Distribution of New Delhi metallo-beta-lactamase producing *Acinetobacter baumannii* in patients with ventilator associated respiratory tract infection

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**Abstract**

**Background and objectives:** Ventilator-associated respiratory tract infection (VARTI) is a major cause of morbidity and mortality among the critically ill patients of intensive care units (ICU). *Acinetobacter baumannii*, an important offending pathogen in VARTI, has been found to be resistant to several antibiotics including carbapenems. The present study was conducted to determine the rate of New Delhi metallo-β-lactamase 1 (NDM-1) producing *A. baumannii* causing VARTI among the patients admitted in an ICU of a large tertiary care hospital.

**Methods:** The study was conducted from July 2013 to June 2014. Endotracheal aspirates (ETA) were collected from patients with clinically suspected VARTI. Samples were collected from patients who were on mechanical ventilation for more than 48 hours. ETA samples were cultured aerobically and isolated *A. baumannii* were tested for susceptibility to carbapenem. Presence of NDM-1 encoded by the *bla*<sub>NDM-1</sub> gene was detected by polymerase chain reaction (PCR).

**Results:** A total of 138 VARTI cases were included in the study. Total 107 (77.5%) bacteria were isolated from 138 ETA samples of which 38 were *A. baumannii*. Out of 38 isolated *A. baumannii*, 35 (92.1%) were resistant to imipenem/meropenem and 33 (86.8%) were positive for *bla*<sub>NDM-1</sub> gene by PCR.

**Conclusion:** The present study demonstrated that high proportion of *A. baumannii* isolated from VARTI cases in ICU were carbapenem resistant and *bla*<sub>NDM-1</sub> positive. Careful infection control program should be considered to contain the spread of this multi-resistant organism to other hospital and community.


**Introduction**

Ventilator-associated respiratory tract infections (VARTI) in ICU patients include ventilator-associated pneumonia (VAP) and tracheobronchitis (VAT). The incidence of VAP and VAT in ICU patients ranges from 7% to 70% and 3% to 10% respectively [1-6]. Most cases of VAP are caused by bacterial pathogens that normally colonize upper respiratory tract and gastrointestinal tract of the patient. External sources like transmission from caregivers, environmental surfaces or other patients have been implicated. Detection of causative organisms and their antibiotic susceptibility is crucial for diagnosis and effective treatment of VAP [7].

Several Gram positive and negative organisms namely methicillin-resistant *Staphylococcus aureus* (MRSA), *Pseudomonas aeruginosa*, ESBL producing *Enterobacteriaceae* and multi-resistant *A. baumannii* have been isolated from cases of VARTI [8,9,4]. Besides in *Klebsiella pneumoniae* and *Escherichia coli*, metallo-β-lactamase (MBL) producing *bla*<sub>NDM-1</sub> gene conferring resistance to carbapenem has recently been identified in *A. baumannii* in different countries of the world [10-14]. In view of the above, the present study was conducted to determine the presence of *bla*<sub>NDM-1</sub> gene in *A. baumannii* isolated from ICU patients with VARTI of a tertiary care hospital in Dhaka city.

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Materials and methods

The study was carried out at the ICU of Dhaka Medical College Hospital, Dhaka from July, 2013 to June 2014. All patients suspected to have either VAP or VAT were included in the study. The study was approved by the Institutional Review Board of Dhaka Medical College.

Study population and sample collection: Patients at ICU on mechanical ventilator for more than 48 hours with suspected VAP and VAT were enrolled in the study. Criteria for suspected VAP include a presence of new and persistent (>48 hours) or progressive radiographic pulmonary infiltrate plus two of the following: temperature of >38°C or <36°C, blood leukocyte count of >10,000 cells/µl or <5,000 cells/µl, purulent tracheal secretions, and gas exchange degradation [5]. VAT was suspected in intubated patients with clinical signs of lower respiratory tract infection (such as fever, leukocytosis and purulent sputum), presence of bacteria within neutrophils in tracheal aspirate by Gram stain and growth of significant bacteria by semi-quantitative culture method in the absence of a new or progressive infiltrate on chest radiography [4].

Endotracheal tube aspirates (ETA) were collected from clinically suspected VAP and VAT cases by gently introducing a 50cm/14Fr suction catheter through the endotracheal tube for a distance of approximately 25-26 cm. The ETA was obtained by suction, without instilling saline. Two milliliters of sterile phosphate buffered saline (PBS) was injected into the lumen of the catheter with a sterile syringe to flush the exudates. The exudates were collected into a sterile 50 ml Falcon tube and transported immediately to the laboratory for further processing. Only one ETA sample was collected from each patient [15,16].

Isolation of A. baumannii and antibiotic susceptibility test: ETA was mechanically liquefied and homogenized by vortexing for one minute with glass beads (1-2 glass beads). After vortexing sample was centrifuged at 2000 rpm for 10 minutes. Supernatant was discarded using a sterile pipette and the deposit was further mixed by vortexing. The processed specimen was used for Gram staining and culture in recommended media. A. baumannii was identified by standard biochemical tests [17]. All isolated A. baumannii were tested for susceptibility to imipenem (10 µg), meropenem (10 µg), piperacillin-tazobactam (30 µg), ceftazidime (30 µg), ceftriaxone (30 µg), cefepime (30 µg), amoxicillin-clavulanic acid (20/10 µg), TMP-SMX (1.25/23.75 µg), ciprofloxacin (5 µg), gentamicin (10 µg), amikacin (30 µg) and colistin (10 µg) by disc diffusion technique [18,19]. The zone of inhibition around the antibiotic disc was measured after 18 hours of incubation of plates at 37°C. The zone of inhibition was interpreted as sensitive and resistant according to CLSI guideline [19]. Potency of the disks and antimicrobial agents were standardized using the reference strain E. coli ATCC 25922.

Detection of blaNDM-1 gene by PCR: The isolates were screened for the presence of blaNDM-1 MBL gene by PCR with the primers reported previously [20]. The sequence of the primers is shown in Table-1. In brief, PCR was performed in a final reaction volume of 25µl in a PCR tube, containing 10µl of master mix (mixture of dNTP, taq polymerase, MgCl₂ and PCR buffer), 4µl primers (Promega corporation, USA), 3 µl extracted DNA and 8µl of nuclease free water. PCR assay was performed in Eppendorf AG thermal cycler. After initial denaturation at 94°C for 10 minutes, the reaction was subjected to 36 cycles. Each cycle consisted of denaturation at 94°C for one minute, annealing at 60°C for one minute and elongation at 72°C for 90 seconds followed by final extension at 72°C for 10 minutes. The product was analyzed by electrophoresis in 1.5% agarose gel containing ethidium bromide (0.5 µg/ml) in TBE buffer (0.04 M Tris acetate, 0.001 M EDTA; pH 8.6) and photographed under UV illumination. DNA of known imipenem sensitive K. pneumonia was used as negative control.

### Table-1: The sequence of primers used for detection of blaNDM-1 gene in A. baumannii by PCR [20]

<table>
<thead>
<tr>
<th>Gene</th>
<th>Sequence</th>
<th>Product size</th>
</tr>
</thead>
<tbody>
<tr>
<td>blaNDM-1 F</td>
<td>GCGCAACACAGGCCTGACTTT</td>
<td>155</td>
</tr>
<tr>
<td>blaNDM-1 R</td>
<td>CAGCCTACCAAAAGCCGATGTC</td>
<td></td>
</tr>
</tbody>
</table>

Result

Total 138 VARTI cases were enrolled in the study of which 65 (47.1%) and 73 (52.9%) were VAP and VAT cases respectively. A total of 107
(77.5%) bacteria were isolated from ETA samples of which 38 were A. baumannii. Of the 38 isolates, 17 (26.2%) were isolated from VAP cases while 21 (28.8%) were from VAT cases. Antimicrobial susceptibility of A. baumannii to different antibiotics is shown in Table-2. The resistance to imipenem/meropenem, aminoglycosides, quinolones and third generation cephalosporins ranged from 92.1% to 100%. However, only 13.2% A. baumannii were resistant to colistin. PCR revealed presence of MBL blaNDM-1 gene in 33 (86.9%) out of 38 isolated A. baumannii (Table-3 and Fig-1). All of them were resistant to carbapenem.

**Table-2: Resistance pattern of A. baumannii to different antibiotics (n=38).**

<table>
<thead>
<tr>
<th>Antibiotic</th>
<th>Number resistant (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Meropenem</td>
<td>35 (92.1)</td>
</tr>
<tr>
<td>Imipenem</td>
<td>35 (92.1)</td>
</tr>
<tr>
<td>Ceftriaxone</td>
<td>35 (92.1)</td>
</tr>
<tr>
<td>Cefazidime</td>
<td>36 (94.7)</td>
</tr>
<tr>
<td>Cefepime</td>
<td>36 (94.7)</td>
</tr>
<tr>
<td>Piperacillin/Tazobactam</td>
<td>32 (84.2)</td>
</tr>
<tr>
<td>Amoxiclav</td>
<td>36 (94.7)</td>
</tr>
<tr>
<td>Gentamicin</td>
<td>38 (100)</td>
</tr>
<tr>
<td>Amikacin</td>
<td>37 (97.4)</td>
</tr>
<tr>
<td>Cotrimoxazole</td>
<td>37 (97.4)</td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td>37 (97.4)</td>
</tr>
<tr>
<td>Azithromycin</td>
<td>38 (100)</td>
</tr>
<tr>
<td>Colistin</td>
<td>5 (13.2)</td>
</tr>
</tbody>
</table>

**Fig.1:** PCR analysis of A. baumannii isolates from VARTI cases showing presence of 155 bp blaNDM-1 gene (Lane 2, 3, 5, 6, 7); Negative control (L1 and 8); L4: 100 bp DNA ladder.

**Table-3: Distribution of blaNDM-1 gene in A. baumannii (n=38).**

<table>
<thead>
<tr>
<th>Organism</th>
<th>Positive Number</th>
<th>Positive %</th>
<th>Negative Number</th>
<th>Negative %</th>
</tr>
</thead>
<tbody>
<tr>
<td>A. baumannii</td>
<td>33</td>
<td>86.9</td>
<td>5</td>
<td>13.16</td>
</tr>
</tbody>
</table>

**Discussion**

Infection by MBL producing organism containing blaNDM-1 gene are increasing in the last few years in Bangladesh [12,21,22]. In 2011, about 3.5% blaNDM-1 positive Escherichia coli, K. pneumoniae, A. baumannii, Providencia rettgeri and Citrobacter freundii were reported from Bangladesh [21]. In 2013, another study from Bangladesh, reported the presence of blaNDM-1 gene in 22% of the imipenem resistant A. baumannii [22]. However, the present study has revealed that over 86% of A. baumannii isolated from high risk ICU patients were positive for blaNDM-1 gene and were resistant to several groups of antibiotics apart from carbapenem. MBL containing organisms are usually sensitive to polymyxins and tigecycline [23]. In the present study, though majority (>90%) of our blaNDM-1 positive A. baumannii were resistant to several classes of antibiotics, but 86.9% of them were sensitive to colistin.

Therefore, the results of present study emphasize the necessity of strong infection control program and continuous monitoring of antibiotic susceptibility of offending organisms to contain the spread of multi-drug resistant blaNDM-1 positive A. baumannii in high risk areas of the hospitals. Also, strict and judicious use of effective antibiotic like colistin is necessary.

**Author’s contributions**

SA designed the study, performed the experiments and wrote the manuscript. SMS conceived, designed and supervised the study.

**Competing interest**

Authors declare no conflict of interest.

**Funding**

None
References


producing super-bugs in Bangladesh. 21st European Congress of Clinical Microbiology and Infectious Diseases (ECCMID) 27th International Congress of Chemotherapy (ICC). Milan, Italy May 2011.
