PHENOTYPIC DETECTION OF METALLO-β-LACTAMASE AMONG THE CLINICAL ISOLATES OF IMIPENEM RESISTANT *PSEUDOMONAS* AND *ACINETOBACTER* IN TERTIARY CARE HOSPITALS OF DHAKA CITY

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Abstract

The rapid spread of Metallo-β-lactamase (MBL) producing Gram negative bacilli represents a matter of great concern worldwide. The study analyzed the occurrence of MBL production in carbapenem resistant *Pseudomonas* and *Acinetobacter* isolates over one year period. A total of 132 *Pseudomonas* and 76 *Acinetobacter* isolates were obtained from two tertiary care hospitals of Dhaka city. A total of 53 *Pseudomonas* and 29 *Acinetobacter* isolates were selected because of their resistance to carbapenem specially imipenem (IPM). Screening for MBL production was performed in these isolates by IPM-EDTA microdilution MIC method. 44 (83%) IPM resistant *Pseudomonas* and 19 (65.5%) *Acinetobacter* isolates were MBL producer by IPM-EDTA microdilution MIC method. These results suggest that MBL producing *Pseudomonas* and *Acinetobacter* isolates are emerging in our country and it is essential to screen carbapenem resistant isolates for MBL production.

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Introduction

Carbapenem, a beta-lactam antibiotic, is used as a last resort of treatment in multidrug resistant Gram negative bacilli infection. It is a β lactam antibiotic with a broad spectrum antibacterial activity and is stable to almost all clinically relevant extended spectrum beta lactamases. However, since last 10 years, acquired resistance to this life saving antimicrobial agent has been increasingly reported worldwide among non-fermenting Gram negative bacilli specially *Pseudomonas* and *Acinetobacter* isolates.[1]

There are several mechanisms of resistance for carbapenem such as lack of drug penetration due to mutation in the porin channel, loss of outer membrane proteins, efflux mechanisms and Ambler class B carbapenemase or Metallo-β-lactamases (MBL). MBL require divalent cations of zinc as cofactor for their enzymatic activity and they are susceptible to inhibition by metal chelators such as EDTA and thiol based compounds like 10-phenanthroline, 2-mercaptopropionic acid (2-MPA), etc. MBLs are most important because they confer high resistance to all β lactams except aztreonam. They are not inhibited by beta-lactamase inhibitors like clavulunate, salbactam, tazobactam. MBL production is typically associated with resistance to aminoglycoside and quinolones further compromising the therapeutic options. They are often expressed in combinations with other β lactamases like AmpC β lactamase and extended spectrum β lactamases (ESBL). The genes for MBL are inserted in integron structures that reside on mobile genetic elements like plasmid, transposons having the potential for rapid and generalized dissemination.[2]

Based on the fact that MBL activity is blocked by chelating agents several screening methods have been

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developed. The microdilution MIC method detects presence of MBL by comparing minimum inhibitory concentration (MICs) of IPM with and without chelators like-EDTA or 2-MPA.

In view of the above, the present study was undertaken to determine the prevalence of MBL producing *Pseudomonas* and *Acinetobacter* isolates in two tertiary care hospitals of Dhaka city by IPM-EDTA MIC method.

Materials and Methods

Study samples

All the 208 isolates (132 *Pseudomonas* and 76 *Acinetobacter*) from sputum, urine, tracheal aspirate, blood, wound swab were obtained from the patient admitted in ICU, ward and outpatient department of Bangabandhu Sheikh Mujib Medical university (BSMMU) and Bangladesh Institute of Research and Rehabilitation for Diabetes, Endocrine and Metabolic Disorders (BIRDEM). Samples were collected from January 2009 to December 2009.

Isolation, identification and antimicrobial susceptibility testing of organisms

All the samples were routinely cultured in MacConkey agar media and blood agar plates. All the suspected colonies of *Pseudomonas* and *Acinetobacter* isolates were identified by Gram staining, colony characteristics, pigment production, motility test and other biochemical reactions.[3]

All the isolates were tested for susceptibility to IPM by disk diffusion method of Kirby-Bauer[4] and as per the recommendations of the NCCLS.[5] The antibiotic testing disks were obtained from Oxoid Ltd (Basingstoke, Hampshire, UK). Antibiotic potency of the disks were standardized against the reference *Pseudomonas* ATCC 25853 strain.

Tests for MBL-production by EDTA-IPM microdilution MIC test

The IPM-EDTA microdilution MIC test was a modification of EPI microdilution MIC test as described by Migliavacca *et al*.[6] MIC of IPM were determined with a standard microdilution assay in 96 well microtiter plates using Mueller Hinton broth (MHB) and a bacterial inoculums of $5x10^4$ CFU per well, in a final volume of 100μl. IPM concentrations in the range of 512 to 0.5 μg/ml were tested in the study. The MICs of IPM were determined with IPM alone and with IPM plus EDTA. The best results of MIC of IPM were observed with a concentration of EDTA of 0.4mM. One well containing the bacterial suspension alone and another well containing EDTA alone were used as control. Results were recorded by visual inspection of microtiter plates after 18 hour of incubation at 37^0C. With a minimum fourfold reduction in the MIC of IPM in presence of EDTA in comparison to IPM alone is designated as the cutoff value for detection of MBL producers.

Results

A total of 132 *Pseudomonas* and 76 *Acinetobacter* were studied of which 90 *Pseudomonas* were isolated from BSMMU (53 non ICU and 37 ICU) and 42 were from BIRDEM (13 non ICU and 29 ICU). Out of 76 *Acineobacter* 62 (36 non ICU and 26 ICU) from BSMMU and 14 were from BIRDEM (6 non ICU and 8 ICU). Amongst them, 53 (40.1%) *Pseudomonas* and 29 (38.1%) *Acinetobacter* isolates were resistant to IPM. These IPM resistant isolates were tested for MBL production by IPM-EDTA microdilution MIC method.

Among 53 IPM resistant *Pseudomonas* and 29 IPM resistant *Acinetobacter*, 44 (83.1%) *Pseudomonas* and 19 (65.5%) *Acinetobacter* were found positive for MBL by EDTA-IPM microdilution MIC test. Among the

Table 1: *Rate of IPM resistance among Pseudomonas and Acinetobacter collected from different hospitals and detection of their MBL production by EDTA-IPM microdilution MIC test*

Isolates collected from	No. of *Pseudomonas*	No. of IPM resistant *Pseudomonas*	Positive for MBL by EDTA-IPM microdilution MIC	No. of *Acinetobacter*	No. of IPM resistant *Acinetobacter*	Positive for MBL by EDTA-IPM microdilution MIC
BSMMU	90	33 (36.6)		62	24 (38.7)	
BIRDEM	42	20 (47.6)	44(83.1%)	14	05 (35.7)	19(65.5%)
Total	132	53 (40.1)		76	29 (38.1)	

MBL positive *Pseudomonas* and *Acinetobacter*, 59.1% *Pseudomonas* and 57.8% *Acinetobacter* exhibited high IPM MICs (≥256μg/ml). In this study 55.5% MBL negative *Pseudomonas* and 70% MBL negative *Acinetobacter* isolates also showed high MIC (≥ 256μg/ml).

Discussion

In this study 53 (40.1%) *Pseudomonas* and 29 (38.1%) *Acinetobacter* were found to be IPM resistant. This finding was consistent with other studies. Noyal *et al* showed high prevalence of imipenem resistant *Pseudomonas* spp (31.1%) and *Acinetobacter* spp (59%) in India in 2008.[7] Indiscriminate use of carbapenems could have resulted in the increase in carbapenem resistant *Pseudomonas* and *Acinetobacter* spp.[7]

Among the IPM resistant *Pseudomonas* and *Acinetobacter,* MBL was found positive in 44 (83%) and 19 (65.5%) respectively by EDTA-IPM microdilution MIC method. Altoparlak *et al*. (2005) in Turkey showed that 56.8% of IPM resistant *Pseudomonas* spp and 33.3% *Acinetobacter* spp isolates were found to be MBL producer.[8]

The IPM resistant MBL negative isolates also showed high MIC, the reason for their resistance may be due to mechanism other than MBL production, like hyper production of serine beta lactamases and /or a change in the membrane permeability in the bacteria, efflux pump or mutation in the porin.[9] In this study, one *Pseudomonas* and one *Acinetobacter* isolate having MIC of 4μg/ml and 8μg/ml respectively were MBL positive though both were within sensitive range for IPM. But both were considered as IPM resistant by disk diffusion method. It has been reported that over 30% MBL carrying isolates, particularly *Enterobacteriaceae*, were found to be IPM sensitive by MIC method though they were IPM resistant in disk diffusion method.[2] These carbapenem sensitive MBL producer may carry "hidden" MBL gene or they may be low level MBL producer. As a result of difficulties in their detection, these organisms may pose a significant risk due to their unnoticed spread within the hospital and their ability to transfer resistant gene to other organisms.[2]

The study documents that MBL producing organisms are already present in our country. To provide correct antibiotics to the patients infected with MBL producer and to prevent their spread, all microbiology laboratories must routinely screen for carbapenem resistance due to MBL. Selected use of carbapenem and infection control programs for nosocomial infection should also be practiced.

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