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Emergence of Metallo-β-Lactamase NDM-1-Producing Multidrug-Resistant *Escherichia coli* in Australia▽

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A multidrug-resistant *Escherichia coli* isolate recovered in Australia produced a carbapenem-hydrolyzing β-lactamase. Molecular investigations revealed the first identification of the bla_{NDM-1} metallo-β-lactamase gene in that country. In addition, this *E. coli* isolate expressed the extended-spectrum β-lactamase CTX-M-15, together with two 16S rRNA methylases, namely, ArmA and RmtB, conferring a high level of resistance to aminoglycosides.

Metallo-β-lactamases (MBLs) are reported increasingly in Gram-negative organisms and are identified mostly in *Pseudomonas* species (6, 13). MBLs hydrolyze all β-lactams, including carbapenems (except aztreonam) (19). Among *Enterobacteriaceae*, the bla_{IMP} and bla_{VIM} genes have been identified worldwide; in addition, the KHM-1 enzyme has been reported from a *Citrobacter freundii* isolate from Japan (17). Recently, a novel MBL named NDM-1 (New Delhi metallo-β-lactamase) was identified from *Klebsiella pneumoniae* (strain 05-506) and *Escherichia coli* isolates recovered from a Swedish patient transferred from India (20). A recent study reported NDM-1-producing *K. pneumoniae*, *E. coli*, *C. freundii*, *Morganella morganii*, *Providencia* species, and *Enterobacter cloacae* isolates in the United Kingdom, scattered in various hospitals (8). That study identified NDM-1 producers in India and Pakistan, evidencing a link between the emergence of NDM-1 producers in the United Kingdom and a possible reservoir identified in the Indian subcontinent (8). More recently, two *K. pneumoniae* isolates producing NDM-1 were isolated in the Netherlands from two patients returning from India (4). NDM-1 is distantly related to other MBLs, sharing only 32% amino acid identity with the most closely related enzymes VIM-1 and VIM-2.

Our study was initiated by the recovery of a multidrug-resistant *E. coli* isolate from a urine sample of a 67-year-old man who had been hospitalized at St. George Hospital, Sydney, Australia, following a medical transfer from Bangladesh, where he had been hospitalized for pneumonia over a period of 12 days. MICs were determined by Etest (AB bioMérieux, Solna, Sweden) on Mueller-Hinton (MH) agar plates at 37°C, and results of susceptibility testing were recorded according to CLSI guidelines (3) (Table 1). *E. coli* isolate 271 was resistant to all β-lactams (including carbapenems), all aminoglycosides, fluoroquinolones, nitrofurantoin, and sulfonamides, remaining susceptible only to tetracycline, tigecycline, fosfomycin, and colistin. MBL detection performed by using Etest MBL strips (AB Biodisk, Solna, Sweden) was positive.

Shotgun cloning experiments performed as described previously (14), followed by sequencing, revealed that the genetic structures surrounding the bla_{NDM-1} gene diverged significantly from those observed in *K. pneumoniae* 05-506 (20). A novel insertion sequence element, namely, IS*Ec33* (http://www-is.biotoul.fr), was identified upstream of the bla_{NDM-1} gene. IS*Ec33* shared 88% nucleotide identity with the most closely related element IS*630* (belonging to the IS*630* family and previously identified in *Shigella sonnei*), with their respective transposases sharing 93% amino acid identity. IS*Ec33* was bracketed by a 2-bp duplication (TA), as observed for other IS*630*-like elements, therefore suggesting an independent transposition of that mobile element.

Detailed analysis of the 194 bp separating the bla_{NDM-1} start codon from IS*Ec33* revealed a promoter that was made of −35 (TTGAAT) and −10 (TACAGT) sequences separated by an optimal 17-bp distance. It is noteworthy that no obvious promoter that could play a role in bla_{NDM-1} expression was identified in IS*Ec33* (Fig. 1A). The locations of these promoter sequences were further analyzed by mapping the bla_{NDM-1} transcription start site using 5′ rapid amplification of cDNA ends (5′ RACE) (version 2.0; Invitrogen/Life Technologies, Cergy-Pontoise, France), as described previously (9). The +1 transcription site of bla_{NDM-1} was identified 7 bp downstream of the putative −10 sequence indicated above, thus confirming our *in silico* analysis. This result indicated that the expression of bla_{NDM-1} was not driven by a promoter provided by IS*Ec33*. The same promoter sequences were identified upstream of the bla_{NDM-1} gene in *K. pneumoniae* 05-506 (Fig. 1A and B). The bla_{NDM-1} upstream sequences diverged exactly at the IS*Ec33* location. Downstream from the bla_{NDM-1} gene, another novel IS element, namely, IS*Sen4* (belonging to the IS*3* family, subgroup IS*407*), previously identified in *Salmonella enterica* serovar Choleraesuis (GenBank no. EU219534) and not bracketed by any target site duplication, was identified in *E. coli* 271 (Fig. 1A). This IS*Sen4* element was absent in the sequence

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TABLE 1. MICs of β-lactams for the *E. coli* 271 clinical isolate, *E. coli* TOP10 harboring recombinant plasmid p271A expressing NDM-1, and the *E. coli* TOP10 reference strain

β-Lactam(s)[a]	MIC (μg/ml) for:		
	E. coli 271	*E. coli* TOP10(p271A)	*E. coli* TOP10
Amoxicillin	>512	>512	4
Amoxicillin + CLA	256	256	4
Ticarcillin	>512	>512	4
Ticarcillin + CLA	512	512	4
Piperacillin	>512	512	1
Piperacillin + TZB	512	512	1
Cefoxitin	512	256	4
Ceftazidime	256	128	0.06
Cefotaxime	512	256	0.12
Cefepime	64	16	0.06
Aztreonam	64	0.25	0.12
Imipenem	6	4	0.06
Meropenem	16	3	0.06
Ertapenem	32	3	0.06

[a] CLA, clavulanic acid at a fixed concentration of 4 μg/ml; TZB, tazobactam at a fixed concentration of 4 μg/ml.

identified from *K. pneumoniae* 05-506. It seems, therefore, that the mobilization events that were at the origin of acquisition of the bla_{NDM-1} gene in *E. coli* 271 and *K. pneumoniae* 05-506 differed significantly.

Since *E. coli* 271 was resistant to all β-lactams, including aztreonam, which is not a substrate for MBLs, an additional extended-spectrum β-lactamase (ESBL) was sought. PCR followed by sequencing using specific primers for bla_{TEM}, bla_{SHV}, bla_{PER-1}, bla_{VEB-1}, bla_{GES-1}, and bla_{CTX-M} ESBL genes (12) identified the ESBL CTX-M-15, together with penicillinase TEM-1. In addition, screening of 16S rRNA methylase-encoding genes was performed by using a multiplex PCR approach as described previously (1) and identified two methylase genes, namely, *armA* and *rmtB*.

Plasmid analysis performed using the Kieser technique (7) revealed that *E. coli* 271 harbored four plasmids of ca. 160, 130, 80, and 50 kb. By using a PCR-based replicon typing (PBRT) method as described previously (2), we showed that these plasmids belong to incompatibility groups IncI1 and IncF, respectively. Transfer of the β-lactam resistance markers from *E. coli* 271 to *E. coli* J53 (azide resistant) was performed by mating assays, with selection based on different and amoxicillin, 100 μg/ml) (9). The *E. coli* TOP10(p271A) transconjugant showed an MBL phenotype and was susceptible to non-β-lactam antibiotics. Plasmid analysis revealed that *E. coli* TOP10(p271A) harbored a bla_{NDM-1}-positive 50-kb plasmid, which could not be typed. The $bla_{CTX-M-15}$ gene was carried on an 80-kb IncF plasmid, although the *rmtB* gene together with bla_{TEM-1} was carried on a 130-kb IncFII plasmid. Since the incompatibility group of plasmid p271A carrying bla_{NDM-1} was not determinable, attempts to evaluate a possible broad host range were performed. For that purpose, DNA of plasmid p271A was electrotransformed in *Pseudomonas aeruginosa* and *Acinetobacter baumannii* recipient strains, and selection was performed with ticarcillin (50 μg/ml)-containing Mueller-Hinton (MH) plates, as described previously (11, 15), but no transformant was obtained in either species, suggesting that plasmid p271A might have a narrow host range.

Since the worldwide spread of CTX-M-15-producing *E. coli* isolates has been demonstrated to be associated with the clonal dissemination of an *E. coli* strain belonging to sequence type 131 (ST131) (5, 10), multilocus sequence typing (MLST) was performed as described previously (10, 18) to identify the genotype of *E. coli* 271. PCR and sequencing of the seven different alleles followed by computer analysis on the MLST website (www.mlst.net) revealed that *E. coli* 271 belonged to ST101, which corresponds to a phylogenetic lineage different from that of ST131 (data not shown).

Conclusion. This study further emphasizes the spread of the novel MBL determinant NDM-1 and its first identification in Australia, which corresponds to a geographical area distantly related to the Indian subcontinent. Previous hospitalization of the patient in Bangladesh suggests that *E. coli* isolate 271 originates from that country. The hypothesis of a foreign origin

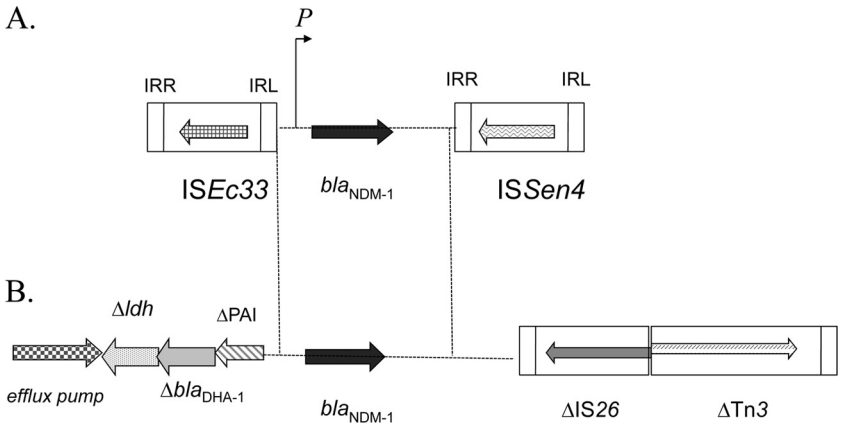

FIG. 1. Schematic map representing the bla_{DIM-1}-surrounding genetic sequences in *E. coli* 271 (this study) (A) compared to those in *K. pneumoniae* 05-506 (20) (B). The vertical dotted lines indicate the locations at which the sequences diverge. *P* corresponds to the location of the promoter for bla_{DIM-1} expression. The insertion sequences IS*Ec33* and IS*Sen4* and their respective right (IRR) and left (IRL) inverted repeats are shown. Horizontal arrows indicate the different open reading frames, including the insertion sequence transposase genes, the genes encoding the efflux pump and lactate dehydrogenase (*ldh*), the short part of the bla_{DHA-1} β-lactamase gene, and the DNA fragment encoding the 5′ end of a phosphoribosyl anthalinate isomerase (ΔPAI). Truncated insertion sequence IS*26* (ΔIS*26*) and transposon Tn*3* (ΔTn*3*) are also indicated.

of the strain is reinforced by the fact that no other similar multiresistant *E. coli* strain had been isolated previously at St. George Hospital in Sydney. It seems, therefore, that the current emergence of NDM-1 in a distantly related geographical area may be superimposed with the scattering of Indian populations worldwide.

Nucleotide sequence accession number. The nucleotide sequence of the bla_{NDM-1}-surrounding structure has been registered in GenBank under accession no. HQ162469.

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