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Detection of New Delhi Metallo-β-Lactamase (Encoded by bla_{NDM-1}) in *Acinetobacter schindleri* during Routine Surveillance

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A carbapenem-resistant *Alcaligenes faecalis* strain was isolated from a surveillance swab of a service member injured in Afghanistan. The isolate was positive for bla_{NDM} by real-time PCR. Species identification was reevaluated on three identification systems but was inconclusive. Genome sequencing indicated that the closest relative was *Acinetobacter schindleri* and that bla_{NDM-1} was carried on a plasmid that shared >99% identity with one identified in an *Acinetobacter lwoffii* isolate. The isolate also carried a novel chromosomally encoded class D oxacillinase.

In response to global concerns over the spread of bla_{NDM} (1–3), the Multidrug-resistant Organism Repository and Surveillance Network (MRSN) implemented routine monthly screening for this gene in all carbapenem-resistant Gram-negative organisms in 2010 (4). From this surveillance initiative, we previously described the first incidence of bla_{NDM-1} in the U.S. military health care system from a strain of *Providencia stuartii*, isolated from a local Afghan national treated at a military facility in Afghanistan (5). Timely feedback was provided to the submitting facility, which resulted in increased surveillance and enhanced infection control policies.

In June 2012, following a blast injury involving shrapnel in Afghanistan, a 22-year-old male received cefazolin for routine prophylaxis and was evacuated to the United States via Germany. During that escalation of care, a routine groin surveillance swab revealed mixed microbial flora. An isolate identified as *Alcaligenes faecalis* using the BD Phoenix automated microbiology system (BD Diagnostics Systems, Sparks, MD) displayed resistance to all β-lactam antibiotics tested (intermediate to ceftriaxone), including the carbapenems and the monobactam aztreonam (Table 1). A modified Hodge test (MHT) was negative for meropenem. For ertapenem, the MHT was positive but the clover leaf-like growth indentation of *Escherichia coli* ATCC 25922 was significantly reduced when grown with the test isolate, designated MRSN 10319, compared to its growth alongside the bla_{KPC}-positive control strain *Klebsiella pneumoniae* ATCC BAA-1705. The isolate was forwarded to the MRSN, a College of American Pathologists (CAP)-certified laboratory, for further evaluation. The identification was reevaluated using three automated identification systems: the Vitek 2 (bioMérieux, Durham, NC), the BD Phoenix, and the Microscan WalkAway (Siemens Healthcare Diagnostics, Inc., Deerfield, IL). The MRSN employs the three most common automated instruments, as these instruments are used throughout the Military Health System (MHS), and discrepancies between their results have been noted (for a comprehensive review, see reference 6). Both Vitek 2 and Microscan identified the organism as *Acinetobacter lwoffi*, whereas the Phoenix was in agreement with the initial identification of *Alcaligenes faecalis*. 16S rRNA sequencing was performed (7) and indicated that the isolate shared 99% identity with *Acinetobacter schindleri*/*Acinetobacter johnsonii*.

TABLE 1 Antibiotic susceptibility profile of *A. schindleri* MRSN 10319

Antibiotic	MIC (μg/ml), phenotype[a]	Interpretation (μg/ml)[b]
Arbekacin	≤0.25[c]	NA
Amikacin	≤8, S	≤16, 32, ≥64
Ampicillin-sulbactam	>16/8, R	≤8/4, 16/8, ≥32/16
Aztreonam	>16[d]	NA
Cefepime	>16, R	≤8, 16, ≥32
Ceftazidime	>16, R	≤8, 16, ≥32
Ceftriaxone	32, I	≤8, 16–32, ≥64
Ciprofloxacin	≤0.5, S	≤1, 2, ≥4
Colistin	0.25,[e] S	≤2, —, ≥4
Gentamicin	≤1, S	≤4, 8, ≥16
Imipenem	>8, R	≤4, 8, ≥16
Levofloxacin	≤1, S	≤2, 4, ≥8
Meropenem	≥32, R[f]	≤4, 8, ≥16
Piperacillin-tazobactam	>64/4, R	≤16/4, 32/4–64/4, ≥128/4
Tetracycline	≤2, S	≤4, 8, ≥16
Tobramycin	≤1, S	≤4, 8, ≥16
Trimethoprim-sulfamethoxazole	≤0.5/9.5, S	≤2/38, —, ≥4/76

[a] MICs were determined using three automated systems (see the text), except for arbekacin, colistin, and meropenem. All results were consistent across the three instruments. The MICs and resulting interpretations are presented using the Phoenix output for clarity. R, resistant; I, intermediate; S, susceptible.
[b] As recommended by the Clinical and Laboratory Standards Institute (CLSI) (18). NA, no CLSI interpretative guidelines are available.
[c] MICs for arbekacin represent the average of three independent broth microdilution assays as described previously (19).
[d] No CLSI interpretive guidelines for aztreonam are available for *Acinetobacter* species.
[e] Average of three independent Etest assays performed as described by the manufacturer (bioMérieux). Etest results were consistent across replicates.
[f] As meropenem is not reported by the BD Phoenix Automated Microbiology System, MICs were performed in triplicate by Etest as described by the manufacturer (bioMérieux). No variation in Etest results was evidenced.

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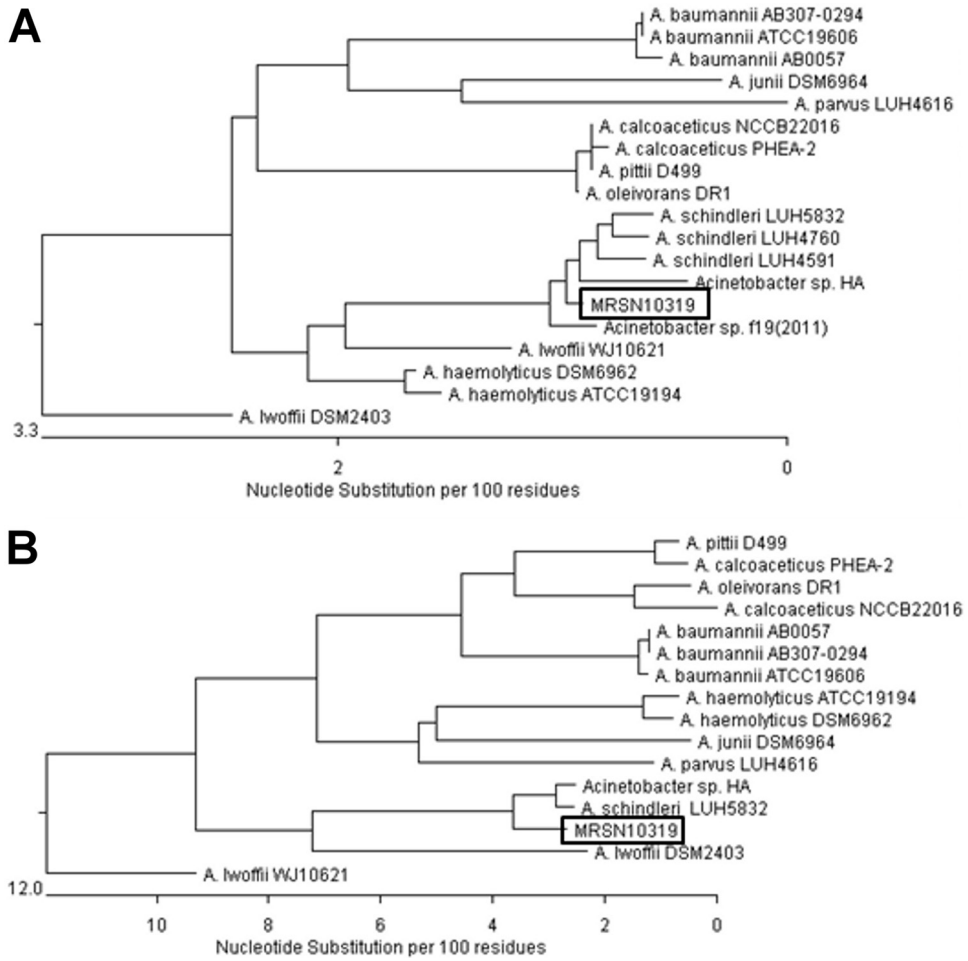

FIG 1 Dendrograms showing the relationship between *A. schindleri* MRSN 10319 and other *Acinetobacter* species based on (A) 16S rRNA and (B) *rpoB* gene sequences. *Acinetobacter* species 16S rRNA and *rpoB* gene sequences were retrieved from GenBank (http://www.ncbi.nlm.nih.gov/pubmed; last accessed 23 August 2012) and aligned using MegAlign (DNASTAR, Madison, MI). Dendrograms based on the numbers of nucleotide changes were generated using the MegAlign program (DNASTAR).

Whole-genome sequencing (WGS) using an Ion Torrent PGM (Ion Torrent Systems, Inc., Guilford, CT), which provided 87× coverage of the 16S rRNA gene, demonstrated 99.5% identity to *Acinetobacter schindleri* 16S rRNA sequences deposited at GenBank (Fig. 1A) (8). Phylogenetic analysis using the *rpoB* gene sequence confirmed this identification (Fig. 1B).

MRSN 10319 was tested by real-time PCR for carbapenemase genes (9) and was positive for bla_{NDM}. The gene was found to contain a single-nucleotide polymorphism (guanine to adenine) at position 468 compared to bla_{NDM-1}, resulting in a synonymous mutation. The gene was located on a 47.3-kb plasmid that shared >99% identity with pNDM-BJ02, a plasmid identified in an isolate of *Acinetobacter lwoffi* cultured from the urine of a 62-year-old female patient in Beijing in November 2010 (10). Hu and colleagues identified *A. lwoffi* by Vitek 2 but make no mention of the 16S rRNA sequence to verify this result. In accordance with the discrepancies noted in this report, Dortet and colleagues have noted that the Vitek 2 identifies rare species of *Acinetobacter*, including *A. schindleri*, as *A. lwoffi* (11).

Plasmid pNDM-BJ02 has 46 open reading frames, and there is no plasmid sequence in GenBank that shares more than 15% homology with it. Furthermore, the plasmid cannot be assigned to any of the described incompatibility groups using the PCR replicon typing method developed by Carattoli and colleagues (12). The plasmid harbors a type IV secretion system (T4SS) gene cluster and a single copy of *aphA6*, which encodes resistance to some aminoglycosides. However, MRSN 10319 was susceptible to all aminoglycosides tested (Table 1). Sequence comparison to pNDM-BJ02 suggests that the original promoter sequence for this gene has been disrupted by an upstream transposition event, as previously noted (10). Hu and colleagues demonstrated that the plasmid had a relatively high transfer frequency (9.1×10^{-3} to 1.3×10^{-2} per donor cell) to *E. coli* J53 Azi^r, suggesting that this plasmid has a high propensity for horizontal transmission (10).

Analysis of the chromosomal sequence revealed just a single locus with homology to known antibiotic resistance genes. This locus encoded a class D oxacillinase that shares its closest homology to the recently described $bla_{OXA-237}$ gene (13) but has 18 amino acid differences and represents a novel bla_{OXA} allele. There is no evidence (i.e., no transposons or insertion sequences) in the surrounding genetic environment to suggest horizontal acquisition of this gene. Based on the antibiotic profile of $bla_{OXA-237}$ (13)

and the lack of any other antibiotic resistance genes, including other β-lactams, aztreonam resistance in this strain is most likely due to this class D oxacillinase. A complete analysis of the MRSN 10319 genome is ongoing and will provide further information.

This report highlights the limitations of automated identification systems when working with unusual species. Commonly used clinical laboratory identification systems do not include *A. schindleri* or *A. johnsonii* on identification panels (manufacturer literature), which can lead to erroneous identification. Due to the high correlation between bla_{NDM} carriage and the *Enterobacteriaceae*, surveillance strategies for this gene have primarily focused on this group of bacteria. However, given the association of this gene with highly promiscuous plasmids, as well as documented horizontal dissemination of this gene, it is critical that surveillance efforts continue to test all carbapenem-resistant Gram-negative organisms for bla_{NDM}. A number of good techniques exist for detecting NDM-producing *Enterobacteriaceae* (14), but false-negative and weakly positive results have been observed in this family with the popular MHT (15, 16). Detection in *Acinetobacter* species remains a challenge due to the potential failure of many techniques, including the MHT and Etest MBL strip (17). Bonnin and colleagues have suggested that for carbapenem-resistant *Acinetobacter baumannii*, isolates should first be screened using EDTA inhibition-based techniques, followed by further PCR-based techniques in a reference laboratory (17). We suggest that the same method be applied to all other carbapenem-resistant *Acinetobacter* species isolated from clinical specimens.

Nucleotide sequence accession number. The novel bla_{OXA} locus described in this study has been assigned the designation bla_{OXA278} (http://www.lahey.org/studies/; last accessed March 2013), and the complete gene sequence has been deposited at GenBank (accession number KC771279).

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