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

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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 Tn*2006*, Tn*2007*, and Tn*2008*, have been identified as genetic structures harboring the bla_{OXA-23} gene, Tn*2006* 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.

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

Characteristic	Value or description for isolate[a]	
	K08-39	Th01-06
Species	*A. nosocomialis*	*A. nosocomialis*
Locality	South Korea	Thailand
Source	Endotrachial 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.

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