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Bacterial Peritonitis Due to *Acinetobacter baumannii* Sequence Type 25 with Plasmid-Borne New Delhi Metallo-β-Lactamase in Honduras

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A carbapenem-resistant *Acinetobacter baumannii* strain was isolated from the peritoneal fluid of a patient with complicated intra-abdominal infection and evaluated at the Multidrug-resistant Organism Repository and Surveillance Network by whole-genome sequencing and real-time PCR. The isolate was sequence type 25 and susceptible to colistin and minocycline, with low MICs of tigecycline. bla_{NDM-1} was located on a plasmid with >99% homology to pNDM-BJ02. The isolate carried numerous other antibiotic resistance genes, including the 16S methylase gene, *armA*.

Carbapenem-resistant Gram-negative organisms are emerging pathogens that threaten global public health and seriously challenge infection control and therapy efforts (1–3). Though still relatively uncommon, the carbapenemase encoded by bla_{NDM} has quickly established itself as one of the most important (4). First described in 2009 (5), the gene has since been identified in nearly every continent and has been detected in species as diverse as *Acinetobacter baumannii* and *Vibrio cholerae* (6–8). Furthermore, the gene has been associated with a diverse range of plasmids that often harbor multiple antibiotic resistance genes (9).

In 2012, the Multidrug-resistant Organism Repository and Surveillance Network (MRSN) (10) received a request from a Ministry of Health hospital in Tegucigalpa, Honduras, for assistance in identifying the genetic mechanism associated with an extensively drug-resistant (XDR) isolate of *A. baumannii*. The isolate was recovered from a 76-year-old male patient admitted for ongoing dialysis in the setting of dysfunctional hemodialysis access. On the second day of admission, a peritoneal catheter was placed and the patient was prescribed empirical ciprofloxacin (250 mg) and a single dose of cephalothin (2 g). Two days later, the patient developed a fever (38.2°C) and experienced right lower abdominal pain. Peritoneal fluid cultures revealed moderate growth of Gram-negative bacilli. The patient was prescribed ceftriaxone (2 g/day) followed by intravenous ciprofloxacin (200 mg every 12 h) and intraperitoneal ceftazidime (250 mg). The organism was identified as *A. baumannii* with resistance to the fluoroquinolones and β-lactams, including carbapenems. Prompt clinical improvement followed removal of the peritoneal dialysis catheter and the initiation of tigecycline (50 mg every 12 h). He was discharged 17 days later and completed a 10-day course of therapy with tigecycline.

The isolate was forwarded to the MRSN and tested for carbapenemase genes by real-time PCR (11). Identification and antibiotic susceptibility tests were performed on three automated systems: the Vitek 2 (bioMérieux, Inc., NC), the BD Phoenix (BD Diagnostics Systems, MD), and the Microscan Walk-Away (Siemens Healthcare Diagnostics Inc., IL). MICs of colistin, minocycline, polymyxin B, and tigecycline were determined by Etest; colistin susceptibility was also assayed using broth microdilution. Whole genome sequencing was performed using an Ion Torrent Personal Genome Machine (PGM) with 200-bp chemistry.

The isolate (MRSN 12227) was identified as *A. baumannii*. It was resistant to amikacin, ampicillin-sulbactam, aztreonam, cefepime, cefotaxime, ceftazidime, ceftriaxone, ciprofloxacin, gentamicin, imipenem, levofloxacin, meropenem, tetracycline, tobramycin, and trimethoprim-sulfamethoxazole using CLSI breakpoints (12). The isolate was susceptible to minocycline (MIC = 3 μg/ml) and to colistin by broth microdilution (MIC = 0.25 μg/ml) and Etest (MIC = 0.125 μg/ml). It had low MICs of tigecycline (1.5 μg/ml) and polymyxin B (0.25 μg/ml), which correlate with the successful treatment of the patient with tigecycline.

MRSN 12227 was assigned to sequence type 25 (ST25) (Pasteur scheme), an ST that has been sporadically identified in a number of countries, including The Netherlands, Turkey, Greece, Italy, Sweden, and Singapore, and is not associated with any clonal complex (13, 14). Though sporadic, ST25 appears to be a stable clone and has been implicated in clinical infections since 1985 (14).

Mean coverage depth for genome assembly was 129-fold. MRSN 12227 carried bla_{NDM-1} on a plasmid that shared >99% homology to pNDM-BJ02, a plasmid initially identified in a clinical isolate of *Acinetobacter lwoffii* in Beijing, China (15). Interestingly, we also identified this plasmid in a clinical isolate of *A. schindleri* recovered from a surveillance groin swab of a U.S. service member wounded in Afghanistan (16). This plasmid is unclassifiable by PCR-based replicon typing, has a novel plasmid backbone sequence, and carries genes that encode a type IV secretion system (T4SS) that facilitates horizontal transmission (15). The composition of the genetic region surrounding bla_{NDM} shares

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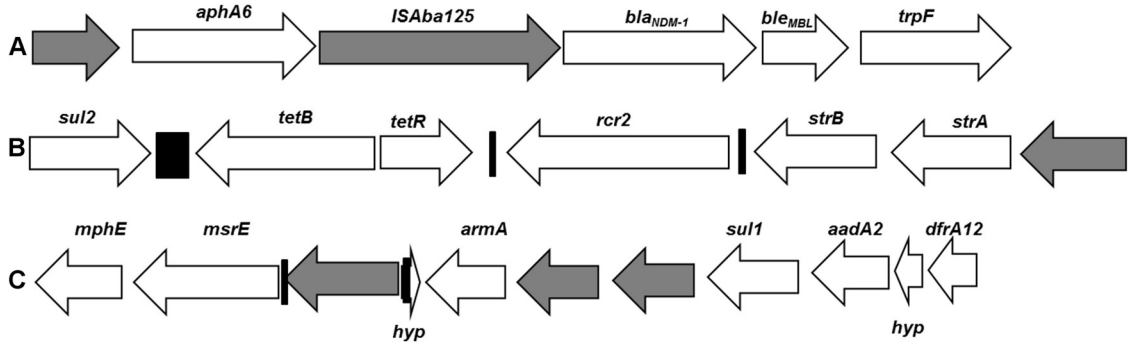

FIG 1 Composition of the genetic environment surrounding bla_{NDM-1} (A), the AbaR resistance island (B), and the 16S methylase gene *armA* (C). Arrows indicate gene orientation from 5′ to 3′. Relative gene sizes are proportional to the lengths of the arrows. White arrows indicate confirmed or putative functional genes with hypothetical genes, marked as *hyp*. Gray arrows indicate transposons, and black bars indicate repeat regions.

common elements with other plasmids carrying bla_{NDM} in *Enterobacteriaceae* (17), including the putative bleomycin resistance gene ble_{MDL}, the *N*-(5′-phosphoribosyl)anthranilate isomerase gene *trpF*, and the insertion sequence ISAba125 (Fig. 1). pNDM-BJ02 also carries a nonfunctional copy of *aphA6*, which normally encodes an aminoglycoside-modifying enzyme (AME) that confers resistance to amikacin. The same genetic architecture is apparent in the plasmid from MRSN 12227, with an upstream transposition event seemingly disrupting the promoter region of *aphA6*. However, unlike the *A. lwoffii* (15) and *A. schindleri* (16) isolates that also carried this plasmid, MRSN 12227 is resistant to amikacin. This discrepancy was resolved when further analysis of the MRSN 12227 genome using ResFinder (18) revealed a chromosomal copy of the 16S methylase gene, *armA*, which confers resistance to all aminoglycosides (19) (Table 1). pNDM-BJ02 has been reported to have a very high frequency of transfer (9.1×10^{-3} to 1.3×10^{-2} per donor cell) to *Escherichia coli* J53 Azi^r (15). Our experience with bla_{NDM}-carrying strains of *Acinetobacter* provides further evidence for the promiscuity of this plasmid. In both instances in which the MRSN has encountered bla_{NDM}-carrying strains of *Acinetobacter*, pNDM-BJ02 has been its vehicle of transmission.

MRSN 12227 also carries plasmid pCTXM360, which harbors the β-lactamase gene $bla_{CTX-M-15}$ (20), and has two additional chromosomal insertions carrying multiple antibiotic resistance genes (Table 1 and Fig. 1). The first of these carries six antibiotic resistance loci, including the aforementioned 16S methylase gene *armA* and the macrolide resistance genes *mphE* and *msrE* (Table 1

TABLE 1 Antibiotic resistance genes carried by MRSN 12227[a]

Gene[b]	Contig[c]	Location[d]	Function[e]	Notes
bla_{OXA-64}	1	Chromosome	Class D β-lactamase	bla_{OXA-51}-like
mphE	1	Chromosome	Macrolide phosphotransferase	Confers resistance to macrolides
msrE	1	Chromosome	Macrolide efflux protein	Confers resistance to macrolides
armA	1	Chromosome	16S methylase	Confers resistance to all aminoglycosides
sul1	1	Chromosome	Dihydropteroate synthase	Confers resistance to sulfonamides
ant(3″)-Ia	1	Chromosome	AME	*aadA2*; confers resistance to gentamicin and tobramycin
dfrA-12	1	Chromosome	Dihydrofolate reductase	Confers resistance to trimethoprim
ceoA-like	6	Chromosome	RND efflux pump	
ceoB	6	Chromosome	RND efflux pump	98% identity to CeoB (protein)[f]
opcM-like	6	Chromosome	RND efflux pump	
sul2	38	Chromosome	Dihydropteroate synthase	Confers resistance to sulfonamides
tetB	38	Chromosome	Efflux pump	Confers resistance to tetracycline
aph(6)-Id	38	Chromosome	AME	*strB*; confers resistance to streptomycin
aph(3″)-Ib	38	Chromosome	AME	*strA*; confers resistance to streptomycin
$bla_{CTX-M-15}$	44	pCTXM360	ESBL	Confers resistance to cephalosporins and monobactams
aph(3′)-VIa	50	pNDM-BJ02	AME	Nonfunctional
bla_{NDM-1}	50	pNDM-BJ02	Class B β-lactamase	Confers resistance to all β-lactams except aztreonam
aac(3)-IIa	56	Putative Plasmid	AME	Confers resistance to gentamicin and tobramycin
bla_{TEM-1}	59	Unknown	ESBL	3 synonymous SNPs[g]; resistance to early cephalosporins

[a] Abbreviations: AME, aminoglycoside-modifying enzyme; ESBL, extended-spectrum β-lactamases; SNP, single-nucleotide polymorphism; RND, resistance/nodulation/division.
[b] Based on closest match to BLAST (http://blast.ncbi.nlm.nih.gov) search.
[c] The MRSN 12227 whole-genome sequence was assembled into 95 contigs. The relative sizes of the reported contigs (in base pairs) are as follows: contig 1, 383,184; contig 6, 197,316; contig 38, 23,159; contig 44, 13,686; contig 50, 8,127; contig 56, 1,720; and contig 59, 1,375.
[d] Putative or confirmed location of the respective antibiotic resistance gene based on whole-genome sequencing.
[e] Confirmed or putative function of protein.
[f] Confers resistances to chloramphenicol, trimethoprim, and ciprofloxacin.
[g] bla_{TEM-1} contains 3 synonymous mutations compared to the reference bla_{TEM-1} sequence (26). pCTXM-360 contains bla_{TEM} in close proximity to $bla_{CTX-M-15}$, but the exact location of bla_{TEM-1} is still uncertain.

and Fig. 1). This structure has been identified on plasmids associated with *Klebsiella oxytoca* and *Citrobacter freundii* (GenBank accession numbers CP003684.1 and JX182975.1, respectively) and on the chromosome of *A. baumannii* MDR-TJ and TYTH-1 (21, 22). The second region also includes multiple antibiotic resistance genes, including the tetracycline efflux gene *tetB*, the streptomycin resistance genes *strA* and *strB*, and the dihydropteroate synthase gene *sul2*. This region most closely resembles the *Acinetobacter* resistance island AbaR1 (23), which is widely distributed in *Acinetobacter baumannii* strains. MRSN 12227 carries the chromosomally encoded $bla_{\text{OXA-51}}$-like gene $bla_{\text{OXA-64}}$ but does not carry any acquired class D carbapenemase genes, indicating that carbapenemase resistance in this strain is probably due to $bla_{\text{NDM-1}}$.

This is the first report of bla_{NDM} from Honduras, and it follows the recent identification of this gene in a strain of *Klebsiella pneumoniae* in neighboring Guatemala (24). The current epidemiological evidence from the hospital supports nosocomial transmission of MRSN 12227, but further investigations are ongoing. The modified Hodge test and the Carba NP tests were both negative for MRSN 12227, but this is not unexpected for *Acinetobacter* (25). Finally, pNDM-BJ02 appears to be emerging as a common vehicle for the horizontal transmission of bla_{NDM}, particularly in *Acinetobacter* species. *In vitro* evidence suggests that this plasmid is highly promiscuous, and further research is warranted to see if this plasmid is capable of being transmitted and stably maintained in other clinically important bacteria.

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