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Prediction of Putative Resistance Islands in a Carbapenem-Resistant *Acinetobacter baumannii* Global Clone 2 Clinical Isolate

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Background: We investigated the whole genome sequence (WGS) of a carbapenem-resistant *Acinetobacter baumannii* isolate belonging to the global clone 2 (GC2) and predicted resistance islands using a software tool.

Methods: *A. baumannii* strain YU-R612 was isolated from the sputum of a 61-yr-old man with sepsis. The WGS of the YU-R612 strain was obtained by using the PacBio RS II Sequencing System (Pacific Biosciences Inc., USA). Antimicrobial resistance genes and resistance islands were analyzed by using ResFinder and Genomic Island Prediction software (GIPSy), respectively.

Results: The YU-R612 genome consisted of a circular chromosome (ca. 4,075 kb) and two plasmids (ca. 74 kb and 5 kb). Its sequence type (ST) under the Oxford scheme was ST191, consistent with assignment to GC2. ResFinder analysis showed that YU-R612 possessed the following resistance genes: four β-lactamase genes bla_{ADC-30}, bla_{OXA-66}, bla_{OXA-23}, and bla_{TEM-1}; *armA*, *aadA1*, and *aacA4* as aminoglycoside resistance-encoding genes; *aac(6')Ib-cr* for fluoroquinolone resistance; *msr(E)* for macrolide, lincosamide, and streptogramin B resistance; *catB8* for phenicol resistance; and *sul1* for sulfonamide resistance. By GIPSy analysis, six putative resistant islands (PRIs) were determined on the YU-R612 chromosome. Among them, PRI1 possessed two copies of Tn2009 carrying bla_{OXA-23}, and PRI5 carried two copies of a class I integron carrying *sul1* and *armA* genes.

Conclusions: By prediction of resistance islands in the carbapenem-resistant *A. baumannii* YU-R612 GC2 strain isolated in Korea, PRIs were detected on the chromosome that possessed Tn2009 and class I integrons. The prediction of resistance islands using software tools was useful for analysis of the WGS.

Key Words: *Acinetobacter baumannii*, Whole genome sequence, GIPSy, Global clone 2

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INTRODUCTION

Acinetobacter baumannii is an opportunistic pathogen that causes hospital-acquired infections, especially in intensive care units [1-3]. The incidence and worldwide dissemination of carbapenem-resistant *A. baumannii* isolates named global clone (GC)1 and GC2 have increased [4-6]. Carbapenem resistance in *A. baumannii* has mainly been ascribed to degradation by carbapenem-hydrolyzing oxacillinases including OXA-23, OXA-24, OXA-58, OXA-51, OXA-143, and their variants [2, 7].

Large DNA sequences (6-200 kb) are termed genomic islands when they are acquired from other organisms through plasmids, transposons, and other mobile genetic elements [8, 9]. Among the genomic islands of *A. baumannii*, multiple antibi-

otic resistance regions that have inserted into the target ATPase gene (*comM*) are termed resistance islands (AbaR) [10]. There are several known AbaRs, which differ according to the clone type [11, 12]. The AbaR3-type resistance islands are the major resistance islands of GC1 isolates, and the AbaR4-type islands are associated with GC2 isolates [13-15]. Some AbaR4-type islands of *A. baumannii* harbor transposons such as Tn2009, Tn2006, and Tn6008 carrying the bla_{OXA-23} gene [16].

Because *in vitro* approaches to investigate genomic islands are time-consuming and expensive, several software tools for identifying putative genomic islands have been developed. Some useful software tools and resources for identification of antimicrobial resistance genes have also been reported, such as ResFinder from the Center for Genomic Epidemiology and the Comprehensive Antibiotic Research Database (CARD) [17, 18]. Recently, an island prediction software tool (Genomic Island Prediction software [GIPSy]) was introduced for the prediction of genomic islands in bacteria [9]. Here, we investigated the whole genome sequence (WGS) of a carbapenem-resistant *A. baumannii* isolate belonging to GC2 and predicted the resistance islands using software tools.

METHODS

1. Bacterial strains and antimicrobial susceptibility tests

The *A. baumannii* YU-R612 strain was isolated from the sputum of a 61-yr-old man with sepsis in Severance Hospital, Seoul, Korea. The species was identified by conventional methods and the VITEK 32 GN system (bioMérieux, Marcy l'Etoile, France). Antimicrobial susceptibility testing was performed by using the VITEK II N211 system (bioMérieux). The YU-R612 strain was resistant to multiple antibiotics including piperacillin (minimum inhibitory concentration [MIC] ≥128 μg/mL), ceftazidime (≥64 μg/mL), cefotaxime (≥64 μg/mL), cefepime (≥64 μg/mL), imipenem (≥16 μg/mL), meropenem (≥16 μg/mL), ampicillin/sulbactam (≥32 μg/mL), ciprofloxacin (≥4 μg/mL), and gentamicin (≥16 μg/mL); however, it was susceptible to minocycline (≤1 μg/mL) and colistin (≤0.5 μg/mL). Modified Hodge tests and EDTA plus sodium mercaptoacetic acid (SMA) double-potentiation tests were carried out to screen carbapenemase and metallo-β-lactamase activity, respectively. Both tests showed negative results, which suggested the absence of metallo-β-lactamase genes in this strain.

2. Whole genome sequencing and PCR experiments

The WGS of the YU-R612 strain was obtained by using the PacBio RS II sequencing system (Pacific Biosciences Inc. Menlo Park, CA, USA) in a commercial laboratory (Macrogen, Seoul, Korea). PCR was performed by using in-house primers (Table 1).

Table 1. Nucleotide sequences of primers designed for this study

Primer	Sequence 5′-3′
P1	ACCCCTTCATCATCTTCTGCA
P2	TGAACAATCTGACTCGGGGT
P3	GTCAGTTGCACTTGGTCGAA
P4	GGCGTTCTTTGGAGTGGAAG
P5	GGAATCGTTGCTGTTGGTCA
P6	TGCAAAGTTAGCGATGAGGC
P7	TCCAAGAGCAACGTACGACT
P8	GGCCATTGACCTTCACGTAG

3. Identification of the resistome and resistance islands

Antimicrobial resistance genes were analyzed by using ResFinder, the resources from the Center for Genomic Epidemiology (www.genomicepidemiology.org). A ResFinder threshold of ID=98% was selected. Genomic islands were determined by using resources from Genomic Island Prediction software (GIPSy) as previously described [9].

RESULTS

The *A. baumannii* strain YU-R612 genome consisted of a circular chromosome (ca. 4,075 kb) and two plasmids (ca. 74 kb and 5 kb). WGS analysis showed that the sequence type (ST) under the Oxford scheme was ST191 (1-3-3-2-2-94-3) and under the Institute Pasteur Multi Locus Sequence Typing (MLST) scheme was ST2, which was consistent with the assignment of this strain to GC2. ResFinder analysis showed that the YU-R612 strain possessed the following resistance genes on its chromosome: four β-lactamase genes, namely bla_{ADC-30} (identity, 100%), bla_{OXA-66} (100%), bla_{OXA-23} (100%), and bla_{TEM-1} (100%); *armA* (100%), *aadA1* (100%), and *aacA4* (99.6%) as aminoglycoside resistance-encoding genes; *aac(6')Ib-cr* (99.2%) for fluoroquinolone resistance; *msr(E)* (100%) for macrolide, lincosamide, and streptogramin B resistance; *catB8* (100%) for phenicol resistance; and *sul1* (100%) for sulfonamide resistance.

Six putative resistant islands (PRIs) were determined on the chromosome of the YU-R612 strain by using the GIPSy tool (Fig. 1). PRI1 possessed two copies of Tn2009 carrying the bla_{OXA-23} gene (Fig. 2A). PRI5 carried two copies of a class I integron including 5′ conserved sequence (CS) and 3′CS, and their gene

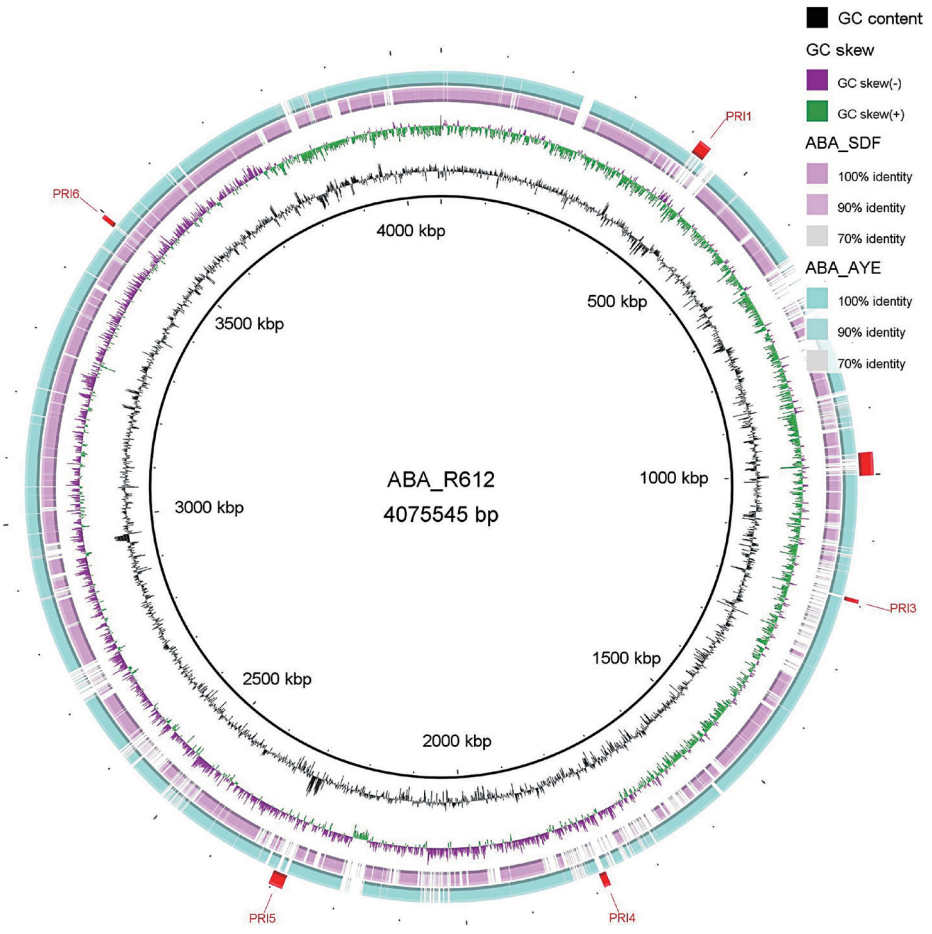

Fig. 1. Circular genome comparison showing putative resistance islands predicted for *Acinetobacter baumannii* strain YU-R612 by GIPSy. The figure was plotted by using *A. baumannii* AYE strain as a reference and generated by using the Basic Local Alignment Search Tool (BLAST) Ring Image Generator (BRIG) software.
Abbreviations: PRI, putative resistance island; GIPSy, Genomic Island Prediction software.

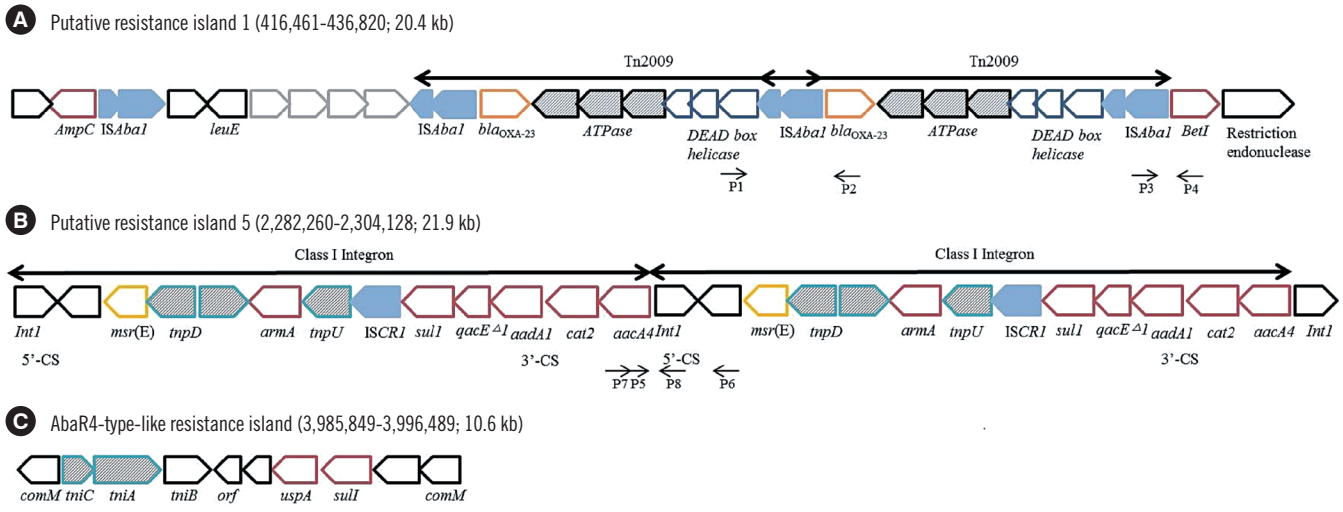

Fig. 2. Structures of putative resistance island 1 (A) and putative resistance island 5 (B) identified by using GIPSy and an AbaR4-like resistance island (C) in the YU-R612 strain. The short arrows indicate the primers (P1 to P8).

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cassettes possessed *sul1* and *armA* resistance genes (Fig. 2B). PRI2, PRI3, PRI4, and PRI6 carried several hypothetical proteins that could not be further annotated. Antimicrobial resistance genes detected by ResFinder were not found on PRI2, PRI3, PRI4, or PRI6.

Interestingly, in addition to the PRIs, a resistance island was identified that was inserted in the *comM* gene on the YU-R612 chromosome. The resistance island was 10.6 kb in size and very similar to AbaR4-type resistance islands, especially Tn6022, which consists of the *tniC-tniA-tniB-tinE-orf-uspA-sul1* genes bracketed by a partial *comM* gene (Fig. 2C). There were two plasmids in the YU-R612 strain, which did not harbor PRIs or antimicrobial resistance genes.

This draft genome sequencing project has been deposited at the GenBank nucleotide database (GenBank nos. CP014215, CP014216, and CP014217) under BioProject ID PRJNA309091.

DISCUSSION

Strain YU-R612 is a multiple antibiotic-resistant isolate recovered from a patient with pneumonia. Resistance to multiple antibiotics correlated with the presence of antimicrobial resistance genes detected by ResFinder: bla_{OXA-23} for resistance to imipenem and meropenem, *armA* for gentamicin, and *aac(6')Ib-cr* for ciprofloxacin, which are major resistance genes for *A. baumannii* in Korea [19, 20].

Under the Oxford scheme, the YU-R612 strain was shown to be ST191, indicating that it is a member of the GC2, which accounts for the majority of isolates recovered in Korea [19]. GC2, which includes clonal complex 92 in the MLST Oxford scheme, has also been identified among carbapenem-resistant *A. baumannii* strains in Asian and European countries [19]. Among the resistance islands of *A. baumannii*, AbaR4-type resistance islands are common in GC2 isolates [12]. Clonal dissemination and diversification of GC92 and the predominance of AbaR4-type resistance islands among these isolates have been reported in Korea [15].

AbaR4-type islands that shared a core structure similar to AbaR4 appeared to be distributed in GC2 isolates via integration into a distinct genomic site at *comM*. The backbone of AbaR4 comprised five open reading frames constituting the so-called transposition module with two other genes encoding the universal stress protein (*uspA*) and sulfate permease (*sul1*) [21]. In this study, a 10.6-kb AbaR4-type resistance island was detected in the YU-R612 strain. While some AbaR4-type resistance islands possess transposons such as Tn2006 and Tn1213 carrying antibiotic resistance genes such as bla_{OXA-23}, the YU-Y612 strain did not carry any transposons or antibiotic resistance genes on its AbaR4-type-like resistance island. However, the bla_{OXA-23} gene on the Tn2009 of YU-R612 strain was detected in PRI1.

In this study, six PRIs were identified on the chromosome of the YU-R612 strain by using the GIPSy tool. Among them, two PRIs (PRI1 and PRI5) possessed transposons and class I integrons that carried important resistance genes: bla_{OXA-23}, *armA*, and *sul1*. The duplication of Tn2009 in PRI1 and class I integrons in PRI5 was confirmed by using PCR to supplement a limitation of deep sequencing for the replication.

The GIPSy software facilitated the prediction of resistance islands and resistance genes in the WGS of this strain. Although some PRIs detected by software tools are different from known resistance islands, this software may be useful to identify novel genomic islands carrying clusters of resistance genes. Software tools provide a convenient way of identifying PRIs as well as acquired antimicrobial resistance genes, and they may simplify the complex steps in analyzing the WGS of bacteria in clinical microbiology laboratories.

In conclusion, the prediction of resistance islands in the WGS of a carbapenem-resistant *A. baumannii* strain belonging to GC2 in Korea revealed PRIs on the chromosome that carried Tn2009 and class I integrons. The predicted resistance islands using software tools helped in the analysis of the WGS in this strain.

Authors' Disclosures of Potential Conflicts of Interest

No potential conflicts of interest relevant to this article were reported.

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