Original Article

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*$_{NDM-1}$ 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*$_{NDM-1}$ 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*$_{NDM-1}$ positive. Careful infection control program should be considered to contain the spread of this multi-resistant organism to other hospital and community.

IMC J Med Sci 2018; 12(1): 37-41. DOI: https://doi.org/10.3329/imcjms.v12i1.35177

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*$_{NDM-1}$ 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*$_{NDM-1}$ 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 48hours 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 $>38^0$C or $<36^0$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 37^0C.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 bla*$_{NDM-1}$ *gene by PCR: The isolates were screened for the presence of *bla*$_{NDM-1}$ 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, $MgCl_2$ 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 94^0C for 10 minutes, the reaction was subjected to 36 cycles. Each cycle consisted of denaturation at 94^0C for one minute, annealing at 60^0C for one minute and elongation at 72^0C for 90 seconds followed by final extension at 72^0C 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 bla*$_{NDM-1}$ gene in *A. baumannii by PCR [20]*

Gene	Sequence	Product size
bla$_{NDM-1}$ F	GCGCAACACAGCCTGACTTT	155
bla$_{NDM-1}$ R	CAGCCACCAAAAGCGATGTC	

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 *bla*NDM-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).*

Antibiotic	Number resistant (%)
Meropenem	35 (92.1)
Imipenem	35 (92.1)
Ceftriaxone	35 (92.1)
Ceftazidime	36 (94.7)
Cefepime	36 (94.7)
Piperacillin/Tazobactam	32 (84.2)
Amoxiclav	36 (94.7)
Gentamicin	38 (100)
Amikacin	37 (97.4)
Cotrimoxazole	37 (97.4)
Ciprofloxacin	37 (97.4)
Azithromycin	38 (100)
Colistin	5 (13.2)

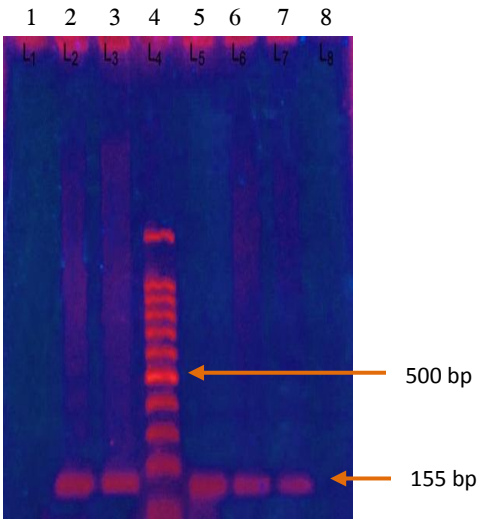

Fig.1: *PCR analysis of A. baumannii isolates from VARTI cases showing presence of 155 bp bla*NDM-1 *gene (Lane 2, 3, 5, 6, 7); Negative control (L1 and 8); L4: 100 bp DNA ladder.*

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

Organism	*bla*NDM-1			
	Positive		Negative	
	Number	%	Number	%
A. baumannii	33	86.9	5	13.16

Discussion

Infection by MBL producing organism containing *bla*NDM-1 gene are increasing in the last few years in Bangladesh [12,21,22]. In 2011, about 3.5% *bla*NDM-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 *bla*NDM-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 *bla*NDM-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 *bla*NDM-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 *bla*NDM-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

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