

# Variations of AbaR4-Type Resistance Islands in *Acinetobacter baumannii* Isolates from South Korea

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**AbaR resistance islands in *Acinetobacter baumannii* isolates from South Korea were investigated. AbaR4-type resistance islands, including *bla*<sub>OXA-23</sub>-containing Tn2006, interrupted the *comM* gene in *A. baumannii* ST75 isolates. However, Tn2006 was not identified within AbaR resistance islands of ST138 isolates, although the *bla*<sub>OXA-23</sub> gene was detected in them. The similar structures of resistance islands suggest that most carbapenem-resistant *A. baumannii* isolates in South Korea have originated from the same ancestor with a globally disseminated clone, GC II.**

*Acinetobacter baumannii* is an opportunistic pathogen that primarily causes nosocomial infections (5). Such infections are often associated with resistance to many antimicrobial agents, including carbapenems (12). In addition to carbapenem resistance, a resistance island, termed AbaR1, was identified by comparison of whole-genome sequences (6). AbaR1 is integrated into the ATPase gene (now called *comM*) and contains a large cluster of antimicrobial and heavy-metal resistance genes. Further studies have revealed that there is significant diversity among the AbaR-type resistance islands in the same region of *comM* in *A. baumannii* isolates (1, 8–10, 13, 14), and AbaR transposons have been found in *A. baumannii* since the 1970s (10). Although AbaR-type resistance islands have been reported in *A. baumannii* isolates from many regions, little is known regarding the composition of different AbaRs in isolates from East Asia. In this study, we investigated the structural variations of AbaR-type resistance islands in multidrug-resistant (MDR) *A. baumannii* isolates from South Korea and their evolution in related clones during a short period.

Forty-four isolates of *A. baumannii* were analyzed (Table 1). All of them were isolated from blood obtained from patients in South Korean hospitals from 2003 to 2010. Antimicrobial susceptibility testing was performed by the broth microdilution method according to the CLSI guidelines (4). Multilocus sequence typing (MLST) according to the Oxford scheme was performed as previously described (2). Of the clones, 19 ST92, seven ST75, five ST138, nine ST69, two ST71, one ST70, and one ST220 clone were included (Table 1). The ST69, ST71, and ST70 clones included only carbapenem-nonsusceptible *A. baumannii* isolates, but ST220 included all carbapenem-susceptible isolates. In the ST92, ST75, and ST138 clones, both carbapenem-nonsusceptible and -susceptible isolates were included (Table 1).

The insertions of a transposon into the *comM* gene were investigated using previously published primers for all *A. baumannii* isolates, including both carbapenem-nonsusceptible and -susceptible isolates (14, 15). AbaR mapping was performed using two steps (Fig. 1). The first step included nine primer sets, including those for detection of the insertion of AbaR in the *comM* gene. When a *yeeA*-*bla*<sub>OXA-23</sub> or *bla*<sub>OXA-23</sub>-*ISAb1* fragment was amplified in the first step (primer sets II

and III), the second step of PCR, including five primer sets (1 to 5), was performed (Fig. 1). If the resistance island was I positive and II negative in the first step, primer set 6 in the second step was used as a confirmation.

All *A. baumannii* isolates, including carbapenem-susceptible isolates of ST92, ST75, ST138, and ST220, failed to produce a *comM* amplicon, indicating the interruption of the *comM* gene. In addition, all isolates with an interrupted *comM* gene produced an amplicon for the *uspA* gene, indicating that the gene has not been interrupted by an *ISPPu12* composite transposon as in other AbaR types (14).

The structure of the AbaR-type resistance islands indicated that all *comM* inserts of carbapenem-resistant isolates corresponded largely to two types (Fig. 2). While isolates of the ST75 clone showed a typical AbaR4 island (7), isolates of the other clones, such as ST92, ST69, ST71, ST220, ST70, and ST138, possessed resistance islands similar to AbaR4, which lacks Tn2006. AbaR4 was initially found in a GC1 isolate, but it was not inserted into the *comM* gene but rather found in a different location (1). Interestingly, Tn2006 including *bla*<sub>OXA-23</sub> was not present in the resistance island but Tn2006 or the *bla*<sub>OXA-23</sub> gene was present in other regions of ST138 isolates (Fig. 2). Isolate H10-103 was positive for primer set I in the first step and negative for primer sets 1 and 5 in the second step but gave amplicons for primer sets 2 to 4 in the second step. In addition, four isolates of ST138 were positive for primer set III in the first step but were negative with primer sets 1 to 5 in the second step.

So far, two subtypes of AbaR4 have been reported. One, produced by primer set b in the first step, is a 3,238-bp amplicon that spans *tniB* to *tniE* (referred to as the D36 type) (15). The other, produced by the same primer set, is a 388-bp amplicon referred to as the AB210 type (Fig. 1 and 2) (7). In this study, three isolates of clone ST75 and one isolate of ST92 showed the D36-type AbaR4,

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TABLE 1 *A. baumannii* isolates analyzed in this study

Sequence type (allelic profile <sup>a</sup> )	Isolate	Isolation yr	Resistance (MIC, mg/liter) <sup>b</sup>					
			Imipenem	Meropenem	Tetracycline	Amikacin	Cefepime	Ampicillin-sulbactam
ST92 (1-3-3-2-2-7-3)	C003	2003	S (2)	S (2)	R (>64)	R (>128)	R (32)	R (64/32)
	C011	2003	I (8)	R (16)	R (>64)	R (>128)	R (64)	R (64/32)
	C022	2004	S (1)	S (2)	R (>64)	R (>128)	R (64)	R (64/32)
	C032	2004	S (1)	S (2)	R (>64)	R (>128)	R (32)	R (>64/32)
	C043	2005	S (1)	S (2)	R (>64)	R (>128)	R (64)	R (>64/32)
	C052	2005	S (2)	S (4)	R (>64)	R (>128)	R (32)	R (64/32)
	C100	2006	S (1)	S (2)	R (>64)	R (>128)	R (32)	R (>64/32)
	H06-454	2006	S (2)	R (16)	R (>64)	R (>128)	R (32)	R (64/32)
	H07-129	2007	S (2)	R (16)	I (8)	R (>128)	R (>64)	R (>64/32)
	H07-332	2007	R (32)	R (16)	R (>64)	R (>128)	R (32)	R (64/32)
	H07-395	2007	R (32)	I (8)	R (>64)	R (>128)	R (32)	R (>64/32)
	E07-111	2007	S (2)	S (4)	R (>64)	R (>128)	R (64)	R (64/32)
	H08-796	2008	R (16)	R (>64)	R (>64)	R (>128)	R (>64)	R (>64/32)
	E08-46	2008	R (16)	R (>64)	R (>64)	R (>128)	R (>64)	R (>64/32)
	H09-169	2009	R (16)	R (>64)	R (>64)	R (>128)	R (>64)	R (>64/32)
	H09-256	2009	S (4)	I (8)	I (8)	R (>128)	R (>64)	R (64/32)
	H09-421	2009	R (32)	R (64)	R (>64)	R (>128)	R (>64)	R (>64/32)
	H09-422	2009	R (16)	R (64)	R (>64)	R (>128)	R (>64)	R (>64/32)
	E09-228	2009	S (2)	S (4)	R (>64)	R (>128)	R (>64)	I (16/8)
	ST69 (1-46-3-2-2-58-3)	C018	2004	R (16)	R (>64)	R (16)	R (64)	R (>64)
C026		2004	R (32)	R (64)	R (16)	R (64)	R (>64)	R (64/32)
C046		2005	I (8)	R (16)	R (16)	R (64)	R (>64)	R (32/16)
C067		2005	R (32)	R (32)	I (8)	I (32)	R (>64)	R (>64/32)
C093		2006	I (8)	R (16)	R (16)	S (16)	R (>64)	R (>64/32)
H06-855		2006	R (16)	R (16)	R (16)	R (>128)	R (>64)	R (>64/32)
H07-130		2007	R (32)	R (>64)	R (16)	R (>128)	R (>64)	R (64/32)
E07-334		2007	R (16)	R (>64)	R (16)	R (128)	R (64)	I (16/8)
H09-347		2009	R (32)	R (64)	R (16)	R (>128)	R (>64)	R (64/32)
ST75 (1-3-3-2-2-11-3)	E08-600	2008	R (64)	R (64)	R (>64)	I (32)	R (>64)	R (>64/32)
	E08-650	2008	R (>64)	R (>64)	R (>64)	I (32)	R (>64)	R (>64/32)
	H09-94	2009	R (>64)	R (>64)	R (>64)	I (32)	R (>64)	R (>64/32)
	H09-146	2009	R (>64)	R (>64)	R (>64)	I (32)	R (>64)	R (>64/32)
	H09-504	2009	R (>64)	R (>64)	R (>64)	R (128)	R (>64)	R (>64/32)
	H09-526	2009	R (>64)	R (>64)	R (>64)	I (32)	R (>64)	R (>64/32)
	E10-93	2010	S (0.25)	S (≤0.0625)	R (64)	S (8)	S (0.5)	R (>64/32)
ST138 (1-3-3-2-2-50-3)	H09-400	2009	R (>64)	R (>64)	S (4)	S (8)	R (>64)	R (>64/32)
	H09-401	2009	R (>64)	R (>64)	S (4)	S (8)	R (>64)	R (>64/32)
	H09-454	2009	R (>64)	R (>64)	S (4)	R (>128)	R (>64)	R (>64/32)
	H09-465	2009	R (>64)	R (>64)	S (4)	R (>128)	R (>64)	R (>64/32)
	H10-103	2009	S (0.25)	S (≤0.0625)	R (>64)	R (>128)	R (>64)	R (>64/32)
ST71 (1-46-3-2-2-58-4)	C077	2005	I (8)	R (16)	R (16)	S (16)	R (>64)	R (>64/32)
	C099	2006	R (16)	R (64)	I (8)	I (32)	R (>64)	R (>64/32)
ST70 (1-46-3-2-1-58-3)	C083	2005	R (16)	R (32)	I (8)	R (64)	R (>64)	R (>64/32)
ST220 (1-3-3-2-1-7-3)	H07-988	2007	S (2)	S (4)	I (8)	I (8)	R (>64)	R (32/16)

<sup>a</sup> *gltA-gyrB-gdhB-recA-cpn60-gpi-rpoD*.<sup>b</sup> R, resistant; I, intermediate; S, susceptible.

and the AbaR4 of the remaining clones was the AB210 type (Fig. 2). In addition to the findings that *bla*<sub>OXA-23</sub> is found in carbapenem-resistant GC1 isolates (11), the diverse pattern of *bla*<sub>OXA-23</sub> in this study may support a previous suggestion that Tn2006-

mediated acquisition of *bla*<sub>OXA-23</sub> may occur as independent events or that Tn2006 is a mobile structure in a given genome (11). However, it is now unclear whether clones with a *bla*<sub>OXA-23</sub>-positive AbaR4 resistance island have been introduced recently in



South Korea or whether the transposon or a portion of it has inserted into the genomes of ST75 and ST138.

One of the interesting results in this study is the similarity of AbaR4-type resistance islands between carbapenem-nonsusceptible and -susceptible *A. baumannii* isolates belonging to the same clone. An isolate of an antimicrobial-susceptible clone, ST220, was found to have the same resistance island structure as that of isolates of several resistant clones, including ST92, ST69, ST71, and ST70. In addition, both carbapenem-nonsusceptible and -susceptible isolates were included in ST92, ST75, and ST138, but the structure of a resistance island was not correlated with the carbapenem susceptibility. Tn6021 forming an AbaR backbone has been identified in a susceptible *A. baumannii* reference strain, ATCC 17978 (6). Our result also indicates that the identification of an AbaR4-like element is not always correlated with carbapenem resistance or MDR, in confirmation of a previous report (3).

In summary, an AbaR4-type resistance island or resistance islands similar to it were identified in *A. baumannii* isolates from South Korea. ST75 isolates possessed AbaR4 islands containing a Tn2006 insert, but isolates of other clones lacked Tn2006 in their resistance islands. ST138 isolates may have the *bla*<sub>OXA-23</sub> gene in loci other than the resistance island. The AbaR4-like island or a portion of it was found even in susceptible clones with an intact *comM* gene.

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