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LETTER TO THE EDITOR

Mobilization of bla_{BKC-1} by IS*Kpn23*?

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The BKC-1 (Brazilian *Klebsiella* carbapenemase-1) class A β-lactamase, a weak carbapenemase, was recently reported in *Klebsiella pneumoniae* ST1781 isolates from São Paulo, Brazil (1). bla_{BKC-1} was found between the novel insertion sequence IS*Kpn23* (IS*1380* family) and an *aphA6* [*aph(3)-VI*] gene on an IncQ plasmid, p60136 (GenBank accession no. KP689347) (1). As in some other IncQ plasmids (2, 3), the resistance segment is located between two *oriV* regions (Fig. 1A). Nicoletti et al. (1) proposed transposition of a genetic element carrying IS*Kpn23*, bla_{BKC-1}, and *aphA6*.

In addition to being similar to IS*Apr9* (1), IS*Kpn23*, as defined in ISfinder (4; https://www-is.biotoul.fr/) (positions 1450 to 1 and 9786 to 9566 in GenBank accession no. KP689347), is ~89% identical to IS*1247*. Like the better-known IS*1380* family element IS*Ecp1* (5, 6), IS*1247* is able to mobilize sequences adjacent to its right inverted repeat (IR_R) by using alternative downstream sequences in conjunction with its left inverted repeat (IR_L) (5, 7, 8). The inserted transposition unit (TU; or transposable module [TMo]) is flanked by 4- or 5-bp direct repeats (DRs), and IS*1247* carries an outward-facing promoter (8).

A BLASTn search with the p60136 sequence downstream of IS*Kpn23* revealed two sequences with a single nucleotide difference: an *Acinetobacter guillouiae* chromosome (GenBank accession no. AP014630) (9), known to be the source of *aphA6* genes (10), and a short sequence from *K. pneumoniae* (KU922932), where the *aphA6* variant [12 nucleotide differences from *aph(3′)-VIa* (11)] was named *aph(3′)-VIi*. The match starts at TTACT 104 to 108 bp beyond the stop codon of bla_{BKC-1}, and a potential DR of TTACT is also present immediately adjacent to the proposed IR_L of IS*Kpn23* (Fig. 1A and B). The match to *A. guillouiae* and the KU922932 sequence continues upstream of IS*Kpn23*, suggesting insertion of a 2.737-kb IS*Kpn23*-bla_{BKC-1} TU. The *aph(3′)-VIi* coding sequence remains intact, and it is possible that both *aph(3)-VIi* and bla_{BKC-1} are expressed from a promoter in IS*Kpn23*, as the original *K. pneumoniae* isolate KP60136 and an *Escherichia coli* transformant were resistant to amikacin and kanamycin in addition to β-lactams (1).

Searches of the NCBI whole-genome shotgun (WGS) contig database with IS*Kpn23* identified several examples of almost identical elements in different species, including one with 5-bp DRs, and several potential IS*Kpn23*-mediated TUs flanked by DRs, in some cases with an uninterrupted version of the flanking sequence available, e.g., carrying different *aac(3)* genes (Fig. 1C). This IS*Kpn23* has five nucleotide differences from IS*Kpn23* in p61036 in the right-hand end, giving an IR_R more similar to that of IS*1247* (Fig. 1D).

The mechanism by which *aph(3′)-VIi* was acquired by an IncQ plasmid is currently unclear. The KU922932 sequence matches p60136 before the start of the match to *A. guillouiae* (Fig. 1E), but the available sequence ends before an identifiable IncQ (or other plasmid) backbone sequence. Resistance genes in IncQ plasmids are often associated with only, at most, remnants of mobile elements (2, 3), and acquisition of some genes (12) and/or subsequent fragmentation of mobile elements (13) may be due to recombination. Identification of additional sequences containing *aph(3′)-VIi* with context information may reveal more about the mobilization of this gene. bla_{BKC-1} has been reported in only six *K. pneumoniae* isolates from São Paulo, Brazil, to date (1, 14), and it will be interesting to see how successful this gene becomes, in comparison with *bla* genes associated with IS*Ecp1*.

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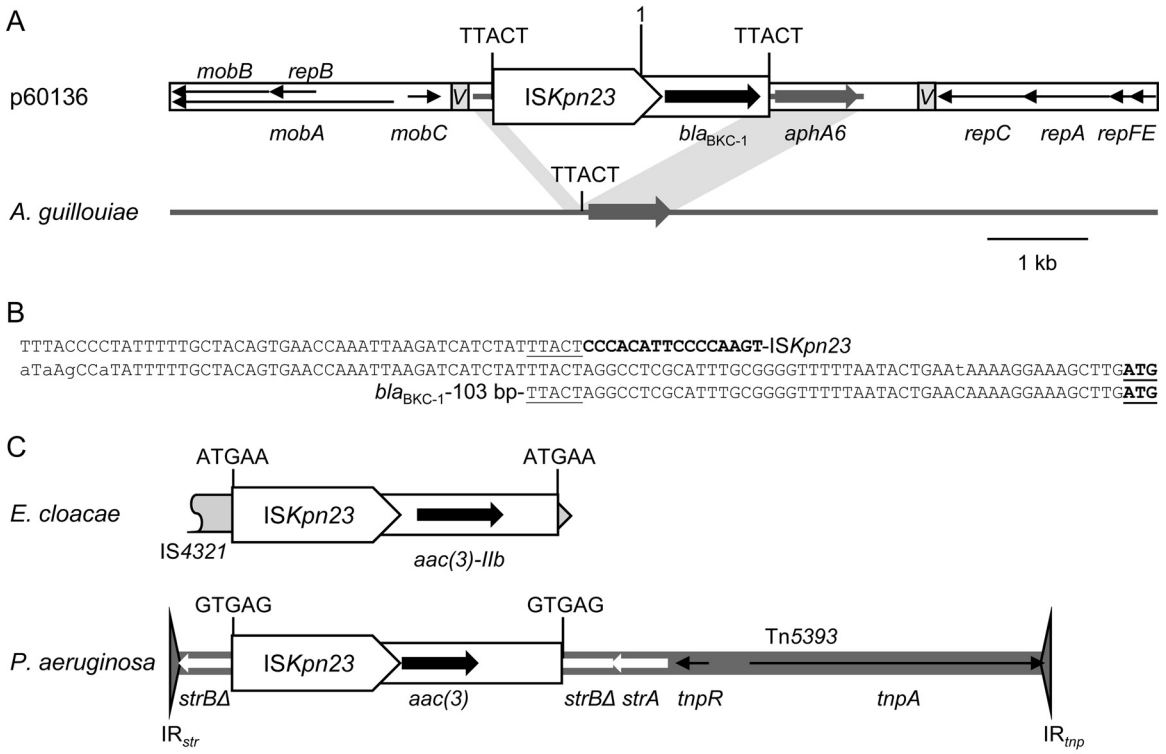

FIG 1 (A) Diagram of the complete sequence of p60136 (GenBank accession no. KP689347) showing the proposed IS*Kpn23-bla*$_{BKC-1}$ TU, compared with *aphA6* in the *A. guillouiae* chromosome (AP014630). "1" indicates position 1 in the p60136 sequence in KP689347, which was reversed to draw the diagram. IS*Kpn23* is shown as a box, with the pointed end indicating IR$_R$. Different types of labeled arrows show the extents and directions of resistance and plasmid backbone genes. *oriV* regions are indicated by shaded boxes labeled "*V*." The regions common to both sequences are indicated by shading, and the positions of the 5-bp DRs (TTACT) flanking the proposed TU in p60136 and the single copy of this sequence in *A. guillouiae* are shown. (B) Sequences around the insertion site in *A. guillouiae* (middle line) and flanking the proposed TU in p60136 (top and bottom lines). Differences are shown in lowercase letters in the *A. guillouiae* sequence. The proposed IR$_L$ of IS*Kpn23* is shown in bold typeface, the *aphA6* start codon is bold and underlined, and the proposed DRs are underlined. (C) Potential IS*Kpn23*-mediated TU in *Enterobacter cloacae* (JCKL01000012), carrying *aac(3)-IIb* inserted in a partial insertion sequence, IS*4321* (gray), and in *Pseudomonas aeruginosa* (JTQL01000147) (15), carrying a putative *aac(3)* gene inserted in Tn*5393*. (D) Comparison of the ends of IS*Kpn23* in p60136, IS*Kpn23* in the NCBI WGS database (differences are underlined in each sequence), and IS*1247* (as proposed in reference 8), with IR$_L$ and IR$_R$ in uppercase letters (16 bp, as listed in ISfinder). (E) Comparison of sequences upstream of the point of insertion of the proposed TU in p60136.

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