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Emergence of Carbapenem-Resistant Non-*baumannii* Species of *Acinetobacter* Harboring a bla_{OXA-51}-Like Gene That Is Intrinsic to *A. baumannii*

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The bla_{OXA-51}-like gene, originally intrinsic to *Acinetobacter baumannii*, had been detected in two clones of *Acinetobacter nosocomialis* and one clone of *Acinetobacter* genomic species "Close to 13TU." These bla_{OXA-51}-like genes, all preceded by IS*Aba1*, were located on plasmids that might have originated with *A. baumannii*. The plasmid-borne IS*Aba1*--bla_{OXA-51}-like confers a high level of carbapenem resistance and affects the accuracy of using bla_{OXA-51}-like detection as a tool for differentiating *A. baumannii* from other *Acinetobacter* species.

The most common mechanism of carbapenem resistance in *Acinetobacter* species is the production of carbapenem-hydrolyzing class D β-lactamases (CHDLs) (16). Among these CHDL genes, the bla_{OXA-51}-like gene is intrinsic to *Acinetobacter baumannii* and originally was confined on the chromosome of this species (7, 14, 17). Therefore, its detection has been used as a method of *A. baumannii* identification (17). However, the genetic structure IS*Aba1*–bla_{OXA-51}-like has integrated into plasmids, probably via a transposition event (2, 9). The plasmids carrying IS*Aba1*–bla_{OXA-51}-like had disseminated into *A. baumannii* isolates in Taiwan (2). In addition, the plasmid-borne bla_{OXA-51}-like gene ($bla_{OXA-138}$) had also been detected in an *Acinetobacter nosocomialis* (formerly *Acinetobacter* genomic species 13TU) isolate (12). In this study, we aim to characterize the non-*Acinetobacter baumannii* species carrying bla_{OXA-51}-like genes.

Among the nonduplicate bacteremic *Acinetobacter calcoaceticus-A. baumannii* (Acb) complex isolates collected from Taipei Veterans General Hospital (TVGH) from January 1996 through December 2007, 676 isolates were identified as non-*A. baumannii* species by a multiplex PCR method (3) and 6 (0.9%) of them had bla_{OXA-51}-like genes. Among 74 other nonduplicate isolates of non-*A. baumannii* species that were consecutively collected from various clinical specimens from 10 medical centers (up to 40 isolates from each center) in Taiwan during the period from July through October 2007 (2), 4 (5.5%) isolates had bla_{OXA-51}-like genes.

The clinical characteristics of the patients who carried non-*A. baumannii* species harboring bla_{OXA-51}-like genes are summarized in Table 1. Nine of these *Acinetobacter* isolates were pathogens of nosocomial infection (infection developed more than 48 h after hospitalization), and 8 of them were isolated from patients during their stay in different intensive care units in TVGH. The 10 *Acinetobacter* isolates were identified as *A. nosocomialis* or *Acinetobacter* genomic species "Close to 13TU" by amplified ribosomal DNA restriction analysis (13) (Table 2). The *A. nosocomialis* isolates belonged to two clones (pulsotypes B and C), and all the *Acinetobacter* genomic species "Close to 13TU" isolates belonged to a single clone (pulsotype A), as determined by pulsed-field gel electrophoresis (10). Three isolates (one from each pulsotype) were selected for multilocus sequence typing (MLST) (6), and they fell into sequence type 74 (ST74) and ST90, corresponding to *A. nosocomialis* and *Acinetobacter* genomic species "Close to 13TU," respectively (13). All of them were nonsusceptible to imipenem or meropenem, accounting for 11.0% and 13.9% of imipenem- and meropenem-resistant non-*A. baumannii* isolates collected in the same period, respectively.

The bla_{OXA-24}-like, bla_{OXA-23}-like, $bla_{OXA-143}$-like, bla_{IMP}-like, bla_{VIM}-like, bla_{SIM}-like, bla_{GIM}-like, and bla_{SPM}-like genes were not detected in the isolates (8, 11). Two isolates of pulsotype B carried bla_{OXA-58} genes, which were flanked by IS*1006*–ΔIS*Aba3*-like (upstream) and IS*Aba3* (downstream) (1). The genetic structure IS*1006*–ΔIS*Aba3*-like–bla_{OXA-58}–IS*Aba3* has been described for an *A. nosocomialis* isolate (1). Most of the bla_{OXA-51}-like genes were $bla_{OXA-194}$ ($n = 4$), followed by $bla_{OXA-138}$ ($n = 2$). The bla_{OXA-51}-like alleles were different from each other in one to three amino acids (Table 2).

PCR mapping with different primer sets showed that all of the 10 isolates had a similar genetic arrangement around IS*Aba1*--bla_{OXA-51}-like (Fig. 1). This genetic structure has been found in pAbSK-OXA-82, which is a widely disseminated plasmid carrying IS*Aba1*--bla_{OXA-51}-like in *A. baumannii* in Taiwan (2). Although the isolates had different plasmid patterns, a Southern blot analysis revealed that they had a plasmid of similar size carrying bla_{OXA-51}-like genes. The size of the plasmids was approximately 50 kb, comparable to that of pAbSK-OXA-82 (data not shown).

The non-*A. baumannii* species carrying plasmid-borne IS*Aba1*–bla_{OXA-51}-like genes may have emerged in three ways. First, they may have acquired the plasmids carrying IS*Aba1*-bla_{OXA-51}-like genes from different *A. baumannii* strains independently. Second, the plasmids may have disseminated among different clones of non-*A. baumannii* species, since the plasmids were similar in size and had similar genetic structures surrounding the bla_{OXA-51}-like allele. Third, clonal propagation of *Acinetobacter* may have also participated in the emergence of isolates carrying plasmid-borne IS*Aba1*--bla_{OXA-51}-like

Received 4 May 2011 Returned for modification 17 July 2011
Accepted 6 November 2011
Published ahead of print 14 November 2011
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doi:10.1128/AAC.00622-11

TABLE 1 Clinical characteristics of patients who carried non-*Acinetobacter baumannii* species harboring bla_{OXA-51}-like genes[a]

Case no.	Hospital	Ward/day[b]	Age (yr)/sex	APACHE II score	Underlying diseases	Invasive devices	Source/ infection[c]	Concomitant isolate[d]	Treatment/appropriate antimicrobial therapy[e]	Outcome
1	TVGH	NSCU/11	77/M	22	Traumatic ICH, SDH, SAH	Tracheostomy, CVC, Foley, ventilator	Blood/I	*Pseudomonas aeruginosa*	Ticarcillin/clavulanate for 18 days/no	Survived
2	TVGH	RCU/25	75/F	21	COPD, DM, hypertension, recent CVA	Tracheostomy, CVC, Foley, ventilator	Blood/I	None	Cefmetazole for 2 days, piperacillin-tazobactam for 3 days, and imipenem for 13 days/no	Survived
3	TVGH	ICUB/7	21/M	19	Pulmonary contusion with pulmonary hemorrhage and hemothorax	Swan-Ganz catheter, CVC, chest tube, ventilator	Blood/I	None	Ciprofloxacin for 14 days/yes	Survived
4	TVGH	CCU/14	87/M	18	Recent myocardial infarction	None	Blood/I	None	Flomoxef plus netilmycin for 9 days/no	Survived
5	TVGH	ICUA/7	24/F	13	Systemic lupus erythematosus	Arterial line, CVC, Foley, ventilator	Sputum/C	*Pneumocystis jirovecii*	—/—[f]	Died of other causes
6	TVGH	ICUA/13	81/F	28	Parkinson's disease, DM, hypertension	Arterial line, CVC, Foley, ventilator	Sputum/I	*Stenotrophomonas maltophilia*	Piperacillin-tazobactam for 21 days/no	Died of other causes
7	TVGH	ICUC/5	69/M	39	Multiple myeloma, old CVA	Arterial line, CVC, Foley, ventilator	Sputum/I	None	Imipenem for 14 days/no	Died of infection
8	NTUH	NA	NA	NA	NA	NA	Blood/I	NA	NA	NA
9	TVGH	CCU/26	80/F	33	Congestive heart failure, asthma, DM, hypertension	Arterial line, CVC, Foley, ventilator, HD via FVC	Blood/I	None	Levofloxacin for 10 days/yes	Died of other causes
10	TVGH	ER	62/M	11	Lung cancer, chemotherapy	None	Blood/I	*Chryseobacterium meningosepticum*	Cefoperazone plus sulbactam for 5 days/no	Survived

[a] Abbreviations: TVGH, Taipei Veterans General Hospital; NTUH, National Taiwan University Hospital; NSCU, neurosurgical care unit; RCU, respiratory care unit; ICU, intensive care unit; CCU, cardiac care unit; NA, data not available; ER, emergency room; ICH, intracerebral hemorrhage; SDH, subdural hemorrhage; SAH, subarachnoid hemorrhage; COPD, chronic obstructive pulmonary disease; DM, diabetes mellitus; CVA, cerebrovascular accident; CVC, central venous catheter; HD, hemodialysis; FVC, femoral vein catheter; Foley, Foley catheter; I, infection; C, colonization.

[b] Ward at which the patient resided and days of admission when the first isolate of non-*A. baumannii* species harboring bla_{OXA-51}-like genes was collected.

[c] Infection (I) or colonization (C).

[d] Organisms isolated or identified from the same site and at the same time with non-*A. baumannii* species.

[e] Appropriate antimicrobial therapy was defined as therapy with at least one antimicrobial agents that had *in vitro* activity against the causative pathogen and administrated within 48 h after the acquisition of index clinical sample for culture.

[f] —/—, since the patient was colonized with non-*A. baumannii* species, antimicrobial therapy for acinetobacters was not warranted.

TABLE 2 Characteristics of non-*Acinetobacter baumannii* species harboring bla_{OXA-51}-like genes[a]

Isolate	Date of isolation	ARDRA profiles	Identification by ARDRA	MLST[b]	Pulsotype	Plasmid pattern	bla_{OXA-51}-like allele	Amino acid at position[c]:								Other carbapenemase gene[d]	MIC,[e] μg/ml							
								4	5	7	69	167	192	214	264		IPM	MEM	CAZ	CFP	TZP	SUL	COL	TGC
Reference								K	A	L	L	L	F	I	T									
1059	Nov. 2004	31113	"Close to 13TU"		A	I	$bla_{OXA-138}$		T			V				None	32	64	64	128	64	2	2	1
1075	Jan. 2005	21111	*A. nosocomialis*		B	III	$bla_{OXA-194}$			F		V				bla_{OXA-58}	32	32	8	4	>128	8	4	0.75
1104	Mar. 2005	31113	"Close to 13TU"		A	I	$bla_{OXA-195}$			F		V	S	M		None	16	64	64	128	64	2	2	1.5
2311	Nov. 2006	31113	"Close to 13TU"	ST90	A	II	$bla_{OXA-194}$			F		V				None	32	64	64	128	128	4	2	1.5
1890	July 2007	31113	"Close to 13TU"		A	II	$bla_{OXA-194}$			F		V				None	64	16	64	128	>128	8	2	3
1892	Aug. 2007	31113	"Close to 13TU"		A	II	$bla_{OXA-196}$	Q			H	V				None	64	64	64	128	>128	8	2	3
1897	Aug. 2007	31113	"Close to 13TU"		A	II	$bla_{OXA-197}$			F		V			A	None	32	64	64	128	>128	8	2	3
2019	Aug. 2007	21113	*A. nosocomialis*	ST74	C	IV	$bla_{OXA-194}$			F		V				None	8	64	8	4	>128	16	1	0.25
1522	Dec. 2007	31113	"Close to 13TU"		A	I	bla_{OXA-82}					V				None	32	64	32	128	>128	1	2	1
1704	Dec. 2007	21113	*A. nosocomialis*	ST74	B	III	$bla_{OXA-138}$		T			V				bla_{OXA-58}	32	64	8	4	>128	32	1	0.38

[a] Abbreviations: ARDRA, amplified ribosomal DNA restriction analysis; *A. nosocomialis*, *Acinetobacter nosocomialis*; MLST, multilocus sequence typing; IPM, imipenem; MEM, meropenem; CAZ, ceftazidime; CFP, cefepime; TZP, piperacillin-tazobactam; SUL, sulbactam; COL, colistin; TGC, tigecycline.

[b] The sequences of the STs are available at www.pasteur.fr/mlst.

[c] The reference amino acids and their positions are from bla_{OXA-66}.

[d] Including bla_{OXA-58}-like, bla_{OXA-24}-like, bla_{OXA-23}-like, $bla_{OXA-143}$-like, bla_{IMP}-like, bla_{VIM}-like, bla_{SIM}-like, bla_{GIM}-like and bla_{SPM}-like.

[e] *In vitro* testing of susceptibilities to imipenem and meropenem was done using Etest (AB BIODISK, Solna, Sweden). *In vitro* testing of susceptibilities to other antibiotics was done using agar dilution methods. The results were interpreted according to the recommendations made by the Clinical and Laboratory Standards Institute (5) or the Food and Drug Administration (for TGC, breakpoints used for the *Enterobacteriaceae*).

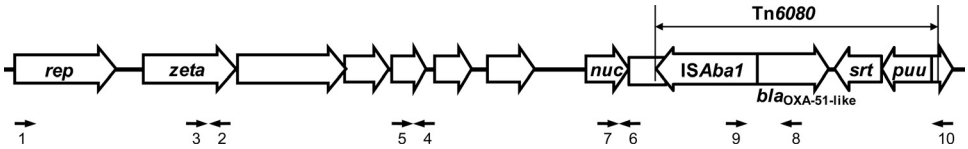

FIG 1 Schematic representation of the surrounding structures of plasmid-borne *bla*$_{OXA-51}$-like. Genes and their corresponding transcription orientations are indicated by horizontal white arrows. The black horizontal arrows indicate the locations of primers used for PCR mapping. *rep*, gene encoding a plasmid replicase; *zeta*, gene encoding a zeta toxin family protein; *nuc*, gene encoding a thermonuclease protein; *srt*, gene encoding a sortase; *puu*, gene encoding a transcriptional regulator. The putative transposon Tn*6080* contained IS*Aba1*--*bla*$_{OXA-51}$-like *srt-puu*. Other details of the genetic structures are described under GenBank accession no. GQ352402.

genes, especially for isolates 1890, 1892, and 1897. These three *Acinetobacter* genomic species "Close to 13TU" isolates were isolated in nearby intensive care units (ICUs) (Table 1) in the same period, belonged to clone A, and had similar plasmid patterns.

The plasmids carrying IS*Aba1*-*bla*$_{OXA-138}$ (from isolate 1704, susceptible to cefepime) or IS*Aba1*-*bla*$_{OXA-194}$ (from isolate 2019, susceptible to kanamycin) were both self-transferable to an *A. baumannii* 218 isolate (susceptible to carbapenem but resistant to cefepime and kanamycin), as demonstrated in mating-out assays (15), in which the transconjugants were selected on agar plates containing imipenem (4 μg/ml) plus either cefepime (16 μg/ml) or kanamycin (50 μg/ml). The plasmids carrying IS*Aba1*-*bla*$_{OXA-138}$ and IS*Aba1*-*bla*$_{OXA-194}$ conferred an increase in the imipenem MIC (from 0.5 μg/ml to 64 μg/ml and 8 μg/ml, respectively) to *A. baumannii* transconjugants, respectively. To determine the contribution of plasmid-borne IS*Aba1*–*bla*$_{OXA-51}$-like, without other possible carbapenem resistance determinants in the original plasmids, to carbapenem resistance, a recombinant plasmid carrying IS*Aba1*-*bla*$_{OXA-138}$ was constructed and electrotransformed into an *A. nosocomialis* reference strain, ATCC 17903, using previously described methods (4). The transformants demonstrated an increase in the imipenem MIC (from 0.12 to 32 μg/ml). Taken together, these results indicated that non-*A. baumannii* species can be a reservoir for the dissemination of the carbapenem resistance determinant, the plasmid-borne IS*Aba1*--*bla*$_{OXA-51}$-like.

In conclusion, plasmids carrying IS*Aba1*–*bla*$_{OXA-51}$-like emerged in carbapenem-resistant isolates of *A. nosocomialis* and the *Acinetobacter* genomic species "Close to 13TU." The emergence of these plasmids is due primarily to plasmid propagation between different clones of non-*A. baumannii* species and dissemination of the *Acinetobacter* clones. The plasmid-borne IS*Aba1*–*bla*$_{OXA-51}$-like in non-*A. baumannii* species not only contributes to a high level of carbapenem resistance but also affects the accuracy of using *bla*$_{OXA-51}$-like detection as a tool for differentiating *A. baumannii* from other *Acinetobacter* species.

Nucleotide sequence accession numbers. The nucleotide sequences of *bla*$_{OXA-194}$ to *bla*$_{OXA-197}$ were assigned accession numbers HQ425492 to HQ425495 in the GenBank database.

ACKNOWLEDGMENTS

The isolates used in this study form part of a collection of the Surveillance of Multicenter Antimicrobial Resistance in Taiwan (SMART) Program, 2007. All of the contributors of the isolates are highly appreciated.

This study was supported by grants from the Taipei Veterans General Hospital (V100C-025 and V10E4-005), the National Science Council (NSC98-2314-B-010-010-MY3), and the Yen Tjing Ling Medical Foundation (CI-99-18).

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