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Brief communication

Characterization of the genetic environment of the bla_{KPC-2} gene among *Klebsiella pneumoniae* isolates from a Chinese Hospital

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ABSTRACT

Infection caused by carbapenem-resistant *Klebsiella pneumoniae* has become a major healthcare threat and KPC-2 enzyme is a dominant factor mediating carbapenems resistance in *K. pneumoniae*. This study was designed to determine the genetic environment of bla_{KPC-2}, which prevailed in clinical *K. pneumoniae* isolates recovered in Huashan Hospital, Shanghai, China. Forty-two clinical isolates were included in this study by bla_{KPC-2} screening. After multilocus sequence typing and plasmid analyses of PCR-based replicon typing (PBRT), junction PCR, mapping PCR and crossing PCR assays, primer walking, and amplicon sequencing were used to analyze the genetic environment of the bla_{KPC-2} gene. ST423, ST65, ST977, and ST11 were all detected in KPC-2-producing *K. pneumoniae*. Two types of bla_{KPC-2}-bearing genetic structure were found: Tn*1721*-bla_{KPC-2}-Tn3 and Tn*1721*-bla_{KPC-2}-ΔTn3-IS*26*; and were carried in IncX and IncFII plasmids, respectively. In conclusion, the genetic environment of the bla_{KPC-2} gene was diverse and Tn*1721*-bla_{KPC-2}-ΔTn3-IS*26* was dominant in clinical *K. pneumoniae* isolates in Huashan Hospital. This study sheds some light on the genetic environment and should foster further studies about the mechanism of the bla_{KPC-2} dissemination.

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Introduction

Carbapenem-resistant *Enterobacteriaceae*, especially *Klebsiella pneumoniae*, have emerged as important causes of morbidity and mortality among hospital acquired and long-term care-associated infections such as bacteremia and pulmonary infections.[1,2] KPC-2, the most common variant of KPC (*K. pneumoniae* carbapenemases) enzymes, is a dominant factor mediating carbapenems resistance in *Enterobacteriaceae*.[3]

In most countries and regions, such as Europe,[4] the United States,[5] and Brazil[6,7] bla_{KPC-2} is mainly located on Tn*4401*. It is an active transposable element capable of mobilizing this drug-resistant gene at high frequency among *Enterobacteriaceae*.[8] In Chinese initial reports, the bla_{KPC-2} gene was located on a Tn3-based transposon, Tn*1721*, on the

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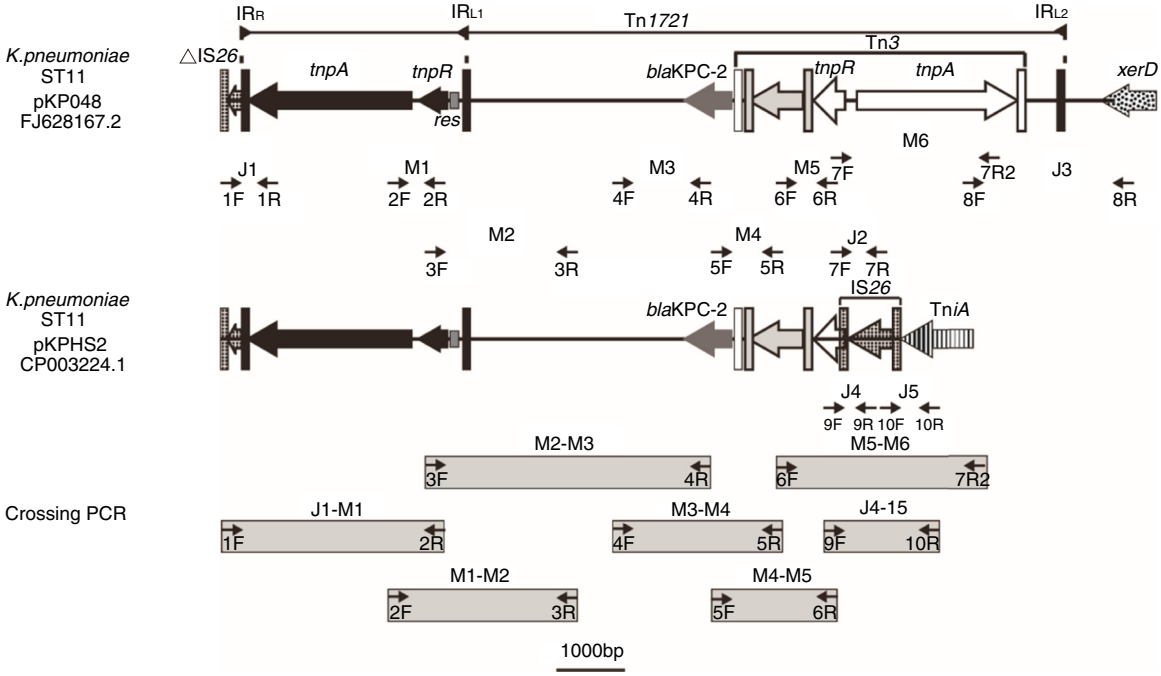

Fig. 1 – Schematic map of primer annealing sites for junction PCR (J1, J2, J3, J4 and J5); mapping PCR (M1, M2, M3, M4, M5 and M6); and crossing PCR assays. All primers were designed based upon pKP048 (GenBank accession no. FJ628167.2) sequence data, with the exception of the primers corresponding to J4 and J5, which were designed based upon pKPHS2 (GenBank accession no. cp003224.1).

plasmid pKP048 (GenBank accession no: FJ628167.2).[9] Then, Liu et al. described a truncated form of Tn*1721* composed of IS*26* inserted into $tnpR_{Tn3}$ which was located on pKPHS2 (GenBank accession no: CP003224.1) from a clinical *K. pneumoniae* HS11286 (Fig. 1).[10] Despite a matching sequence in Tn*4401* that exhibits identity to the 3 kb bla_{KPC-2}-bearing region within Tn*1721*, Tn*4401* did not carry bla_{KPC-2} in any of the Chinese isolates up to now.[9–11] Obviously, distinct genetic environment of bla_{KPC-2} suggests that the mechanism to mobilize bla_{KPC-2} in China is different from that in other regions.

This study reports a nosocomial outbreak of 42 KPC-2-producing *K. pneumoniae* isolates at our hospital and presented the genetic environment of bla_{KPC-2} in these isolates using a series of PCR assays and amplicon sequencing instead of the tedious process of plasmid fully sequenced.

Materials and methods

Forty-two, non-duplicated, bla_{KPC-2}-postive *K. pneumoniae* isolates were included in this study by bla_{KPC-2} screening as described previously,[12] after routine identification and antimicrobial susceptibility testing by the Microbiology Laboratory, Huashan Hospital, Fudan University (Shanghai, China) between August 2006 (when the first bla_{KPC-2}-positive clinical *K. pneumoniae* isolate was detected) and October 2010 (when the bla_{KPC-2}-positive isolates were detected continuously and steadily). The multilocus sequence types (ST) were determined by analyzing seven housekeeping genes (i.e., *gapA*, *infB*, *mdh*, *pgi*, *phoE*, *rpoB* and *tonB*) as reported previously.[13] The results were compared with the MLST databases available at http://www.pasteur.fr/recherche/genopole/PF8/mlst/Kpneumoniae.html.

Conjugation was performed using donor and recipient *Escherichia coli* J53 cells mixed at a 1:1 ratio in broth cultures as described previously.[13] Transconjugants were selected on MacConkey agar containing imipenem (0.5 mg/L) and sodium azide (100 mg/L; Sigma Chemical Co.). When resistance plasmid transfer failed in mating experiments, a transformation was used. Plasmids from wild-type isolates were extracted by using a Qiagen Plasmid Midi kit (Qiagen, Germany). *E. coli* ElectroMAX DH5α competent cells were transformed with the extracted plasmids DNA by electroporation (Micro-Pulser electroporator; Bio-Rad, USA). Transconjugants were selected on MacConkey agar containing imipenem (0.5 mg/L). Putative transconjugant colonies were selected and identified by the Vitek system, and further confirmed in a bla_{KPC-2} PCR assay. Plasmids were classified following the PCR-based replicon typing (PBRT) scheme targeting replicons of the major Inc plasmids.[14]

Based upon previous reports,[9,10] we predicted the genetic environment of bla_{KPC-2} would be the same as that found in pKP048 and pKPHS2, or present a new structure different from the two structures mentioned above. Junction PCR (J1, J2, J3, J4, and J5) and mapping PCR (M1, M2, M3, M4, M5, and M6) assays were performed for all of the isolates using the relevant primers (Table S1). Junction PCR was mainly used to determine the boundaries of bla_{KPC-2}-bearing genetic structure and to distinguish the sequence of the structure between pKP048 and pKPHS2. Mapping PCR was mainly used for amplifying

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