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Original Article

Metallo-ß-Lactamase Producing Pseudomonas species in a Tertiary Care Hospital of Dhaka City

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ABSTRACT

Among the various beta-lactam antibiotics, carbapenems are the most potent and have been reserved for use in treating infections caused by multi-drug resistant (MDR) Gram negative bacilli, especially Pseudomonas. They are effective even against Extended Spectrum beta-lactamase (ESBL) and Amp C b-lactamase producing bacteria. The clinical utility of carbapenems is under threat with the emergence of carbapenem resistant bacteria due to production of carbapenem hydrolyzing metallo-beta-lactamase (MBL) which confers high-level resistance to all b-lactam antibiotics except aztreonam. The prevalence of MBLs have been studied in many countries but not been reported in Bangladesh. The purpose of the study to determine the presence of MBLs producing *Pseudomonas* in clinical samples from a tertiary care hospital. MBLs producing *Pseudomonas* in various clinical samples of an urban hospital of Dhaka city was investigated over a 6-month period (January 2009-June 2009). EDTA-IMP agar dilution minimum inhibitory concentration (MIC) reduction method was employed to detect MBL producing Pseudomonas sp. Out of 44 Pseudomonas isolates 08 (18.2%) were sensitive and 23 (52.3%) were resistant to imipenem while 13 (29.5%) were intermediate resistant (MIC = 8 µg/ml) to imipenem. All Pseudomonas showing intermediate resistance to imipenem were found sensitive by disc diffusion method. MBL phenotype was detected in 43%(10 out of 23) imipenem resistant Pseudomonas spp. while the rate was 61%(08 out of 13) is intermediate resistant strains by EDTA-IMP agar dilution MIC method. The results of the study indicated high prevalence of MBL producing Pseudomonas spp. in our hospital environment. Early detection of these MBL producing Pseudomonas is necessary to institute appropriate treatment and effective infection control measures.

Key words: Metallo-ß-lactamase, Pseudomonas species, Gram negative bacilli.

Introduction

Development of antibiotic resistance is a major concern in the management of bacterial infections. Carbapenems are often used as antibiotics of last resort for treating infections due to multi-drug resistant Gram-negative bacilli. They are stable against extended spectrum beta-lactamase (ESBL) and AmC beta-lactamase. However, this scenario is changing with the emergence of metallo- -lactamase (MBL) producing strains. The MBLs can efficiently hydrolyze all beta-lactam antibiotics except aztreonam.[1] MBL producing Gram-negative bacilli, specially Pseudomonas sp, have been increasingly reported in Asia, Europe, Latin American and the United States.[2-5] Therefore, detection of MBL-producing Gram negative bacilli is crucial for the optimal treatment of patients and to control the spread of resistance.

At present no data is available on MBL producing organisms in Bangladesh. Therefore, the present study was undertaken to detect the prevalence of MBL-producing Pseudomonas in a tertiary care hospital of Dhaka city.

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Methods

Study population and specimens

All samples were collected from hospitalized patients of Bangladesh Institute of Research and Rehabilitation in Diabetes, Endocrine and Metabolic Disorder (BIRDEM). The

study was carried out during December, 2008 to June, 2009. The specimens included were pus, blood, urine and tracheal aspirates. Production of MBL was tested in Pseudomonas sp only.

Microbiological methods

All samples were routinely cultured on MacConkey and blood agar plates. Blood culture was done by lytic centrifugation method.[6] All suspected colonies of Pseudomonas were identified by Gram staining, colony characteristics, positive oxidase test, motility and standard biochemical reactions. [7]

Antibiotic susceptibility testing

Antimicrobial susceptibility testing of the isolated organisms was done by a disk diffusion method using the Kirby–Bauer technique [8] and as per the recommendations of the NCCLS.[9] All disks were obtained from Oxoid Ltd., Basingstoke, Hampshire, UK. Antibiotic potency of the disks was standardized against the reference strain, *Pseudomonas aeuruginosa* ATCC 25853.

Detection of MBL production

Production of MBL by Pseudomonas spp. is determined by EDTA-imipenem (EDTA-IMP) agar dilution MIC reduction method. [10] The EDTA-IMP agar dilution MIC reduction test was a modification of EPI microdilution MIC test as described by Migliavacca *et al.*[11] The test was used as gold standard for detection of MBLs production in this study.

Briefly, MIC of imipenem of isolated Pseudomonas sp was determined with or without EDTA of defined concentration. First, MIC of imipenem of test organisms was performed by agar dilution method with imipenem concentration between 0.125-1024 µg/ml. Then, again MIC of imipenem of test organism was determined in presence of combination of imipenem and 0.4mM EDTA. Muller-Hinton agar plates were prepared with suspensions of 0.125µg/ml, 0.25µg/ml, 0.5µg/ml, 1µg/ml, 2µg/ml, 8µg/ml, 16µg/ml, 32µg/ml, 64µg/ml, 128 µg/ml, 256 µg/ml, 512 µg/ml, 1024 µg/ml imipenem plus 0.4mM EDTA. A fixed inoculum of 10^4 cfu of the test strains was inoculated on these plates. The reading was taken after 24 hours of incubation. The highest dilution that inhibited the growth of the organism was taken as MIC of the test organism. An organism was considered MBL positive if the MIC of imipenem was reduced by fourfold or more in presence of EDTA compared to MIC of imipenem alone.

Result

A total of 44 Pseudomonas were isolated from various clinical samples of which 08 (18.2%) were sensitive, while 13 (29.5%) and 23 (52.3%) were intermediate resistant (MIC = 8 µg/ml) and resistant (MIC 16 µg/ml) to imipenem respectively (Table-1). All 44 *Pseudomonas* isolates were tested for production of MBL by EDTA-IMP agar dilution MIC method.

Table 1: Rate of isolation of MBL-producing Pseudomonas sp (n=44) by IMP (imipenem) agar dilution MIC method.

MBLs	No. of total tested Pseudomonas 44	Imipenem MICs resistant MIC= 16 µg/ml	Imipenem MICs intermediate resistant MIC = 8 µg/ml	Imipenem MICs sensitive MIC= 4 µg/ml
Positive	18	10	8	0
Negative	26	13	5	8

MBL detected in 43% (10 out of 23) imipenem resistant spp. while 61% (08 out of 13) in intermediate resistant strains as they showed ≥ fourfold reduction of imipenem MIC in presence of chelating agents EDTA (Table: 2).

Table 2: Rate of isolation of MBL-producing Pseudomonas sp by IMP-EDTA agar dilution MIC method.

MBLs	Imipenem MICs resistant MIC =16 µg/ml (Total= 23)	Imipenem MICs intermediate resistant MIC = 8 µg/ml (Total= 13)
Positive	10 (43%)	8 (61%)
Negative	13	5

Discussion

In the present study, about 43% of Pseudomonas isolated from various clinical samples were MBL producers. But it is interesting to note that 61% of MBL producing Pseudomonas were detected among those which showed intermediate resistance to imipenem by MIC. Therefore it appears that these strains could be low level producers of MBL. The clinical outcome of patients infected with such imipenem sensitive, as shown by routine disc diffusion test, but low level MBL producing organisms remains unknown. Similar observation was made in other countries where *Klebsiella pneumoniae* and E*scherichia coli* isolated from clinical samples were found carbapenem sensitive but positive for MBL gene. [12-15]

The high rate of MBL positive Pseudomonas in our study was probably due to the fact that majority of our strains were isolated from Intensive Care Units (ICU) samples. In Japan, the rate of resistance to carbapenems increased from 19.3 % in 1998 to 38 % in 2002.[5] A study in a tertiary-care teaching hospital in southern Brazil reported high rates (56.7-58.3%) of imipenem resistance among *P. aeruginosa.*[15] In Italy, about 20% of all *P. aeruginosa* and 70% of carbapenem resistant strains contained MBLs.[10] We are unable to determine the trends of MBL producing Pseudomonas in clinical specimens as our study was the first to detect MBLs producing organisms from clinical specimens in Bangladesh.

The results of the study indicated a high prevalence of MBL producing Pseudomonas in tertiary care hospital of Dhaka city.

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