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Complete Sequence of a Novel 178-Kilobase Plasmid Carrying bla_{NDM-1} in a *Providencia stuartii* Strain Isolated in Afghanistan

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In response to global concerns over the spread of the New Delhi metallo-β-lactamase gene 1, bla_{NDM-1}, a monthly surveillance program was initiated in September 2010. All carbapenem-resistant Gram-negative strains forwarded to our facility are screened for this gene. To date, 321 carbapenem-resistant isolates, encompassing 11 bacterial species, have been tested. In February 2011, two strains of *Providencia stuartii*, submitted from a military hospital in Afghanistan, tested positive for bla_{NDM-1}. Both strains were identical by pulsed-field gel electrophoresis (PFGE). bla_{NDM-1} was carried on a large plasmid, pMR0211, which was sequenced by emulsion PCR and pyrosequencing. pMR0211 is 178,277 bp in size and belongs to incompatibility group A/C. The plasmid consists of a backbone with considerable homology to pAR060302 from *Escherichia coli*, and it retains many of the antibiotic resistance genes associated with it. The plasmid also shares common elements with the pNDM-HK plasmid, including bla_{NDM-1}, *armA*, and *sul1*. However, gene orientation is reversed, and a 3-kb fragment from this region is absent from pMR0211. pMR0211 also contains additional genes, including the aminoglycoside-modifying enzyme loci *aadA* and *aac*(6′), the quinolone resistance gene *qnrA*, a gene with highest homology to a U32 family peptidase from *Shewanella amazonensis*, and the bla_{OXA-10} gene. The finding of this gene in an intrinsically colistin-resistant species such as *Providencia stuartii* is especially worrisome, as it renders the organism resistant to nearly every available antibiotic. The presence of multiple insertion sequences and transposons flanking the region containing the bla_{NDM-1} gene further highlights the potential mobility associated with this gene.

During the past 2 decades, metallo-β-lactamases have risen from relative obscurity to their current status at the forefront of antibiotic resistance research (8). Potent carbapenemase activity and resistance to clinical β-lactamase inhibitors, combined with increasing association with highly mobile genetic elements (8, 13, 22), makes them one of the most serious challenges facing infection control programs in recent years (17, 19, 23).

The gene of New Delhi metallo-β-lactamase 1 (bla_{NDM-1}) constitutes a case study in the epidemiology of metallo-β-lactamase transmission. Since its initial identification in 2008 (25), bla_{NDM-1} has been identified in many countries (1, 11, 22), and the global distribution of this gene is imminent (20). bla_{NDM-1} is found in an ever-widening number of bacterial species and is strongly associated with large, highly mobile plasmids that carry numerous other antibiotic resistance genes (22, 25). Like the VIM-type metallo-β-lactamases, NDM-1 confers resistance to all β-lactam agents except the monobactams, but mobile DNA elements associated with bla_{NDM-1} transmission routinely carry numerous other antibiotic resistance genes, including those encoding resistance to monobactams (12, 13, 22, 23).

With increasing surveillance for bla_{NDM-1}, a concomitant increase in the variety of plasmids associated with its transmission has been reported (12, 13, 22, 23). Plasmid sizes vary considerably, ranging from 50 to >400 kb (23), but some common associations have been noted. For example, Walsh and coworkers identified 12 plasmids from 20 bacterial species in an environmental survey of New Delhi, and all five of the *Enterobacteriaceae* in that study carried bla_{NDM-1} on a 140-kb plasmid of the incompatibility group A/C (IncA/C) type (23). In contrast, bla_{NDM-1} was found on an 89-kb plasmid of incompatibility group IncL/M from a strain of *Escherichia coli* recovered in Hong Kong (12).

In September 2010, a high-throughput real-time PCR assay for detecting bla_{NDM-1} was developed, and monthly screening of carbapenem-resistant clinical isolates from within the Military Health System was implemented at the Multidrug-Resistant Organism Repository and Surveillance Network (MRSN) at the Walter Reed Army Institute of Research (15, 16). To date, 13 hospitals, including 5 in war zones, submit an average of 550 isolates each month, including carbapenem-resistant Gram-negative organisms. In February 2011, a strain of *Providencia stuartii* submitted from a military hospital in Afghanistan tested positive for bla_{NDM-1}. Sequencing revealed 100% homology to the archetypal bla_{NDM-1} gene from *Klebsiella pneumoniae* (13), and plasmid analyses indicated that the gene was present on a large plasmid termed pMR0211.

In this report, we describe the structure of pMR0211, a 178-kb plasmid carrying the bla_{NDM-1} gene. The plasmid shares considerable homology to plasmid pAR060302 from *E. coli* (4), as well as to genes previously described in pNDM-HK, including bla_{NDM-1}, *armA*, and *sul1* (12). The plasmid also contains additional open reading frames, including the aminoglycoside-modifying enzyme loci *aadA* and *aac*(6′), the quinolone resistance gene *qnrA*, a U32 family peptidase gene, and the bla_{OXA-10} gene.

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MATERIALS AND METHODS

Case report. On 4 March 2011, two isolates of *Providencia stuartii* obtained on 26 January from the blood of a burn patient in a U.S./Coalition medical facility in Afghanistan were found to harbor the bla_{NDM-1} gene by real-time PCR as part of an ongoing monthly surveillance for this gene. The patient was an Afghan national who was transferred from a local national facility in Kabul 5 days after injury to the intensive care unit 25 km north of Kabul for the treatment of severe burns and inhalational injury following a gas explosion. The patient received unspecified, broad-spectrum antibiotics at the local national hospital, which were changed to levofloxacin, piperacillin-tazobactam, and vancomycin upon arrival at the U.S./Coalition medical facility. No history of prior illness or travel exposures was obtained. The patient was coinfected with a carbapenem-resistant, bla_{NDM-1}-negative *Pseudomonas aeruginosa* strain as well as a less resistant *P. aeruginosa* strain and *Proteus mirabilis*. Computed tomography imaging of the brain showed global effacement and herniation. The patient died from complications due to severe injuries and central nervous system infections 12 days after injury.

Surveillance isolates. MRSN participants submitted an average of 26 carbapenem-resistant Gram-negative clinical isolates each month since September 2010. Resistance to carbapenems in *Enterobacteriaceae* is defined according to the updated Clinical and Laboratory Standards Institute guidelines (7) (for imipenem, ≤ 1 μg ml^{-1} is sensitive and ≥ 4 μg ml^{-1} is resistant; for meropenem, ≤ 1 μg ml^{-1} is sensitive and ≥ 4 μg ml^{-1} is resistant; for ertapenem, ≤ 0.25 μg ml^{-1} is sensitive and ≥ 1 μg ml^{-1} is resistant; and for doripenem, ≤ 1 μg ml^{-1} is sensitive and ≥ 4 μg ml^{-1} is resistant). Identification and antibiotic susceptibilities of isolates were confirmed at the central facility using three commercially available platforms: the Vitek II (bioMérieux, Inc., NC), the BD Phoenix automated microbiology system (Becton Dickinson, NJ), and the Microscan WalkAway (Siemens, PA).

To date (May 2011), 213 carbapenem-resistant isolates have been submitted through the surveillance initiative for bla_{NDM-1} testing. Isolates were from blood, surveillance, respiratory, urine, and wound cultures from health care facilities in Washington, DC, Maryland, Virginia, North Carolina, Hawaii, Iraq, and Afghanistan. *Acinetobacter baumannii* and *P. aeruginosa* were the predominant species (52.1 and 38%, respectively). Other species included *Aeromonas veronii* (0.9%), *Burkholderia cepacia* (0.5%), *Citrobacter freundii* (0.5%), *Enterobacter cloacae* (0.9%), *E. coli* (2.8%), *K. pneumoniae* (2.3%), *Pseudomonas stutzeri* (0.5%), *P. stuartii* (0.9%), and *Serratia marcescens* (0.5%).

Processing of bacterial samples. All bacterial isolates were cultured overnight on blood agar, and single colonies were resuspended in 200 μl of sterile phosphate-buffered saline (PBS). Ten-μl aliquots were treated with 20 μl Lyse-and-Go PCR reagent in a 96-well plate format per the manufacturer's instructions for the isolation of total DNA (Thermo Fisher Scientific, MA). Two-μl aliquots of the resulting lysate were used directly for real-time PCR and sequencing.

Real-time PCR assay for the detection of bla_{NDM-1}. Real-time PCR primers targeting the bla_{NDM-1} gene were designed and validated using MIQE (for minimum information for the publication of quantitative real-time PCR experiments) guidelines (3) with Primer Express 2.0 (Applied Biosystems, CA) from the bla_{NDM-1} sequence deposited at GenBank under accession number FN396876 by Yong et al. (25). All isolates were tested for the presence of bla_{NDM-1} using primer pair NDMRT1-F (GGC CAC ACC AGT GAC AAT ATC A) and NDMRT1-R (CAG GCA GCC ACC AAA AGC) on a Light Cycler 480 (Roche Applied Science, IN). To ensure efficient lysis, isolates were tested in parallel using a universal 16S real-time primer pair (18). Reaction conditions were performed in 20-μl volumes in 96-well plates with an annealing temperature of 56°C. Positive (*K. pneumoniae* CDC1000529 and *K. pneumoniae* NCTC 13443) and negative (*K. pneumoniae* ATCC 8049 and *K. pneumoniae* ATCC 1708) controls were included in duplicate on every plate. All samples were tested in triplicate from three biological replicates (i.e., three separate Lyse-and-Go preparations).

The bla_{NDM-1} genes from both *P. stuartii* strains (MRSN 2154 and MRSN 2155) were sequenced by Macrogen Corp. (Rockville, MD) using the primer pair NDMPCR-F (CCA TGC GGG CCG TAT GAG TGA TT) and NDMPCR-R (AAG CTG AGC ACC GCA TTA GCC G), which amplifies a 763-bp region of the bla_{NDM-1} gene. The amplification of the same region from *K. pneumoniae* CDC1000529 was sequenced in parallel as a control. Sequences were assembled using SeqMan and aligned using MegAlign (DNAStar Inc., WI).

DNA extraction and pyrosequencing. Twenty colonies were collected and used for the extraction of total DNA (chromosomal and extrachromosomal). DNA was purified using the bacterial genomic DNA purification kit (EdgeBio, MD) with the following modifications. Following the isopropanol precipitation and pelleting of the DNA by centrifugation, the supernatant was centrifuged for an additional 15 min at 4°C to ensure the full recovery of plasmid DNA. Both pellets were processed separately and combined after dissolution in 10 mM Tris-HCl.

One microgram of the total DNA extract was subjected to DNA fragmentation using the Covaris S2 system (Covaris, Inc., MA), and a rapid ligation (RL) genomic shotgun library was prepared using the GS FLX Titanium rapid library preparation kit (Roche 454 Life Sciences, CT). After purification, the RL library was resolved on a 0.8% agarose gel. Fragments ranging in size from 600 to 800 bp were collected and subjected to DNA gel extraction. Subsequent emulsion PCR and pyrosequencing using the Genome Sequencer FLX system (Roche 454 Life Sciences) were performed as described by the manufacturer.

Pyrosequencing data analysis and assembly of pMR0211. Sequencing reads were assembled to consensus *de novo* assembly contigs using the Roche Genome Sequencer FLX software GSAssembler, version 2.5.3. All *de novo* contigs were subjected to direct megablast (http://blast.ncbi.nlm.nih.gov/) against GenBank's nucleotide collection (nr/nt) with the Entrez query set to bacterial plasmids. Two plasmids, pAR060302 and pNDM-HK, showed the highest identities with contig sequences and were used as query sequences to align all contigs. A draft plasmid sequence for pMR0211 was created using the sequences of pAR060302 and pNDM-HK as larger scaffolds from the information generated by the contig branching structure. The scaffold assembly and draft completed sequence were verified by mapping against the sequence reads to ensure contiguous coverage crossing adjacent contigs. The authenticity of the assembly was further tested by PCR amplification using a set of primer pairs and the total DNA extract. Amplification product sizes were estimated using 1% agarose gel electrophoresis and compared to the expected sizes.

Conjugation. Conjugation experiments were performed using *E. coli* J53Az^R as the recipient as described previously (23), except that MacConkey agar containing 100 μg/ml sodium azide and 8 μg/ml arbekacin was used in addition to MacConkey agar containing 100 μg/μl sodium azide and 0.5 μg/ml of meropenem for selection. Plasmids were extracted using S1 nuclease digestion and pulsed-field gel electrophoresis (PFGE) as described previously (13). Plasmid bands were excised from agarose gels, and the detection of bla_{NDM-1}, bla_{OXA-10}, and *armA* was performed by PCR and real-time PCR. Plasmid extracts were also tested for *uidE*, *ompF*, and the 16S gene, which are chromosomally encoded in *E. coli* using species-specific primers developed previously (unpublished results).

Nucleotide sequence accession numbers. The nucleotide sequences determined in the course of this work have been deposited in GenBank under accession numbers JF826283 (MRSN 2154), JF826284 (MRSN 2155), and JF826285 (MRSNKpNDM). The sequence of plasmid pMR0211 has been deposited at GenBank with accession number JN687470.

RESULTS

Characterization of clinical isolates. *P. stuartii* MRSN 2154 and MRSN 2155 were resistant to carbapenems (ertapenem, imipenem, and meropenem) and susceptible to the monobactam aztreonam. The isolates also were resistant to all other antibiotics

TABLE 1 Antibiotic susceptibilities for NDM-1-positive and -negative isolates from the same patient

Antibiotic[b]	MIC[a] (μg/ml)			
	P. stuartii (MRSN 2154/2155)	*P. aeruginosa* (MRSN 2152)	*P. aeruginosa* (MRSN 2153)	*P. mirabilis* (MRSN 2150)
Tobramycin	>8	>8	>8	≤1
Ampicillin-sulbactam	>16/8	>16/8	>16/8	16/8
Piperacillin-tazobactam	>64/4	>64/4	>64/4	≤2/4
Cefepime	>16	>16	>16	≤2
Cefoxitin	>16	>16	>16	≤4
Ceftazidime	>16	>16	>16	≤2
Ceftriaxone	>32	>32	>32	≤2
Aztreonam	≤2	>16	>16	≤2
Colistin[c]	>256	-	2	-
Ertapenem	>4	-	-	≤0.5
Imipenem	>8	≤4	>8	≤4
Meropenem	>8	≤4	≧16	≤4
Ciprofloxacin	>2	2	>2	>2
Levofloxacin	>4	4	>4	>4
Tetracycline	>8	>8	>8	-
Tigecycline[c]	4	-	-	-
Trimethoprim-sulfamethoxazole	>2/38	-	-	>2/38

[a] MICs were confirmed and replicated on Vitek2, Phoenix, and Microscan automated systems.
[b] All isolates were resistant to gentamicin. A dash indicates not tested or inherently resistant.
[c] Etest result.

tested, including colistin (MIC > 256 μg/ml via Etest) (Table 1). Pulsed-field gel electrophoresis (PFGE) revealed 99.5% identity between both strains.

Two strains of *P. aeruginosa* also were isolated, one from the blood and one from a wound of the patient. One was of particular concern, because it was resistant to all 15 antibiotics tested (Table 1) but was bla_{NDM-1} negative. The other *P. aeruginosa* isolate displayed multiple resistance also, but it retained carbapenem susceptibility and intermediate susceptibility to fluoroquinolones (ciprofloxacin and levofloxacin). The *P. mirabilis* strain was susceptible to 10 of the 15 antibiotics tested (Table 1).

Conjugation of plasmid pMR0211 with J53Az^R. Plasmid pMR0211 was readily transferred from *P. stuartii* MRSN 2154 to *E. coli* J53Az^R with a frequency of $(3.4 \pm 0.7) \times 10^{-5}$ per donor cell at 37°C. All transconjugants displayed resistance to meropenem, imipenem, and arbekacin relative to the recipient parent strain. Plasmid analyses showed that all transconjugants contained a plasmid of ~180 kb in size which was positive by PCR for the *armA*, bla_{NDM-1}, and bla_{OXA-10} genes but negative for chromosomally carried *uidE*, *ompF*, and the 16S gene.

Pyrosequencing, assembly, and annotation of pMR0211. An average coverage depth of 18.5×, with an average quality of QV64 for the host *P. stuartii* genome and the pMR0211 plasmid sequence, were obtained using Roche GS FLX Titanium pyrosequencing of the shotgun RL library for isolate MRSN2154. Seventeen *de novo* assembly contigs which were homologous to plasmid pAR060302 or pHK-NDM1 were used to assemble the complete nucleotide sequence for plasmid pMR0211. Seventeen regions which covered individual or two consecutive repeats and junctions were amplified to confirm correct assembly. Sizes of the PCR products determined on agarose gel are unambiguously consistent with expected sizes based on the assembled complete pMR0211 sequences (see Table S1 and Fig. S1 in the supplemental material).

pMR0211 was functionally annotated using the xBASE server (www.xbase.ac.uk) (6), with *E. coli* strain K-12, substrain DH10B, serving as the reference genome. The 148 proteins predicted computationally (see Table S2 in the supplemental material) were compared against the set of proteins encoded by pAR060302 using BLAST (26). Homologs for predicted proteins that did not match a product from pAR060302 were identified via blastp or blastx analysis using the nonredundant protein data set maintained by the National Center for Biotechnology Information (NCBI).

Structure of pMR0211. The total length of pMR0211 is 178,277 bases, with an average GC content of 51.44% (Fig. 1 and 2). *In silico* PCR with the pMR0211 sequence and the replicon typing primers constructed by Carattoli and colleagues indicated that plasmid pMR0211 belongs to incompatibility group A/C (5).

Regions of homology to pAR060302 and pNDM-HK (Fig. 1) were defined using the BLAST and ClustalW software (14). The orthologous regions between pAR060203 and pMR0211 retain the genes involved in plasmid maintenance and transfer as well as multiple antibiotic resistance genes, including the chloramphenicol resistance gene *floR*, the tetracycline resistance gene *tetA*, the streptomycin resistance genes *strA* and *strB*, the sulfonamide resistance genes *sul1* and *sul2*, the class C β-lactamase *ampC*, and the quaternary ammonium resistance gene *sugE1* (see Table S2 in the supplemental material) (4).

Sequence analysis reveals several alterations that distinguish pMR0211 from pAR060302. First, nucleotides 6838 to 8524 of pAR060302 are absent from pMR0211. This 1,687-bp region contains an IS*91* element containing a gene encoding an IS*1274* transposase protein. In addition, pMR0211 has a 53-bp deletion corresponding to nucleotides 23178 to 23229 of pAR060302. This deletion affects a pair of inverted *insB2* sequences. pMR0211 also differs from pAR060302 with respect to two prominent insertion/deletion events (Table 2). The first is the deletion of 8,342 bp from pAR060302 (positions 115225 to 123566) that encodes the *aadA*, *aacC*, *groS*, and *groEL* loci. This region is replaced by a 5,983-bp insert in pMR0211-containing genes encoding a chloramphenicol acetyltransferase (*cmlA7*), a class D carbapenemase (bla_{OXA-10}), and an aminoglycoside resistance protein (*aadA1*) (Table 2 and

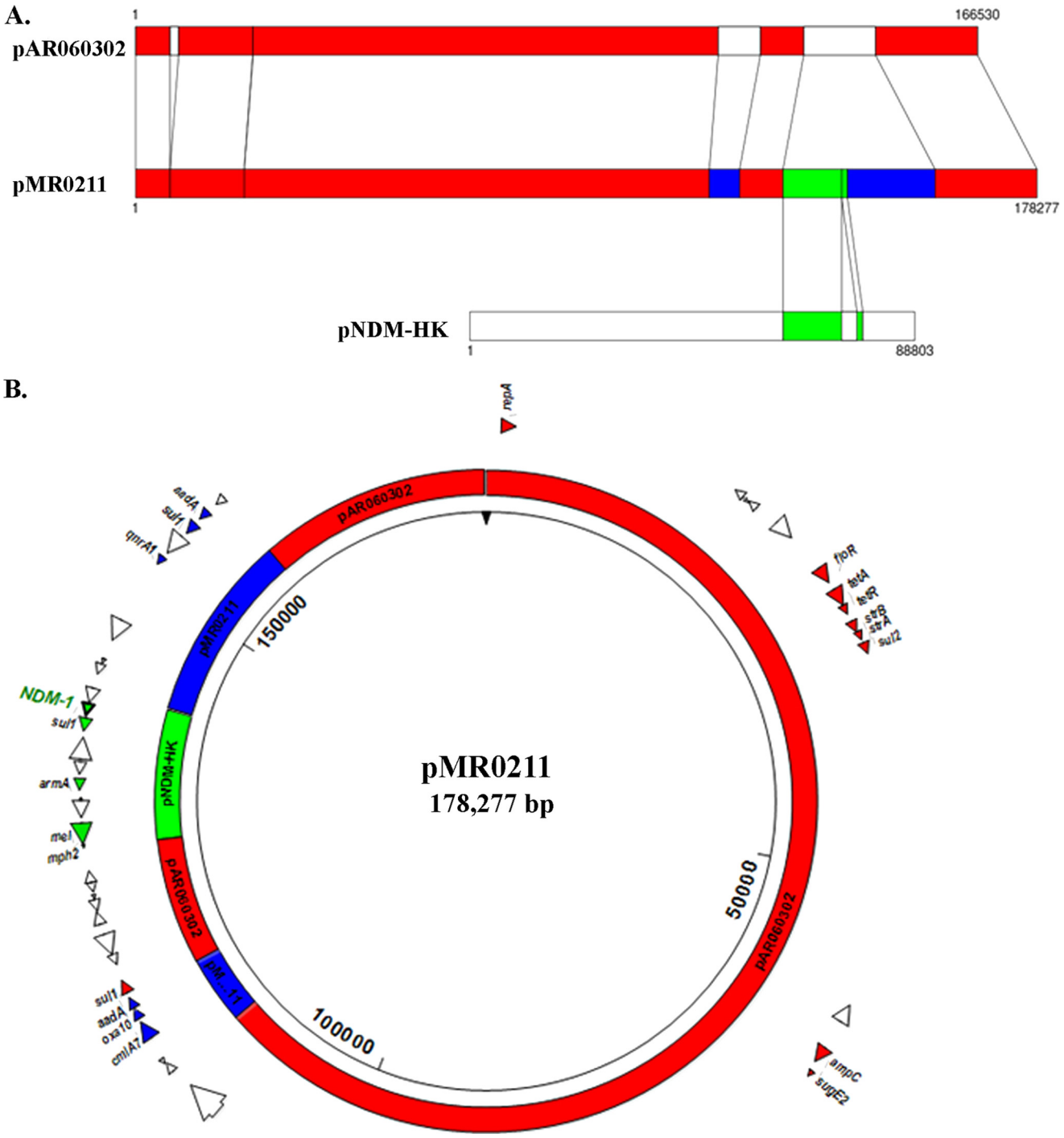

FIG 1 Structure of pMR0211. (A) Alignment of pAR060302 (4) and pNDM-HK (12) with pMR0211 showing areas of homology between all three plasmids. White shading (on pAR060302 and pNDM-HK only) indicates areas with no homology to pMR0211. Red shading indicates regions with homology to pAR060302. Green shading indicates regions with homology to pNDM-HK. Blue shading (on pMR0211 only) indicates regions with no orthologs in pAR060302 and pNDM-HK. Solid lines connecting the plasmids indicate insertion and deletion events in pMR0211 compared to the other plasmids. The sizes of the respective plasmids are indicated at the 3′ terminus. (B) Circular representation of plasmid pMR0211. Colored shading represents genes and regions with homology to pAR060302, pNDM-Hk, and pMR0211 as described above. The *repA* gene and putative antibiotic resistance genes with their respective orientations are indicated in the outermost region by block arrows with the same color scheme. White block arrows indicate the orientation and position of putative insertion sequence elements and transposons.

Fig. 1). The second event is a deletion of 14.2 kb from pAR060302 (132151 to 146351), which includes a cluster of mercury resistance genes (4). In pMR0211, this region is replaced by two distinct insertions of 12.7 and 17.3 kb. The 12.7-kb insertion shares close homology to a portion of plasmid pNDM-HK that contains the bla_{NDM-1} gene. The orientation of this region is reversed in pMR0211, and this region also has a 3-kb deletion that removes the bla_{DHA-1}, *ampR*, and *hypA* loci and part of the *qacΔ1* gene

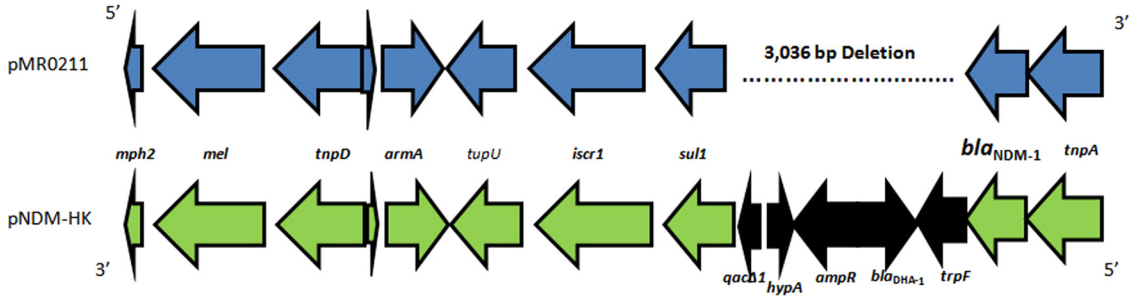

FIG 2 Structure of the bla_{NDM-1} gene and surrounding regions in pMR0211 and pNDM-HK. Gene orientation is reversed in both plasmids, as indicated. A 3,036-bp deletion in pMR0211 is indicated by the dotted line on pMR0211 and corresponds to the genes shaded in black on pNDM-HK. Putative gene annotations were assigned using xBASE (www.xbase.ac.uk) (6).

found in pNDM-HK (Fig. 1B). The 17.3-kb insertion encodes 13 predicted open reading frames, including genes with homology to the aminoglycoside-modifying enzyme loci *aadA* and *aac*(6′), a gene encoding a product similar to a protein of unknown function (DUF1696) from *Shewanella woodyi* ATCC 51908, a gene encoding a U32 family peptidase with homology to the collagenase PrtC, the quinolone resistance gene *qnrA*, and the sulfonamide resistance gene *sul1* (Table 2 and Fig. 1). These regions are surrounded by multiple insertion elements and transposons, including elements from the *ins* and *isc* family of insertion sequences (Table 2; also see Table S2 in the supplemental material).

DISCUSSION

In this study, we report on the complete sequence of a plasmid carrying the bla_{NDM-1} gene belonging to incompatibility group A/C. The plasmid was isolated from a strain of *P. stuartii* that was recovered from a patient undergoing treatment for extensive burn injuries at a hospital in Afghanistan. Although *P. stuartii* is not considered a priority ESKAPE pathogen as defined by the Infectious Disease Society of America (2), the finding of a highly mobile bla_{NDM-1}-carrying plasmid in *Providencia* that is intrinsically resistant to polymixins (21) is of great concern, because such strains thus become resistant to virtually all currently available antibacterials. This pan-resistant phenotype is evident in the strain of *Providencia* described herein, as evidenced by resistance to all antibiotics except aztreonam.

The sequence of pMR0211 further demonstrates the remarkable mobility of the bla_{NDM-1} gene and the ever-expanding number of plasmid backbones associated with its carriage. The plasmid backbone shares extensive homology (>98%) with pAR060302, a plasmid isolated from a dairy calf in Illinois (4), and is a distinct component of a larger lineage of plasmids that are distributed among *E. coli*, *Yersinia pestis*, *Y. ruckeri*, *Photobacterium damselae*, and *Salmonella enterica* (4). pAR060302 contains multiple insertion sequences and transposons, harbors an array of antibiotic resistance genes, and is readily transferable to *E. coli* recipient cells, all characteristics that are shared by pMR0211. In pMR0211, the plasmid backbone has retained two of the four antibiotic resistance regions, the *floR* region and the *aadA* region identified by Call and coworkers (4), and it carries 22 putative transposons and associated insertion sequences. pMR0211 contains three other regions that mark its divergence from pAR060302. The first is a 5.9-kb region containing genes encoding a chloramphenicol

TABLE 2 Open reading frames present in pMR0211 but absent from pAR060302 and pNDM-HK

pMR0211 locus	Function[a]	Position		Direction[b]
		Start	End	
0100	Transposase, *insB4*	113534	114250	+
0101	Integrase, *intI1*	114284	114679	−
0102	Chloramphenicol resistance gene, *cmlA7*	116271	117578	+
0103	Beta-lactamase OXA-10 precursor, bla_{OXA-10}	117843	118643	+
0104	Aminoglycoside-modifying enzyme, *aadA*	118660	119451	+
0124	3′-Aminoglycoside phosphotransferase type VI, *aac*(6′)	141819	142598	−
0125	Transposase, *insB*	142704	143444	−
0126	Transposase IS*3*/IS*911* family	143531	143848	−
0127	Transposase, *iscR1*	145261	146802	−
0128	Protein of unknown function, DUF1696	147246	147620	−
0129	U32 family peptidase (related to collagenase *prtC*)	147631	148410	−
0130	No known homology	148420	149265	+
0131	No known homology	149954	150109	−
0132	Quinolone resistance gene, *qnrA1*	151427	152083	−
0133	Transposase, *iscR1*	152482	154023	−
0134	Sulfonamide resistance gene, *sul1*	154428	155354	−
0135	Aminoglycoside-modifying enzyme, *aadA*	155777	156571	−
0136	Transposase, *insB3*	157351	158067	−

[a] Functional categories were assigned as described in Results.
[b] Gene orientation is relative to that of the *repA* sequence: +, 5′ to 3′; −, 3′ to 5′.

acetyltransferase (*cmlA7*), a class D carbapenemase (bla_{OXA-10}), and an aminoglycoside resistance protein (*aadA1*) flanked by two transposons. BLAST analysis of this sequence reveals that it is composed of two overlapping sequences with 100% homology to regions in the *K. pneumoniae* subsp. *pneumoniae* plasmid pKPN5; a class 1 integron in *Salmonella enterica*, *P. aeruginosa*, and *A. veronii*; *E. coli* plasmid R751; and in the genome of *A. baumannii* AYE. The second region is a 12.7-kb insertion that shares almost 100% homology to a region in pNDM-HK and harbors the class B metallo-β-lactamase bla_{NDM-1}, the 16S methylase *armA*, the macrolide resistance genes *mel* and *mph*, and the sulfonamide resistance gene *sul1*. The region has a 3-kb deletion in pMR0211 compared to pNDM-HK that removes the bla_{DHA-1}, *ampR*, and *hypA* loci and part of the *qacΔ1* gene. The final region encompasses a 17.3-kb insert with some notable features. In addition to carrying the quinolone resistance gene *qnrA*, the region also contains genes encoding two aminoglycoside-modifying enzymes, *aadA* and *aac*(6′), and an additional copy of *sul1* (bringing the total copy number of this gene to 3 in the entire plasmid). Two additional loci, pMR0211_0130 and pMR0211_0131, have no known homology to any sequence in GenBank. Interestingly, a BLAST analysis of this region indicates that the sequence with no known homology has been inserted into mobile elements associated with other bacterial species, including plasmids pKP96 and pNICED61 from *K. pneumoniae* and *Vibrio fluvialis*, respectively, a class I integron associated with plasmids in *C. freundii*, *K. pneumoniae*, *Enterobacter cloacae*, *E. coli*, *Aeromonas punctate*, and *P. mirabilis* and with a chromosomal region in *A. baumannii* AYE.

To date (August 2011), the sequences of two bla_{NDM-1}-carrying plasmids have been submitted to GenBank: an 88.8-kb plasmid, designated pNDM-HK, isolated from a strain of *E. coli* in Hong Kong (HQ451074) (12), and p271A, a 35.9-kb plasmid isolated from *E. coli* (JF785549). Both sequences show that the bla_{NDM-1} gene is associated with insertion sequences and transposons, although the plasmid backbone and structure of the mobile elements associated with bla_{NDM-1} vary. Similarly, the sequencing of the regions surrounding bla_{NDM-1} in *E. coli* DVR22 (JF922606) and *Acinetobacter baumannii* strain 161/07 (HQ857107) revealed the presence of multiple insertion sequences and transposons. These structural features are evident in pMR0211 also, with five different transposons and insertion sequences surrounding the region containing the bla_{NDM-1} gene.

In recent years, plasmids belonging to the IncA/C incompatibility group have received increased attention, primarily due to their broad host range and ability to confer resistance to a diverse group of antimicrobial agents (9). They have been identified in numerous bacterial species, and sequence analysis of these plasmids has revealed that they share considerable homology (4, 10, 24). Interestingly, the IncA/C plasmids are increasingly associated with bla_{NDM-1} carriage (9, 11, 23), and a recent study has shown that plasmids carrying bla_{NDM-1} among *Enterobacteriaceae* isolated from New Delhi all were from incompatibility group A/C and were approximately 140 kb in size (23). The sequencing of these plasmids would provide valuable information on the epidemiology of incompatibility group A/C plasmids in this region and their association with *Enterobacteriaceae*.

In conclusion, pMR0211 represents a vivid example of a highly mobile megaplasmid that encodes an extensive arsenal of antibiotic resistance genes. Like other plasmids isolated in this geographical region (13, 23), it belongs to the IncA/C family, further highlighting the role of these plasmids in the dissemination of bla_{NDM-1}. These plasmids present a serious and growing challenge to health care workers and patients throughout the world.

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