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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*<sub>NDM-1</sub> 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 relatively uncommon, the carbapenemase encoded by *bla*<sub>NDM</sub> 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 (500 mg every 12 h) and intraperitoneal ceftazidime (250 mg). 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 = 0.25 μg/ml) and to colistin by broth microdilution (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*<sub>NDM-1</sub> 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*<sub>NDM</sub> shares
common elements with other plasmids carrying \textit{bla}_{\text{NDM}} in \textit{Enterobacteriaceae} (17), including the putative bleomycin resistance gene \textit{ble}_{\text{MDL}}, the N-\textit{\textbf{5}}'-phosphoribosyl)anthranilate isomerase gene \textit{trpF}, and the insertion sequence IS\textit{Aba}125 (Fig. 1). p\textit{NDM-BJ02} also carries a nonfunctional copy of \textit{aphA6}, which normally encodes an aminoglycoside-modifying enzyme (AME) that confers resistance to amikacin. This discrepancy was resolved when further analysis of isolates that also carried this plasmid, MRSN 12227 is resistant to amikacin. However, unlike the \textit{A. lwoffii} (15) and \textit{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, \textit{armA}, which confers resistance to all aminoglycosides (19) (Table 1). p\textit{NDM-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 \textit{Escherichia coli} J53 Azr' (15). Our experience with \textit{bla}_{\text{NDM}}-carrying strains of \textit{Acinetobacter} provides further evidence for the promiscuity of this plasmid. In both instances in which the MRSN has encountered \textit{bla}_{\text{NDM}}-carrying strains of \textit{Acinetobacter}, p\textit{NDM-BJ02} has been its vehicle of transmission.

MRSN 12227 also carries plasmid p\textit{CTXM360}, which harbors the \textit{\beta}-lactamase gene \textit{bla}_{\text{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 \textit{armA} and the macrolide resistance genes \textit{mphE} and \textit{msrE} (Table 1).

\begin{table}[h]
\centering
\caption{Antibiotic resistance genes carried by MRSN 12227$^a$}
\begin{tabular}{lllll}
\hline
Gene$^b$ & Contig$^c$ & Location$^d$ & Function$^e$ & Notes \\
\hline
\textit{bla}_{\text{OXA-51}} & 1 & Chromosome & Class D \textit{\beta}-lactamase & \textit{bla}_{\text{OXA-51}}-like \\
\textit{mphE} & 1 & Chromosome & Macrolide phosphotransferase & Confers resistance to macrolides \\
\textit{msrE} & 1 & Chromosome & Macrolide efflux protein & Confers resistance to macrolides \\
\textit{armA} & 1 & Chromosome & 16S methylase & Confers resistance to all aminoglycosides \\
\textit{sal1} & 1 & Chromosome & Dihydropteroate synthase & Confers resistance to sulfonamides \\
\textit{ant(3')-Ia} & 1 & Chromosome & AME & \textit{aadA2}; confers resistance to gentamicin and tobramycin \\
\textit{dfrA-12} & 1 & Chromosome & Dihydrofolate reductase & Confers resistance to trimethoprim \\
\textit{ceoA}-like & 6 & Chromosome & RND efflux pump & 98\% identity to \textit{CeoB} (protein)$^f$ \\
\textit{ceoB} & 6 & Chromosome & RND efflux pump & \\
\textit{opcM}-like & 6 & Chromosome & RND efflux pump & \\
\textit{sal2} & 38 & Chromosome & Dihydropteroate synthase & Confers resistance to sulfonamides \\
\textit{tetB} & 38 & Chromosome & Efflux pump & Confers resistance to tetracycline \\
\textit{aph(6')-Ia} & 38 & Chromosome & AME & \textit{strB}; confers resistance to streptomycin \\
\textit{aph(3')-Ib} & 38 & Chromosome & AME & \textit{strA}; confers resistance to streptomycin \\
\textit{bla}_{\text{CTX-M-15}} & 44 & p\textit{CTXM360} & ESBL & Confers resistance to cephalosporins and monobactams \\
\textit{ap1(3')-Vla} & 50 & p\textit{NDM-BJ02} & AME & Nonfunctional \\
\textit{bla}_{\text{NDM-1}} & 50 & p\textit{NDM-BJ02} & Class B \textit{\beta}-lactamase & Confers resistance to all \textit{\beta}-lactams except aztreonam \\
\textit{aac(3)-IIa} & 56 & Putative Plasmid & AME & Confers resistance to gentamicin and tobramycin \\
\textit{bla}_{\text{TEM-1}} & 59 & Unknown & ESBL & 3 synonymous \textit{SNP}s; resistance to early cephalosporins \\
\hline
\end{tabular}
\footnotesize{$^a$ Abbreviations: AME, aminoglycoside-modifying enzyme; ESBL, extended-spectrum \textit{\beta}-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 46, 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 resistance to chloramphenicol, trimethoprim, and ciprofloxacin.}
\end{table}
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 blaCTX-M-51 gene in blader, but does not carry any acquired class D carbapenemase genes, indicating that carbapenemase resistance in this strain is probably due to blander.

This is the first report of blander 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, pNMD-BJ02 appears to be emerging as a common vehicle for the horizontal transmission of blander, 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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Material has been reviewed by the Walter Reed Army Institute of Research. There is no objection to its presentation. The opinions or assertions contained herein are the private views of the authors and are not to be construed as official, or reflecting the views of the Department of the Army or the Department of Defense.

REFERENCES