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A Novel Insertion Sequence, IS*Aba10*, Inserted into IS*Aba1* Adjacent to the bla_{OXA-23} Gene and Disrupting the Outer Membrane Protein Gene *carO* in *Acinetobacter baumannii*▽†

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We investigated an outbreak caused by carbapenem-resistant *Acinetobacter baumannii* carrying the bla_{OXA-23} gene. A novel insertion sequence (IS), named IS*Aba10*, was found to be inserted into the IS*Aba1* element preceding the bla_{OXA-23} gene in a group of isolates showing higher carbapenem MICs. The presence of IS*Aba10* was associated with increased OXA-23 expression, likely by providing additional promoter sequences. IS*Aba10* was also inserted into the *carO* outer membrane protein gene in most of these isolates.

Multidrug-resistant *Acinetobacter baumannii* causes serious infections associated with high mortality rates, including septicemia, pneumonia, and urinary tract infections, especially in intensive care units (4, 12, 14). Carbapenem resistance in *A. baumannii* has mostly been ascribed to plasmid- and chromosome-encoded carbapenemases such as OXA carbapenemases and metallo-β-lactamases (MBLs) (10, 14). Loss of outer membrane proteins (OMPs), efflux pump overexpression, and alteration of penicillin-binding proteins have also been found to play roles in acquiring carbapenem resistance in *A. baumannii* (1, 5, 9).

Insertion sequences (ISs) may enhance β-lactamase gene expression by providing promoters. IS*Aba1* has frequently been found upstream of the ADC AmpC β-lactamase and OXA carbapenemase genes in *A. baumannii* (16). IS*Aba2*, IS*Aba3*, and IS*Aba4* elements have also been found to precede the bla_{OXA-58} and the bla_{OXA-23} genes in clinical isolates of *A. baumannii* (2, 15, 17).

In this work, we investigated an outbreak of carbapenem-resistant *A. baumannii* and identified a new IS that could be involved in carbapenem resistance by multiple mechanisms.

Consecutive nonreplicate carbapenem-resistant *A. baumannii* isolates were recovered from 23 patients hospitalized at a tertiary care hospital in Korea between May and July 2007. All isolates showed positive results with the modified Hodge test, suggesting carbapenemase production, but negative results with the EDTA-sodium mercaptoacetic acid double-disk synergy test for the screening of MBLs (6). All isolates were nonsusceptible to multiple drugs, including ampicillin, cefoxitin, ceftazidime, cefotaxime, cefepime, levofloxacin, amikacin, gentamicin, and tobramycin, by the CLSI disk diffusion method (3). SmaI macrorestriction anaylsis of 23 isolates exhibited genetic similarities of 70% to 100% by the unweighted pair group method with arithmetic average method (data not shown) (8, 18). Thirteen of the isolates (group I) showed identical SmaI macrorestriction patterns (Table 1).

Genes encoding known carbapenemases were investigated as described previously (20). The naturally occurring bla_{OXA-66} gene, a member of the bla_{OXA-51} cluster, was detected in all 23 *A. baumannii* isolates. Furthermore, 20 of the 23 isolates showed positive results in PCR experiments for the detection of the bla_{OXA-23} gene. I-CeuI mapping experiments showed that a probe specific for bla_{OXA-23} hybridized with an approximately 500-kb I-CeuI chromosomal fragment that was also recognized by a probe specific for 16S rRNA genes, revealing a chromosomal location of the bla_{OXA-23} gene, as observed in recent studies (8, 11). Genes encoding OXA-24-like and OXA-58-like carbapenemases or MBLs such as IMP-1-like, VIM-2-like, and SIM-1 were not detected in any of the isolates.

PCR experiments detected IS*Aba1* upstream of the bla_{OXA-23} gene in all the bla_{OXA-23}–positive isolates (19, 20). However, the PCR product from 13 isolates (group I) was about 2.5 kb, which was larger than the expected size (1.4 kb), suggesting the insertion of additional DNA. Direct sequencing of PCR products showed the presence of a novel IS, named IS*Aba10*, inserted at the 167th nucleotide from the right inverted repeat of the IS*Aba1* element. Although insertion of IS*Aba10* disrupted the IS*Aba1* element, promoter sequences for the bla_{OXA-23} gene within IS*Aba1* remained intact. The new IS*Aba10* element was 1,023 bp long, contained a 927-bp open reading frame (ORF) encoding a putative transposase, and was bounded by imperfect 18-bp inverted repeat sequences, which are common to members of the IS*903* group (Fig. 1). A 9-bp duplication (5′-TGTTTGCTT-3′) flanked IS*Aba10* at the predicted insertion site. Amino acid sequence alignment using an online tool (http://www-is.biotoul.fr) showed that the ORF of

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TABLE 1. Phenotypic and genotypic characteristics of carbapenem-nonsusceptible *A. baumannii*[a]

Group (no. of isolates)	Isolate	IS preceding the bla_{OXA} gene			MIC (mg/liter)		Expression level of bla_{OXA-23}[b]	Status of CarO
		IS*Aba1*-bla_{OXA-23}	ΔIS*Aba1*-IS*Aba10*-bla_{OXA-23}	IS*Aba1*-bla_{OXA-66}	IMP	MER		
Group I (13)	SC0701	−	+	−	32	32	4.6	Intact
	SC0702	−	+	−	32	32	3.2	Lost
	SC0703	−	+	−	32	32	2.2	Lost
	SC0704	−	+	−	32	32	4.9	Lost
	SC0705	−	+	−	32	32	3.4	Lost
	SC0706	−	+	−	32	32	4.0	Lost
	SC0707	−	+	−	32	32	2.3	Lost
	SC0708	−	+	−	32	32	2.0	Lost
	SC0709	−	+	−	32	32	4.6	Lost
	SC0710	−	+	−	32	32	1.8	Lost
	SC0711	−	+	−	32	32	1.5	Lost
	SC0712	−	+	−	32	32	2.2	Lost
	SC0713	−	+	−	32	64	2.7	Lost
Group II (7)	SC0721	+	−	−	16	16	1.0	NT
	SC0722	+	−	−	16	16	1.1	NT
	SC0723	+	−	−	16	16	1.4	NT
	SC0724	+	−	−	16	16	1.3	NT
	SC0725	+	−	−	8	16	1.4	NT
	SC0726	+	−	−	16	16	1.1	NT
	SC0727	+	−	−	16	16	1.0	NT
Group III (3)	SC0731	NT	NT	+	8	16	NT	NT
	SC0732	NT	NT	+	8	16	NT	NT
	SC0733	NT	NT	+	8	16	NT	NT
	ATCC 19606^T	NT	NT	−	0.25	1	NT	Intact

[a] Abbreviations: ΔIS*Aba1*, disrupted IS*Aba1*; IMP, imipenem; MER, meropenem; +, positive; −, negative; NT, not tested.
[b] Expression levels of the bla_{OXA-23} gene were measured by real-time quantitative PCR and normalized against the 16S rRNA gene.

the IS*Aba10* element shared some similarities to the transposases of ISs such as IS*GNB1-1* (identity, 55%), IS*Jsp1* (52%), IS*Rusp5* (51%), IS*Aba7* (48%), and IS*Aba5* (45%) in the IS*903* group.

The remaining seven bla_{OXA-23}-positive isolates (group II) carried an intact IS*Aba1* upstream of the bla_{OXA-23}-like gene. In all group I and group II isolates, bla_{OXA-66} was not preceded by the IS*Aba1* element, while in the three bla_{OXA-23}-negative isolates (group III) the bla_{OXA-66} gene was preceded by IS*Aba1*.

Notably, group I isolates showed two to eight times higher MICs (32 to 64 mg/liter) for both imipenem and meropenem, compared to group II isolates, by agar dilution. Real-time quantitative PCR experiments with the primers and probes listed in Table 2 showed 2- to 5-fold higher bla_{OXA-23} gene expression in group I isolates than in group II isolates (Table 1) (8). Based on this, we speculated that the IS*Aba10* element may play a role in higher-level carbapenem resistance by conferring additional promoter sequences to the bla_{OXA-23} gene. Analyses using the online tool BPROM (Softberry, Inc., Mount Kisco, NY) suggested the presence of a putative promoter within the IS*Aba10* element (Fig. 1).

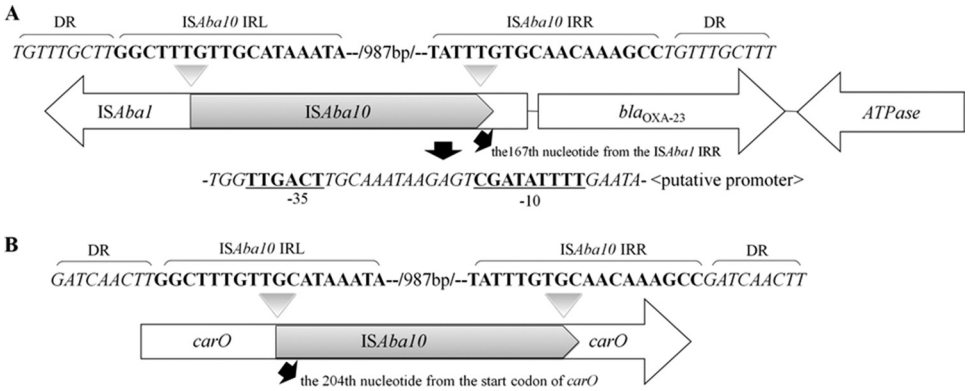

FIG. 1. Schematic representation of the genetic organization of IS*Aba10* disrupting IS*Aba1* adjacent to the bla_{OXA-23} gene in *A. baumannii* SC0701 (A) and IS*Aba10* disrupting the *carO* gene in *A. baumannii* SC0702 (B). Arrows designate transcription directions of genes. IRR, right inverted repeats; IRL, left inverted repeats; DR, direct repeat.

TABLE 2. Primer and probe sequences used for real-time PCR in this study

Primer or probe	Sequence (5′→3′)	Accession no. of reference gene
bla_{OXA-23} forward	TCTGGTTGTACGGTTCAG	FJ6281701
bla_{OXA-23} reverse	TTTTTATCTGTTTGAATAACCAG	
bla_{OXA-23} probe	CCCGAGTCAGATTGTTCA	
16S rRNA forward	AGCTAGAGTATGGGAGAGGATGG	EU030641
16S rRNA reverse	TTCGTACCTCAGCGTCAGTATTAG	
16S rRNA probe	TGCCTTCGCCATCGGTATTCCTCCAGA	

Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) experiments were performed as previously described (7). *A. baumannii* ATCC 19606 was used as a reference strain. All group I isolates, except one (isolate SC0701), lacked the 29-kDa CarO-like OMP, while the other OMPs, such as 33- to 36- and 43-kDa porins, were apparently similar to the wild-type strain (data not shown). In the 12 isolates lacking the 29-kDa CarO-like protein, PCR experiments for the *carO* gene yielded a PCR product about 1.7 kb in size, which was larger than the expected size (741 bp), suggesting the insertion of additional DNA (9). Direct amplicon sequencing confirmed the presence of the IS*Aba10* element within the *carO* gene in these isolates. IS*Aba10* inserted at the 204th base from the *carO* gene start codon (Fig. 1).

Disruption of the *carO* gene by the IS*Aba1*, IS*Aba125*, or IS*Aba825* element has previously been described (13, 16). Loss of the CarO OMP likely plays a role in carbapenem resistance in *A. baumannii*. Interestingly, however, the isolate SC0701, which carried the intact *carO* gene, exhibited similar MIC levels for imipenem and meropenem compared to the other 12 clonally related group I isolates. Our results suggest that, under similar conditions, loss of the CarO OMP had only a minor effect on carbapenem resistance in *A. baumannii*.

Nucleotide sequence accession numbers. The nucleotide sequence data reported in this paper are available in the GenBank nucleotide database under accession numbers FJ998184 and GQ379223.

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