JCM Journals.ASM.org

Tn*4401* Carrying bla_{KPC} Is Inserted within Another Insertion in pKpQIL and Related Plasmids

Sally R. Partridge

Centre for Infectious Diseases and Microbiology, Westmead Millennium Institute and Westmead Hospital, Westmead, New South Wales, Australia

The bla_{KPC-2} gene, encoding the KPC-2 carbapenemase, was first identified in the United States in 1996, and bla_{KPC} variants now appear to be endemic in several countries, including the United States, Israel, Greece, and China, with sporadic reports in other locations (1). bla_{KPC} genes have generally been found flanked by the insertion sequences IS*Kpn7* and IS*Kpn6* within the Tn*3*-family transposon Tn*4401* (2) or truncated versions of this structure. Tn*4401* has been detected in plasmids from several incompatibility groups, including $IncFII_K$ plasmids related to pKpQIL (3), IncN, ColE (4), IncI2 (5), and IncX3 (6) plasmids.

A recent paper linked an ~11,109-Da peak detected by matrix-assisted laser desorption ionization–time of flight mass spectrometry (MALDI-TOF MS) with the presence of bla_{KPC} and used it to track pKpQIL-like plasmids (7). The peak corresponds to the cleavage product of a hypothetical protein designated p019 in the pKpQIL sequence (GenBank accession no. NC_014016.1). The authors noted that although p019 "appears to be fairly closely linked to the bla_{KPC} gene," "genetic events in the plasmid may change the association of the pKpQIL_019 MALDI-TOF MS peak with the presence of functional carbapenemase" (7).

Detailed analysis of the pKpQIL sequence suggests that the p019 gene is part of an insertion into which Tn*4401* has then been inserted. This insertion is flanked by 10-bp direct repeats (DR) and is 2,328 bp in length (excluding one 5-bp DR flanking Tn*4401*) (Fig. 1A). As noted previously (8), part of this region is related to the insertion sequence IS*As12* (~91% nucleotide identity), which creates 10 bp (ISfinder, https://www-is.biotoul.fr/) (9). The ends of this putative IS, designated IS*Kpn31* by ISfinder, resemble the terminal inverted repeats (IR) of IS*As12*. However, the adjacent region containing the p019 gene and other IS fragments, which has no equivalent in IS*As12*, also ends in a similar sequence (Fig. 1C). It is possible that this region had previously become incorporated as an internal part of an IS*Kpn31*-derived mobile element or that it was mobilized in a way similar to that of IS*Ecp1*-mediated capture of regions adjacent to the right IR (IR_R) (10), except that it lies adjacent to the left end of IS*Kpn31*.

Editor: G. V. Doern

Address correspondence to sally.partridge@health.nsw.gov.au.

For the author reply, see doi:10.1128/JCM.02614-14.

doi:10.1128/JCM.02426-14

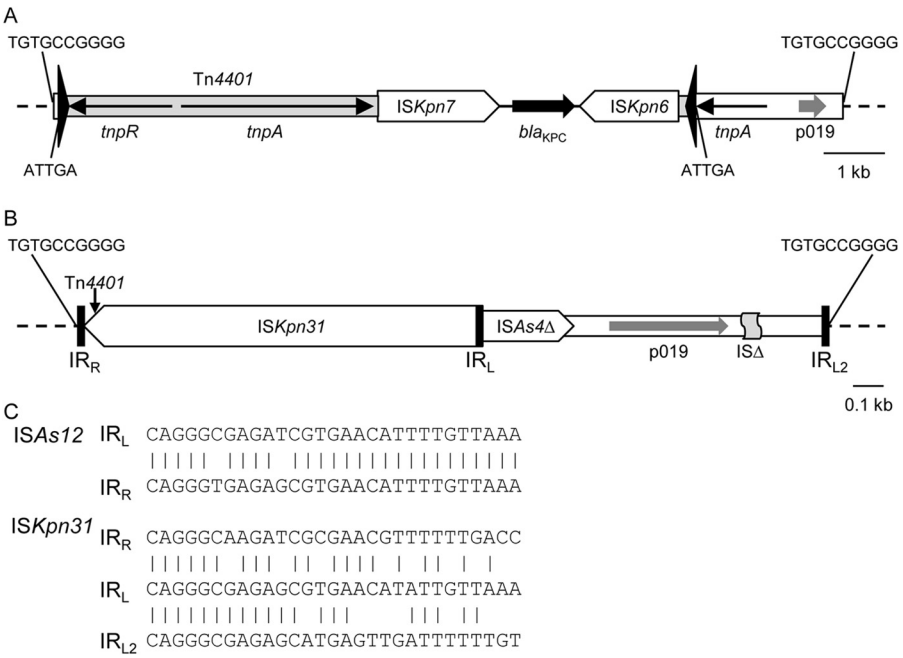

FIG 1 Insertion in pKpQIL-like plasmids. (A) Complete insertion (positions 8823 to 21062 in GenBank accession no. NC_014016.1). (B) Expanded diagram showing details of the inserted region with Tn*4401* and one 5-bp DR removed (positions 8823 to 8881 and 18794 to 21062). Inverted repeats (IR) of Tn*4401* are shown as tall black triangles, and IR of IS*Kpn31* are indicated by black bars. IS are shown by boxes, with the pointed end indicating IR_R. Labeled arrows indicate the directions and extents of selected genes. The sequences of DR flanking insertions are shown. ISΔ is a fragment of an IS*21*-like IS. (C) Comparison of IR sequences. (IS*As12* IR are from the 16 copies in the *Aeromonas media* chromosome in GenBank accession no. CP007567).

Although BLAST searches indicate that the insertion containing p019 has been found only in plasmids carrying bla_{KPC} to date, these two genes are part of different mobile elements. Independent movement is already evident for Tn*4401*, which has been found in different plasmid backbones flanked by 5-bp DR, indicative of direct insertion, e.g., in p15S (GenBank accession no. FJ223606) (4) and pCOL-1 (KC609323) (11). The presence of p019 and bla_{KPC} on $IncFII_K$ plasmids less closely related to pKpQIL (e.g., pKPN-101-IT) (12) or those that belong to other Inc groups, e.g., IncI2 (pBK15692) (5) or IncX3 (pKPC-NY79) (6), also means that detecting p019 does not always indicate closely related or even similar plasmids. Thus, caution needs to be used in correlating the presence of p019 with the presence of bla_{KPC} or a particular plasmid, and understanding the genetic contexts of markers apparently linked to resistance genes is important.

REFERENCES

1. **Nordmann P, Naas T, Poirel L.** 2011. Global spread of carbapenemase-producing *Enterobacteriaceae*. Emerg. Infect. Dis. **17:**1791–1798. http://dx.doi.org/10.3201/eid1710.110655.
2. **Naas T, Cuzon G, Villegas MV, Lartigue MF, Quinn JP, Nordmann P.** 2008. Genetic structures at the origin of acquisition of the β-lactamase bla_{KPC} gene. Antimicrob. Agents Chemother. **52:**1257–1263. http://dx.doi.org/10.1128/AAC.01451-07.
3. **Leavitt A, Chmelnitsky I, Carmeli Y, Navon-Venezia S.** 2010. Complete nucleotide sequence of KPC-3-encoding plasmid pKpQIL in the epidemic *Klebsiella pneumoniae* sequence type 258. Antimicrob. Agents Chemother. **54:**4493–4496. http://dx.doi.org/10.1128/AAC.00175-10.
4. **Gootz TD, Lescoe MK, Dib-Hajj F, Dougherty BA, He W, Della-Latta P, Huard RC.** 2009. Genetic organization of transposase regions surrounding bla_{KPC} carbapenemase genes on plasmids from *Klebsiella* strains isolated in a New York City hospital. Antimicrob. Agents Chemother. **53:**1998–2004. http://dx.doi.org/10.1128/AAC.01355-08.
5. **Chen L, Chavda KD, Al Laham N, Melano RG, Jacobs MR, Bonomo RA, Kreiswirth BN.** 2013. Complete nucleotide sequence of a bla_{KPC}-harboring IncI2 plasmid and its dissemination in New Jersey and New York hospitals: a hidden threat. Antimicrob. Agents Chemother. **57:**5019–5025. http://dx.doi.org/10.1128/AAC.01397-13.
6. **Ho PL, Cheung YY, Lo WU, Li Z, Chow KH, Lin CH, Chan JF, Cheng VC.** 2013. Molecular characterization of an atypical IncX3 plasmid pKPC-NY79 carrying bla_{KPC-2} in a *Klebsiella pneumoniae*. Curr. Microbiol. **67:**493–498. http://dx.doi.org/10.1007/s00284-013-0398-2.
7. **Lau AF, Wang H, Weingarten RA, Drake SK, Suffredini AF, Garfield MK, Chen Y, Gucek M, Youn JH, Stock F, Tso H, DeLeo J, Cimino JJ, Frank KM, Dekker JP.** 2014. A rapid matrix-assisted laser desorption ionization-time of flight mass spectrometry-based method for single-plasmid tracking in an outbreak of carbapenem-resistant *Enterobacteriaceae*. J. Clin. Microbiol. **52:**2804–2812. http://dx.doi.org/10.1128/JCM.00694-14.
8. **Moura A, Pereira C, Henriques I, Correia A.** 2012. Novel gene cassettes and integrons in antibiotic-resistant bacteria isolated from urban wastewaters. Res. Microbiol. **163:**92–100. http://dx.doi.org/10.1016/j.resmic.2011.10.010.
9. **Siguier P, Perochon J, Lestrade L, Mahillon J, Chandler M.** 2006. ISfinder: the reference centre for bacterial insertion sequences. Nucleic Acids Res. **34:**D32–D36. http://dx.doi.org/10.1093/nar/gkj014.
10. **Poirel L, Lartigue MF, Decousser JW, Nordmann P.** 2005. IS*Ecp1B*-mediated transposition of bla_{CTX-M} in *Escherichia coli*. Antimicrob. Agents Chemother. **49:**447–450. http://dx.doi.org/10.1128/AAC.49.1.447-450.2005.
11. **Naas T, Bonnin RA, Cuzon G, Villegas MV, Nordmann P.** 2013. Complete sequence of two KPC-harbouring plasmids from *Pseudomonas aeruginosa*. J. Antimicrob. Chemother. **68:**1757–1762. http://dx.doi.org/10.1093/jac/dkt094.
12. **Frasson I, Lavezzo E, Franchin E, Toppo S, Barzon L, Cavallaro A, Richter SN, Palu G.** 2012. Antimicrobial treatment and containment measures for an extremely drug-resistant *Klebsiella pneumoniae* ST101 isolate carrying pKPN101-IT, a novel fully sequenced bla_{KPC-2} plasmid. J. Clin. Microbiol. **50:**3768–3772. http://dx.doi.org/10.1128/JCM.01892-12.