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Comparative genomics of IncL/M-type plasmids; evolution by acquisition of resistance genes and insertion sequences

Sir,

IncL/M-type plasmids R446b and R471a were originally isolated from *Morganella morganii* (formerly *Proteus morganii*) as the first members of the IncL/M group of multidrug resistance (MDR) plasmids (6, 7). IncL/M plasmids are now commonly identified among environnemental and clinical isolates (1, 3, 10). This group of plasmids can be considered as an emerging threat since it has been increasingly identified as a source of broad-spectrum ß-lactam resistance, encoding either the metallo-β-lactamase NDM-1 or the class D carbapenemase OXA-48, and being also responsible for the dissemination of extended-spectrum ß-lactamase genes such as $bla_{\text{CTX-M-3}}$ (5, 8-10). In this study, we analyzed the sequence of plasmid pNDM-OM, harboring the $bla_{\text{NDM-1}}$ gene, recovered from a clinical *Klebsiella pneumoniae* isolate from Sultanate of Oman, providing new insights into the evolution of MDR IncL/M-type plasmids.

The complete sequencing work flow of the Illumina Genome Analyzer IIx system (Illumina Inc., San Diego, CA) was performed by the DNAVision company (Gosselles, Belgium). The size of plasmid pNDM-OM was 87,185-bp in-size with an average GC content of 52%, and contained 98 open reading frames. It possessed a complete array of genes involved in replication, conjugation, and partition (Table S1). The plasmid architecture observed in pNDM-OM was similar to that of other IncL/M plasmids previously sequenced (Fig. 1). It exhibited a very high gene synteny with plasmid pNDM-HK also encoding NDM-1 with only very few differences, consisting in one to four nucleotide changes/deletions at

three different locations, together with the lack of two insertion sequences identified in pNDM-HK, namely IS*26* and IS*186* (Fig. 1). In addition, part of the *tnpA* gene of Tn2 (563 bp) were missing in pNDM-HK when compared to pCTX-M-3 and pNDM-OM.

Overall, in-silico analysis of the Genbank databases revealed that IncL/M plasmids have evolved through the acquisition of resistance genes and insertion sequences. The 60,145-bp plasmid pEL60, isolated from *Erwinia amylovora*, can be considered as the typical IncL/M backbone since it does not possess any resistance gene or insertion sequence (Table S1) (Fig. 1 and 2) (3). Plasmid pOXA-48a differed from pEL60 by two main additional elements; i) the integration of composite transposon Tn*1999* carrying the $bla_{\text{OXA-48}}$ carbapenemase gene in the *tir* gene that encodes a transfer inhibitory protein, and ii) a recombination event resulting in the exchange of three gene within the *tra* operon (namely *traX*, *traY* and *excA*) (9). Plasmid pCTX-M3 differs from pEL60 (12) by the acquisition of the IS*Ecp1*-$bla_{\text{CTX-M-3}}$-carrying transposon and by acquisition of a large resistance region constituted by transposon Tn*2* in which a Tn*1548*-like transposon, carrying the 16S rRNA methylase *armA* gene, had inserted. The structure of pCTX-M360 can be considered as an intermediate between plasmids pEL60 and pCTX-M3 since it possesses the typical IncL/M backbone with the insertion of IS*Ecp1*-$bla_{\text{CTX-M-3}}$ and the insertion of a native Tn*2* (Fig. 2) (12). On the other hand, plasmids pNDM-HK and pNDM-OM possess a similar synteny compared to pCTX-M3, including the insertion of Tn*2* and the insertion of a Tn*1548*-like transposon within the Tn*2* resolvase gene, but a likely recombination event resulted in the insertion of the NDM-module within the Tn*1548*-borne class 1 integron, leading to the replacement of the integrase gene and the gene cassette array by that module.

The integration hotspot we identified among the IncL/M-type plasmids was located between the replication locus and the *trbC* gene that encodes part of the Trb transfer operon (Fig. 2). First, transposon Tn*2* carrying the $bla_{\text{TEM-1}}$ β-lactamase gene has inserted within this

region and then IS*26*-mediated insertions may have occurred, leading to a deletion of the resolvase gene and the 5'-end of the Tn*2* transposase gene. These events gave rise to a complex structure carrying many resistance determinants including those compromising the activity of carbapenems, broad-spectrum cephalosporins, aminoglycosides, quinolones, trimethoprim, and sulfonamides (2, 4, 5, 8, 9, 11). The acquisition of the bla_{NDM-1} gene in pNDM-OM resulted from a recombination event leading to an exchange of a class 1 integron within the Tn*1548* transposon by the NDM module. This acquisition was probably recent since the resistance loci of the pNDM-OM, pNDM-HK, and pCTX-M-3 derive from a common ancestor (Fig. 1). A second integration hotspot has been identified near the *pemI/pemK* locus in which the IS*Ecp1*-$bla_{CTX-M-3}$ transposon has inserted. This transposon conferring resistance to broad-spectrum cephalosporins was widely distributed among clinical isolates (10). Surprisingly, the pNDM-OM and pNDM-HK did not harbor the IS*Ecp1*-bla_{CTX-M} transposon despite the fact that they are very likely derivatives of pCTX-M3 based on the synteny and phylogenetic analysis of the transfer operon.

This further strengthens the evolutive potential of IncL/M-type plasmids that constitute currently efficient vectors for emerging and clinically-relevant resistance genes.

Nucleotide sequence accession number. The nucleotide and protein sequences corresponding to plasmid pNDM-OM have been registered in GenBank under accession no. JX988621.

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Legend to the figure.

Figure 1. Major structural features of plasmid pNDM-OM in comparison with IncL/M-type plasmids pCTX-M3 (Genbank AF550415), pEL60 (Genbank AY422214), pCTX-M360 (Genbank EU938349), pOXA-48a (Genbank accession number JN626286) and pNDM-HK (Genbank HQ451074). White boxes indicated plasmid scaffold regions that are in common among plasmids. The *tra* locus is indicated within the box. Resistance genes are indicated by orange coloured boxes, except the ß-lactamase genes which are indicated by blue boxes. Transposon-related genes [*tnpA*, *tnpR*, *tnpM*] and insertion sequences are indicated by red boxes. Replicase genes are indicated by purple boxes.

Figure 2. Schematic representation of insertions in IncL/M backbone. A proposed backbone namely pEL60 is represented as in figure 1. pOXA48a specific insertion or recombination are shown below the backbone while specific insertion and recombination of pCTX-M360, pCTX-M3, pNDM-HK and pNDM-OM are shown above the backbone. Type of modification either transposition or recombination are indicated in the figure.

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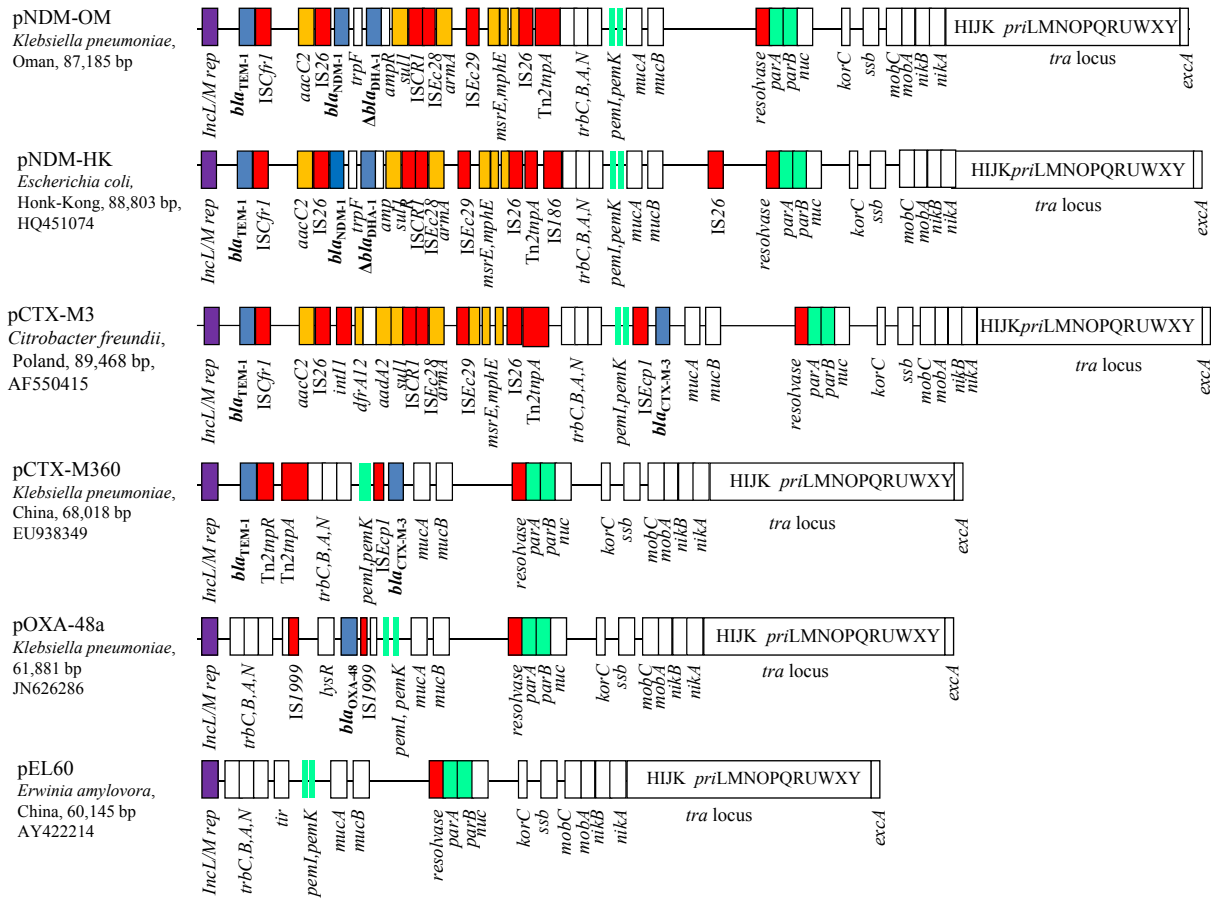

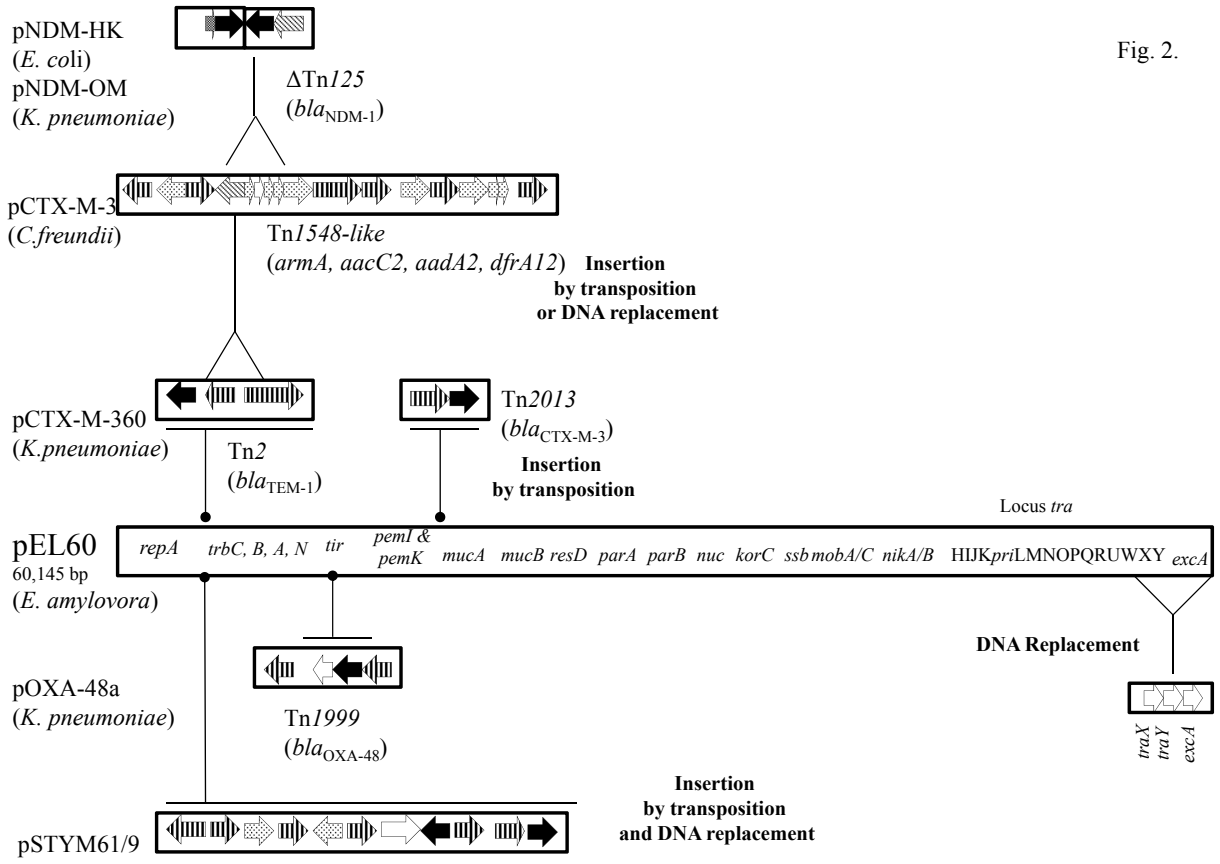

Fig. 2.