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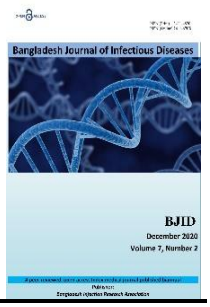

Status of Carbapenemase Encoding Genes among Imipenem Resistant *Acinetobacter baumannii* Isolated from Different Samples at a Tertiary Care Hospital in Bangladesh

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Abstract

Background: Nosocomial infections caused by carbapenem-resistant *Acinetobacter baumannii* are a global health problem. **Objectives:** The aim of this study was to investigate the molecular mechanisms of carbapenem resistance in *Acinetobacter baumannii* clinical isolates recovered from a tertiary care hospitals in Bangladesh. **Methodology:** This cross-sectional study was conducted in the Department of Microbiology of Dhaka Medical College and Hospital, Dhaka, Bangladesh over a period of one year from July 2015 to June 2016. *Acinetobacter baumannii* was isolated from different specimens and was identified and were screened for carbapenemase production using imipenem discs. Phenotypic identification of carbapenemase production was done by the double disc synergy (DDS) test, combined disc (CD) assay, and modified Hodge test (MHT). The minimum inhibitory concentration (MIC) of imipenem was determined by the agar dilution method. Genes encoding *bla*NDM-1, *bla*IMP, *bla*VIM and *bla*KPC were identified by polymerase chain reaction (PCR). **Results:** Twenty-one (80.8%) imipenem resistant *Acinetobacter baumannii* were detected among 26 isolates. Among 21 imipenem resistant *Acinetobacter baumannii*, 20(95.2%) carbapenemase producers were detected by PCR, 16(76.2%) by DDS test, 18 (85.7%) by CD assay and 5(23.8%) were detected by MHT. The *bla*NDM-1 gene was most prevalent 18 (85.7%) followed by *bla*VIM 14 (66.7%), *bla*IMP 8(38.1%) and *bla*KPC 5(23.8%). The minimum inhibitory concentration of imipenem of the imipenem resistant *Acinetobacter baumannii* ranged from ≥256 µg/ml to 8 µg/ml. **Conclusion:** This study shows a significant proportion (95.2%) of imipenem resistant *Acinetobacter baumannii* were carbapenemase producers. Genes encoding carbapenemase enzyme including *bla*NDM-1, *bla*VIM, *bla*IMP and *bla*KPC are responsible for imipenem resistance. [*Bangladesh Journal of Infectious Diseases, June 2025;12(1):27-33*]

Keywords: Carbapenemase; imipenem resistant; *Acinetobacter baumannii*

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Introduction

Acinetobacter baumannii is an important opportunistic pathogen and is often involved in various nosocomial infections, such as bacteremia, urinary tract infection, surgical site infection, and nosocomial and ventilator associated pneumonia, especially in patients admitted to ICU[1]. *Acinetobacter baumannii* is notable for its remarkable innate and acquired resistance to multiple antimicrobial classes, including extended-spectrum cephalosporins and carbapenems. Resistance to carbapenems is the most concerning, as carbapenems have a potent activity against *Acinetobacter* species and are often used as a last resort for the treatment of infections due to multidrug resistant *Acinetobacter baumannii* isolates[2-3].

Carbapenem resistance is mainly due to the expression of a carbapenemase enzyme, efflux pump, or porin loss. The most important and difficult mechanism is the production of the carbapenemase enzyme, because it is present on mobile genetic elements, which are easily transferable from one bacterium to another bacterium such as *Pseudomonas* species*, Acinetobacter* species*, Escherichia coli (E. coli), and Klebsiella* species, which the World Health Organization (WHO) has designated as high priority organisms in 2017[4-6].

The vast majority of acquired carbapenemases belong to three of the four known classes of β-lactamases, namely Ambler class A enzymes such as *Klebsiella pneumoniae* carbapenemase (KPC) types, Ambler class B enzymes, or metallo-β-lactamases such as VIM, IMP, NDM-1 types and Ambler class D enzymes or oxacillinases such as OXA-23, OXA-48, OXA-181 types[7-8].

Misuse and overuse of antibiotics is extremely common in Bangladesh due to lack of implication of proper guideline regarding the use of antibiotics. Selective pressure of antibiotics in Bangladeshi hospitals allows the preservation of MDR determinants not only in nosocomial pathogens but also in hospital environment[9-10].

Bangladesh is the prevalent zone of antimicrobial resistance, which has been evidenced by previous studies. Previous reports suggest *Acinetobacter baumannii* as one of the important nosocomial pathogens in Bangladesh and the resistance determinants are incredibly frequent among the species[11-14]. To explore the present situation of nosocomial antimicrobial resistance in Bangladesh, this study evaluated the genetic background of carbapenem resistance among the nosocomial *Acinetobacter baumannii* in Bangladesh.

Methodology

Study Settings and Population: This cross-sectional study was carried out at Department of Microbiology in Dhaka Medical College (DMC), Dhaka, Bangladesh over a period of one year which was from July 2015 to June 2016. Tracheal aspirate, blood, urine and wound swab samples were collected from all recruited patients for microscopy, culture and sensitivity testing. Samples were collected from patients of all age groups, both sexes, who were critically ill and suspected for pneumonia, urinary tract infection, septicaemia, skin and soft tissue infection.

Study Procedure: Samples were inoculated on Blood Agar and MacConkey Agar plates under strict aseptic conditions. Plates were incubated at 37°C for 24 to 48 hours.

Isolation of *Acinetobacter baumannii* and antibiotic susceptibility test: *Acinetobacter baumannii* was identified and confirmed by Gram staining as Gram negative coccobacilli or cocci in pairs, non-motile, oxidase negative, Alkaline/Alkaline (K/K) reaction in Triple Sugar Iron (TSI) slant, catalase positive, Indole negative, Citrate utilization test positive, urease test negative. It showed Oxidative–Fermentative (O/F) test – oxidative[15-17]. All isolated *A. baumannii* were tested for susceptibility to imipenem (10 µg) by disc diffusion method. Minimum inhibitory concentration (MIC) of imipenem was determined by agar dilution method[18-19].

Phenotypic Detection of Carbapenemase Producers: Carbapenemase producers were phenotypically detected by DDS test and CD assay for MBL producers and MHT was used for detection of all carbapenemase producers among the isolated imipenem resistant *Acinetobacter baumannii*[20-22].

Detection of carbapenemase encoding genes by Polymerase chain reaction (PCR): Carbapenepase encoding genes (*bla*KPC, *bla*VIM, *bla*IMP, *bla*NDM-1) were detected among imipenem resistant *Acinetobacter baumannii* by PCR with the primers reported previously[23-25]. 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 12.5µl of master mix (mixture of dNTP, taq polymerase, MgCl2 and PCR buffer), 2µl of forward primer, 2µl of reverse primer (Promega Corporation, USA), 2µl of DNA template and 6.5 µl of sterile distilled water. PCR assay was performed in Eppendorf AG thermal cycler. After initial denaturation at 94℃ for 10 minutes, the reaction was subjected to 36 cycles. Each cycle consisted of denaturation at 94℃ for 30 seconds, annealing at 52℃ for 40 seconds and elongation at 72℃ for 1 minute followed by final extension at 72℃ for 10 minutes. The amplified DNA were loaded into a 1.5% agarose gel, electrophoreses at 100 volts for 35 minutes, stained with 1% ethidium bromide, and visualized under UV light.

Table 1: Sequence of Primers used for Detection of Carbapenemase Encoding Genes in *Acinetobacter baumannii* by PCR[23-25]

Genes	Sequence (5´-3´)	bp
NDM1-F NDM1-R	GCGCAACACAGCCTGACTTT CAGCCACCAAAAGCGATGTC	155
VIM-F VIM-R	GATGGTGTTTGGTCGCATA CGAATGCGCAGCACCAG	390
IMP-F IMP-R	GGAATAGAGTGGCTTAATCTC CCAAACACTAGTTATCT	188
KPC-F KPC-R	CGTCTAGTTCTGCTGTCTTG CTTGTCATCCTTGTTAGGCG	Variable

Statistical Analysis: Statistical analysis was performed by Windows based software named as Statistical Package for Social Science (SPSS), versions 22.0 (IBM SPSS Statistics for Windows, Version 22.0. Armonk, NY: IBM Corp.). Continuous data were expressed as mean, standard deviation, minimum and maximum. Categorical data were summarized in terms of frequency counts and percentages. Chi-square test was used for comparison of categorical variables and Student t test was applied for continuous variables. Every efforts were made to obtain missing data. A two-sided P value of less than 0.05 was considered to indicate statistical significance. Differences between case and control were tested.

Ethical Consideration: All procedures of the present study were carried out in accordance with the principles for human investigations (i.e., Helsinki Declaration 2013) and also with the ethical guidelines of the Institutional research ethics. Formal ethics approval was granted by the local authority. Participants in the study were informed about the procedure and purpose of the study and confidentiality of information provided. All participants consented willingly to be a part of the study during the data collection periods. All data were collected anonymously and were analyzed using the coding.

Results

Of the total 300 samples, 26(8.7%) *Acinetobacter baumannii* were isolated. Out of 26(8.7%) *Acinetobacter baumannii*, 21(80.8%) imipenem resistant strains were detected by disc diffusion method. Of them, 2(7.7%) were isolated from wound swab, 18(69.2%) from ETA and one (3.8%) from blood samples. No imipenem resistant *Acinetobacter baumannii* was isolated from urine samples (Table 2). MIC of imipenem among 21 imipenem resistant *Acinetobacter baumannii* ranged from ≥256 µg/ml to 8 µg/ml.

Table 2: Distribution of Imipenem Resistant *Acinetobacter baumannii* Isolated From Different Samples (N=26)

Type of samples	Frequency	Percent
Wound swab	2	7.7
Urine	0	0.0
Endotracheal aspirate	18	69.2
Blood	1	3.8
Total	**21**	**80.8**

Among 21 imipenem resistant *Acinetobacter baumannii*, 16(76.2%) carbapenemase producers were detected by DDS test, 18(85.7%) by CD assay, 5(23.8%) by MHT and 20(95.2%) carbapenemase producers were detected by PCR (Table 3).

Table 3: Detection of Carbapenemase Producers Among Imipenem Resistant *Acinetobacter baumannii* by Phenotypic Methods and PCR (N=21)

Method	Positive	Negative
DDS test	16 (76.1%)	5 (23.8%)
CD assay	18 (85.7%)	3 (14.3%)
MHT	5 (23.8%)	16 (76.2%)
PCR	20 (95.2%)	1 (4.8%)

Out of 21 imipenem resistant strains, 18(85.7%) were positive for $bla_{\text{NDM-1}}$, 14(66.7%) for bla_{VIM}, 8(38.1%) for bla_{IMP} and 5(23.8%) were positive for bla_{KPC}. Most of the genes were isolated from endotracheal aspirate (Table 4 and Figure I).

Table 4: Carbapenemase Encoding Genes Among Imipenem Resistant *Acinetobacter baumannii* by PCR Isolated from Different Samples (N=21)

Type of Samples	NDM-1	VIM	IMP	KPC
Wound swab (N=2)	2(9.5%)	1 (4.8%)	2 (9.5%)	0 (0.0%)
Urine (N=0)	0(0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)
Endotracheal aspirate (N=18)	15(71.4%)	13 (61.9%)	5 (23.8%)	5 (23.8%)
Blood (N=1)	1(4.8%)	0 (0.0%)	1 (4.8%)	0 (0.0%)
Total (N=21)	**18(85.7%)**	**14 (66.7%)**	**8 (38.1%)**	**5 (23.8%)**

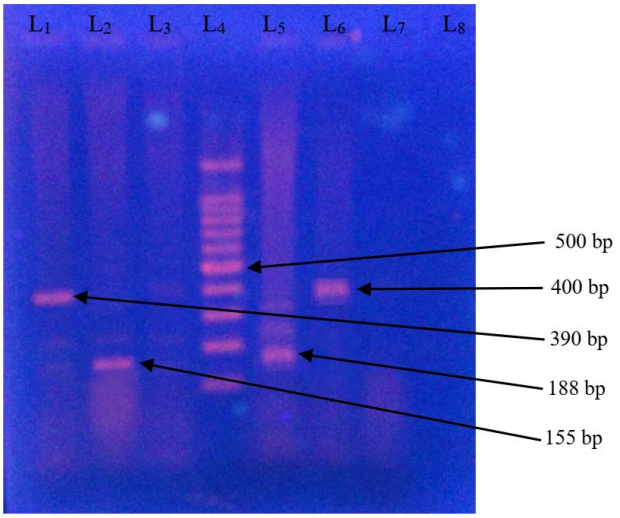

Figure I: Photograph of gel electrophoresis of amplified DNA of 390 bp for *bla*VIM gene (Lane 1), DNA of 155 bp for *bla*NDM-1 gene (Lane 2), Hundred bp DNA ladder (Lane 4), DNA of 188 bp for *bla*IMP gene (Lane 5), DNA of 400 bp for *bla*KPC gene (Lane 6). Negative control without DNA (Lane 3 and 7).

Discussion

During the last decades, *Acinetobacter baumannii* has emerged globally as an important nosocomial pathogen that gives rise to outbreaks of colonization and infection in critically ill, hospitalized patients[26].One of the main problems facing by hospitals, clinicians and health care personnel in regards to *Acinetobacter baumannii* is multidrug resistance. Continuous monitoring and rapid detection of this virulent organism may check their spread and play a vital role in infection control. To address this rising resistant determinant, this study has been observed the occurrence of carbapenemase encoding genes among imipenem-resistant *Acinetobacter baumannii.*

In this study, most (80.8%) of the *Acinetobacter baumannii* are resistant to imipenem. High resistance rate for *Acinetobacter baumannii* strains to imipenem has been reported by Joshi et al[27]. which is 29.0%. Another study in India has been showed 59.0% meropenem resistance among *Acinetobacter species* which reflect the evolving scenario in India[28]. In a study, Lone et al[29] has been demonstrated that 98.5% of the *Acinetobacter baumannii* are sensitive to imipenem. In Bangladesh, studies have been showed 88.0% and 92.1% of the *Acinetobacter* are imipenem resistant[30-31]. Since last 10 years, acquired resistance to imipenem has been increasingly reported worldwide in non-fermenting gram-negative bacilli (NFGNB) including *Acinetobacter* species[32]. High antibiotic pressure due to indiscriminate use of carbapenems could have resulted in the increase in carbapenem resistant *Acinetobacter* isolates[28].

In the present study, among the 21-imipenem resistant *Acinetobacter baumannii*, 16(76.2%) carbapenemase producers are detected by DDS test, 18(85.7%) by CD assay and 5(23.8%) by MHT. In a previous study, 24.4% and 25.4% carbapenemase producers have been detected by DDS test and CD assay[33]. Since evaluation of phenotypic methods have been described in several studies from the discovery of MBLs producers[20-21,34], no method gave satisfactory results to detect all the carbapenemase producers. So, from this point it can be concluded that PCR is the most reliable and acceptable method for early and accurate identification of carbapenemase producers.

In this study, 20 (95.2%) carbapenemase producers are detected by PCR out of 21 imipenem resistant *Acinetobacter baumannii*. A study in Bangladesh has been revealed 100.0% MBL producers among the imipenem resistant *Acinetobacter* species[11]. The findings of the present study reveal that the prevalence of MBL producing *Acinetobacter* species is increasing in Bangladesh.

This study observes 18(85.7%) NDM-1 positive isolates detected by PCR among 21 imipenem resistant *Acinetobacter baumannii*. Previous studies in Bangladesh have been demonstrated 92.0% and

88.8% NDM-1 positive strain among the imipenem resistant *Acinetobacter baumannii* which are almost similar to the present study[30-31]. A study by Kumarasamy et al[35] has been reported NDM-1 producing bacteria from India, Pakistan and the UK. NDM-1 producers have also been detected from Europe, Australia and the USA[36-38]. Interestingly, most of the cases from Europe, Australia and the USA have a history of recent travel or hospital admission in the Indian subcontinent. *bla*$_{NDM-1}$ containing organisms are now alarmingly rising worldwide and pose therapeutic failure[35-36]. Another study in Bangladesh have been demonstrated 3.5% and 22.9% NDM-1 producers among the imipenem resistant organisms[11,13]. The present study have found high proportion of NDM-1 producers in Bangladesh than the previous study. The increasing percentage of this new resistance mechanism is due to healthcare associated acquisition of *bla*NDM-1 in hospitalized patient in different part of the world[36]. Conversely, in India, NDM-1 producing organisms are mostly acquired from the community[35]. It reflects that though most NDM-1 producers have been isolated from hospitalized patients, originally this resistance mechanism may have extended from the community. Inappropriate and non-prescribed antibiotics use might be the probable cause of development of this new resistance mechanism in this subcontinent[39]. MBL encoding genes have been detected from several gram-negative bacilli belonging to the family *Enterobacteriaceae* and also in *Acinetobacter* species[40]. Acquired MBLs in gram-negative bacteria are becoming an emerging resistant determinant worldwide[41].

This study also observed 14 (66.7%) VIM positive isolates detected by PCR among 21 imipenem resistant *Acinetobacter baumannii*. Khatun et al[30] have been reported 72.0% VIM producers among the imipenem resistant *Acinetobacter baumannii*. Findings of the present study is almost similar to the previous study.

Current study has found 8 (38.1%) IMP positive isolates detected by PCR among 21 imipenem resistant *Acinetobacter baumannii*. A study in Bangladesh has been reported 40.0% IMP producers among the imipenem resistant *Acinetobacter baumannii* which support the present study[40].

In present study, 5(23.8%) of imipenem resistant *Acinetobacter baumannii* are KPC positive and all of them are isolated from ETA. Mostofa et al[40] have been reported that from a total of 20 imipenem resistant *Acinetobacter baumannii* isolates, 6 (30%) are identified as KPC positive. The presence of this gene suggests the possibility of horizontal transmission, as this carbapenemase has been associated with mobile genetic elements (transposons) which can be transferred from one bacterium to another[42-43].

Conclusions

In this study *bla*NDM-1, *bla*KPC, *bla*VIM and *bla*IMP were predominant carbapenemase encoding genes among imipenem resistant *Acinetobacter baumannii*. This study reflects that *bla*NDM-1 positive *Acinetobacter baumannii* are increasing in Bangladesh. Early detection of this resistance mechanism, implementation of strict microbial policies and infection control programs may prevent the rapid dissemination of this organism.

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Conflict of Interest
Authors declared no conflict of interest.

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Authors' contributions
Sultana S conceived and designed the study, analyzed the data, interpreted the results, and wrote up the draft manuscript. Begum, Rahman T and Mostofa HA contributed to the analysis of the data, interpretation of the results and critically reviewing the manuscript. Jahan T, Yusuf MA and Asifudduza M involved in the manuscript review and editing. All authors read and approved the final manuscript.

Data Availability
Any inquiries regarding supporting data availability of this study should be directed to the corresponding author and are available from the corresponding author on reasonable request.

Ethics Approval and Consent to Participate
Ethical approval for the study was obtained from the Institutional Review Board. As this was a prospective study the written informed consent was obtained from all study participants. All methods were performed in accordance with the relevant guidelines and regulations.

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