

AbaR4-Type Resistance Island Including the *bla*_{OXA-23} Gene in *Acinetobacter nosocomialis* Isolates

Dae Hun Kim,^a Ji Young Choi,^a Sook-In Jung,^b Visanu Thamlikitkul,^c Jae-Hoon Song,^{d,e} and Kwan Soo Ko^{a,e}

Department of Molecular Cell Biology, Samsung Biomedical Research Institute, Sungkyunkwan University School of Medicine, Suwon, South Korea^a; Division of Infectious Diseases, Chonnam National University Medical School, Gwangju, South Korea^b; Siriraj Hospital, Mahidol University, Bangkok, Thailand^c; Division of Infectious Diseases, Samsung Medical Center, Sungkyunkwan University School of Medicine, Seoul, South Korea^d; and Asia Pacific Foundation for Infectious Diseases (APFID), Seoul, South Korea^e

This study reports for the first time the AbaR4-type resistance island with the *bla*_{OXA-23} gene in two carbapenem-resistant *A. nosocomialis* isolates from South Korea and Thailand.

OXA-23, an acquired class D β-lactamase with carbapenemase activity, confers carbapenem resistance in *Acinetobacter baumannii*. The presence of the *bla*_{OXA-23} gene has increasingly been reported worldwide and has emerged as a serious threat (5). Although several transposons, such as Tn2006, Tn2007, and Tn2008, have been identified as genetic structures harboring the *bla*_{OXA-23} gene, Tn2006 is part of the IS*Aba1*-linked AbaR4-type resistance island of *A. baumannii* related to clonal dissemination of the carbapenem-resistant *A. baumannii* European clone II, ST92, in many countries (5). *Acinetobacter radioresistens* was identified as a source of the *bla*_{OXA-23}-like genes (8), but an AbaR4-type resistance island including the *bla*_{OXA-23}-like gene has not been found in species other than *A. baumannii*. In this article, we report the AbaR4-type resistance island with the *bla*_{OXA-23} gene in two *Acinetobacter nosocomialis* (formerly *Acinetobacter* genomospecies 13TU) isolates.

In a molecular epidemiology study of *Acinetobacter* sp. isolates from Asian countries, we found two *A. nosocomialis* isolates harboring the *bla*_{OXA-23}-like gene, which was identified by multiplex PCR (11). This species was identified using sequences of 16S rRNA and partial *rpoB* genes (2). The sequences displayed 100% similarity to the reference strains of *A. nosocomialis* LMG 10619^T and RUH503 in the 16S rRNA and *rpoB* gene analyses, respectively. Two *bla*_{OXA-23}-positive *A. nosocomialis* isolates, K08-39 and Th01-06, were isolated from South Korea and Thailand, respectively. Both were isolated from patients who developed hospital-acquired pneumonia: K08-39 from the endotracheal aspirate and Th01-06 from the sputum. *In vitro* susceptibility testing was performed by measuring the MIC using the broth microdilution method according to Clinical and Laboratory Standards Institute (CLSI) guidelines (1). K08-39 from South Korea and Th01-06 from Thailand were resistant to both imipenem and meropenem (Table 1). K08-39 showed very high MICs for imipenem and meropenem (>64 and 64 mg/liter, respectively), while Th01-06 did not. K08-39 was susceptible to antimicrobial agents other than carbapenem, but Th01-06 was resistant to piperacillin-tazobactam and intermediate in resistance to cefepime.

The *comM* gene was evaluated for the presence of a transposon containing the *bla*_{OXA-23} gene. Both the Th01-06 and K08-39 isolates failed to produce a *comM* amplicon, indicating the interruption of the *comM* gene. They were PCR positive for the AbaR-*comM* junction, confirming the presence of an AbaR-type resistance island. The structure of the AbaR-type resistance

islands was determined by several PCR procedures and sequencing using previously published primers (9, 10). Both showed the AbaR4 type, although their subtypes were also identified (see Fig. S1 in the supplemental material). While a primer set spanning *tniB*-*tniE* produced a 388-bp amplicon in the K08-39 isolate (referred to as the AB210 type), a 3,238-bp amplicon was produced by the same primer set in the Th01-06 isolate (referred to as the D36 type). In K08-39, the AB210 type, the *tniB* gene was 500 bp in size, while the *tniD* gene was absent. In Th01-06, the D36 type, however, the *tniB* gene was 921 bp in size, while the *tniD* gene was present. These AbaR4-type resistance islands have been identified nearly exclusively in the global clone II (GC II; formerly European clone II) of *A. baumannii* (9, 10), which is the most frequently identified clone in Asian countries, including South Korea (6). Thus, we hypothesize that AbaR4-type resistance islands, including the *bla*_{OXA-23} gene in *A. nosocomialis*, have been transferred from *A. baumannii* GC II clones. Although the resistance islands of both isolates belonged to the AbaR4 type, they might have been transferred independently because they are of different subtypes and different localities.

The expression of OXA-23 in the two *A. nosocomialis* isolates was evaluated by quantitative reverse transcription-PCR (qRT-PCR) and normalized against the housekeeping gene *rpoB*. To compare the expression of OXA-23, two *bla*_{OXA-23}-positive carbapenem-resistant, one *bla*_{OXA-23}-negative carbapenem-resistant, and one *bla*_{OXA-23}-negative carbapenem-susceptible *A. baumannii* isolate were also included in the qRT-PCR. Both *bla*_{OXA-23}-positive *A. nosocomialis* isolates showed high mRNA levels of OXA-23 (see Fig. S2 in the supplemental material). Since we know that the *bla*_{OXA-23} gene confers resistance to carbapenems in *A. baumannii* isolates, the high expression of *bla*_{OXA-23} genes in the two *A. nosocomialis* isolates likely contributes to the resistance to carbapenems as well.

Received 3 May 2012 Returned for modification 6 May 2012

Accepted 27 May 2012

Published ahead of print 11 June 2012

Address correspondence to Kwan Soo Ko, ksko@skku.edu.

Supplemental material for this article may be found at <http://aac.asm.org/>.

Copyright © 2012, American Society for Microbiology. All Rights Reserved.

doi:10.1128/AAC.00923-12

TABLE 1 Characteristics of two *A. nosocomialis* isolates with *bla*_{OXA-23}

Characteristic	Value or description for isolate ^a	
	K08-39	Th01-06
Species	<i>A. nosocomialis</i>	<i>A. nosocomialis</i>
Locality	South Korea	Thailand
Source	Endotracheal aspirate	Sputum
MIC of antimicrobial agent, mg/liter (susceptibility)		
Gentamicin	2 (S)	0.25 (S)
Ceftazidime	4 (S)	2 (S)
Cefotaxime	4 (S)	8 (S)
Cefepime	2 (S)	16 (I)
Ciprofloxacin	0.12 (S)	0.12 (S)
Imipenem	>64 (R)	16 (R)
Meropenem	64 (R)	16 (R)
Trimethoprim-sulfamethoxazole	0.12, 2.37 (S)	2, 38 (S)
Piperacillin-tazobactam	≤0.25, 4 (S)	256, 4 (R)
Colistin	0.5 (S)	1 (S)
Polymyxin B	0.5 (S)	1 (S)

^a R, resistant; I, intermediate; S, susceptible.

A. nosocomialis, which was long referred to as *Acinetobacter* genomospecies 13TU, belongs to the *A. calcoaceticus*-*A. baumannii* (Acb) complex or *A. baumannii* group along with *Acinetobacter pittii* (formerly *Acinetobacter* genomospecies 3) because they could not be differentiated easily by phenotypic or biochemical methods. *A. nosocomialis* is the second-most-frequent species isolated from blood among the genus *Acinetobacter* in South Korea and displays a low carbapenem resistance rate but a high polymyxin resistance rate, unlike *A. baumannii* (6). In our recent study, two carbapenem-resistant *A. nosocomialis* isolates from South Korea contained the *bla*_{SIM-1} gene, which has been identified in other *Acinetobacter* species, including *A. baumannii* (3, 4, 7, 12). Although *bla*_{OXA-23} has been identified in *A. radioresistens* and *Acinetobacter baylyi* (9, 12), it was first identified in *A. nosocomialis*. Thus, our findings in this study suggest that interspecies transfer of the resistance island may occur, which could contribute

to the dissemination of the antimicrobial-resistant isolates of *Acinetobacter* species.

ACKNOWLEDGMENTS

Acinetobacter isolates used in this study were obtained from the Asian Bacterial Bank (ABB) of the Asia Pacific Foundation for Infectious Diseases (APFID) (Seoul, South Korea).

This study was supported by a Samsung Biomedical Research Institute grant (no. BA-90012).

REFERENCES

1. Clinical and Laboratory Standards Institute (CLSI). 2011. Performance standards for antimicrobial susceptibility testing, 19th informational supplement, M100-S21. CLSI, Wayne, PA.
2. Ko KS, et al. 2007. High rates of resistance to colistin and polymyxin B in subgroups of *Acinetobacter baumannii* isolates from Korea. *J. Antimicrob. Chemother.* 60:1163–1167.
3. Lee K, et al. 2010. Characteristics of clinical isolates of *Acinetobacter* genomospecies 10 carrying two different metallo-beta-lactamases. *Int. J. Antimicrob. Agents* 36:259–263.
4. Lee K, et al. 2005. Novel acquired metallo-beta-lactamase gene, *bla*_{SIM-1}, in a class 1 integron from *Acinetobacter baumannii* clinical isolates from Korea. *Antimicrob. Agents Chemother.* 49:4485–4491.
5. Mugnier PD, Poirel L, Naas T, Nordmann P. 2010. Worldwide dissemination of the *bla*_{OXA-23} carbapenemase gene of *Acinetobacter baumannii*. *Emerg. Infect. Dis.* 16:35–40.
6. Park YK, et al. 2012. Changes in antimicrobial susceptibility and major clones of *Acinetobacter calcoaceticus-baumannii* complex isolates from a single hospital in Korea over 7 years. *J. Med. Microbiol.* 61:71–79.
7. Park YK, Jung SI, Park KH, Kim SH, Ko KS. 2012. Characteristics of carbapenem-resistant *Acinetobacter* spp. other than *Acinetobacter baumannii* in South Korea. *Int. J. Antimicrob. Agents* 39:81–85.
8. Poirel L, Figueiredo S, Cattoir V, Carattoli A, Nordmann P. 2008. *Acinetobacter radioresistens* as a silent source of carbapenem resistant for *Acinetobacter* spp. *Antimicrob. Agents Chemother.* 52:1252–1256.
9. Post V, White PA, Hall RM. 2010. Evolution of AbaR-type genomic resistance islands in multiply antibiotic-resistant *Acinetobacter baumannii*. *J. Antimicrob. Chemother.* 65:1162–1170.
10. Turton JF, Baddal B, Perry C. 2011. Use of accessory genome for characterization and typing of *Acinetobacter baumannii*. *J. Clin. Microbiol.* 49:1260–1266.
11. Woodford N, et al. 2006. Multiplex PCR for genes encoding prevalent OXA carbapenemases in *Acinetobacter* spp. *Int. J. Antimicrob. Agents* 27: 351–353.
12. Zhou Z, et al. 2011. Clinical carbapenem-resistant *Acinetobacter baylyi* strain coharboring *bla*_{SIM-1} and *bla*_{OXA-23} from China. *Antimicrob. Agents Chemother.* 55:5347–5349.