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KPC-2-encoding plasmids from *Escherichia coli* and *Klebsiella pneumoniae* in Taiwan

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Objectives: Two plasmids carrying bla_{KPC-2} isolated from carbapenem-resistant *Escherichia coli* (CR-EC) and carbapenem-resistant *Klebsiella pneumoniae* (CR-KP), respectively, were completely sequenced. The CR-KP strain was selected from an outbreak in 2012, and the CR-EC strain was the first bla_{KPC-2}-carrying *E. coli* identified in the same carbapenem resistance monitoring programme in Taiwan.

Methods: Antimicrobial susceptibility tests, multilocus sequence typing (MLST) and the conjugal transfer of plasmids were performed. Complete sequencing of the plasmids was performed using a shotgun approach.

Results: The CR-EC and CR-KP strains in this study were determined to be ST410 and ST11, respectively, by MLST. From CR-EC, we identified a 145 kb conjugative plasmid that carries bla_{KPC-2}, bla_{CMY-2}, $bla_{CTX-M-3}$ and bla_{TEM-1}. The plasmid is a chimera composed of three regions related to IncI, IncN and RepFIC replicons. From CR-KP, we identified an 86.5 kb plasmid, pKPC-LK30, which carries bla_{KPC-2} and bla_{SHV-11}. The plasmid is very similar to two bla_{KPC-2}-carrying $IncFII_K$ plasmids, but lacks one of the replication origins and cannot conjugate.

Conclusions: The differences in cross-species transferability of the two plasmids can be explained by genetic differences between their backbones and could have resulted in the confined bla_{KPC-2}-carrying CR-KP outbreak in Taiwan. Plasmid pKPC-LKEc is the first bla_{KPC-2}-carrying plasmid identified from CR-EC in Taiwan. With relatively high transferability it should be closely monitored.

Keywords: $IncFII_K$, carbapenemases, resistance mechanisms

Introduction

The increasing incidence of carbapenem-resistant bacteria, which are resistant to almost all antibiotics, is of great concern.[1] Among the mechanisms that cause carbapenem resistance, carbapenemases, such as NDM or KPC, are frequently studied due to their rapid global spread.[1,2] One intriguing observation is that carbapenem resistance genes, such as those encoding KPC or the OXA-type carbapenemase in *Klebsiella* spp. and *Acinetobacter* spp., respectively, are highly species specific.[3,4] These species-specific carbapenemases, especially KPC, are occasionally observed in other bacteria, such as *Pseudomonas* spp. and *Acinetobacter* spp.[5,6] Previous reports have indicated that the bla_{KPC}-carrying plasmid could be conjugatively transferred within species of *Klebsiella*, but not *Escherichia coli*; the plasmid is lost unless co-cultured with *K. pneumoniae*, suggesting that it is unstable outside of *K. pneumoniae*.[2] Although it is not as common as in *K. pneumoniae*, KPC-producing *E. coli* can be isolated from

patients.[7] However, little is known of the genetic background of bla_{KPC}-encoding plasmids in *E. coli*. In Taiwan, the first bla_{KPC}-carrying *K. pneumoniae* was identified in 2011.[8] We obtained the first bla_{KPC}-encoding *E. coli* isolate in 2012 and the complete sequencing and comparative analysis of the bla_{KPC}-encoding plasmids from *E. coli* and *K. pneumoniae* are reported here.

Materials and methods

bla_{KPC}-carrying E. coli and K. pneumoniae

The bla_{KPC}-carrying *E. coli* and *K. pneumoniae* strains [i.e. carbapenem-resistant *E. coli* (CR-EC) and carbapenem-resistant *K. pneumoniae* (CR-KP)] were identified in a national surveillance of carbapenem resistance among *K. pneumoniae* and *E. coli* in 2012, which was supported by Taiwan Centers for Disease Control.

Multilocus sequencing typing (MLST) was performed according to Woodford *et al*.[9]

Conjugation of plasmids into recipient E. coli or K. pneumoniae

For CR-EC and CR-KP donors, recipient *E. coli* strains such as azide-resistant J-53 or rifampicin-resistant J-995 were used. MacConkey agar plates containing sodium azide or rifampicin (100 mg/L) and imipenem (2 mg/L) were used for the selection of transconjugants. For the CR-KP donor, which was susceptible to streptomycin, a serotype K2 *K. pneumoniae* strain with intrinsic resistance only to ampicillin was used as recipient. The transconjugants were selected using brilliant green agar containing inositol-nitrate-deoxycholate (BIND) supplemented with 2 mg/L imipenem and 100 mg/L streptomycin. For CR-EC, conjugation to K2 *K. pneumoniae* was detected using BIND agar supplemented with 2 mg/L imipenem.

Sequencing and annotation of plasmids

Complete sequencing of the plasmids was performed with a shotgun approach using 454 GS Junior (Roche). The GenBank accession numbers are KC405622 and KC788405 for pKPC-LK30 and pKPC-LKEc, respectively.

Results and discussion

Molecular typing and conjugation of the plasmids

KPC-producing bacteria in Taiwan were first described in 2011 and became an outbreak through the clonal spread of CR-KP.[10] The present CR-EC was identified in 2012 and was the first bla_{KPC-2}-carrying *E. coli* in our locality. MLST indicated that the CR-KP and CR-EC were ST11 and ST410, respectively. Both of these are international multidrug-resistant clones and are well known in association with the clonal spread of CTX-M-type extended-spectrum β-lactamase production in *K. pneumoniae* and *E. coli*.[9] Recent reports have shown that ST11 is also the predominant type of globally disseminated KPC carbapenemase among *K. pneumoniae* and ST410 is an emerging type with KPC among *E. coli*.[9,10] The plasmid pKPC-LKEc from CR-EC was able to transfer into recipient *E. coli* JP-995 but not *E. coli* J-53. The pKPC-LK30 from CR-KP was unable to transfer into recipient serotype K2 *K. pneumoniae*, *E. coli* JP-995 or *E. coli* J-53.

Sequencing and annotation of plasmid pKPC-LKEc from CR-EC

The 145 401 bp plasmid pKPC-LKEc from CR-EC is a chimera formed by three regions similar to different replicons. These include an 82 kb region similar to the IncI plasmid R64, a 20 kb region similar to the IncN plasmid R46 and an 18.6 kb region similar to the RepFIC replicon (Figure 1a). R64 is a conjugative plasmid

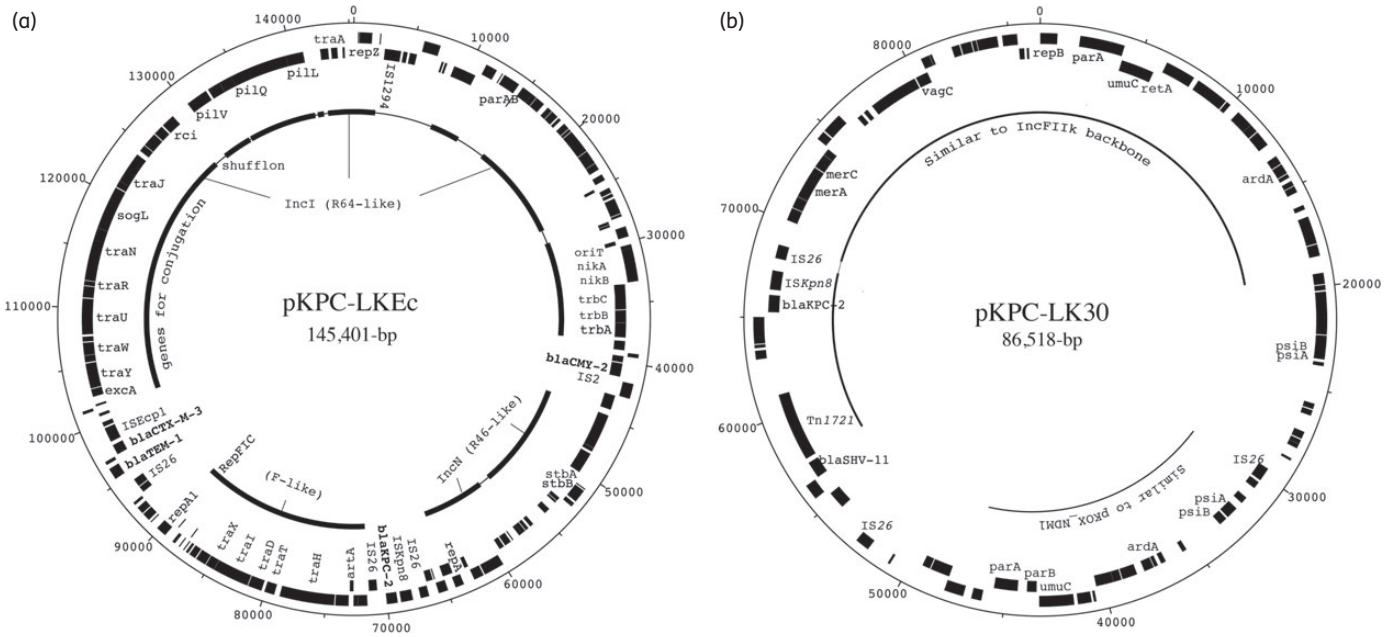

Figure 1. Schematic maps of (a) pKPC-LKEc and (b) pKPC-LK30. Black blocks on the outer circle are the coding DNA sequence of the positive strand, and the black blocks on the inner circle are the coding DNA sequence of the negative strand. The remarkable features are named. The consensus regions similar to the other plasmids/replicons are marked with thick black lines inside the circles with non-consensus sections between the conserved regions shown by thin lines.

originally isolated from *Salmonella enterica*.[11] The 82 kb R64-like region on pKPC-LKEc contains a *repZ* gene for autosomal replication, the pilus genes and *tra* genes for conjugal transfer. The 20 kb R46-like region on pKPC-LEKc contains a *repA* gene and *stbA stbB* plasmid stability genes similar to the IncN plasmid R46 from *S. enterica* serovar Typhimurium.[12] The 18 kb F-like region contains *traF*, *trbA*, *artA*, *finO* and *repA1* genes similar to the RepFIC replicon.[13] The antimicrobial resistance genes bla_{KPC-2}, bla_{CMY-2}, $bla_{CTX-M-3}$ and bla_{TEM-1} were all found next to the IS elements that were located between the three conserved replicon backbone regions (Figure 1a).

The genes responsible for conjugal transfer of the IncI plasmid R64 include the *tra*/*trb* gene clusters, the *pil* genes for the type IV pilus, *oriT* and the *nikAB* gene cluster for DNA processing.[14] A unique shufflon multiple inversion system may alter the C-terminal amino acid sequence of the PilV adhesin of the type IV pilus, thus adjusting the specificity of the recipient cells during conjugation.[15,16] With these genetic features identified in the R64-like region of pKPC-LKEc, it is very likely that pKPC-LKEc can also transfer itself conjugatively to specific hosts.

Sequencing and annotation of plasmid pKPC-LK30 from CR-KP

The carbapenem resistance plasmid pKPC-LK30 from CR-KP is 86 518 bp in length and carries bla_{KPC-2} and bla_{SHV-11} (Figure 1b). The plasmid is very similar to two bla_{KPC-2}-carrying plasmids, pKP048 and pKPN101-IT, reported recently from *K. pneumoniae*.[17,18] Both of these were classified as $IncFII_K$ type, and contained two replication initiation regions, including an FIIK1 region and a *repB* region.[17,18] Comparative analyses revealed several conserved regions between the $IncFII_K$ plasmids and pKPC-LK30, including a *repB* putative replication initiation region. Surprisingly, the FIIK1 region in pKP048 and pKPN101-IT was not found in pKPC-LK30. It seemed that *repB* alone could still promote plasmid replication in *K. pneumoniae*. A 36 kb region containing the transfer operon (locus *tra-trb*) embedded in pKP048 and pKPN101-IT is completely missing in pKPC-LK30. This may explain why pKPC-LK30 from CR-KP does not transfer conjugatively, like pKP048 or pKPN101-IT, to the recipients. Furthermore, absence of the FIIK1 region may hinder the replication and stability of pKPC-LK30 and thus be responsible for the confinement of its spread. In addition to the $IncFII_K$-like region, the plasmid also contained a 16 kb region similar to that of plasmid pKOX_NDM-1 from *Klebsiella oxytoca* E718, which was collected in Taiwan.[19] The 16 kb region contained genes encoding additional plasmid partitioning protein homologues, and those similar to plasmid SOS-inhibition protein homologues and antirestriction protein homologue (Figure 1b). No putative replication initiation regions or transfer locus were identified outside the $IncFII_K$-like region.

Comparative analysis of the bla_{KPC-2} region

The immediate genetic environment of bla_{KPC-2} in pKPC-LK30 and pKPC-LKEc is the same and comparable to pKP048, including an interrupted Tn*3* and an IS*Kpn8* followed by bla_{KPC-2} and the IS*Kpn6*-like element (Figure 2). In these plasmids, the region containing bla_{KPC-2} and part of the downstream IS*Kpn6*-like gene is identical to Tn*4401*, which is presumably the origin of bla_{KPC-2} mobilization to the plasmids.[20] The Tn*3* immediately upstream of bla_{KPC-2} in all these plasmids is interrupted by the insertion of an IS*Kpn8* at an identical location, leaving a short segment of Tn*3* inverted repeats upstream of the bla_{KPC-2}. The Tn*3* in pKPC-LK30 and pKPC-LKEc is interrupted further by the insertion of an IS*26* element at the Tn*3* *tnpR*. In pKPC-LK30 and pKP048, a conserved Tn*1721* is located downstream of bla_{KPC-2} (Figure 2). However, the IS*26* *tnpA* pseudogenes next to Tn*1721* were truncated, presumably by Tn*1721*, at different locations. This suggests that the acquisition of the bla_{KPC-2} region into the $IncFII_{K1}$ backbones, presumably aided by the conserved Tn*1721*, were independent events. In pKPC-LKEc, a 4.8 kb region including bla_{KPC-2} and two flanking IS*26* elements resembled a composite transposon. However, no direct repeats were identified flanking this putative composite transposon in pKPC-LKEc. The bla_{KPC-2} region might have been acquired via homologous recombination mediated by the flanking IS*26*.

Conclusions

The emergence of KPC-producing *E. coli* is of concern because the bla_{KPC-2}-bearing plasmid may carry resistance across species barriers. In this report, although sequencing analysis suggested that pKPC-LKEc is capable of conjugal transfer, and the transferability

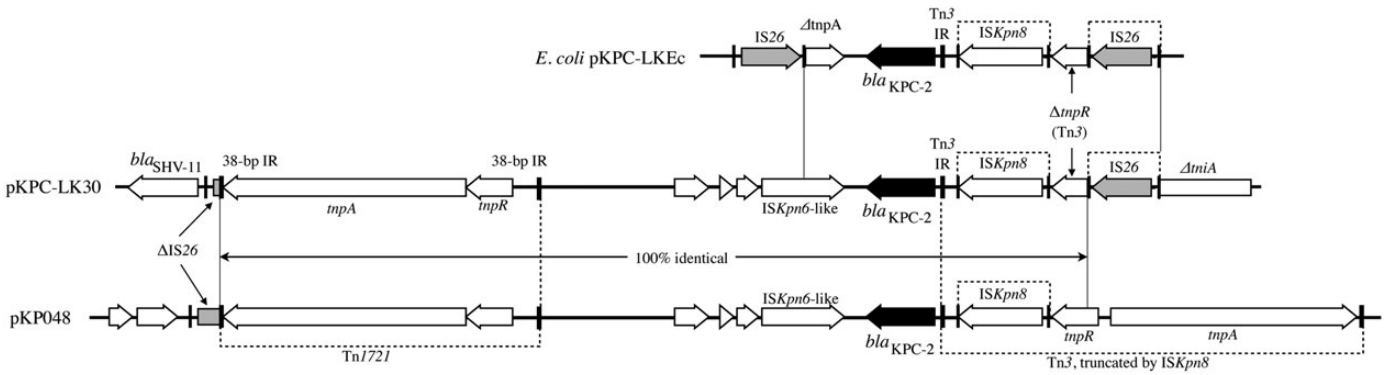

Figure 2. Comparison of the bla_{KPC-2} region between pKPC-LK30, pKPC-LKEc and pKP048. The regions spanning the bla_{KPC-2} gene on the three plasmids are shown, and the genes are depicted as arrows according to the direction of transcription. The bla_{KPC-2} genes are shown in black, the pseudogenes are shown in grey and the inverted repeats are indicated by the vertical bars.

was confirmed in the laboratory using *E. coli* JP-995, conjugal transfer was not successful on all occasions. With high sequence similarity to plasmid R64, it is likely that the conjugation specificity of pKPC-LKEc is also controlled by the shufflon. It was reported that, by altering the C-terminus of the PilV adhesion domain that targets lipopolysaccharide on recipient cells, the specificity for different recipients could be modulated.[14,15] Conversely, the pKPC-LK30 plasmid from CR-KP does not carry genes known for conjugal transfer. This could have led to the confined outbreak of CR-KP in Taiwan. The pKPC-LKEc plasmid isolated from *E. coli* has the potential for transferability and should be closely monitored.

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Transparency declarations

None to declare.

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