Original Article

Occurrence of New Delhi Metallobetalactamase mediated carbapenem resistance among *Acinetobacter* species in intensive care units

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Abstract

Back ground: Carbapenems are the main stay of therapy in the treatment of serious infections caused by Acinetobacter species. Emergence and spread of resistance to carbapenems has resulted in limiting the therapeutic options. Carbapenem resistance is most commonly mediated by production of carbapenemase. The rapidly disseminating New Delhi Metallobetalactamases (NDM), a class B MBL is also being detected in *Acinetobacter* species. This study was done to detect the presence *bla* $_{NDM-1}$ in *Acinetobacter species* in a tertiary care centre. **Materials and Methods**: One hundred and seventeen carbapenem resistant clinical isolates of a total of Acinetobacter species cultured during the study period were screened for the presence of NDM-1by PCR. Clinical characteristics of the NDM-1 positive isolates were studied and outcome was followed up. **Results**: Thirteen of 116 carbapenem resistant *A. baumannii* harboured the *bla* $_{NDM}$ gene. In 12 isolates, NDM was foundalong with either VIM or OXa Carbapenemase encoding genes. Only one isolate harboured *bla* $_{NDM}$ alone. All the 13 isolates exhibited a positive result with MHT and MBL screen test. Their MIC $_{90}$ to imipenem and meropenem were 32mg/l and 64 mg/l respectively. These NDM producers were isolated from specimens such as respiratory secretions (7), blood (5) and cerebrospinal fluid (1). The majority of the isolates were from the multidisciplinary ICU (9) of the hospital followed by 2 each in cardiothoracic and neurosurgery ICU. Of the 13 patients with NDM positive *A. baumannii* infections, 11 patients expired. The lone *A. lwoffii* harboured both *bla* $_{VIM}$ and *bla* $_{NDM}$. **Conclusion:** With limited therapeutic options, NDM mediated resistance in *Acinetobacter* species is a cause for concern in critically ill patients with life threatening infections. Adequate detection of NDM producing *Acinetobacter* is crucial for infection control measures and appropriate choice of antimicrobial therapy.

Keywords: Acinetobacter; Carbapenems; New Delhi Metallo betalactamase; outcome;

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INTRODUCTION

Acinetobacter species are aerobic gram-negative organism associated with infections such as ventilator associated pneumonia, bacteremia, meningitis, urinary tract and wound infections. The most important representative organism is *Acinetobacter baumannii* and the other species such as *Acinetobacter lwoffii, Acinetobacter johnsoni*, and *Acinetobacter junii* are rarely isolated from clinical specimens. Their ability to survive in the hospital environment make them a frequent cause of outbreaks of infection and an endemic health care associated pathogen.[1] Carbapenems are the main stay of therapy in the treatment of serious infections caused by

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Acinetobacter species. But subsequent emergence and spread of resistance to carbapenems has resulted in limiting the therapeutic options to tigecycline and polymyxins. Carbapenem resistance in *Acinetobacter* species is due to a variety of combined mechanisms such as hydrolysis by carbapenemases, alterations in outer membrane protein and penicillin binding proteins and increased activity of efflux pumps.[2,3] The most widespread carbapenemases in *Acinetobacter* species are the Class A Oxacillinases (OXA) and Class B Metallobetalactamases (MBL) namely the VIM and IMP types. All these carbapenemases are encountered either alone or in combination in single isolates of *Acinetobacter*[2]. In addition the rapidly disseminating New Delhi Metallobetalactamases (NDM), a class B MBL which was first reported in Enterobacteriaceae is also being detected in *Acinetobacter* species. The genes encoding NDM are present either on chromosomes or on transmissible plasmids.[4] With limited therapeutic options, NDM mediated resistance in *Acinetobacter* species is a cause for concern in critically ill patients with life threatening infections. Polymyxins (colistin and polymyxin B) and tigecycline are the last line of defence against these Gram negative pathogens. More recently, colistin-resistant and even pan-drug-resistant Acinetobacter are being reported [4, 5]. Adequate detection of NDM producing *Acinetobacter* is crucial for infection control measures and appropriate choice of antimicrobial therapy. This study was therefore undertaken to detect the presence of NDM in *Acinetobacter* species obtained from patients admitted to the intensive care units (ICU) in a tertiary care centre.

MATERIALS AND METHODS

The study protocol was approved by the Institutional ethics committee. The study was conducted in a 1600 bedded University teaching hospital between April and October 2010

Bacterial isolates

The study included 117 clinically significant, non-repetitive carbapenem resistant *Acinetobacter* species isolated from patients admitted to ICU. It included *Acinetobacter baumannii* (116) and *Acinetobacter lwoffii*[1]. Species identification was carried out by Microscan Walkaway 96 - using gram-negative panels (Siemens Health-care Diagnostics Inc. - Sacramento CA, USA). Acinetobacter baumannii were obtained from specimens such as respiratory secretions (62), exudative specimens[25], blood[25] and urine[4].The lone *Acinetobacter lwoffii* was isolated from blood. For isolates obtained from non-sterile sites (respiratory tract, urinary tract and wound swabs), differentiation between commensals and pathogens were done 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.

Antimicrobial susceptibility testing

Antimicrobial susceptibility testing was done by disc diffusion method. The antimicrobial agents tested were ceftazidime (30µg), cefepime (30µg), piperacillin-tazobactam (100/10µg), ciprofloxacin (5µg), amikacin (30µg), imipenem (10µg) and meropenem(10µg) (Hi-Media Laboratories, India). The results were interpreted as per CLSI 2014 guidelines.[6]. Susceptibility to tigecycline was performed using 15µg disc (BBL ™ BD, USA) and interpretation of zone of inhibition was done using the United States, Food and Drug Administration, tigecycline susceptibility breakpoints criteria.[7] Minimum inhibitory concentration (MIC) to imipenem and meropenem was determined by broth microdilution method and results interpreted according to CLSI document M100-S 24. MIC to colistin was determined by the E test (Biomerieux SA,France).[6]

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).[8]

Polymerase Chain Reaction (PCR)

All study isolates were subjected to PCR using primers targeting *bla* $_{NDM,}$ *bla* $_{VIM}$ and *bla* $_{IMP}$[9,10]. Multiplex PCR was done to detect the presence of all MBL encoding genes[11]. OXA carbapenemases were detected by multiplex PCR[12] Positive controls used were the strains previously confirmed by PCR and gene sequencing and negative control was *E. coli* ATCC 25922. The primers used in the study are shown in tables 1A, 1B, 1C

DNA sequencing

PCR products of representative isolates were purified using 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, Foster city, CA, USA).The aligned sequences were analyzed with the Bioedit sequence program and similarities searches for the nucleotide sequences were performed with the BLAST program (http://www.ncbi.nlm.nih.gov).

Clinical Data

Retrospectively the medical record of the study patients were analysed and follow up was done till discharge /death.

RESULTS

Antimicrobial susceptibility profile

All the *Acinetobacter baumannii* study isolates were resistant to amikacin, ciprofloxacin, ceftazidime, piperacillin-tazobactam, imipenem and meropenem. The MIC to imipenem and meropenem ranged from 8-128µg/ml. The MIC $_{50}$ and MIC $_{90}$ for imipenem were 16 µg /ml and 32 µg/ml respectively. For meropenem MIC $_{50}$ and MIC $_{90\ were}$ 32µg/ml and 64 µg /ml. Among the 116 isolates, 97.4% (113) were susceptible to colistin and 93.1% (108) to tigecycline. *Acinetobacter lwoffii* isolate from blood stream infection was also resistant to amikacin, ciprofloxacin, ceftazidime, piperacillin tazobactam, imipenem and meropenem. The MIC to imipenem and meropenem were 16 µg/ml and 64 µg/ml respectively. It was susceptible to both tigecycline and colistin

Phenotypic tests

Of 116 *A. baumannii,* the modified Hodge test was positive in 113(97.4%) of isolates. MBL screening test with EDTA was positive in 92(79.3%). Both the screen tests were positive in *A. lwoffii.*

PCR

Thirteen of 116 carbapenem resistant *A. baumannii* harboured the *bla* $_{NDM}$ gene. Amongst them, 6 also had the *bla* $_{VIM}$, *bla* $_{OXA\ -23}$ like and *bla* $_{OXA\ -51}$ like genes, while six others had *bla* $_{OXA-23}$ and *bla* $_{OXA-51}$ without VIM along with the *bla* $_{NDM.}$ One isolates harboured *bla* $_{NDM}$ alone. All the 13 isolates exhibited a positive result with MHT and MBL screen test. Their MIC $_{90}$ to imipenem and meropenem were 32mg/l and 64 mg/l respectively. These NDM producers were isolated from specimens such as respiratory secretions[7], blood[5] and cerebrospinal fluid[1]. One or more carbapenemase encoding gene was carried by 95.7% (111/116) of *A. baumannii*. MBL encoding genes were harboured by 63 isolates with *bla* $_{VIM}$ being the most common type. *Bla* $_{OXA-23}$ -like, *bla* $_{OXA-24}$ -like, *bla* $_{OXA-51}$ -like and *bla* $_{OXA}$-58 like were detected in the majority (106) of isolates. MBL and OXA coexisted in 58 isolates. The distribution of carbapenemase encoding genes in *Acinetobacter baumannii* is shown in table-2. The sensitivity of MHT and MBL screen test was 100%. None of the genes were found in five isolates. In three of these isolates both MHT and MBL screen test were negative and two isolates exhibited positive results. The majority of the isolates were from the multidisciplinary ICU (9) of the hospital followed by 2 each in cardiothoracic and neurosurgery ICU. Of the 13 patients with NDM positive *A. baumannii* infections, 11 patients expired. The clinical characteristics of the study patients with NDM positive *A. baumannii* infections is shown in table-3. The lone *A. lwoffii* isolated from a child with septicemia in cardiothoracic ICU harboured both *bla* $_{VIM}$ and *bla* $_{NDM.}$ MBL screen test and MHT were positive in the isolate. The clinical characteristics of this patients is also shown in table-3

Table 1 A: Primers used in the study

Primer	Primer sequence (5'-3')	Product size (bp)
Bla $_{NDM-1}$ - F	GGG CAG TCG CTT CCA ACG GT	475
Bla $_{NDM-1}$ –R	GTA GTG CTC AGT GTC GGC AT	
Bla $_{VIM}$-F	TTTGGTCGCATATCGCAACG	500
Bla $_{VIM}$-R	CCATTCAGCCAGATCGGCAT	
Bla $_{IMP}$-F	GTTTATGTTCATACWTCG	432
Bla $_{IMP}$-R	GGTTTAAYAAAACAACCAC	

Table 1 B: Multiplex PCR for MBL genes

Primer	Primer sequence (5'-3')	Product size (bp)
IMP family- F	GGA ATA GAG TGG CTT AAY TCT C	188
IMP family- R	CCA AAC YAC TAS GTT ATC T	
VIM family- F	GAT GGT GTT TGG TCG CAT A	390
VIM family- R	CGA ATG CGC AGC ACC AG	
GIM-1- F	TCG ACA CAC CTT GGT CTG AA	271
GIM-1- R	AAC TTC CAA CTT TGC CAT GC	
SPM-1-F	AAA ATC TGG GTA CGC AAA CG	477
SPM-1-R	ACA TTA TCC GCT GGA ACA GG	
SIM-1-F	TAC AAG GGA TTC GGC ATC G	570
SIM-1-R	TAA TGG CCT GTT CCC ATG TG	

Table 1C: Multiplex PCR for OXA carbapenemases

Primer	Primer sequence (5'-3')	Product size (bp)
Bla $_{OXA-23}$ like – F	GATCGGATTGGAGAACCAGA	501
Bla $_{OXA-23}$ like – R	ATTTCTGACCGCATTTCCAT	
Bla $_{OXA-51}$ like – F	TAATGCTTTGATCGGCCTTG	353
Bla $_{OXA-51}$ like – R	TGGATTGCACTTCATCTTGG	
Bla $_{OXA-24}$ like – F	GGTTAGTTGGCCCCCTTAAA	246
Bla $_{OXA-24}$ like – R	AGTTGAGCGAAAAGGGGATT	
Bla $_{OXA-58}$ like – F	AAGTATTGGGGCTTGTGCTG	599
Bla $_{OXA-58}$ like – R	CCCCTCTGCGCTCTACATAC	

Table 2: Distribution of Carbapenemase encoding genes in *Acinetobacter baumannii*

Carbapenemase encoding gene	No of isolates
OXA + MBL (58)	
OXA +NDM	6
OXA+VIM	45
OXA+VIM+NDM	6
OXA+VIM+IMP	1
MBL alone (5)	
VIM	4
NDM	1
OXA carbapenemases (OXA-23,24,51,58)	48
None	5

Table 3: Clinical Characteristics of the patients infected with NDM-1 producing Acinetobacter species

Isolate no and organism	Specimen type	Imp MIC µg/ml	Mem MIC µg/ml	Carbapenemase	No of days of hospital stay	Diagnosis	Antimicrobials used for treatment	Outcome
MS1833 *A.baumannii*	Blood	32	64	NDM, VIM, OXA-23, OXA_51	11	Sepsis	Linezolid, metronidazole, cefoperazone sulbactam, imipenem	Expired
MS2203 *A.baumannii*	Endotracheal secretion	8	32	NDM, VIM, OXA-23, OXA_51	59	Pneumonia, cardiac arrest	Linezolid, cefoperazone sulbactam, imipenem	Expired
MS2173 *A.baumannii*	Bronchial wash	16	32	NDM, VIM, OXA-23, OXA_51	10	Chronic renal failure	Levofloxacin, imipenem	Expired
MS2242 *A.baumannii*	Bronchial wash	32	128	NDM, VIM, OXA-23, OXA_51	12	Alcoholic liver disease, urosepsis	Amoxycillin clavulanate, imipenem	Expired
MS5048 *A.baumannii*	Blood	32	32	NDM	22	Sepsis, Chronic renal failure	Linezolid, cefoperazone sulbactam,	Expired
MS5360 *A.lwoffii*	Blood	16	64	NDM, VIM	30	Congenital heart disease, Pneumonia	Amoxycillin clavulanate, ceftriaxone, polymyxin B, amikacin	Improved
MS5660 *A.baumannii*	Blood	32	128	NDM, OXA-23, OXA-51	19	Road traffic accident fracture pelvis, sepsis	Linezolid, metronidazole, cefoperazone sulbactam, ciprofloxacin	Expired
MS5656 *A.baumannii*	Bronchial wash	16	64	NDM, OXA-23, OXA-51	50	Carcinoma rectum	Piperacillin tazobactam, meropenem, metronidazole, amikacin,	Expired
MS5679 *A.baumannii*	Bronchial wash	32	64	NDM, OXA-23, OXA-51	3	Pulmonary edema	Levofloxacin, cefoperazone sulbactam	Expired
MS5969 *A.baumannii*	Endotracheal secretion	64	128	NDM, OXA-23, OXA-51	39	Ventilator associated pneumonia, coronary heart disease	Piperacillin tazobactam, amikacin,	Expired
MS5980 *A.baumannii*	Cerebro spinal fluid	16	64	NDM, OXA-51	24	Road traffic accident fracture mandible	Piperacillin tazobactum, metronidazole, amikacin, Polymyxin -B	Improved
MS6215 *A.baumannii*	Endotracheal secretion	32	64	NDM, OXA-23, OXA-51	40	Road traffic accident fracture, subdural hemorrhage	Piperacillin tazobactam, metronidazole, amikacin, polymyxin B, tigecycline	Improved
MS6178 *A.baumannii*	Blood	64	64	VIM, NDM, OXA-51	32	sepsis, acute pancreatitis	Polymyxin B, metronidazole, amikacin, Piperacillin tazobactam vancomycin	Expired
MS4844 *A.baumannii*	Blood	32	16	NDM, OXA-23, OXA-51	5	sepsis, pyelonephritis	cefoperazone sulbactam, ciprofloxacin, Vancomycin	Expired

DISCUSSION

Although the most widespread carbapenemases in *A. baumannii* are the Class D OXA types and the MBLs namely VIM and IMP, more recently NDM is being reported from many countries.[3,4]. NDM-1 was first identified in *Enterobacteriaceae* and subsequently reported in *Acinetobacter baumannii*.[9,13]. The new NDM-2 variant was first detected in *A. baumannii* from a patient transferred from Egypt to Germany[14].Lat er a series of further variants differing in single amino acid sequences (designated NDM-3–NDM-10) have been reported.[4,15,16]. NDM production in *A. baumannii* has serious implications since it is an important nosocomial pathogen. Noticeably, NDM-1-producing strains harbour multiple plasmids and/or chromosomes that encode for resistance to other antimicrobial agents also which explains their multidrug resistance pattern. Most NDM-1 producers remain susceptible only to tigecycline and colistin.[13,17]. In the present study, 13 of 116 (11.2%) carbapenem resistant *A. baumannii* harboured the *bla* NDM

gene. In India, three NDM positive A. *baumannii* were reported from a hospital in Chennai. In contrast to our study, a high prevalence of 32% (20/62) was reported from Pune[18,19]. Later there were several published reports of NDM in *A. baumannii* and other species namely *A. lwoffìi, A.junii* and *A. pittii*[4,13]. In two studies from China, the prevalence of the *bla* $_{\text{NDM-1}}$ gene in *A. baumannii* and *A. pittii* were 0.18% (4/2109) and 0.86% (27/3114) respectively[19,20]. In the present study, in 12 isolates NDM coexisted with VIM or OXA carbapenamases. Only in one isolate NDM was found alone. Coexistence of NDM with other carbapenemase such as OXA has also been previously reported[18]. Clonal spread of NDM-2 producing *A. baumannii* strains have been described in a rehabilitation ward in Israel and in The United Arab Emirates[21,22]. Since the study isolates were from patients admitted during different time periods, this study did not ascertain the clonality of the NDM positive isolates. The NDM producers were susceptible only to colistin and tigecycline. Treatment with colistin was instituted in one patient and in another patient a combination of colistin and tigecycline was used. Both these patients survived, while the remaining 11 patients succumbed to the infection. *Acinetobacter lwoffii* isolate which produced both NDM and VIM was from the blood stream infection of a 4 years old child who underwent a cardiac surgery for closure of ventricular septal defect and was in the cardiac ICU on ventilator support. The child responded to treatment with colistin. While *bla* $_{\text{NDM}}$ has commonly been found on plasmids in Enterobacteriaceae, it is notable that there are only a few reports of plasmid-mediated NDM in *A. baumannii*[19], although diverse plasmids encoding NDM have been found in other species of *Acinetobacter*[20,23]. In all other reported isolates of *A. baumannii*, the *bla* $_{\text{NDM}}$ gene was located on the chromosome[13,14]. However, the present study did not focus on the location of the NDM gene. The occurrence of NDM genes were encountered only in the critically ill ICU patients in this study. Noticeably the mortality was also high among these patients. Transfer of patients in and out of the ICU may lead to their occurrence outside the ICU as well. The risk factors for acquisition of NDM producing Acinetobacter infections and their outcomes could not be assessed in the present study because most of the isolates harboured multiple carbapenemase encoding genes. Prospective case controlled study with adequate sample size and a primary objective to identify the risk factors for these infection are needed to have a clear understanding of this problem.

CONCLUSION

Though not as prevalent as other MBLs such as IMP and VIM, a strict vigilance and continuous surveillance of NDM is essential considering the difficulties in therapeutic management and control. Since acquisition of multidrug resistant *A. baumannii* is related to environmental contamination and colonisation in health care providers, control measures should address the source of infection. Continued careful attention to hand hygiene, contact isolation, barrier precautions, adequate environmental cleaning and careful disinfection of patient care equipments along with surveillance is essential to prevent outbreak of infections caused by these multidrug resistant strains

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