

# AbaR-Type Genomic Islands in Non-*baumannii* *Acinetobacter* Species Isolates from South Korea

Dae Hun Kim, Kwan Soo Ko

Department of Molecular Cell Biology, Samsung Biomedical Research Institute, Sungkyunkwan University School of Medicine, Suwon, South Korea

**To investigate the presence and structure of AbaR-type genomic islands (GIs) in non-*Acinetobacter baumannii* isolates, a total of 155 non-*baumannii* *Acinetobacter* isolates from a South Korean hospital were analyzed. GIs were found in three *Acinetobacter nosocomialis* and two *Acinetobacter seifertii* isolates. Their structures were similar to those in *A. baumannii* isolates from Asian countries, including South Korea. The existence of AbaR-type GIs in non-*baumannii* *Acinetobacter* isolates is believed to be due to interspecies transfer of GI.**

Recently, *Acinetobacter* spp. have emerged as important opportunistic nosocomial pathogens. In addition to *Acinetobacter baumannii*, which is the most important species in clinical settings, especially in intensive care units, other *Acinetobacter* spp., such as *A. nosocomialis* and *A. pittii*, are frequently isolated in hospitals (1, 2). A resistance island, termed AbaR1, was identified by whole-genome sequence comparisons with a multidrug-resistant (MDR) *A. baumannii* strain, AYE (3). AbaR1 is integrated into the ATPase gene (now called *comM*) and contains a large cluster of antimicrobial and heavy metal resistance genes. Some studies have revealed diverse related AbaR resistance islands in the same region of *comM* in *A. baumannii* isolates (4–8). Although most AbaR resistance islands have been reported in *A. baumannii* isolates, recently, AbaR4 was reported in *A. nosocomialis* isolates from South Korea and Thailand (9). However, the prevalence and characteristics of AbaR-type genomic islands (GIs) in non-*baumannii* *Acinetobacter* isolates are not well known.

In the present study, the prevalence of AbaR-type GIs was investigated among non-*baumannii* *Acinetobacter* isolates from a South Korean hospital. In addition, the structure of AbaR-type GIs found in non-*baumannii* *Acinetobacter* isolates was analyzed.

In a previous study (10), 155 non-*baumannii* *Acinetobacter* strains were isolated from patients with bloodstream infections admitted to a tertiary care hospital in South Korea between August 2003 and February 2010. Species identification of the isolates using *rpoB* and 16S rRNA gene sequences revealed 93 *A. nosocomialis*, 28 *Acinetobacter seifertii* (formerly *Acinetobacter* genomic species “close to 13TU”), 15 *A. pittii*, three *Acinetobacter calcoaceticus*, six *Acinetobacter bereziniae*, four *Acinetobacter* genomic species 16, three *Acinetobacter ursingii*, two *Acinetobacter parvus*, and one *Acinetobacter junii* strain. *In vitro* antimicrobial susceptibility testing was also performed using a broth microdilution method, according to CLSI guidelines, in a previous study (10, 11). All of these 155 isolates were used in the present study.

Transposon insertion into *comM* was investigated using previously published primers for all *Acinetobacter* isolates (12, 13). Amplification of intact *comM* (982 bp) indicated that no GI interrupted *comM*, while no amplification indicated the possibility of GI interrupting *comM*. The integration of GI was confirmed by using two primer sets amplifying AbaR-*comM* (RH927 and RH797) and *comM*-AbaR (RH916 and RH928) (12). The structure of AbaR-type GIs in non-*baumannii* *Acinetobacter* isolates

was identified by sequential PCR amplification (amplicon sizes, 4 to 5 kb) and sequencing using additional 20 primers.

Among the 155 non-*baumannii* *Acinetobacter* isolates, GIs were identified in five isolates, of which three were *A. nosocomialis* and two were *A. seifertii*. On the other hand, the *comM* gene was yielded by primers used in this study in *A. pittii*, *A. calcoaceticus*, *A. junii*, *A. parvus*, *A. ursingii*, *A. bereziniae*, and *Acinetobacter* genomic species 16. Among the five GI-positive non-*baumannii* *Acinetobacter* isolates, three and four were resistant to imipenem and meropenem, respectively (Table 1). All GI-positive non-*baumannii* *Acinetobacter* isolates were resistant to ciprofloxacin, piperacillin-tazobactam, and ampicillin-sulbactam. *A. nosocomialis* strain H06-681 was resistant to all antimicrobial agents, excluding imipenem, polymyxins, and tigecycline. Only *A. seifertii* strain C066 was resistant to colistin.

The structure of AbaR-type GIs in three *A. nosocomialis* and two *A. seifertii* isolates was determined (Fig. 1). *A. nosocomialis* H06-681 and *A. seifertii* C066 carried Tn6022 with a deletion of *tniD* (Tn6022Δ*tniD*), although they belong to different species (Fig. 1). Although a *bla*<sub>OXA-23</sub>-like gene was identified with IS*Aba1* in *A. nosocomialis* H06-681, it was not detected within the GI. Thus, the *bla*<sub>OXA-23</sub>-like gene was assumed to be located in another region of the chromosome of *A. nosocomialis* H06-081, along with IS*Aba1*. On the other hand, no *bla*<sub>OXA-23</sub>-like gene was identified in *A. seifertii* C066. *A. nosocomialis* strains H09-1045 and E09-34, which showed very high MICs for carbapenems (Table 1), harbored AbaR4, which is composed of Tn6022 with Tn2006 (14). In *A. seifertii* strain C044, Tn6166 was also identified (Fig. 1). However, the Tn6166 identified in this isolate, which lacked *tniD* and Tn2006, included *tetA*(B), *tetR*(B),

Received 18 May 2015 Returned for modification 6 June 2015

Accepted 12 June 2015

Accepted manuscript posted online 22 June 2015

Citation Kim DH, Ko KS. 2015. AbaR-type genomic islands in non-*baumannii* *Acinetobacter* species isolates from South Korea. *Antimicrob Agents Chemother* 59:5824–5826. doi:10.1128/AAC.01175-15.

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

Copyright © 2015, American Society for Microbiology. All Rights Reserved.

doi:10.1128/AAC.01175-15

TABLE 1 AbaR-type genomic island and antimicrobial resistance in non-*baumannii* *Acinetobacter* isolates harboring genomic islands

Species	Isolate	Type	MIC (mg/liter) of <sup>a</sup> :													
			IMP	MEM	TET	CIP	RIF	AMK	CPM	CTR	CAZ	P/T	A/S	PB	COL	TIG
<i>A. nosocomialis</i>	H06-681	Tn6022Δ <i>tniD</i>	2	16	>64	>64	4	>128	>64	>128	>64	>256/4	64/32	1	2	4
	H09-1045	AbaR4	>64	>64	64	64	2	32	64<	16	8	>256/4	64/32	2	2	4
	E09-34	AbaR4	>64	>64	>64	8	4	32	64<	16	8	>256/4	>64/32	2	2	4
<i>A. seiffertii</i>	C044	Tn6166	1	1	>64	>64	4	>128	16	>128	>64	256/4	64/32	1	2	2
	C066	Tn6022Δ <i>tniD</i>	16	32	8	>64	2	16	>64	>128	>64	>256/4	64/32	2	8	4

<sup>a</sup> IMP, imipenem; MEM, meropenem; TET, tetracycline; CIP, ciprofloxacin; RIF, rifampin; AMK, amikacin; CPM, cefepime; CTR, ceftriaxone; CAZ, ceftazidime; P/T, piperacillin-tazobactam; A/S, ampicillin-sulbactam; PB, polymyxin B; COL, colistin; TIG, tigecycline. MICs in bold indicate resistance. The breakpoints of resistance are from CLSI guidelines (11) for most antimicrobial resistance (resistance defined as  $\geq 16$  mg/liter for both imipenem and meropenem). The criteria recommended by the CLSI for staphylococci were applied for rifampin (resistance defined as  $\geq 4$  mg/liter), and the criteria of the U.S. Food and Drug Administration (FDA) for *Enterobacteriaceae* were used for tigecycline (resistance defined as  $\geq 8$  mg/liter) (18).

*CR2*, *strB*, *strA*, and *orf4b* instead and did not interrupt the *comM* gene.

Since the discovery of AbaR1 in *A. baumannii* strain AYE in 2006 (3), it has been known that the resistance island may play a significant role in the antimicrobial resistance of *A. baumannii*. Since then, several of its variants have been identified and used in epidemiological studies (4–8, 12, 15–17).

One of the most interesting findings in this study was that GIs were identified in five isolates of *A. nosocomialis* and *A. seiffertii*, which belong to the *A. calcoaceticus/A. baumannii* (ACB) complex or *A. baumannii* complex. None of them are clonal, judging from 16S rRNA and *rpoB* gene sequences. Non-*baumannii* *Acinetobacter* species of *A. baumannii* complex, such as *A. nosocomialis*, *A. pittii*, and *A. seiffertii*, are increasingly reported to cause

human infections with the introduction of molecular identification tools (2). The AbaR-type GIs of five non-*baumannii* *Acinetobacter* isolates identified in this study were shared with those of *A. baumannii* isolates. Tn6022Δ*tniD*, which was detected in *A. nosocomialis* H06-681 and *A. seiffertii* C066, contains a Tn6022 backbone and is the simplest GI (14, 16). The same GI structure found in different non-*baumannii* *Acinetobacter* species may be evidence that horizontal transfer of GIs occurred several times. AbaR4, which was identified in two isolates of *A. nosocomialis*, has been reported in *A. baumannii* sequence type 75 (ST75) isolates from South Korea, in *A. nosocomialis* strain Th01-06 from Thailand, and in many *A. baumannii* isolates from South Korea (9, 15). In addition, the Tn6166 structure of C044 is identical to that described by Nigro and Hall (6). These data imply that the GIs are

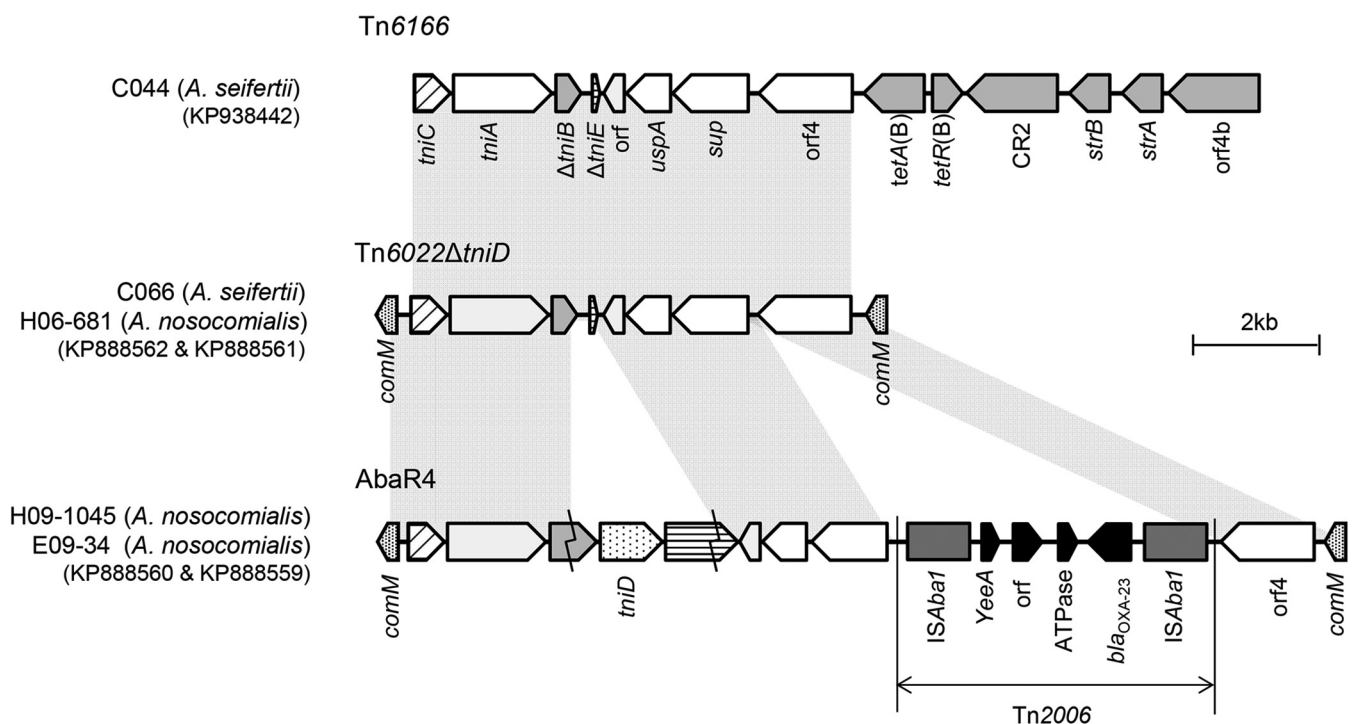


FIG 1 Structure of GIs in five non-*baumannii* *Acinetobacter* isolates from Korea. Tn6166 of C044 is different from Tn6022Δ*tniD* of C066 and H06-681 in that six genes, *tetA*(B), *tetR*(B), *CR2*, *strB*, *strA*, and *orf4b*, are present at the 5' end of *orf4*. In AbaR4 of H09-1045 and E09-34, *tniD* was intact and Tn2006, including *bla*<sub>OXA-23</sub>, was incorporated, comparing it with Tn6022Δ*tniD*.

possibly transferred among *Acinetobacter* species and suggest the increased frequency of AbaR-type GI in non-*baumannii* *Acinetobacter* isolates in the future.

In this study, we identified the AbaR-type GIs in five non-*baumannii* *Acinetobacter* isolates, and their structures were determined. The structure of GIs in non-*baumannii* *Acinetobacter* isolates suggests the interspecies transfer of GIs.

**Nucleotide sequence accession numbers.** Sequences determined in this study have been deposited in GenBank under accession no. KP938442, KP888559, KP888560, KP888561, and KP888562.

#### ACKNOWLEDGMENTS

The *Acinetobacter* isolates used in this study were provided by Sook-In Jung (Chonnam National University Medical School, Gwangju, South Korea).

This research was supported by the Basic Science Research Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Science, ICT and Future Planning (grant NRF-2013R1A2A2A0101413).

#### REFERENCES

1. Wisplinghoff H, Paulus T, Lugenheim M, Stefanik D, Higgins PG, Edmond MB, Wenzel RP, Seifert H. 2012. Nosocomial bloodstream infections due to *Acinetobacter baumannii*, *Acinetobacter pittii* and *Acinetobacter nosocomialis* in the United States. *J Infect* 64:282–290. <http://dx.doi.org/10.1016/j.jinf.2011.12.008>.
2. Park YK, Jung SI, Park KH, Kim DH, Choi JY, Kim SH, Ko KS. 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. <http://dx.doi.org/10.1099/jmm.0.033852-0>.
3. Fournier PE, Vallenet D, Barbe V, Audic S, Ogata H, Poirel L, Richet H, Robert C, Mangenot S, Abergel C, Nordmann P, Weissenbach J, Raoult D, Claverie JM. 2006. Comparative genomics of multidrug resistance in *Acinetobacter baumannii*. *PLoS Genet* 2:e7. <http://dx.doi.org/10.1371/journal.pgen.0020007>.
4. Krizova L, Dijkshoorn L, Nemeč A. 2011. Diversity and evolution of AbaR genomic resistance island in *Acinetobacter baumannii* strains of European. *Antimicrob Agents Chemother* 55:3201–3206. <http://dx.doi.org/10.1128/AAC.00221-11>.
5. Nigro SJ, Hall RM. 2012. Tn6167, an antibiotic resistance island in an Australian carbapenem-resistant *Acinetobacter baumannii* GC2, ST92 isolate. *J Antimicrob Chemother* 67:1342–1346.
6. Nigro SJ, Hall RM. 2012. Antibiotic resistance islands in A320 (RUH134), the reference strain for *Acinetobacter baumannii* global clone 2. *J Antimicrob Chemother* 67:335–338. <http://dx.doi.org/10.1093/jac/dkr447>.
7. Šepūtienė V, Povilonis J, Sužiedėlienė E. 2012. Novel variants of AbaR resistance islands with a common backbone in *Acinetobacter baumannii* isolates of European clone II. *Antimicrob Agents Chemother* 56:1969–1973. <http://dx.doi.org/10.1128/AAC.05678-11>.
8. Kochar M, Crosatti M, Harrison EM, Rieck B, Chan J, Constantinidou C, Pallen M, Ou HY, Rajakumar K. 2012. Deletion of TnAbaR23 results in both expected and unexpected antibiogram changes in a multidrug-resistant *Acinetobacter baumannii* strain. *Antimicrob Agents Chemother* 56:1845–1853. <http://dx.doi.org/10.1128/AAC.05334-11>.
9. Kim DH, Choi JY, Jung SI, Thamlikitkul V, Song JH, Ko KS. 2012. AbaR4-type resistance island including the *bla*<sub>OXA-23</sub> gene in *Acinetobacter nosocomialis* isolates. *Antimicrob Agents Chemother* 56:4548–4549. <http://dx.doi.org/10.1128/AAC.00923-12>.
10. 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.
11. Clinical and Laboratory Standards Institute. 2013. Performance standards for antimicrobial susceptibility testing; 21st informational supplement. CLSI document M100-S23. Clinical and Laboratory Standards Institute, Wayne, PA.
12. 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.
13. Turton JF, Baddal B, Perry C. 2011. Use of accessory genome for characterization and typing of *Acinetobacter baumannii*. *J Clin Microbiol* 49:1260–1266. <http://dx.doi.org/10.1128/JCM.02335-10>.
14. Hamidian M, Hall RM. 2011. AbaR4 replaces AbaR3 in a carbapenem-resistant *Acinetobacter baumannii* isolate belonging to global clone 1 form an Australian hospital. *J Antimicrob Chemother* 66:2484–2491. <http://dx.doi.org/10.1093/jac/dkr356>.
15. Kim DH, Park YK, Ko KS. 2012. Variations of AbaR4-type resistance island in *Acinetobacter baumannii* isolates from South Korea. *Antimicrob Agents Chemother* 56:4544–4547. <http://dx.doi.org/10.1128/AAC.00880-12>.
16. Kim DH, Choi JY, Kim HW, Kim SH, Chung DR, Peck KR, Thamlikitkul V, So TM, Yasin RM, Hsueh PR, Carlos CC, Hsu LY, Buntaran L, Lalitha MK, Song JH, Ko KS. 2013. Spread of carbapenem-resistant *Acinetobacter baumannii* global clone 2 Asia and AbaR-type resistance. *Antimicrob Agents Chemother* 57:5239–5246. <http://dx.doi.org/10.1128/AAC.00633-13>.
17. Saule M, Samuelsen Ø, Dumpis U, Sundsfjord A, Karlson A, Balode A, Miklasevics E, Karah N. 2013. Dissemination of a carbapenem-resistant *Acinetobacter baumannii* strain belonging to international clone II/sequence type 2 and harboring a novel AbaR4-like resistance island in Latvia. *Antimicrob Agents Chemother* 57:1069–1072. <http://dx.doi.org/10.1128/AAC.01783-12>.
18. Wyeth Pharmaceuticals, Inc. 2014. Tygacil—tigecycline injection, powder, lyophilized, for solution. Wyeth Pharmaceuticals, Inc., Philadelphia, PA. <http://www.pfizerpro.com/hcp/tygacil>.