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A comparative study of class 1 integrons in *Acinetobacter baumannii*

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ABSTRACT

Multidrug resistance (MDR) in *Acinetobacter baumannii* is increasingly reported and has become a significant public concern. The method responsible for the acquisition of resistance genes via integrons from the environment or intra-species in *A. baumannii* remains to be understood. This study was performed to investigate the transmission route of these integrons using a comparative analysis of published *A. baumannii* complete genomes. The phylogenetic analysis of *A. baumannii* type 1 integrases (IntI1) showed that the integrons could be transferred across the two evolutionary lineages, the international clone I (IC I) and clone II (IC II) strains. In addition, the integrons in *A. baumannii* strains were mainly responsible for the transfer of resistance genes for two types of long-term usage antibiotics and antiseptics, such as aminoglycosides, chloramphenicol and the quaternary-ammonium-compound family. The in silico comparative analysis of known integron integrases revealed that the *intI* genes were phylogenetically related among *A. baumannii* strains and some microorganisms living in a sediment community, implicating that the integrons of *A. baumannii* might have originated from those microorganisms belonging to the β-preoteobacterial class in the sediment environment. The data suggest that the gain of class 1 integrons in *A. baumannii* strains may have started before the antibiotic era. This report shows that the origins of *A. baumannii* class 1 integrons may be the soil environment and that the resistance genes included in integrons are horizontally transferred across all the *A. baumannii* genomes, including IC I and IC II.

1. Introduction

Acinetobacter baumannii is recognized as an important cause of nosocomial infections worldwide. *Acinetobacter* infections may pose chemotherapeutic difficulties, as nosocomial isolates are typically resistant to a wide variety of antimicrobial agents, such as aminoglycosides, fluoroquinolones, cephalosporins and carbapenems (Kempf and Rolain, 2012; Perez et al., 2007). The horizontal gene transfer (HGT) of antibiotic resistance genes within a shared gene pool may be one of the most important means by which the spread of antimicrobial resistance in the hospital environment is enhanced (Valenzuela et al., 2007).

The most prevalent multidrug resistant (MDR) determinants in *A. baumannii* include genes for efflux pumps (Vila et al., 2007), class B β-lactamase (metallo-beta-lactamase) (Castanheira et al., 2004), class C chromosomal β-lactamase Amp C (Bou and Martinez-Beltran, 2000), class D β-lactamase (OXA-type carbapenemase) (Afzal-Shah et al., 2001), integrons (Lee et al., 2005), and associated insertion sequence (IS) elements. Recently, several studies have revealed that the chromosome of some *A. baumannii* strains harbors large clusters of horizontally transferred genes conferring resistance to multiple antibiotics and heavy metals, termed antibiotic resistance islands (AbaRs) (Post et al., 2010). Evolutionary studies have shown that these AbaRs in the *A. baumannii* strains of the same lineage shared the same origin (Krizova et al., 2011). Although AbaRs play important roles in the resistance of *A. baumannii* strains, the diversity and origin of the resistance genes in addition to the AbaRs remain to be understood.

Integrons are mobilizable platforms — DNA elements that capture and express genes found in their environment as part of small mobile elements, termed gene cassettes (Mazel, 2006; Stokes and Hall, 1989). Approximately 17% of organisms carry an integron integrase (207 out of the 1189 complete bacterial genomes currently available at NCBI) (Cambray et al., 2010). Most of the integrons are contained in transposons and plasmids outside AbaRs and have been found to include small arrays of up to six cassettes, which mainly encode antibiotic-resistant

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Abbreviations: DNA, deoxyribonucleic acid; MDR, multidrug resistance; IntI, integrase; HGT, horizontal gene transfer; IS, insertion sequence; AbaRs, antibiotic resistance islands.

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determinants (Mazel, 2006). Of the five classes of integrons, class 1 integrons are the most prevalent class found among clinical isolates, followed by class 2. However, class 3 is rarely detected (Partridge et al., 2009). And hundreds of accessory genes that confer resistance are known. These integrons share their core structures with an integrase gene (*intI*) and a recombination site (*attI*) (Hall and Collis, 1995). Despite the divergent gene cassettes found in integrons, a phylogeny of integron integrases has been described, demonstrating the evolutionary history that enabled these elements to succeed in a wide variety of hosts and environments (Mazel, 2006).

The dissemination and characterization of integrons among *A. baumannii* have been described in previous studies (Lin et al., 2010; Yamamoto et al., 2011). Particularly, integrons are useful markers for epidemic strains of *A. baumannii*, and integrase typing provides valuable information for epidemiological and evolutionary studies (Lin et al., 2010). The aim of the study was to investigate the structure of integrons among 11 complete *A. baumannii* genomes. A comparative study of integrase sequences will provide evolutionary insights concerning the acquisition of resistance genes from the environment or intra-species in *A. baumannii*.

2. Results

2.1. Comparative genomics

A phylogenetic tree was constructed based on the CVTree approach using 11 finished *A. baumannii* genomes and three reference strains, *Escherichia coli* K-12 substr. MG1655, *Pseudomonas aeruginosa* PAO1, and *Acinetobacter baylyi* ADP1, and the result is shown in Fig. 1. According to the phylogenetic tree, nine MDR *A. baumannii* strains were grouped into two clusters, one belonging to IC I, including AYE, AB0057, and AB307-0294, and the other belonging to IC II, including ACICU, 1656-2, TCDC-AB0715, MDR-TJ, MDR-ZJ06, and TYTH-1.

2.2. Phylogenetic analysis of integron integrases in A. baumannii

The integrons, together with the gene cassettes they display, are listed in Table 1. All integrases and integrons detected belong to class 1. Fig. 2 shows the results of a phylogenetic analysis (A) and alignment (B) of the integrases in eight *A. baumannii* strains. Excluding AB307-0294, ATCC17978, and SDF, five strains (TYTH-1, MDR-ZJ06, AB0057, 1656-2, and ACICU) included one integron, and three strains (AYE, MDR-TJ, and TCDC-AB0715) included two integrons. Eleven integrases were grouped into two clusters, one belonging to the IC II strains, including MDR-TJ, TYTH-1, and 1656-2, and another belonging to both the IC I and IC II strains, including AYE, AB0057, TCDC-AB0715, MDR-ZJ06, and ACICU. The alignment of the 11 type 1 integrases showed that the amino acids of the integrases were variable from residue 267 to the C-terminal, grouping into two clusters. In addition, the integrase of $\text{In}_{\text{AB-2-MDR-TJ}}$ was truncated from amino acid residue 253 to the C-terminal. Compared with the result shown in Figs. 1 and 2, the integrases in the eight *A. baumannii* strains were evolutionarily distinct from IC I and IC II.

2.3. Integron cassette arrays in A. baumannii strains

Six types of integron cassette arrays among eight *A. baumannii* strains were analyzed, and the results are shown in Fig. 3. Except for $\text{In}_{\text{AB-6}}$ that was included in the AYE strains, the cassette arrays in the other five integrons were composed of two to five gene cassettes. All the integrons contained resistance genes for aminoglycosides (*aadA33*, *aacC1*, *aadA1*, and *aadB*), chloramphenicol (*catB8*) or the quaternary-ammonium-compound family (*qacEdelta1*). In addition, resistance genes for the β-lactam antibiotic (*verb-1*, *oxa-10*), trimethoprim (*dfrA1*), rifampin (*arr2*) and efflux pumps (*cmlA2*) were observed in $\text{In}_{\text{AB-6}}$. The gene cassette array *aacA33-catB8-aadA1-qacEdelta1* (previously termed *aacA4-catB8-aadA1-qacE*) was widely distributed in the IC II strains, including MDR-TJ, MDR-ZJ06, TYTH-1, and TCDC-AB0715. Notably, only one integron cassette array *aacC1-ORF-ORF-aadA1-qacEdelta1* was distributed in both the IC I (AYE) and IC II (MDR-TJ, TCDC-AB0715) strains.

2.4. Comparative analysis of known integron integrases (IntI)

A comparative analysis of integrases in *A. baumannii* with four types of integrases was performed, and the result is shown in Fig. 4. Four

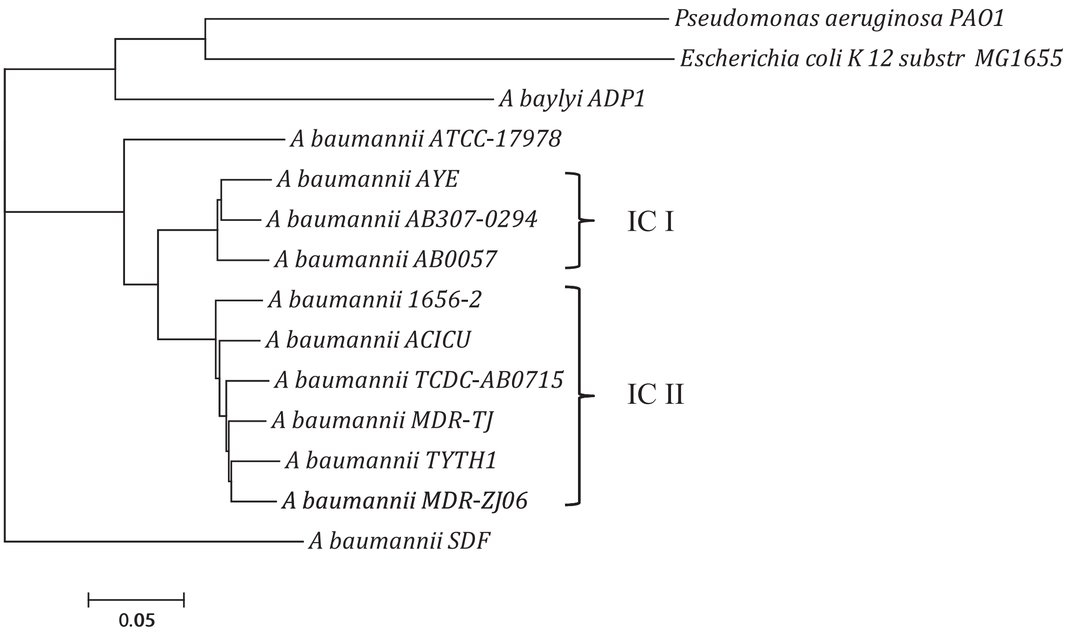

Fig. 1. Phylogenetic analysis of all complete *A. baumannii* genomes. The phylogenetic tree of 11 complete *A. baumannii* genome sequences was constructed using CVTree, and the neighbor-joining tree was constructed using the MEGA5 program. The genomes of *E. coli* K12 substr MG1655, *P. aeruginosa* PAO1 and *Acinetobacter baylyi* ADP1 were used as reference strains to the tree. IC, international clone.