Detection of New Delhi Metallo-β-Lactamase (blaNDM-1) in Acinetobacter schindleri during routine surveillance

Patrick McGann1*, Michael Milillo1, Robert J. Clifford1, Erik Snesrud1, Lindsay Stevenson2, Michael G. Backlund2, Helen B. Viscount2, Reyes Quintero1, Yoon I Kwak1, Michael J. Zapor3, Paige E Waterman1, Emil P Lesho1
1 Multidrug-resistant Organism Repository and Surveillance Network, Walter Reed Army Institute of Research, Silver Spring, Maryland
2 Department of Pathology, Walter Reed National Military Medical Center, Bethesda, Maryland
3 Infectious Diseases Service, Walter Reed National Military Medical Center, Bethesda, Maryland

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* Corresponding author
Patrick McGann, PhD
Multidrug-resistant Organism Repository and Surveillance Network
Walter Reed Army Institute of Research, Building 503, 2S35
Silver Spring, Maryland 20910, USA
Ph: (301) 319 9912
Fax (301) 319 9548
Email: patrick.mcgann@amedd.army.mil
Abstract. A carbapenem-resistant *Alcaligenes faecalis* was isolated from a surveillance swab of a service member injured in Afghanistan. The isolate was positive for *bla*<sub>NDM</sub> by real-time PCR. Species identification was re-evaluated on three identification systems, but was inconclusive. Genome sequencing indicated the closest relative was *Acinetobacter schindleri* and *bla*<sub>NDM-1</sub> was carried on a plasmid that shared >99% identity with one identified in an *Acinetobacter lwoffi*. The isolate also carried a novel chromosomally-encoded class D oxacillinase.
In response to global concerns over the spread of bla\textsubscript{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\textsubscript{NDM-1} in the US military healthcare system from a strain of \textit{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 U.S. via Germany. During that escalation of care, a routine groin surveillance swab revealed mixed microbial flora. An isolate identified as \textit{Alcaligenes faecalis} using the BD Phoenix Automated Microbiology System (BD Diagnostics Systems, Sparks, MD) displayed resistance to all tested \textit{B-lactam} antibiotics (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 \textit{E.coli} ATCC 25922 was significantly reduced when grown with the test isolate, designated MRSN 10319, compared to growth alongside the bla\textsubscript{KPC}-positive control strain \textit{Klebsiella pneumoniae} ATCC BAA-1705. The isolate was forwarded to the MRSN, a College of American Pathologists (CAP) certified laboratory, for further evaluation. Identification was re-evaluated on three automated identification systems: the VITEK 2 (bioMerieux, Durham, NC), the BD Phoenix, and the Microscan Walkway (Siemens Healthcare Diagnostics Inc, Deerfield, IL). The MRSN employs the three most common automated instruments as these instruments are used throughout the Military Healthcare System (MHS) and discrepancies between their results have been noted (for
a comprehensive review see (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 the isolate shared

99% identity with *Acinetobacter schindleri/Acinetobacter johnsonii*. Whole genome sequencing

(WGS) using an Ion Torrent PGM (Ion Torrent Systems, Inc., Guilford, CT), which provided

87X coverage of the 16S rRNA gene, demonstrated 99.5% identity to *Acinetobacter schindleri*

16S rRNA sequences deposited at GenBank (Figure 1A) (8). Phylogenetic analysis using the

*rpoB* gene sequence confirmed this identification (Figure 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 Carattolli 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
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 x 10\(^{-3}\) to 1.3 x 10\(^{-2}\) per donor cell) to *E. coli* J53 Azi\(^8\), 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 closest homology to the recently described *bla\(_{OXA-237}\)* gene (13), but has 18 amino acid differences and represents a novel *bla\(_{OXA}\)* allele. The final nomenclature for this gene is currently being assigned by the Lahey group ([www.lahey.org](http://www.lahey.org); last accessed March 2013) and the complete gene sequence has been deposited at GenBank (Accession number KC771279). 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 blaNDM. 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 A. 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.
Acknowledgements

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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 Defense.
References


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

<table>
<thead>
<tr>
<th>Antibiotic</th>
<th>MIC (µg/ml)(^1)</th>
<th>Interpretation (µg/ml)(^2)</th>
</tr>
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<tbody>
<tr>
<td>Arbekacin</td>
<td>≤0.25</td>
<td>NA</td>
</tr>
<tr>
<td>Amikacin</td>
<td>≤8, S</td>
<td>≤16, 32, ≥64</td>
</tr>
<tr>
<td>Ampicillin-sulbactam</td>
<td>&gt;16/8, R</td>
<td>≤8/4, 16/8, ≥32/16</td>
</tr>
<tr>
<td>Aztreonam</td>
<td>&gt;16(^4)</td>
<td>NA</td>
</tr>
<tr>
<td>Cefepime</td>
<td>&gt;16, R</td>
<td>≤8, 16, ≥32</td>
</tr>
<tr>
<td>Ceftazidime</td>
<td>&gt;16, R</td>
<td>≤8, 16, ≥32</td>
</tr>
<tr>
<td>Ceftriaxone</td>
<td>32, I</td>
<td>≤8, 16-32, ≥64</td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td>≤0.5, S</td>
<td>≤1, 2, ≥4</td>
</tr>
<tr>
<td>Colistin</td>
<td>0.25, S(^5)</td>
<td>≤2, &gt;4, ≥4</td>
</tr>
<tr>
<td>Gentamicin</td>
<td>≤1, S</td>
<td>≤4, 8, ≥16</td>
</tr>
<tr>
<td>Imipenem</td>
<td>&gt;8, R</td>
<td>≤4, 8, ≥16</td>
</tr>
<tr>
<td>Levofloxacin</td>
<td>≤1, S</td>
<td>≤2, 4, ≥8</td>
</tr>
<tr>
<td>Meropenem</td>
<td>≥32, R(^6)</td>
<td>≤4, 8, ≥16</td>
</tr>
<tr>
<td>Piperacillin-tazobactam</td>
<td>&gt;64/4, R</td>
<td>≤16/4, 32/4-64/4, ≥128/4</td>
</tr>
<tr>
<td>Tetracycline</td>
<td>≤2, S</td>
<td>≤4, 8, ≥16</td>
</tr>
<tr>
<td>Tobramycin</td>
<td>≤1, S</td>
<td>≤4, 8, ≥16</td>
</tr>
<tr>
<td>Trimethoprim-sulfamethoxazole</td>
<td>≤0.5/9.5, S</td>
<td>≤2/38, &gt;4/76</td>
</tr>
</tbody>
</table>

\(^1\) Determined using three automated systems (see text) except for arbekacin, colistin, ertapenem, and meropenem. All results were consistent across the three instruments. The MICs and resulting interpretations are presented using the Phoenix output for clarity.
As recommended by the Clinical and Laboratory Standards Institute (CLSI) (18). No CLSI interpretative guidelines are indicated by “NA”.

MICs for arbekacin represent the average of three independent microbroth dilution assays as described (19).

No CLSI interpretive guidelines of aztreonam are available for Acinetobacter species.

Average of three independent Etest assay performed as described by the manufacturer (bioMérieux). Etest results were consistent across replicates.

Ertapenem is not usually reported for Acinetobacter species due to intrinsic resistance but is included due to the rarity of susceptibility data from A. schindleri. 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). Meropenem MICs represents the average of three independent Etests performed as described by the manufacturer (bioMérieux). No variation in Etest results was evidenced.
Figure 1. Dendrogram 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
MegAlign (DNASTAR, Madison, MI). Dendrograms, based on the number of nucleotide
changes were generated using the MegAlign program (DNASTAR).