Detection of New Delhi metallo beta lactamase-1 (NDM-1) carbapenemase in *Pseudomonas aeruginosa* in a single centre in southern India

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**Background & objectives:** New Delhi metallo β-lactamase-1 (NDM-1) producing *Pseudomonas aeruginosa* isolates are potential threat to human health. This study was conducted to detect the presence of *bla*<sub>NDM-1</sub> in carbapenem resistant *P. aeruginosa* in a tertiary care center in southern India.

**Methods:** Sixty one carbapenem resistant clinical isolates of a total of 212 *P. aeruginosa* isolates cultured during the study period were screened for the presence of NDM-1 by PCR. Clinical characteristics of the NDM-1 positive isolates were studied and outcome of the patients was followed up.

**Results:** Of the 61 isolates, NDM-1 was detected in four isolates only. These were isolated from patients in the intensive care units and chest medicine ward. The source specimens were pus, sputum, bronchoalveolar lavage and endotracheal aspirate. The NDM-1 producers were susceptible only to polymyxin B. Only one patient responded to polymyxin B therapy, while the others succumbed to the infection.

**Conclusion:** These findings reveal that NDM-1 is not a major mechanism mediating carbapenem resistance in *P. aeruginosa* in this centre. However, continuous surveillance and screening are necessary to prevent their dissemination.

**Key words** India - NDM-1 - *Pseudomonas aeruginosa*

New Delhi metallo beta lactamase-1 (NDM-1), the recently discovered transferable molecular class B β-lactamase is a growing threat worldwide. First reported in 2008 in Sweden from a patient previously hospitalized in India, NDM-1-producing *Enterobacteriaceae* are now the focus of attention globally\(^{1-3}\). NDM-1-producing strains exhibit multidrug resistant profile because they harbour other genes that encode for resistance to aminoglycosides and fluoroquinolones\(^4\). Till date, reports of NDM-1 in *Pseudomonas aeruginosa* are scarce. There are only two reports of its occurrence in patients from Serbia and none of them had a history of travel to the Indian subcontinent\(^5,6\). Though reports are scarce and sporadic, knowledge of its prevalence

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is essential because *P. aeruginosa* is an environmental pathogen with intense colonization capacity and ability to persist for indefinite periods in the hospital environment. NDM-1 has not been reported in *P. aeruginosa* from any other part of the world. In the present study, we report the presence of NDM-1 in carbapenem resistant *P. aeruginosa* clinical isolates in a university teaching hospital in south India.

**Material & Methods**

The study was conducted in the department of Microbiology, Sri Ramachandra Medical College and Research Institute, a 1600 bedded university teaching hospital in Chennai, South India, from April to October 2010. During this period, a total of 212 clinical isolates of *P. aeruginosa* were collected, of which 61 were carbapenem resistant. All of these were from patients hospitalized for 48 h or more. These were cultured from clinical specimens such as blood (8), urine (21), exudative specimens (11) such as pus, wound swabs, body fluids including cerebrospinal fluid (CSF) and lower respiratory secretions (21) which included bronchoalveolar lavage (BAL), endotracheal aspirates (ETA) and sputum. The organisms were identified upto species level using Microscan Walk Away 96, Gram-negative panels (Siemens Healthcare Diagnostics Inc – Sacramento CA, USA). Commensals were differentiated from pathogens for isolates obtained from non-sterile sites (respiratory tract, urinary tract and wound swabs) by ascertaining their significance based on clinical history, presence of the organism in the Gram stain, presence of intracellular forms of the organism and pure growth in culture with significant colony count. The isolates from sputum and endotracheal aspirates were further verified for their clinical significance by correlation with other respiratory parameters and findings in the chest radiograph. The clinical characteristics of the study patients were noted from records and follow up was done till discharge /death.

The study protocol was approved by the institutional ethics committee.

**Antimicrobial susceptibility testing:** Susceptibility to various classes of antimicrobial agents was determined by disc diffusion method in accordance with Clinical Laboratory Standard Institute (CLSI) guidelines. The antibiotics tested were amikacin (30 µg), ciprofloxacin (5 µg), ceftazidime (30 µg), aztreonam (30 µg), piperacillin-tazobactam (100/10 µg), imipenem (10 µg), meropenem (10 µg), colistin (10 µg) and polymyxin B (300 units) (Hi-media Laboratories, Mumbai, India).

**Determination of minimum inhibitory concentration (MIC):** Minimum inhibitory concentrations of imipenem and meropenem were determined by agar dilution method using cation adjusted Mueller Hinton Agar (MHA) (Hi-media Laboratories, India). Pure forms of imipenem and meropenem (Sigma Aldrich, India) were used. The scheme for preparing dilutions of antimicrobial agents and the methodology was according to CLSI guidelines.

**Phenotypic tests:** Carbapenemase production was screened by the Modified Hodge test (MHT) and MBL production by inhibitor potentiated disk diffusion test with ethylene diamine tetra acetic acid (EDTA). Though CLSI does not advocate the use of MHT for the detection of carbapenemases production in *P. aeruginosa*, several investigators have found this test using meropenem as a useful screening test. For the isolates which were negative on initial testing by the above method, the test was repeated on Mueller-Hinton agar supplemented with zinc sulphate at a concentration of 70 mg/l.

**Polymerase chain reaction (PCR):** All study isolates were subjected to PCR using primers targeting *bla*<sub>NDM-1</sub>, *bla*<sub>VIM</sub> and *bla*<sub>IMP</sub>. Co-existence of other MBL encoding genes namely *bla*<sub>VIM</sub> and *bla*<sub>IMP</sub> were looked for using consensus primer. The primers used in the study are listed in Table I. For optimization of PCR, strains previously confirmed by PCR and gene sequencing were used as positive control and *P. aeruginosa* ATCC 27853 was used as negative control. All isolates were subjected to multiplex PCR for the detection of MBL genes: VIM, IMP, GIM, SIM and SPM.

**DNA sequencing:** PCR products of the isolates that carried the *bla*<sub>NDM-1</sub> gene were purified using the PCR DNA purification kit (QIA quick Gel Extraction Kit, Qiagen, Valencia, CA, USA) and subjected to automated DNA sequencing (ABI 3100, Genetic Analyser, Applied Biosystems, USA). The aligned sequences were analyzed with the Bioedit sequence program and similarity searches for the nucleotide sequences were performed with the BLAST program (http://www.ncbi.nlm.nih.gov).

**Results & Discussion**

Of the 61 isolates tested, *bla*<sub>NDM-1</sub> was found in four isolates only (MS 5639, MS 5641, MS 6196 and R 1780). The PCR screening results were validated by sequencing and the sequence of the *bla*<sub>NDM-1</sub> genes showed 100 per cent identity with...
previously reported genes (Figure). These four NDM-1 positive \textit{P. aeruginosa} were isolated from patients in multidisciplinary intensive care unit (2), neurosurgical intensive care unit (1) and chest medical ward (1). Both the phenotypic tests employed were positive in the NDM-1 producers. The source specimens were BAL, ETA, sputum and pus from post-operative laparotomy wound site. The isolate from pus also carried the \textit{blaVIM}.

\textit{blaNDM-1} was the only gene detected in the other three isolates. The NDM-1 producers were susceptible only to colistin and polymyxin B and resistant to amikacin, ciprofloxacin, aztreonam, piperacillin-tazobactam and ceftazidime. The clinical characteristics of the four patients are depicted in Table II.

Overall, among the study isolates, \textit{blaNDM-1} alone was detected in three, \textit{blaNDM-1} coexisted with \textit{blaVIM} in one isolate, \textit{blaVIM} alone was detected in 33 isolates and two isolates carried the \textit{blaIMP} gene alone. Further, \textit{blaSIM}, \textit{blaSPM} and \textit{blaGIM} were not detected in any of the study isolates. In 22 isolates none of the genes looked for, were detected.

\textit{NDM-1} was first identified in \textit{Enterobacteriaceae} and recently reported in \textit{Acinetobacter baumannii} also. Variants of \textit{NDM-1} namely \textit{NDM-2} to \textit{NDM-7} differing in single aminoacid sequences have also been described\textsuperscript{7,14}. Identification of \textit{blaNDM-1} in many genera and species of Gram-negative bacteria indicate that this gene can spread at a high rate\textsuperscript{2,17}. The genes encoding \textit{NDM} are heterogeneous on the basis of molecular size and location. In \textit{Enterobacteriaceae}, it is plasmid-borne, while in \textit{A. baumannii} both chromosomal and plasmid locations have been confirmed\textsuperscript{7,17}. At the Military Medical Academy in Serbia, routine analysis of carbapenemase producing bacterial isolates revealed NDM-1 in seven clinical isolates of \textit{P. aeruginosa}. The source patients were hospitalized in Serbia and had no history of travel to any other country\textsuperscript{5}. Subsequently, in 2012 recurrent pyelonephritis due to NDM-1 producing \textit{P. aeruginosa} was reported from France. This patient had history of prior hospitalization in Serbia and hence it was hypothesised that the Balkan States may be endemic for NDM-1\textsuperscript{6}.

This study was conducted to detect for the presence of \textit{blaNDM-1} in carbapenem resistant \textit{P. aeruginosa}. Considering that of the 61 carbapenem resistant \textit{P. aeruginosa} isolates tested, only four were found to harbour NDM-1, it can be reasonably assumed that NDM-1 is not a major mechanism mediating carbapenem resistance in \textit{P. aeruginosa} in this hospital. This being a single centre report, further studies are required at national or regional levels to understand the magnitude and prevalence of NDM-1 in \textit{P. aeruginosa}. Additionally, co-existence with other carbapenemase encoding genes was evident in a single isolate. On receipt of the microbiology culture report, all patients were initiated on polymyxin B therapy.

![Fig. PCR for detection of \textit{blaNDM-1}](image-url)

Lane 1: molecular mass marker (100bp DNA ladder); Lane 2: positive control; Lanes 3-6: \textit{blaNDM-1} positive isolates (amplicon size- 475bp); Lane 7: negative control.
Table II. Clinical characteristics of the patients infected with NDM-1 producing *P. aeruginosa*

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Patient -1</th>
<th>Patient -2</th>
<th>Patient -3</th>
<th>Patient -4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Isolate Number</td>
<td>MS 5639</td>
<td>MS 5641</td>
<td>MS 6196</td>
<td>R 1780</td>
</tr>
<tr>
<td>Age (yr)/sex</td>
<td>47, male</td>
<td>63, Female</td>
<td>51, Female</td>
<td>58, Male</td>
</tr>
<tr>
<td>Hospital location</td>
<td>ICU</td>
<td>ICU</td>
<td>ICU</td>
<td>ward</td>
</tr>
<tr>
<td>Admitting unit</td>
<td>Neurosurgery</td>
<td>Neurology</td>
<td>General surgery</td>
<td>Chest medicine</td>
</tr>
<tr>
<td>Underlying disease/diagnosis</td>
<td>Road traffic accident, ventilator associated pneumonia</td>
<td>Meningoencephalitis, ventilator associated pneumonia</td>
<td>Intestinal tuberculosis with obstruction and peritonitis, surgical site infection</td>
<td>Chronic obstructive pulmonary diseases, hospital acquired pneumonia</td>
</tr>
<tr>
<td>Co-morbid conditions</td>
<td>None</td>
<td>Diabetis mellitus, hypothyroidism</td>
<td>None</td>
<td>Corticosteroid therapy</td>
</tr>
<tr>
<td>Days in hospital</td>
<td>31 days</td>
<td>23 days</td>
<td>70 days</td>
<td>16 days</td>
</tr>
<tr>
<td>Surgical procedures, if any</td>
<td>Craniotomy, VP shunt</td>
<td>None</td>
<td>Laprotomy</td>
<td>None</td>
</tr>
<tr>
<td>Antimicrobials used prior to detection of NDM-1</td>
<td>Amikacin, metronidazole, piperclillin-tazobactam, impenem, vancomycin</td>
<td>Ceftriaxone, cefoperazone-sulbactam, amikacin</td>
<td>Amikacin, metronidazole, piperclillin-tazobactam, impenem, vancomycin, fluconazole,</td>
<td></td>
</tr>
<tr>
<td>Indwelling devices</td>
<td>Central line, peripheral venous line, urinary catheter</td>
<td>Peripheral venous line, urinary catheter</td>
<td>Peripheral venous line, drain from surgical site, urinary catheter</td>
<td>Peripheral venous line</td>
</tr>
<tr>
<td>Mechanical ventilation</td>
<td>Intubated</td>
<td>Intubated</td>
<td>Intubated</td>
<td>Nil</td>
</tr>
<tr>
<td>Outcome</td>
<td>Expired</td>
<td>Expired</td>
<td>Expired</td>
<td>Improved</td>
</tr>
<tr>
<td>Source specimen</td>
<td>Endotraechal secretion</td>
<td>Broncho alveolar lavage</td>
<td>Pus from post-operative laparotomy wound site</td>
<td>Sputum</td>
</tr>
<tr>
<td>MIC-IMP (mg/l)</td>
<td>32</td>
<td>32</td>
<td>32</td>
<td>128</td>
</tr>
<tr>
<td>MIC-MEM (mg/l)</td>
<td>64</td>
<td>64</td>
<td>32</td>
<td>128</td>
</tr>
<tr>
<td>MHT</td>
<td>Positive</td>
<td>Positive</td>
<td>Positive</td>
<td>Positive</td>
</tr>
<tr>
<td>MBL screen test</td>
<td>Positive</td>
<td>Positive</td>
<td>Positive</td>
<td>Positive</td>
</tr>
<tr>
<td>Carbapenemase</td>
<td>NDM-1</td>
<td>NDM-1</td>
<td>NDM-1 &amp; VIM</td>
<td>NDM-1</td>
</tr>
</tbody>
</table>

While one patient responded, the others succumbed to the infection. The lone survivor was followed up till discharge after recovery. Of the previously reported Serbian patients infected with NDM-1 *P. aeruginosa*, two expired and the one with pyelonephritis recovered on treatment with colistin\(^5,6\).

None of the MBL encoding genes looked for were detected in 22 isolates. The possible operative mechanisms in these isolates may be hyperproduction of Amp C or other beta lactamases, porin defect and/or upregulation of efflux pumps\(^8\). In this study, however, the presence of these mechanisms was not evaluated.

The ability of NDM-1 producing *P. aeruginosa* to survive under a wide range of environmental conditions and potential to spread in hospital settings make them unique. Though not as prevalent as other MBLs such as IMP and VIM, strict vigilance and continuous surveillance of NDM-1 is essential considering the difficulties in therapeutic management and control. In the present study, though NDM-1 was detected in only four of 61 clinical isolates of *P. aeruginosa* tested, these were associated with a high degree of mortality. Therefore, their identification is crucial for appropriate and early initiation of treatment and also to implement infection prevention measures directed to curtail their dissemination.
References


