A Novel Insertion Sequence, IS$\text{Aba}10$, Inserted into IS$\text{Aba}1$ Adjacent to the bla$\text{OXA-23}$ Gene and Disrupting the Outer Membrane Protein Gene car$O$ in Acinetobacter baumannii$^\dag$$^\ddag$$^\dag$

Yangsoon Lee,$^1$ Chang-Ki Kim,$^2$ Hyukmin Lee,$^3$ Seok Hoon Jeong,$^{1,*}$ Dongeun Yong,$^1$ and Kyungwon Lee$^1$

Department of Laboratory Medicine and Research Institute of Bacterial Resistance, Yonsei University College of Medicine, 250 Seongsanno, Seodaemun-gu, Seoul 120-752, South Korea; Korean Institute of Tuberculosis, 14 Woomyun-dong, Seocho-gu, Seoul 137-900, South Korea; and Kwandong University College of Medicine, 697-24 Hwajeong-dong, Deogyang-gu, Goyang-si, Gyunggi-do, South Korea$^3$

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We investigated an outbreak caused by carbapenem-resistant Acinetobacter baumannii carrying the bla$\text{OXA-23}$ gene. A novel insertion sequence (IS), named IS$\text{Aba}10$, was found to be inserted into the IS$\text{Aba}1$ element preceding the bla$\text{OXA-23}$ gene in a group of isolates showing higher carbapenem MICs. The presence of IS$\text{Aba}10$ was associated with increased OXA-23 expression, likely by providing additional promoter sequences. IS$\text{Aba}10$ was also inserted into the car$O$ 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$\text{Aba}1$ has frequently been found upstream of the ADC AmpC β-lactamase and OXA carbapenemase genes in A. baumannii (16). IS$\text{Aba}2$, IS$\text{Aba}3$, and IS$\text{Aba}4$ elements have also been found to precede the bla$\text{OXA-58}$ and the bla$\text{OXA-23}$ 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 analysis 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$\text{OXA-66}$ gene, a member of the bla$\text{OXA-51}$ 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$\text{OXA-23}$ gene. I-CeuI mapping experiments showed that a probe specific for bla$\text{OXA-23}$ 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$\text{OXA-23}$ 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$\text{Aba}1$ upstream of the bla$\text{OXA-23}$ gene in all the bla$\text{OXA-23}$-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$\text{Aba}10$, inserted at the 167th nucleotide from the right inverted repeat of the IS$\text{Aba}1$ element. Although insertion of IS$\text{Aba}10$ disrupted the IS$\text{Aba}1$ element, promoter sequences for the bla$\text{OXA-23}$ gene within IS$\text{Aba}10$ remained intact. The new IS$\text{Aba}10$ 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 IS903 group (Fig. 1). A 9-bp duplication (5’-TGTATTCTG-3’) flanked IS$\text{Aba}10$ at the predicted insertion site. Amino acid sequence alignment using an online tool (http://www-is.biotoul.fr) showed that the ORF of
the ISaba10 element shared some similarities to the transposases of ISs such as ISGNB1-1 (identity, 55%), ISJsp1 (52%), ISRusp5 (51%), ISAba7 (48%), and ISAba5 (45%) in the IS903 group.

The remaining seven blaOXA-23-positive isolates (group II) carried an intact ISaba1 upstream of the blaOXA-23-like gene. In all group I and group II isolates, blaOXA-66 was not preceded by the ISaba1 element, while in the three blaOXA-23-negative isolates (group III) the blaOXA-66 gene was preceded by ISaba1.

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 blaOXA-23 gene expression in group I isolates than in group II isolates (Table 1) (8). Based on this, we speculated that the ISaba10 element may play a role in higher-level carbapenem resistance by conferring additional promoter sequences to the blaOXA-23 gene. Analyses using the online tool BPROM (Softberry, Inc., Mount Kisco, NY) suggested the presence of a putative promoter within the ISaba10 element (Fig. 1).

![FIG. 1. Schematic representation of the genetic organization of ISaba10 disrupting ISaba1 adjacent to the blaOXA-23 gene in A. baumannii SC0701 (A) and ISaba10 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.](http://aac.asm.org/Downloaded from)

### TABLE 1. Phenotypic and genotypic characteristics of carbapenem-nonsusceptible *A. baumannii*

| Group (no. of isolates) | Isolate | IS preceding the blaOXA gene | MIC (mg/liter) | Expression level of blaOXA-23 | Status of CarO |
|--------------------------|---------|-----------------------------|----------------|-----------------------------|---------------|
|                          |         | ISaba1-blaOXA-23 | ΔISaba1-ISaba10-blaOXA-23 | ISaba1-blaOXA-66 | IMP | MER | carOIS |
| Group I (13)             |         | -     | +     | -                        | 32 32 | 4.6 | Intact |
| SC0701                   |         | -     | +     | -                        | 32 32 | 3.2 | Lost   |
| SC0702                   |         | -     | +     | -                        | 32 32 | 2.2 | Lost   |
| SC0703                   |         | -     | +     | -                        | 32 32 | 4.9 | Lost   |
| SC0704                   |         | -     | +     | -                        | 32 32 | 3.4 | Lost   |
| SC0705                   |         | -     | +     | -                        | 32 32 | 4.0 | Lost   |
| SC0706                   |         | -     | +     | -                        | 32 32 | 2.3 | Lost   |
| SC0707                   |         | -     | +     | -                        | 32 32 | 2.0 | Lost   |
| SC0708                   |         | -     | +     | -                        | 32 32 | 4.6 | Lost   |
| SC0709                   |         | -     | +     | -                        | 32 32 | 1.8 | Lost   |
| SC0710                   |         | -     | +     | -                        | 32 32 | 1.5 | Lost   |
| SC0711                   |         | -     | +     | -                        | 32 32 | 2.2 | Lost   |
| SC0712                   |         | -     | +     | -                        | 32 32 | 2.7 | Lost   |
| SC0713                   |         | -     | +     | -                        | 32 64 | 2.0 | Lost   |
| Group II (7)             |         | +     | -     | -                        | 16 16 | 1.0 | NT     |
| SC0721                   |         | +     | -     | -                        | 16 16 | 1.1 | NT     |
| SC0722                   |         | +     | -     | -                        | 16 16 | 1.4 | NT     |
| SC0723                   |         | +     | -     | -                        | 16 16 | 1.3 | NT     |
| SC0724                   |         | +     | -     | -                        | 8 16  | 1.4 | NT     |
| SC0725                   |         | +     | -     | -                        | 16 16 | 1.1 | NT     |
| SC0726                   |         | +     | -     | -                        | 16 16 | 1.0 | NT     |
| SC0727                   |         | +     | -     | -                        | 8 16  | 1.2 | NT     |
| Group III (3)            |         |         |         | +                        | 8 16  | NT | NT |
| SC0731                   |         | NT    | NT    | +                        | 8 16  | NT | NT |
| SC0732                   |         | NT    | NT    | +                        | 8 16  | NT | NT |
| SC0733                   |         | NT    | NT    | +                        | 8 16  | NT | NT |
| ATCC 19606T              |         | NT    | NT    | +                        | 0.25  | 1  | NT | Intact |

**Abbreviations:** ΔISaba1, disrupted ISaba1; IMP, imipenem; MER, meropenem; +, positive; −, negative; NT, not tested.  
*Expression levels of the blaOXA-23 gene were measured by real-time quantitative PCR and normalized against the 16S rRNA gene.*
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 43-kDa porins, were apparently similar to the wild-type strain. All group I isolates, except one (isolate SC0701), lacked the 29-kDa CarO-like protein, PCR experiments for the *carO* gene start codon (Fig. 1).

Disruption of the *carO* gene by the IS*Abal* element within the *carO* gene in these isolates. IS*Abal* inserted at the 204th base from the *carO* gene start codon (Fig. 1).

Disruption of the *carO* gene by the IS*Abal*, IS*Abal*25, or IS*Abal*825 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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**REFERENCES**

1. Bratu, S., D. Landman, D. A. Martin, C. Georgescu, and J. Quale. 2008. Correlation of antimicrobial resistance with beta-lactamases, the OmpA-like porin, and efflux pumps in clinical isolates of *Acinetobacter baumannii* endemic to New York City. Antimicrob. Agents Chemother. 52:2999–3005.

2. Chen, T. L., R. C. Wu, M. F. Shaio, C. P. Fung, and W. L. Cho. 2008. Acquisition of a plasmid-borne *bla*OXA-58 gene with an upstream IS1008 insertion conferring a high level of carbapenem resistance to *Acinetobacter baumannii*. Antimicrob. Agents Chemother. 52:2573–2580.

3. Clinical and Laboratory Standards Institute. 2010. Performance standards for antimicrobial susceptibility testing: twelfth informational supplement. M100-S20. Clinical and Laboratory Standards Institute, Wayne, PA.

4. Fournier, P. E., and H. Richet. 2006. The epidemiology and control of *Acinetobacter baumannii* in health care facilities. Clin. Infect. Dis. 42:692–699.

5. Hu, W. S., S. M. Yao, C. P. Fung, Y. P. Hsieh, C. P. Lin, and J. F. Lin. 2007. An OXA-66/OXA-51-like carbapenemase and possibly an efflux pump are associated with resistance to imipenem in *Acinetobacter baumannii*. Antimicrob. Agents Chemother. 51:3844–3852.

6. Lee, K., Y. S. Lim, D. Yong, J. H. Yum, and Y. Chong. 2003. Evaluation of the Hodge test and the imipenem-EDTA double-disk synergy test for differentiating metallo-beta-lactamase-producing isolates of *Pseudomonas* spp. and *Acinetobacter* spp. J. Clin. Microbiol. 41:4623–4629.

7. Lee, K., D. Yong, Y. S. Choi, J. H. Yum, J. M. Kim, N. Woodford, D. M. Livermore, and Y. Chong. 2007. Reduced imipenem susceptibility in *Klebsiella pneumoniae* clinical isolates with plasmid-mediated CMY-2 and DHA-1 beta-lactamases co-mediated by porin loss. Int. J. Antimicrob. Agents 29:201–206.

8. Lee, Y., J. H. Yum, C. K. Kim, D. Yong, E. H. Jeon, S. H. Jeong, Y. J. Ahn, and K. Lee. 2010. Role of OXA-23 and AdeABC efflux pump for acquiring carbapenem resistance in an *Acinetobacter baumannii* strain carrying the *bla*OXA-66 gene. Ann. Clin. Lab. Sci. 40:43–48.

9. Lu, P. L., M. Dournath, D. M. Livermore, T. P. Chen, and N. Woodford. 2009. Diversity of carbapenem resistance mechanisms in *Acinetobacter baumannii* from a Taiwan hospital: spread of plasmid-borne OXA-72 carbapenemase. J. Antimicrob. Chemother. 63:61–67.

10. Mendes, R. E., J. M. Bell, J. D. Turnidge, M. Castanheira, and R. N. Jones. 2009. Emergence and widespread dissemination of OXA-23, -24/40 and -58 carbapenemases among *Acinetobacter* spp. in Asia-Pacific nations: report from the SENTRY Surveillance Program. J. Antimicrob. Chemother. 64:55–59.

11. Mognier, P. D., L. Poirel, T. Naas, and P. Nordmann. 2010. Worldwide dissemination of the OXA-23 carbapenemase gene of *Acinetobacter baumannii*. Emerg. Infect. Dis. 16:34–40.

12. Munoz-Price, L. S., and R. A. Weinstein. 2008. *Acinetobacter* infection. N. Engl. J. Med. 358:1274–1281.

13. Musi, M. A., A. S. Limansky, and A. M. Viale. 2005. Acquisition of resistance to carbapenems in multidrug-resistant clinical strains of *Acinetobacter baumannii*: natural insertional inactivation of a gene encoding a member of a novel family of beta-barrel outer membrane proteins. Antimicrob. Agents Chemother. 49:1342–1346.

14. Perez, F., A. M. Hujer, K. M. Hujer, B. K. Decker, P. N. Rather, and R. A. Bonomo. 2007. Global challenge of multidrug-resistant *Acinetobacter baumannii*. Antimicrob. Agents Chemother. 51:3471–3484.

15. Poirel, L., S. Figueiredo, V. Cattoir, A. Carattoli, and P. Nordmann. 2008. *Acinetobacter* radiorestrictas as a silent source of carbapenem resistance for *Acinetobacter* spp. Antimicrob. Agents Chemother. 52:1252–1256.

16. Poirel, L., and P. Nordmann. 2006. Carbapenem resistance in *Acinetobacter baumannii*: mechanisms and epidemiology. Clin. Microbiol. Infect. 12:826–836.

17. Poirel, L., and P. Nordmann. 2006. Genetic structures at the origin of acquisition and expression of the carbapenem-hydrolyzing oxacillinase gene *bla*OXA-58 in *Acinetobacter baumannii*. Antimicrob. Agents Chemother. 50:1442–1448.

18. Tenover, F. C., R. D. Arbeit, R. V. Goering, P. A. Mickelsen, B. E. Murray, D. H. Persing, and B. Swaminathan. 1995. Interpreting chromosomal DNA restriction patterns produced by pulsed-field gel electrophoresis: criteria for bacterial strain typing. J. Clin. Microbiol. 33:2233–2239.

19. Turton, J. F., N. Woodford, J. Glover, S. Yarde, M. E. Kaufmann, and T. L. Pitt. 2006. Identification of *Acinetobacter baumannii* by detection of the *bla*OXA-51-like carbapenemase gene intrinsic to this species. J. Clin. Microbiol. 44:2974–2976.

20. Woodford, N., M. J. Ellington, J. M. Coelho, J. F. Turton, M. E. Ward, S. Brown, S. G. Amyes, and D. M. Livermore. 2006. Multiplex PCR for genes encoding prevalent OXA carbapenemases in *Acinetobacter* spp. Int. J. Antimicrob. Agents 27:351–353.