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Insertion Sequence IS*Aba11* Is Involved in Colistin Resistance and Loss of Lipopolysaccharide in *Acinetobacter baumannii*[▽]

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Infections caused by *Acinetobacter baumannii* are of increasing concern, largely due to the multidrug resistance of many strains. Here we show that insertion sequence IS*Aba11* movement can result in inactivation of the *A. baumannii* lipid A biosynthesis genes *lpxA* and *lpxC*, resulting in the complete loss of lipopolysaccharide production and high-level colistin resistance.

The Gram-negative pathogen *Acinetobacter baumannii* is a leading cause of hospital-acquired infections, including septicemia, pneumonia, and urinary tract infections. The treatment of infections caused by *A. baumannii* is significantly hampered by an increase in multidrug resistance (MDR), including resistance to last-line antibiotics such as colistin (9). Recently, colistin resistance in the *A. baumannii* type strain ATCC 19606 was shown to result from the loss of lipopolysaccharide (LPS), the initial binding target of colistin and the major component of the outer leaflet of the Gram-negative outer membrane (7).

We have previously shown that we can select for colistin-resistant mutants of *A. baumannii* strain ATCC 19606 by growth on Mueller-Hinton agar containing 10 μg/ml colistin sulfate (7). Our initial study reported the characterization of a group of 13 colistin-resistant mutants which each contained point mutations or deletions in one of the first three genes in the lipid A biosynthesis pathway, *lpxA*, *lpxC*, or *lpxD*, resulting in the loss of LPS production and high-level colistin resistance (MIC > 128 μg/ml) (7). In the present study, we report the characterization of a second group of eight colistin-resistant mutants of *A. baumannii* strain ATCC 19606. Carbohydrate-specific silver staining (7) of the proteinase K-treated whole-cell lysates of these strains indicated that they also produced no LPS (Fig. 1), which was confirmed by *Limulus* amebocyte lysate assay (7) (data not shown). However, sequencing analysis of the *lpxA*, *lpxC*, and *lpxD* genes in these strains showed that they contained no point mutations or deletions but rather contained an insertion sequence (IS) element, IS*Aba11* (GenBank accession number JF309050), in either *lpxA* or *lpxC*.

In seven of the strains, the IS*Aba11* element had inserted into *lpxC*, while in the remaining strain, it had inserted into *lpxA* (Table 1). In six of the colistin-resistant strains, IS*Aba11* was bracketed by 5-bp direct repeats, indicating target site duplication. However, in strain AL1838, it was bracketed by 34-bp direct repeats, and in strain AL1837, no direct repeats were observed, but rather, IS*Aba11* was associated with a 27-bp deletion within *lpxC* (Table 1). In four of the colistin-resistant strains, IS*Aba11* had inserted between nucleotides 390 and 393 of the *lpxC* gene, while in three of the strains, it inserted at either nucleotide 420 or 421 of the *lpxC* gene, indicating that these regions may represent hot spots for IS*Aba11* insertion.

IS*Aba11* has previously been identified only as part of the transposon Tn*6021* (http://www-is.biotoul.fr/index.html?is_special_name=ISAba11) from *A. baumannii* ATCC 17978 (10). The 1.1-kb element IS*Aba11* is flanked by perfect 13-bp inverted repeats and is predicted to encode a transposase with a conserved DDE motif, considered essential for transposition (8). IS*Aba11* shows the greatest similarity to insertion sequence elements from the IS*701* and IS*4* families; these elements have inverted repeats of 14 to 24 bp and 13 to

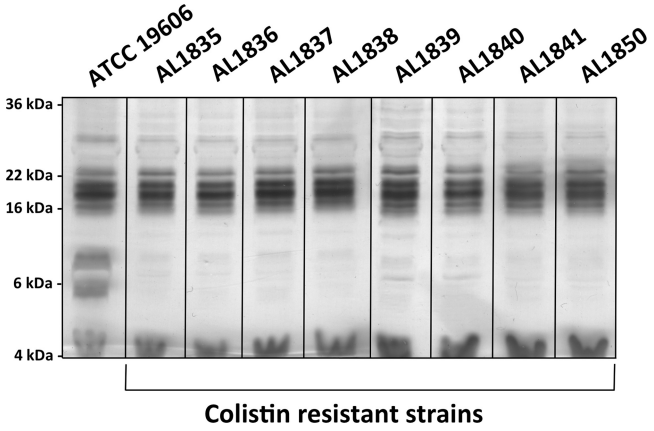

FIG. 1. Colistin-resistant derivatives of ATCC 19606 do not produce LPS. SDS-PAGE separation and carbohydrate-specific silver staining of proteinase K-treated whole-cell lysates of the colistin-sensitive strain ATCC 19606 and eight colistin-resistant derivatives. The positions of standard molecular mass markers are shown on the left.

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TABLE 1. Positions of IS*Aba11* insertions and target site duplications in the colistin-resistant, LPS-deficient derivatives of ATCC 19606

Strain	Gene	Insertion (nucleotide)	Target site duplication
AL1835	*lpxC*	393	AAATA
AL1836	*lpxC*	393	AAATA
AL1837	*lpxC*	391	No duplication[a]
AL1838	*lpxC*	420	AAAAATATTAAAGCCAGTTG AGGCTTTAATTGAT
AL1839	*lpxC*	421	TGATG
AL1840	*lpxC*	420	TTGAT
AL1841	*lpxC*	390	TAAAA
AL1850	*lpxA*	588	GTATA

[a] Deletion (27 bp) within *lpxC* following insertion of IS*Aba11*.

26 bp, respectively, which contain conserved nucleotides and target site duplications of 4 bp (IS*701*) and 10 to 13 bp (IS*4*) (2). The absence of conserved nucleotides in the inverted repeats and the different target site duplication sizes observed here for IS*Aba11* do not fit the defining characteristics of either family (2), and given these key differences, we propose that IS*Aba11* represents an emerging insertion sequence (IS) family.

The number of IS*Aba11* copies present in the ATCC 19606 genome, and the ability of the IS element to replicate and mobilize in this strain, was assessed by Southern hybridization. We compared the number of copies and the position of the element in the parent strain ATCC 19606 with the numbers of copies and the positions of the elements in the genomes of the colistin-resistant, LPS-deficient derivatives. Genomic DNA from the parent strain ATCC 19606 and the colistin-resistant, LPS-deficient derivative strains was digested with DraI (IS*Aba11* does not contain any DraI sites) and separated by gel electrophoresis, and the resulting DNA fragments were transferred to a nylon membrane. The membrane was hybridized under high-stringency conditions using a probe specific for the IS*Aba11* transposase gene (Fig. 2). The 760-bp probe was amplified from ATCC 19606 genomic DNA using the primers BAP6531 (GAAGACTACACACC GCACGA) and BAP6532 (TCCGCTCAAACTGGTTCT TT), with digoxigenin (DIG)-11-dUTP (Roche) incorporation. At least three DNA fragments of approximately 2.1, 1.5, and 1.3 kb in size hybridized with the IS*Aba11* probe in the ATCC 19606 genomic DNA, indicating that this strain contains multiple copies of IS*Aba11*. However, in the lanes containing genomic DNA isolated from the colistin-resistant, LPS-deficient derivatives of ATCC 19606, up to two additional bands hybridized with the probe, indicating that the insertion element had mobilized and that, in most instances, mobilization involved replicative movement of the element (Fig. 2). For the seven mutants in which IS*Aba11* was identified by DNA sequencing as present in *lpxC* (AL1835 to AL1841) (Table 1), one hybridizing fragment of 2.9 kb (Fig. 2) was consistently observed across all strains. This fragment corresponded to the expected size of the *lpxC* DraI fragment containing IS*Aba11*. For the mutant in which IS*Aba11* was inserted into *lpxA* (AL1850) (Table 1), a unique hybridizing fragment that corresponded to the predicted size of the *lpxA* DraI fragment containing IS*Aba11* was observed (1.9 kb) (Fig. 2). In summary, these data indicate that IS*Aba11* in ATCC 19606 is mobile and replicative (Fig. 2).

Further bioinformatic analyses indicated that IS*Aba11* is present in the *A. baumannii* ATCC 17978 genome (11) and in the unclosed genome sequences of *A. haemolyticus*, *A. lwoffii*, and *A. johnsonii*. However, there is no evidence that the element is present in any of the recently sequenced clinical *A. baumannii* isolates, suggesting that IS*Aba11* is not present in the global European clone I and clone II lineages. However, we have recently shown the involvement of a separate novel IS element in the disruption of the lipid A biosynthesis gene *lpxD* of a colistin-resistant clinical *A. baumannii* isolate, B0707-070 (7), thus supporting our hypothesis that IS elements may play a significant role in the mechanism of colistin resistance in *A. baumannii*. Indeed, 15 different IS elements have been described in *A. baumannii* to date, and the mobilization of many of these elements is associated with an increase in antibiotic resistance (1, 3–6). This study highlights the significant role

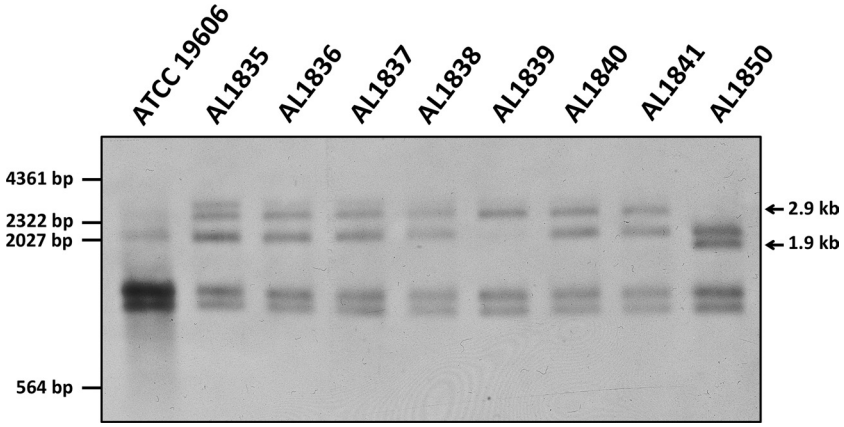

FIG. 2. The insertion sequence IS*Aba11* is mobile and replicative in ATCC 19606. Southern hybridization using a probe specific for the IS*Aba11* transposase gene against DraI-digested genomic DNA isolated from ATCC 19606, AL1835 to AL1841 (*lpxC*::IS*Aba11* mutants), and AL1850 (*lpxA*::IS*Aba11* mutant). The positions of DNA size markers are shown on the left. Arrows shown on the right indicate the positions and sizes of the novel hybridizing fragments present in the LPS-deficient, colistin-resistant strains.

that IS element movement and insertion play in the genome plasticity and increasing antibiotic resistance profile of *A. baumannii* and shows that IS*Aba11* movement can directly result in colistin resistance in ATCC 19606.

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