bla_{\text{OXA–23}}$.

This study showed that all but two of the isolates examined were CRAB (98.31%); this observation is consistent with the findings from neighbouring countries of Iraq where the rate of CRAB ranged from more than 30–90% [29–34]. The rate of CRAB rates was slightly lower in neighboring countries than Iraq; they varied from 84.6 to 88.5% in Iran, 83–84% in Turkey, and 85.18–87.04% in Kuwait [29–31]. Also, resistance to carbapenem ranged from 68.9 to 75.1% in Jordan and was reported 64.6% in Syria, and 32.6% in Saudi Arabia [32–34].

This study further indicates the first analysis of global clones using allele-specific PCR in Iraqi *A. baumannii* isolates and revealed that most of the isolates belong to GC2 as seen in different parts of the world [35–37]. All but one of GC2 isolates examined in this study were resistant to main classes of antibiotics including carbapenems, aminoglycosides, cephalosporins, fluoroquinolones. Interestingly, one of the GC2 isolates was susceptible to carbapenems including imipenem, meropenem, and doripenem. The full-length sequencing of the $bla_{\text{OXA–Ab}}$ of this GC2 isolate revealed that it contains an $bla_{\text{OXA–66}}$ variant of the intrinsic $bla_{\text{OXA–Ab}}$ gene consistent with their assignment to GC2. This isolate was found to belong to ST2, which is consistent of assignment of this isolate to GC2 as represented in majority of strains that belong to GC2 [26]. The isolates belong to ST2 reported from Lebanon [38], and Japan [39], in all of these studies the ST2 isolates were resistant to carbapenems.

While the most of GC1 isolates tested in this study were resistant to different classes of antibiotics including carbapenems, aminoglycosides, cephalosporins, fluoroquinolones, there was one GC1 isolate was susceptible to carbapenems including imipenem, doripenem, and meropenem. The full-length sequencing of the $bla_{\text{OXA–Ab}}$ gene of this GC1 isolate identified CSAB showed an $bla_{\text{OXA–69}}$ variant of the $bla_{\text{OXA–Ab}}$ gene consistent with their assignment to GC1. Using MLST, it was found that the GC1 isolate belong to ST623 that is a Single Locus Variant (SLV) of ST1 (*cpn60-1*, *fusA-1*, *gltA-1*, *pyrG-1*, *recA-5*, *rplB-1* and *rpoB-1*) and is consistent of assignment of this isolate to GC1. ST623 is a sequence type which have been reported in three isolates from Erbil city Kurdistan Region, Iraq [28], suggesting that ST623 might be found in the country. Prior to the study from Kurdistan, ST623 was found in 17 isolates from Nepal and grouped with ST1 in clonal complex 1 (CC1) [40]. Of notable is that the isolates belonging to GC1 and GC2 have so far rarely been found to be susceptible to carbapenem globally. Hence, the CSAB isolates of GC1 and GC2 found in this study, might have undergone unique evolutionary process and need to be investigated by metagenomics study in the future.

The percentages of the $bla_{\text{OXA–23}}$ and $bla_{\text{OXA–24}}$ genes were not statistically different in the clinical and environmental isolates. This study demonstrated that the $bla_{\text{OXA–23}}$ gene, which is present on the ISAba1-bounded transposon Tn*2006*, was present in the majority of GC1 and GC2 CRAB isolates. $bla_{\text{OXA–23}}$ was discovered in Tn*2006* [17, 41] rather than Tn*2007*, Tn*2008*, and Tn*2009* by earlier studies. Since Tn*2006* may migrate about on its own, as was previously shown, it can be found in various genomic places and structures in different *A. baumannii* strains [16].

Conclusions

This study provides evidence for the dissemination of $bla_{\text{OXA–23}}$ on the Tn*2006* in CRAB isolates in Baghdad, Iraq. It appears that this transposon is widespread in GC1 and 2 isolates as in the other parts of the world. Interestingly, one GC1 and one GC2 isolate from Iraq were found to be susceptible to carbapenem while the isolates belonging to GC1 and GC2 have so far rarely been found to be susceptible to carbapenem globally.

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s13104-024-06890-w.

Supplementary Material 1

Supplementary Material 2

Supplementary Material 3

Author contributions

M.W.O. performed the microbiologic and molecular experiments, wrote the draft of the manuscript. I.AK was the advisor of the project. MR.P was the co-supervisor. Gh.N., M.A, and S.Gh did the molecular experiments and bioinformatic analysis. M.D. conceptualized, designed, coordinated, and supported this study. The acquisition of fund, the analysis and interpretation of data and revision of manuscript were also done by M.D. All authors have read and agreed to the published version of the manuscript.

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Data availability
The datasets used and/or analyzed during the current study available from the corresponding author on reasonable request.

Declarations

Competing interests
The authors declare no competing interests.

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