Ann Clin Microbiol Vol. 16, No. 2, June, 2013
http://dx.doi.org/10.5145/ACM.2013.16.2.75
ISSN 2288-0585

Dissemination of an AbaR-type Resistance Island in Multidrug-resistant *Acinetobacter baumannii* Global Clone 2 in Daejeon of Korea

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Background: *Acinetobacter baumannii* resistance islands (AbaRs) are transposons that have the role of important vehicles for the acquisition of antimicrobial resistance genes, and are associated with multidrug resistance (MDR). In this study, we aimed to determine the AbaRs in MDR *A. baumannii* global clone 2 (GC2) clinical isolates obtained from a university hospital in Daejeon, Korea.
Methods: This study included 17 MDR *A. baumannii* strains isolated in Daejeon, Korea. The minimal inhibitory concentrations (MICs) were determined by Etest. *A. baumannii* isolates were characterized using 2 multiplex PCR assays and a multilocus sequence typing (MLST) scheme. To detect and characterize AbaRs, PCR and PCR mapping experiments were performed.
Results: All 17 MDR *A. baumannii* isolates tested in this study belonged to GC2 and contained 5 sequence types (STs): 75, 92, 137, 138, and 357. Tn*6166* that contains antimicrobial resistance genes and is also known as AbaR4a was found in all 17 GC2 strains. This is the first report of Tn*6166* in MDR *A. baumannii* GC2 isolates in Korea. In contrast, AbaR4 was not found in the GC2 isolates.
Conclusion: Tn*6166* has been disseminated among MDR *A. baumannii* GC2 isolates in Korea. Further investigation is needed to recover the various types of AbaRs in MDR *A. baumannii* GC2 isolates in Korea are responsible for the multiple antimicrobial resistance mechanisms. **(Ann Clin Microbiol 2013;16: 75-80)**

Key Words: *A. baumannii*, Transposon, Multilocus sequence typing, PCR

INTRODUCTION

Multidrug-resistant (MDR) *A. baumannii* strains have been reported worldwide usually belonged to one of two globally distributed clonal complexes. These two clones are global clones 1 and 2 (GC1 and GC2) which are also known as European clone I and II, respectively [1]. Most MDR *A. baumannii* isolates belonging to GC1 and GC2 are important opportunistic pathogen associated with nosocomial infections and hospital outbreaks. The evolution of MDR *A. baumannii* strains have been suggested to decrease cell wall permeability, increase constitutive expression of efflux pumps and/or acquire their antimicrobial resistance genes via integrons, transposons and plasmid [2]. In particular, rapid dissemination of acquired resistance genes has led to the widespread of MDR *A. baumannii* strains which give rise to significant therapeutic failures and increase of patient morbidity and mortality [3].

Many MDR *A.baumannii* strains harbor multiple antimicrobial resistance genes, which are integrated in the chromosomal *comM* gene encoding an ATPase domain. Such genomic resistance islands were designated *A. baumannii* resistance islands (AbaRs) [3]. AbaRs are transposons that incorporate themselves into the *comM* gene and have role as important vehicles for the acquisition of antimicrobial resistance genes. The first example of AbaRs was found in strain AYE and designated AbaR1 island, which includes more than 40 antimicrobial and heavy metal resistance genes. Until recently, many types of AbaRs have been fully characterized following detection of AbaR1 and most of them belong to AbaR3-type and AbaR4-type islands [3,4].

AbaR3-type islands found in GC1 isolates consist of a backbone transposon, Tn*6019*, which interrupted by a large compound transposon containing a variable resistance region located

Received 12 September, 2012, Revised 21 November, 2012
Accepted 10 December, 2012
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between two copies of transposon, Tn*6018* [4]. AbaR4-type islands found in both GC1 and GC2 isolates are consisted of a backbone transposon, Tn*6022*, which is most closely related to the transposon Tn*6021* that interrupts the *comM* gene but carries no resistance genes [5,6]. Tn*6022* differs from Tn*6021* in that it includes a short different patch of 90.8% identity in the *tniC-tniA* gene region (shown as a black line in Fig. 1) and lacks of *tniD* gene and part of *tniB* and *tniE* genes [4].

Even though various AbaRs have been recovered in MDR *A. baumanii* isolates worldwide [6,7], there is a relative paucity of reports about AbaRs in MDR *A. baumanii* isolates from Korea. In the present study, we aimed to investigate the AbaRs for understanding of acquired antimicrobial resistances mechanisms in MDR *A. baumannii* GC2 isolates obtained from a university hospital in Daejeon, Korea.

MATERIALS AND METHODS

1. Selection of bacteria isolates

Seventeen MDR *A. baumannii* isolates were collected and characterized in our previous study [8]. The isolates were collected from different patients in one university hospital in Daejeon (Korea) during the period between 2009 and 2011. MDR phenotypes were defined as showing resistance to representative antimicrobial agents of at least three different classes of drugs: aminoglycosides (gentamicin, amikacin), antipseudomonal penicillins (ticarcillin, piperacillin, piperacillin/tazobactam), carbapenems (imipenem, meropenem), antipseudomonal cephalosporins (ceftazidime, cefepime), and fluoroquinolone (ciprofloxacin) [9].

2. DNA extraction and PCR amplification

Whole-cell (genomic) DNA was obtained from each target strain by using a genomic DNA purification kit (SolGent, Daejeon, Korea) according to the manufacturer's instructions. PCR was performed using 50 ng of genomic DNA, 2.5 μL of 10× Taq buffer, 0.5 μL of 10 mM dNTP mix, 20 pmol of each primer, and 0.7 U of Taq DNA polymerase (SolGent), in a total volume of 25 μL. Each target site was amplified in a GeneAmp PCR System 9600 thermal cycler (Perkin-Elmer Cetus Corp., Norwalk, CT, USA). The amplified products were separated via electrophoresis on 1.5% (w/v) agarose gels containing ethidium

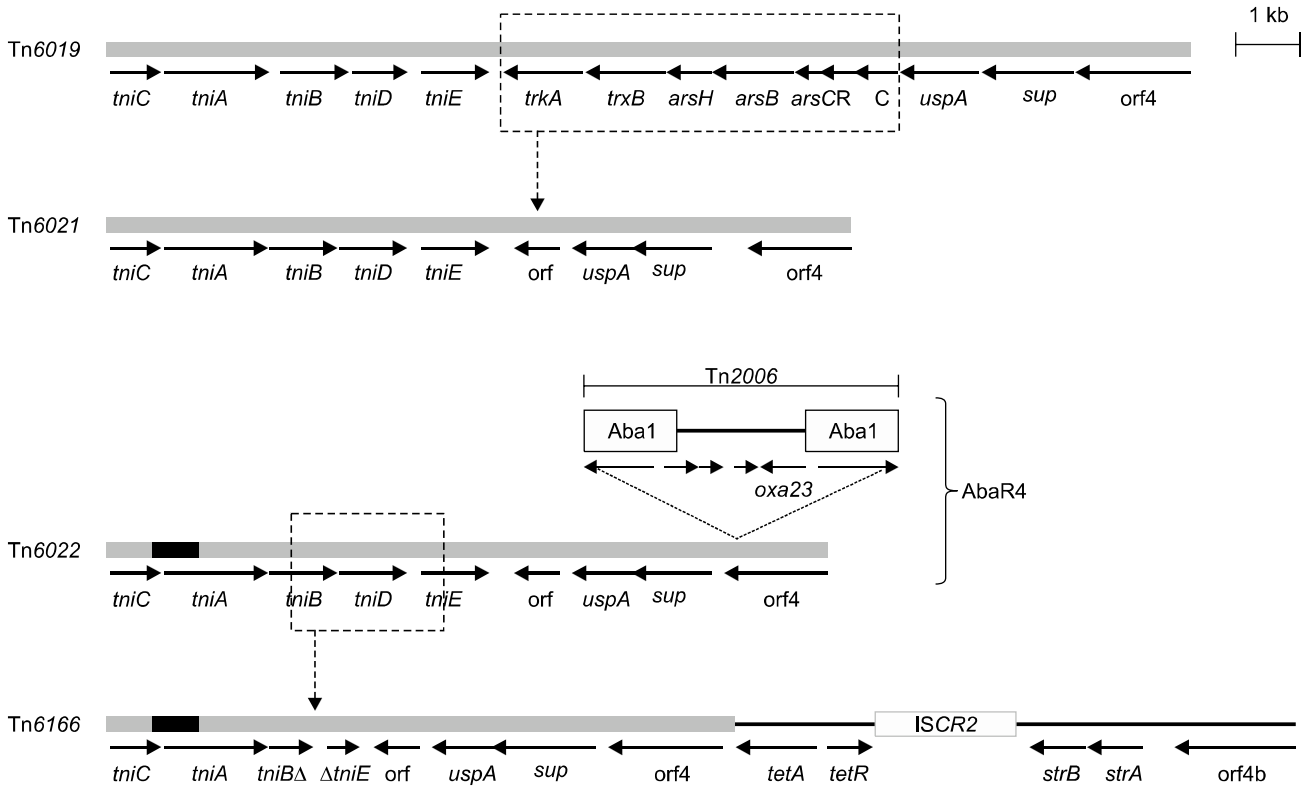

Fig. 1. Schematic representation of transposons isolated from multidrug-resistant *A. baumannii* strains. The dotted rectangle in Tn*6019* represents deletion part in Tn*6021*. A short patch of 90.8% identity in the *tniC-tniA* gene region was shown as a black line in Tn*6022* and Tn*6166*. In Tn*6022*, the dotted rectangle exhibits deletion part in Tn*6166*. The horizontal arrows indicate the translation orientation of the genes.

bromide, and visualized using a BioDoc-14TM Imaging system (UVP, Cambridge, UK). For sequencing, PCR products were purified with a PCR purification kit (SolGent) following the manufacturer's protocols.

3. Characterization of *A. baumannii* isolates

The two multiplex PCR assays were used as previously described [10] to identify members of the GC1 and GC2. The Oxford multilocus sequence typing (MLST) scheme [11], which uses seven housekeeping genes (*gltA, gyrB, gdhB, recA, cpn60, gpi* and *rpoD24*) was used to determine the sequence types (STs). A ST number was assigned by comparing the allele sequences to ones in the MLST site (http://pubmlst.org/abaumannii/).

4. Detections and characterizations of AbaRs

The genes associated with AbaRs were detected by PCR using the published primers (Table 1) [2,3]. The amplification regions are represented in Fig. 2. To investigate the structures of

Table 1. Primer pairs used for the detection and characterization of *A. baumannii* resistance islands

Region	Primer	Sequence 5'-3'	Amplicon length (bp)
comM	RH791	TGCTGCAATGAGCTGAAAGT	982
	RH913	GCCTCTCATTGAGGTTGAGG	
comM-AbaR (J1)	RH927	CAACCCTGTCTTTGCATTTG	846
	RH792	TTCGAGCTTGAAAACTGCAC	
AbaR-*comM* (J2)	RH916	CCCAAATACTGCCATGTTGA	796
	RH928	GCCAGCAAGCTCAGCATAA	
Tn*6018*	RH768	GAATCGCTGGTGATGATGGC	1,630
	RH769	GGTCTGAGACTTCGTGAGCGC	
uspA	RH919	TGTCAAAAATTATTGCATGT	632
	RH793	CCCAAGAGAGCTGATTTTGC	
*comM-tniB*Δ	RH791	TGCTGCAATGAGCTGAAAGT	3,119
	RH909	GCGATTCAAAATATCGGTCAA	
comM-orf/*uspA*	RH791	TGCTGCAATGAGCTGAAAGT	4,632
	RH592	AAGCTTATCGAAAAGGCGTAGA	
tniB-tniE	RH910	GCGATAGTGAACGGATTGAGA	560
	RH587	TTGCCCATTAAGCACAACAG	
*tniB*Δ*-sup*	RH910	GCGATAGTGAACGGATTGAGA	3,267
	RH771	TGTAAAATCTGGTGGTCGTAC	
sup-tetA(B)	RH772	GCAGCCATAGGAATGACTTTTA	3,961
	tetBF	TTGGTTAGGGGCAAGTTTTG	
orf4-CR2	RH916	CCCAAATACTGCCATGTTGA	4,466
	LECR2	CACTGGCTGGCAATGTCTAG	
CR2-*comM*	RH702	CTCGTCAACGATCTGATAGAGAAGGG	5,021
	RH928	GCCAGCAAGCTCAGCATAA	

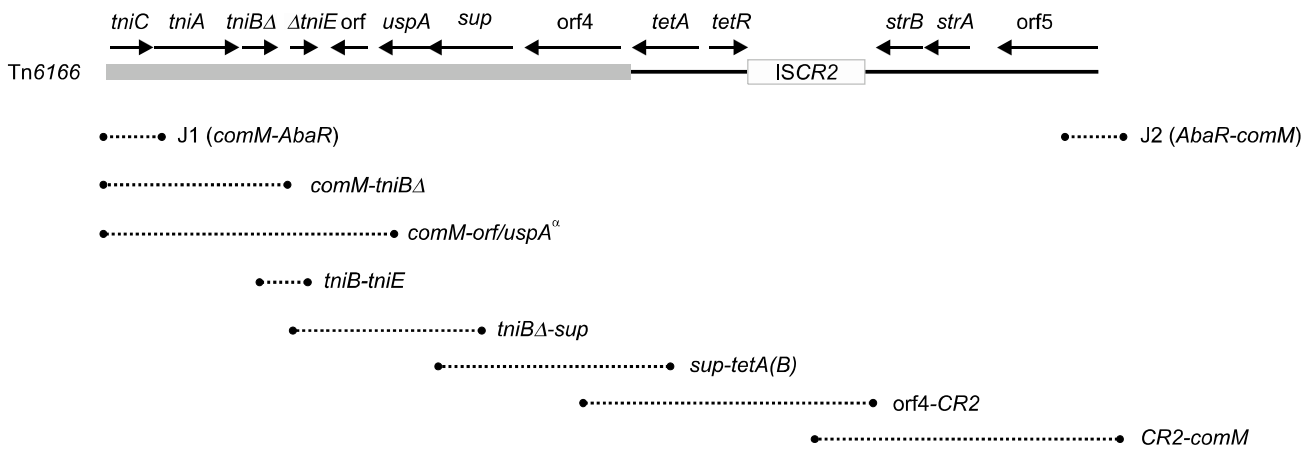

Fig. 2. Schematic representation of transposon, Tn*6166* and the amplification regions used in the diagnostic PCRs. The genes are shown by horizontal arrows with the gene name above. The dotted lines indicate PCR amplification regions with primer name on the right. Abbreviation: Tn, transposon.

Table 2. Properties of multidrug-resistant *A. baumannii* GC2 isolates carrying Tn*6166*

Sequence type	Number of isolates	Global clone	bla_{OXA-51} like	Island junctions				Features of Tn*6166*				
				comM	*comM*-AbaR J1	AbaR-*comM* J2	Tn*6018*	*uspA*	*sul*	*strA, strB*	*tetB, tetR*	ISC*R2*
75	1	2	OXA-66	-	+	+	-	+	+	+	+	+
92	2	2	OXA-66	-	+	+	-	+	+	+	+	+
137	7	2	OXA-66	-	+	+	-	+	+	+	+	+
138	3	2	OXA-66	-	+	+	-	+	+	+	+	+
357	4	2	OXA-66	-	+	+	-	+	+	+	+	+

Abbreviation: AbaR, *A. baumannii* resistance island.

AbaR backbone, PCR mapping experiments were performed as described previously [2,3]. The amplicons were purified and sequenced using a BigDye Terminator Cycle Sequencing Kit (PE Applied Biosystems, Foster City, CA, USA) and an ABI PRISM 3730XL DNA analyzer (PE Applied Biosystems). DNA fragments (up to 1 kb in size) were sequenced using the overlapping PCR technique. The various DNA sequences were confirmed by using the BLAST paired alignment facility (http://blast.ncbi.nlm.gov).

RESULTS

1. Characterization of MDR *A. baumannii*

Association of the GC lineages was tested for 17 MDR *A. baumannii* using multiplex PCRs. All 17 isolates tested in this study belonged to GC2 and carried allele 66 of the intrinsic bla_{OXA-51}-like genes which corresponds with their assignment to GC2. MLST analysis of GC2 isolates revealed 5 STs (75, 92, 137, 138 and 357) (Table 2).

2. MDR *A. baumannii* GC2 strains including AbaRs

To determine whether MDR *A. baumannii* GC2 strains harbored a transposon in *comM* gene, all 17 strains were tested for the presence of *comM* gene. An amplicon of *comM* gene was not obtained from any of our isolates except *Acinetobacter calcoaceticus* control strain. J1 (*comM*-AbaR) and J2 (AbaR-*comM*) segments, which form the boundaries of the AbaR backbone transposon, Tn*6019*, were amplified in all 17 isolates, indicating that transposon related insertions are present at the position of *comM* gene. However, Tn*6018* and an interrupted *uspA* gene were not present in all isolates tested in present study. These results indicated that AbaR3-type islands were not present in GC2 isolates.

3. Characterization of AbaRs

To characterize AbaRs contained in GC2 isolates, we mapped the continuous regions from J1 to J2 segment by overlapping PCRs and the sequencing. The about 18 kb transposon, Tn*6166* (GenBank accession no. JN247441), was identified from the genome of all MDR *A. baumanii* isolates which were tested in this study and belonged to GC2 (Fig. 1). Tn*6166* carries Tn*6022* containing intact universal stress protein (*uspA*) and sulfate permease (*sul*) except for a 2.85 kb deletion region which removes *tniD* gene and part of *tniB* and *tniE* genes. The *tet(B)* determinant and *strA* and *strB* genes are located in Tn*6166* harboring the small mobile element CR2.

DISCUSSION

MDR *A. baumannii* isolates pose therapeutic problems caused by intrinsic antimicrobial resistance mechanisms and capability to acquire multiple antimicrobial resistance genes via integron and transposon. Many researchers tried to find the role of integron and transposon mediated resistance in developing MDR *A. baumannii* strains, and recently recognized AbaRs as transposons containing multiple resistance determinants [2]. Further studies have found the frequent occurrence and worldwide dissemination of various AbaRs among MDR *A. baumannii* isolates [12]. However, much less is known about AbaRs in MDR *A. baumannii* isolates in Korea.

In this study, structure of transposon in MDR *A. baumannii* GC2 isolates was determined by using a strategy based on the structural and sequence homology of the AbaRs [2,3]. All GC2 isolates harbored an interrupted *comM* gene and uninterrupted *uspA* gene. In addition, Tn*6018* was not found in any isolates tested present study. Our results suggest that transposon insertion in GC2 isolates was not closely connected to the

Tn*6018*-containing AbaR3-type islands found in most of GC1 isolates. To characterize the transposons we used published primers for Tn*6166*, which is a type of genomic resistance island found in GC2 isolates and also known as AbaR4a for the structural similarity to Tn*6022*, the backbone of AbaR4 [13]. The Tn*6166* was found in all 17 MDR *A. baumannii* GC2 strains which were collected during the three years and revealed various ST types (ST 75, 92, 137, 138 and 357). This result suggests that Tn*6166* was disseminated in GC2 isolates and have existed for many years, though there have not been any reports as yet concerning Tn*6166* in Korea. This is the first report of Tn*6166* in MDR *A. baumannii* GC2 isolates in Korea.

Tn*6166* was first detected in *A. baumannii* RUH134/A320 which was isolated in 1982 in the Netherlands and has been used as the reference strain for GC2 isolates [4]. This suggests that Tn*6166* has been served as a reservoir for antimicrobial resistance genes in GC2 isolates since long before. The genomic island has a different origin to the AbaR3-type islands found in GC1 isolates and representative of a type of genomic island found in GC2 isolates.

Reportedly, AbaR4 which carries transposon Tn*2006* harboring the $bla_{\text{OXA-23}}$ carbapenem resistance gene have been identified in GC1 and GC2 isolates [14,15]. AbaR4 globally disseminated has been recovered in Australia, UK and Taiwan [5, 16,17], but there is not yet any data about AbaR4 in Korea so far. Similarly, in our examination, AbaR4 was not found in any isolates, although the 5 isolates of them harbored $bla_{\text{OXA-23}}$ gene.

In present study, it was found that various MDR *A. baumannii* GC2 isolates collected during the period between 2009 and 2011 contained Tn*6166* containing antimicrobial resistance genes. This result suggests that Tn*6166*, AbaR4-type island-related transposons, has been disseminated among MDR *A. baumanii* GC2 isolates in Korea before the transposons were determined in GC2 isolates. This finding emphasizes that further investigation is needed to recover various AbaRs in MDR *A. baumannii* GC2 isolates in Korea because AbaRs may seem to play a substantial role in antimicrobial resistance in MDR *A. baumannii* isolates through insertion and homologous recombination of various antimicrobial resistance genes.

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=국문초록=

대전에서 분리된 다제내성 *Acinetobacter baumannii* Global Clone 2에서 AbaR-type Resistance Island의 확산

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배경: *Acinetobacter baumannii* resistance islands (AbaRs)는 *A. baumannii*가 항균제 내성 유전자를 획득하고 다제내성을 갖는데 중요한 역할을 하는 transposons이다. 따라서 본 연구에서는 대전의 한 대학병원에서 분리된 다제내성 *A. baumannii* global clone (GC) 2에서 AbaRs를 검출하여 항균제 내성기전을 분석하고자 하였다.
방법: 대전의 한 대학병원에서 분리된 다제내성 *A. baumannii* 17균주를 대상으로 항균제에 대한 최소발육저지농도를 결정하기 위해 Etest를 수행하였다. 또한 다중중합효소연쇄반응 및 multilocus sequence typing (MLST)을 이용하여 *A. baumannii*의 분자유전학적 특징을 결정하였다. AbaRs는 PCR mapping과 염기서열 분석을 통하여 확인하였다.
결과: 본 연구에서 분리된 다제내성 *A. baumannii* 17균주는 모두 GC2에 속하는 것으로 나타났다. 또한 이들 균주는 모두 AbaR4a로도 알려져 있는 Tn*6166*을 포함하고 있음이 확인되었다. 국내에서는 아직까지 Tn*6166*을 포함하고 있는 *A. baumannii*에 대한 보고가 없었으며 본 연구가 최초의 보고이다.
결론: 본 연구에서 Tn*6166*이 한국에서 분리된 다제내성 *A. baumannii* 사이에 확산되어 있음이 확인되었다. 따라서 다제내성 *A. baumannii*의 내성기전을 밝히기 위해서는 다양한 종류의 AbaRs에 대한 추가적인 연구가 필요할 것으로 사료된다.
[Ann Clin Microbiol 2013;16:75-80]

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