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Emergence of *bla*$_{NDM-11}$ carried by an IncX3 plasmid in *Citrobacter freundii* ST266 in China

Editor: Dr Jon Hobman

Sir,

The global dissemination of carbapenemase-producing Enterobacterales (CPE), especially those producing New Delhi metallo-β-lactamase (NDM) enzymes, has become a serious health threat. Since NDM-1 was first reported, 30 NDM variants have been identified so far (https://www.ncbi.nlm.nih.gov/pathogens/refgene/#). Among them, the *bla*$_{NDM-11}$ gene was first identified on an IncF plasmid from *Escherichia coli* in India [1]. More recently, *bla*$_{NDM-11}$ was also detected in *E. coli* isolates recovered from patients in Egypt [2]. Here we report the first occurrence of NDM-11-producing *Citrobacter freundii* and characterised the corresponding *bla*$_{NDM-11}$-bearing IncX3 plasmid.

Citrobacter freundii isolate C46 was recovered from a urine sample of a hospitalised patient in China in 2019. Species identification was performed using both a VITEK® 2 Compact system (bioMérieux) and 16S rRNA sequencing. Multilocus sequence typing (MLST) indicated that strain C46 belonged to the uncommon sequence type 266 (ST266). To our knowledge, this ST has not yet been described in *C. freundii* carrying carbapenem resistance genes. Antimicrobial susceptibility testing was conducted by broth microdilution and the results were interpreted according to Clinical and Laboratory Standards Institute (CLSI) and European Committee on Antimicrobial Susceptibility Testing (EUCAST) (for tigecycline and colistin) guidelines. The results of antimicrobial susceptibility testing indicated strain C46 was resistant to imipenem, meropenem, ertapenem, ceftazidime, cefepime, ampicillin, tetracycline and colistin but was susceptible to tigecycline (Supplementary Table S1). A PCR assay for carbapenemase genes was performed as previously described [3], resulting in a positive result for *bla*$_{NDM}$. The complete sequence of the *bla*$_{NDM}$ gene was amplified and analysis of the sequence showed that strain C46 harboured a *bla*$_{NDM-11}$ gene. S1 nuclease pulsed field gel electrophoresis (S1-PFGE) and Southern blot hybridisation revealed that *bla*$_{NDM-11}$ was located on an ~54 kb plasmid, designated pC46-NDM11. Conjugation assays showed that plasmid pC46-NDM11 was successfully transferred into the recipient *Escherichia coli* EC600 at a frequency of 2.1×10^{-3}. The transconjugant displayed increased minimum inhibitory concentrations (MICs) to all β-lactam antibiotics tested including carbapenems (Supplementary Table S1). pC46-NDM11 was subjected to plasmid sequencing using both Illumina short-reads and Nanopore long-reads, followed by hybrid assembly using Unicycler [4]. The plasmid sequence was annotated using the RAST server (https://rast.nmpdr.org/) and corrected by BLASTn. The complete sequence of plasmid pC46-NDM11 has been deposited in the GenBank database under accession no. **MW269623**.

Plasmid pC46-NDM11 had a length of 54 035 bp and a GC content of 49.04%. BLASTn analysis showed that pC46-NDM11 shared >99% nucleotide identity with the *bla*$_{NDM-1}$-carrying plasmids p309074-NDM (**MH909346**) and pKP04NDM (**KU314941**) from *Klebsiella pneumoniae* and pP10159-1 (**MF072961**) from *C. freundii*, for which only the sequences are available from GenBank (Fig. 1a). pC46-NDM11 was assigned to incompatibility group IncX3 using PlasmidFinder [5]. pC46-NDM11 had a typical IncX3 plasmid backbone, including replication (*repB*), partitioning (*parA* and *parB*), maintenance (*hns, topB, kikA, dnaJ* and *umuD*) and conjugal transfer (*virB1–virB11, taxA–C*). Analysis of the genetic context showed that *bla*$_{NDM-11}$ was located on a variant of transposon Tn*125*. In contrast to the prototype Tn*125*, the upstream IS*Aba125* element of the Tn*125* variant was interrupted by an IS*5* element in pC46-NDM11, and 5-bp direct target site duplications (5′-TTAGG-3′) were detected. The downstream IS*Aba125* was replaced by an IS*26*-flanked *bla*$_{SHV-12}$-carrying segment in plasmid pC46-NDM11 (Fig. 1b). The *bla*$_{SHV-12}$-carrying segment had IS*26* elements located in opposite orientations at its termini (Fig. 1b). However, no direct repeats were found immediately downstream of the two IS*26* copies. To determine whether a free circular form containing IS*26*-flanking sequences can be formed, a reverse PCR assay was performed but no circular intermediate was detected. Therefore, the result showed that this IS*26*-flanked segment probably does not function as a composite transposon.

In conclusion, the occurrence of *bla*$_{NDM-11}$ together with *bla*$_{SHV-12}$ on a conjugative IncX3 plasmid is of great concern and underlines the need for further efforts aimed at limiting the spread of IncX3-type plasmids.

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Competing interests

None declared.

Ethical approval

Not required.

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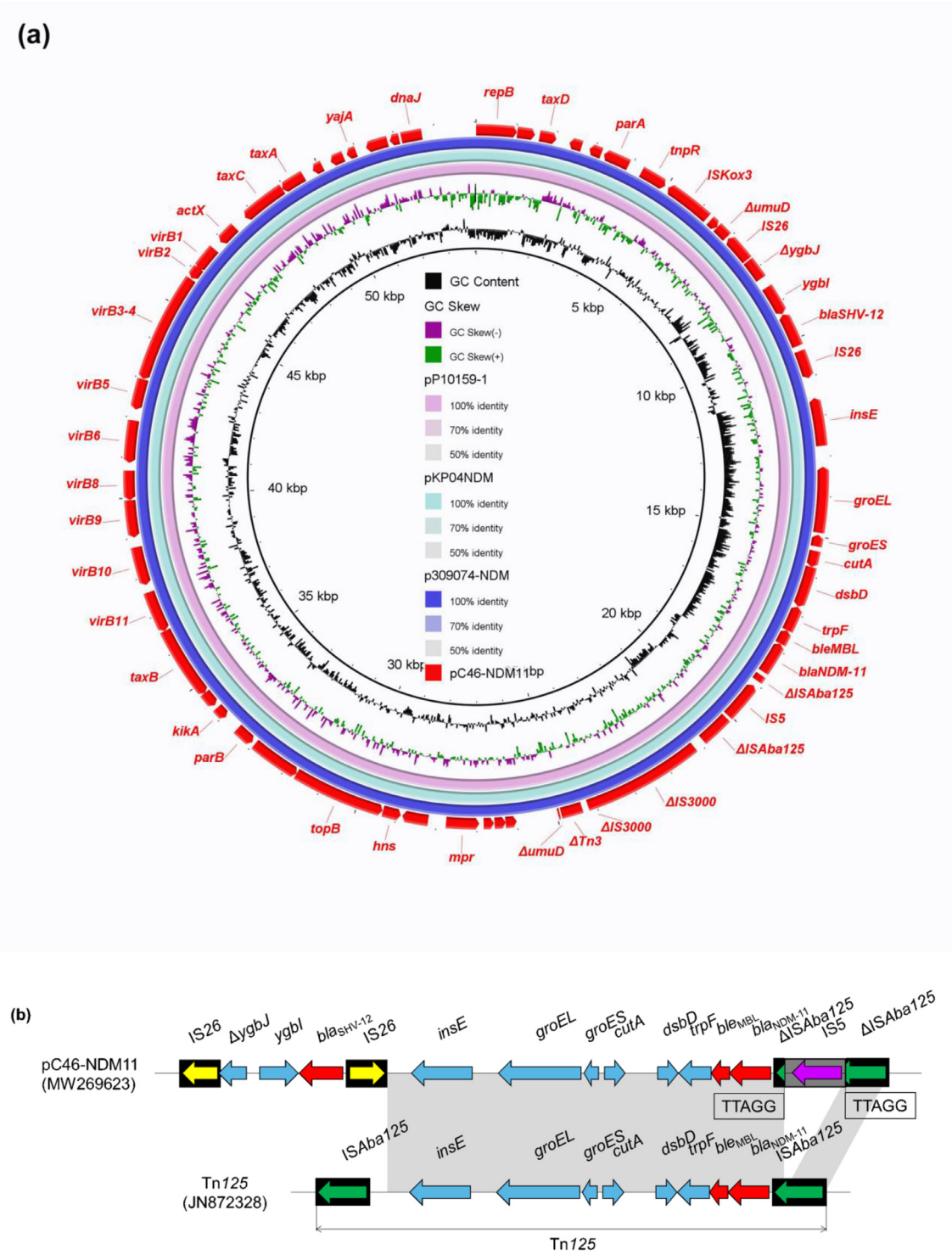

Fig. 1. (a) Circular comparison between plasmid pC46-NDM11 and other reported similar plasmids. The circles (from the outside to inside) indicate plasmid pC46-NDM11 (**MW269623**), p309074-NDM (**MH909346**), pKP04NDM (**KU314941**) and pP10159-1 (**MF072961**); GC skew [(G+C)/(G–C)]; GC content; and scale in kb. Arrows indicate the direction of transcription of genes. (b) Genetic environment of bla_{NDM-11} and bla_{SHV-12} in pC46-NDM11. Light grey shading shows regions with a high degree of homology. Genes are represented by arrows and are coloured depending on the gene function as follows: red, antimicrobial resistance genes; blue, other proteins. The different insertion sequence (IS) elements are displayed as black or grey boxes with the transposase gene in yellow (IS*26*), green (IS*Aba125*) or purple (IS*5*).

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