P1343 Complete sequencing of a novel IncH plasmid carrying the *bla*$_{NDM-1}$, *bla*$_{CTX-M-15}$ and *qnrB1* genes

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Objectives

The current emergence of the *bla*$_{NDM-1}$ gene is not related to the spread of a single epidemic plasmid with a given scaffold but rather to multiple events of acquisitions on different plasmid types. In several environmental strains isolated from seepage water and public tap water collected from New Delhi, India, the *bla*NDM-1 was identified on large (>250 kb) and non-typeable plasmids (Walsh et al. 2011). The complete sequence of pNDM-MAR, a non-typeable plasmid of 250 kb identified from a *K. pneumoniae* from Morocco (Poirel et al. 2011) and carrying the genes encoding NDM-1, CTX-M-15 and *qnrB1*, was determined and analyzed.

Methods

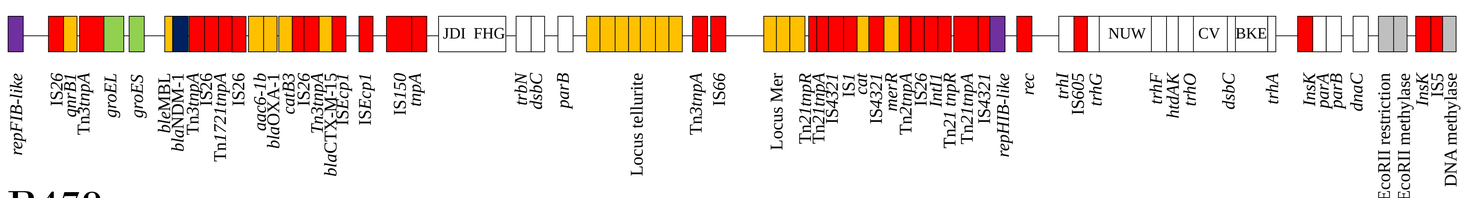
Plasmid sequencing was performed applying the 454-Genome Sequencer FLX procedure on libraries obtained on total plasmid DNA purified from an *E. coli* J53 transconjugant. Contigs with at least 15-fold coverage obtained by GS De Novo Assembler software were assembled in continue plasmid sequences by the PCR-based gap closure method.

Results

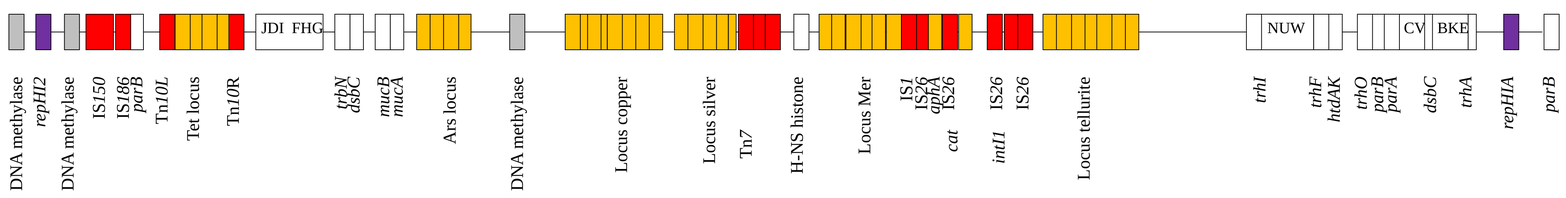
Plasmid pNDM-MAR was 267,242 bp in-size and encoded 177 putative open reading frames coding. BLASTN comparison indicated a completely novel plasmid scaffold, showing only small portions of homology (18-20% coverage) with GenBank data. pNDM-MAR possessed novel replicons and transfer loci, defining a new group within the IncH plasmid family. Plasmid pNDM-MAR carried the *bla*$_{NDM-1}$ gene into a genetic environment that was different from those previously described for other plasmids encoding NDM-1. In addition, it encoded the extended-spectrum beta-lactamase CTX-M-15 and the plasmid-mediated quinolone resistance gene *qnrB1*. That latter was flanked by an IS*26* element and the Tn*3* transposase which proceeded the *groEL-groES* genes, flanking the *bla*$_{NDM-1}$ gene. The *bla*$_{CTX-M-15}$ gene was associated to insertion sequence IS*Ecp1*. Plasmid pNDM-MAR carried additional resistance determinants to tellurite, mercury, chloramphenicol, and aminoglycosides.

pNDM-MAR is a novel member of the IncH plasmid family

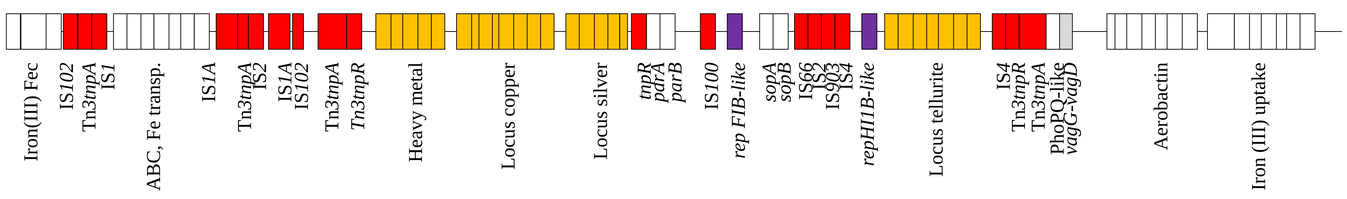
pNDM-MAR, *Klebsiella pneumoniae*, Morocco, 267,242 bp, JN420336



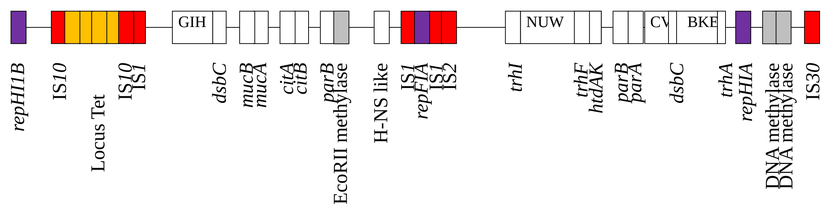
R478, *Serratia marcescens*, Reference IncHI2, 274,762 bp, NC_005211

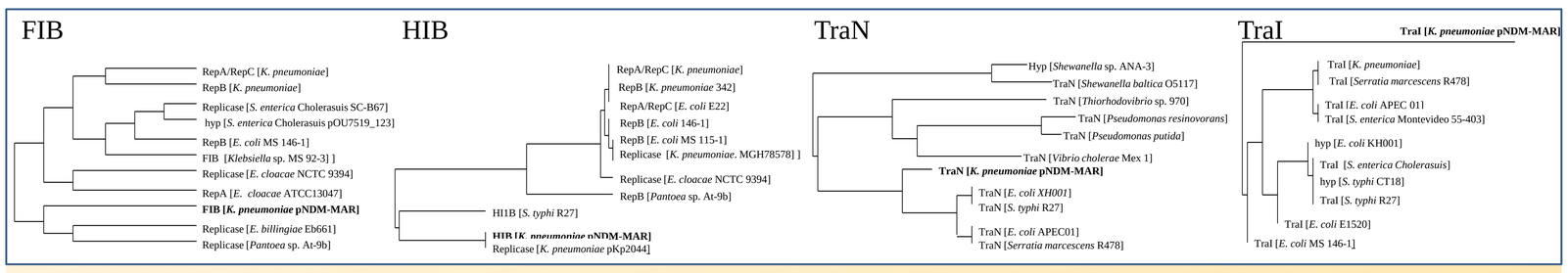


pK2044, *Klebsiella pneumoniae*, China, 224,152 bp, AP006726



R27, *Salmonella enterica* Typhi, Reference IncHI1, 180,461 bp, AF250878



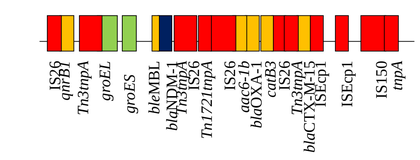


The IncH family includes all plasmids producing the H-pilus, a long and flexible conjugation-pilus similar to the F-pilus. Plasmids of the IncHI1 and IncHI2 type have been fully sequenced; they possess two transfer regions, sharing 71-78% nucleotide identity, have an optimal transfer at 22-30 C, and are multi-replicons. In particular, plasmid R27 possesses three replicons called RepHI1A, RepHI1B and RepFIA-like, plasmid R478 possesses two replicons called RepHI1A and RepHI2. R27 and R478 are each other incompatible since share the common replicon RepHI1A. The RepHI1B replicon is unique of the IncHI1 subgroup, while the RepHI2 replicon is unique of the IncHI2 subgroup.

Plasmid pNDM-MAR harboured two novel replicons, one related to FIB and one related to RepHI1B. Comparative analysis suggested that the first one corresponded to a new member of the FIB replicon type, which is identified here for the first time and could not be detected by the current PBRT scheme. The second replicon identified in pNDM-MAR shared 99% amino acid identity with a replicase protein encoded by pK2044 (pK2044_00935) and 61% identity with the RepHI1B (Swiss-Prot: Q7AQZ8) of plasmid R27.

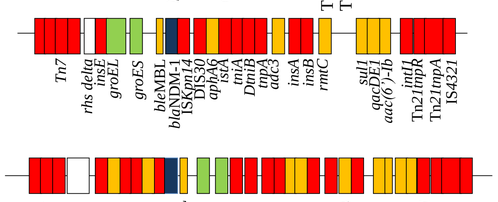
Further regions encoding homologous CDSs were observed among plasmids pNDM-MAR, R27, and R478. In particular, there was a significant level of homology (45-53% amino acid identity) in the conjugal transfer loci with R27, especially for proteins involved in the H-pilus assembly and transfer (i.e. 64% identity for the TraN protein R27_p006). Comparison of the TraI relaxase proteins suggested that plasmid pNDM-MAR constitutes a novel plasmid type , exhibiting a maximum of 38% amino acid identity with previously identified IncHI-type relaxases . However, the presence of a related HIB-like replicon, and the conservation of the proteins involved in the conjugative H-pilus synthesis, strongly suggest that plasmid pNDM-MAR can be included as a novel variant within the IncH plasmid family.

Resistance genes on plasmid pNDM-MAR

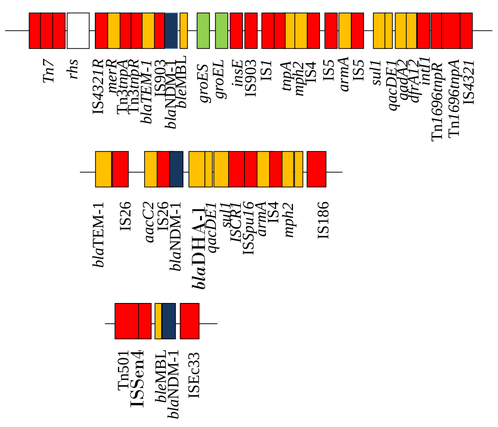


IncH pNDM-MAR, *Klebsiella pneumoniae*, Morocco, JN420336

IncA/C pNDM-KN, *Klebsiella pneumoniae*, Kenya, JN157804



IncA/C pNDM10505, *Escherichia coli* , Canada, JF503991



IncA/C pNDM-1_Dok01, *Escherichia coli* , Japan, AP012208

IncL/M pNDM-HK *Escherichia coli*, Hong-Kong, HQ451074

IncN2 pNDM-1 *Escherichia coli*, Australia, JF785549

Plasmid pNDM-MAR carried the *bla*$_{NDM-1}$ gene into a genetic environment significantly different from those previously identified from other *bla*$_{NDM-1}$-positive plasmids, including the groES and groEL chaperonin-encoding genes previously identified onto other IncA/C-type plasmids. In addition, it harboured the ESBL gene *bla*$_{CTX-M-15}$ together with the quinolone resistance gene *qnrB1*. That latter gene was flanked by an IS*26* element and by the Tn*3* transposase that immediately proceeded the *groES-groEL* genes. The *groEL* gene sequence is partially interrupted presumably by the integration of this Tn*3*-like element. The *bla*$_{CTX-M-15}$ gene was flanked by insertion sequence IS*Ecp1* that is known to be at the origin of *bla*$_{CTX-M-15}$ acquisition through a one-ended transposition process. Noteworthy, such physical association between the *bla*$_{CTX-M-15}$ and *bla*$_{NDM-1}$ genes had never been reported.

Plasmid pNDM-MAR also harboured tellurite, mercury chloramphenicol and aminoglycoside resistance genes. Interestingly, pNDM-MAR did not carry any 16S rRNA methylase gene, such as those encoding ArmA, RmtB, or RmtC, by contrast to most *bla*$_{NDM-1}$-positive plasmids.

References

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Conclusions

pNDM-MAR that has been identified from different isolates recovered from patients in Morocco significantly differed from all known *bla*$_{NDM-1}$-bearing plasmids identified so far. Considering that the Indian subcontinent appears to be a main reservoir for NDM-1 producers, that plasmid might circulate in that geographical area. On the other side, it might be also speculated that this plasmid could correspond to a specific vehicle for *bla*$_{NDM-1}$ only in North Africa. Since IncHI1 and IncHI2 plasmids have a broad host range, being identified among several environmental Gram-negative species, the identification of the *bla*$_{NDM-1}$ gene onto IncH plasmids raises the alarming possibility that they will contribute to the dissemination of the *bla*$_{NDM-1}$ gene among bacterial species in water and soil environments.