Frequency and susceptibility pattern of metallo-beta-lactamase producers in a hospital in Pakistan

Fatima Kaleem, Javaid Usman, Afreenish Hassan, Aslam Khan

Department of Microbiology, Army Medical College, National University of Sciences and Technology, Pakistan

Abstract
Introduction: The rapid spread of acquired metallo-beta-lactamases (MBLs) among major Gram-negative pathogens is an emerging threat and a matter of particular concern worldwide.
Methodology: This descriptive study was conducted between January and August 2009 in the department of Microbiology, Army Medical College, National University of Sciences and Technology, Rawalpindi, to determine the frequency and susceptibility patterns of MBL-producers among carbapenem-resistant Gram-negative rods (GNRs) from clinical isolates of a tertiary care hospital. All clinical samples were processed according to standard microbiological methods. Isolated GNRs were subjected to susceptibility testing against various antibiotics by disc diffusion method according to the Clinical and Laboratory Standards Institute (CLSI) guidelines. Carbapenem-resistant isolates were subjected to the detection of MBL production by the E-test MBL strip method.
Results: Out of 50 carbapenem resistant isolates, 39 (78%) of were confirmed to be MBL producers by the E-strip method. Acinetobacter baumannii were the most frequent MBL producers, followed by Pseudomonas aeruginosa. A total of 19 (37%) of the MBL producers were susceptible to cefoperazone-sulbactam.
Conclusion: The findings strongly suggest that there is a need to track the detection of MBL producers and that judicious use of carbapenems is necessary to prevent the further spread of these organisms.

Key words: carbapenem-resistant Gram-negative rods; metallo-beta-lactamase; antimicrobial resistance; extended-spectrum beta lactamase


(Received 26 March 2010 – Accepted 19 May 2010)

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Introduction
Infection with the metallo-beta-lactamase (MBL) producing organisms is associated with higher rates of mortality, morbidity, and health care costs. MBL producing Pseudomonas aeruginosa was first reported in Japan in 1991 and since then has been found in various parts of the world including Asia, Europe, Australia, South America, and North America [1-8]. The introduction of carbapenems into clinical practice marked a great advance for the treatment of serious bacterial infections caused by beta-lactam-resistant bacteria [9]. Resistance to extended-spectrum beta-lactams has been frequently observed in non-fermenting bacilli such as Pseudomonas aeruginosa and Acinetobacter spp. Resistance is mediated by a lack of drug penetration for example due to porin mutations, efflux pumps or hydrolysis by beta-lactamases [10]. Based on molecular studies, carbapenem hydrolyzing enzymes are classified into four groups: A, B, C and D. The MBLs, which belong to group B, are enzymes requiring divalent cations as cofactors for optimal enzyme activity, being inhibited by the action of a metal ion chelator [10]. The MBLs efficiently hydrolyze all beta-lactams. Several phenotypic methods are available for the detection of MBL-producing bacteria. All these methods are based on the ability of metal chelators such as Ethylenediaminetetraacetic acid (EDTA) and thiol-based compounds to inhibit the activity of MBLs. These tests include the double disc synergy tests using various combinations such as EDTA with imipenem (IPM) or ceftazidime (CAZ) [10-12]; 2-mercaptopropionic acid with CAZ or IPM [15]; the Hodge test [12,13]; a combined disk test using EDTA with CAZ or IPM [14-16]; the MBL E-test (AB BioDisk) [17]; and a micro dilution method using EDTA and 1,10-phenanthroline with IPM [18].

Gram-negative bacilli associated with hospital infections are often difficult to eradicate because they are resistant to drugs. Therefore, detection of MBL-producing Gram-negative bacilli is crucial to control the spread of resistance and for the optimal treatment of patients, particularly the critically ill and hospitalized patients [19].
information concerning MBL-producing isolates available in Pakistan. Therefore, this study was conducted to detect the presence of MBL in carbapenem-resistant isolates obtained from the clinical isolates from a tertiary care hospital, which has intensive care units with a heavy patient turnover and extensive antibiotic use.

Methodology

Duration and setting

This descriptive study was conducted between January and August 2009 in the Department of Microbiology, Army Medical College, National University of Sciences and Technology, Rawalpindi, after approval from the ethical committee of the institute.

Specimens

Routine specimens were used, including nasobronchial lavages, pus swabs, blood, sputum, catheter tips, and urine received from admitted patients in the Military Hospital, Rawalpindi. Most of the specimens were received from patients admitted in medical and surgical intensive care units who had serious underlying diseases and risk factors and who were already on antibiotics. All specimens were processed according to standard microbiological procedures. From a total of 2347 samples, 430 consecutive non-duplicate Gram-negative bacilli were recovered (61%). Isolated Gram-negative rods were subjected to antimicrobial sensitivity testing per the Clinical and Laboratory Standards Institute (CLSI) guidelines [20]. All carbapenem-resistant isolates were subjected to detection of MBLs by the E-test method as described by Yan et al. [14].

E-test

The E-test MBL strip (AB Biodisc, Sweden) contains predefined gradients of imipenem ranging from 1 to 256 µg/ml. Carbapenem-resistant Gram-negative rod colonies that were grown overnight were emulsified in normal saline to achieve a turbidity equivalent to 0.5 McFarland standard (1 McFarland if mucoid). A sterile swab was dipped into the inoculum suspension; excess fluid was removed. The entire Mueller Hinton agar surface was inoculated, and the strip was placed onto the inoculated agar surface. The plates were incubated at 37°C for 16 to 18 hours. When bacterial growth was clearly visible, the MIC values were read where the respective inhibition ellipses intersected the strip in accordance with the manufacturer’s instructions (AB Biodisc, Sweden). Pseudomonas aeruginosa ATCC 27853 was used as a negative control for MBL and an MBL genotype-positive reference strain; Stenotrophomonas maltophilia ATCC 13636 (intrinsic MBL production) was used as a positive control.

Results

Among the 50 carbapenem-resistant isolates, 39 (78%) were found to be MBL positive by the E-test strip method. Twenty-seven (84%) carbapenem-resistant Acinetobacter species were found to be MBL producers, whereas seven (78%) of carbapenem-resistant Pseudomonas aeruginosa were positive for MBL production and five (71%) carbapenem-resistant Escherichia coli were metallo-beta-lactamase producers. (Table 1). Most of the MBL producers were isolated from nasobronchial lavage samples followed in number by urine and pus samples. (Table 2). Nineteen (38%) of the MBL producers were sensitive to the cefoperazone-sulbactam combination and the aminoglycosides. Only seven (14%) MBL-producers were sensitive to the ampicillin-clavulanic acid combination. Thirteen percent of the MBL producers were sensitive to piperacillin-tazobactum combination and only 11% showed sensitivity to tetracycline group.

Table 1. Percentage of MBL producers among carbapenem-resistant organisms

<table>
<thead>
<tr>
<th>Carbapenem resistant Organism</th>
<th>Total</th>
<th>MBL + by Etest</th>
<th>Percentage of MBL production</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acinetobacter baumannii</td>
<td>32</td>
<td>27</td>
<td>84%</td>
</tr>
<tr>
<td>Pseudomonas aeruginosa</td>
<td>9</td>
<td>7</td>
<td>78%</td>
</tr>
<tr>
<td>Escherichia coli</td>
<td>7</td>
<td>5</td>
<td>71%</td>
</tr>
<tr>
<td>Providencia rettegeri</td>
<td>1</td>
<td>0</td>
<td>0%</td>
</tr>
<tr>
<td>Morganella morganii</td>
<td>1</td>
<td>0</td>
<td>0%</td>
</tr>
</tbody>
</table>

(* = 50)*
**Table 2.** Number of MBL producers isolated from different clinical samples containing carbapenems resistant organisms.

<table>
<thead>
<tr>
<th>Specimen</th>
<th>Total</th>
<th>Total MBL+ organisms isolated</th>
</tr>
</thead>
<tbody>
<tr>
<td>NASOBRONCHIAL LAVAGE</td>
<td>22</td>
<td>22</td>
</tr>
<tr>
<td>PUS</td>
<td>10</td>
<td>6</td>
</tr>
<tr>
<td>URINE</td>
<td>6</td>
<td>3</td>
</tr>
<tr>
<td>CATHETER TIPS</td>
<td>4</td>
<td>3</td>
</tr>
<tr>
<td>BLOOD</td>
<td>3</td>
<td>2</td>
</tr>
<tr>
<td>SPUTUM</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>EAR SWAB</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>HIGH VAGINAL SWAB</td>
<td>1</td>
<td>1</td>
</tr>
</tbody>
</table>

**Discussion**

Japan reported the first plasmid-mediated MBL in *Pseudomonas aeruginosa* in 1991. This was followed by a report of transferable metallo enzyme in *Bacteroides fragilis* [1]. In addition to *Pseudomonas aeruginosa*, other Gram-negative bacteria including *Klebsiella pneumoniae*, *Escherichia coli*, *Enterobacter aerogenes*, *Enterobacter cloacae*, *Citrobacter freundii*, *Proteus vulgaris*, *Acinetobacter spp.* and *Alcaligenes xylosoxidans* have also been shown to produce MBL [16]. There are frequent reports of MBL production in *Pseudomonas aeruginosa* and *Acinetobacter* species from different parts of the world, particularly in military settings [21]. MBLs have been identified from clinical isolates worldwide with increasing frequency over the past few years, and strains producing these enzymes have been responsible for prolonged nosocomial outbreaks that were accompanied by serious infections [22].

A total of 56 (87.5%) carbapenem resistant isolates were found to be MBL producers from hospitalized patients in Chennai, India, in 2005. Reports have also described the significant prevalence of MBLs in *Pseudomonas aeruginosa* and *Acinetobacter spp.* from India in a study conducted in 2008 [23]. Carbenapen-resistant Gram-negative bacteria that were isolated from nosocomial infections in patients in a university hospital in Turkey (15 of 52 *Pseudomonas aeruginosa* strains [29%], 5 of 24 *Acinetobacter baumannii* strains [21%], and 2 of 2 *Klebsiella pneumoniae* strains [100%]) were found to be metallo enzyme producers using the E-test MBL technique. *In vitro* antibiotic susceptibility of the MBL-positive organisms was investigated by the E-test method. Of the ten drugs tested, isepamicin was the most active agent against the MBL-producing strains. Overall, the rank order of activity of the ten antibiotics, in terms of the percentages of susceptible strains was as follows: isepamicin: 73%; ciprofloxacin: 64%; amikacin: 59%; aztreonam: 18%; tobramycin: 18%; meropenem: 14%; cefoperazone-sulbactam: 5% [24].

The results are similar to those of a 2008 study conducted at Aga Khan University, Karachi, by Irfan et al., in which 96.6% of the carbapenem resistant *Acinetobacter baumannii* were MBL producers and all the carbapenem-resistant *Pseudomonas spp.* were found to be MBL producers [25].

Emergence of MBL-mediated resistance in Pakistan is of serious concern. Carbapenems are effective therapeutic agents against highly resistant pathogens such as *Pseudomonas spp.* and *Acinetobacter spp.* Spread of this resistance among these pathogens and transfer to other Gram-negative bacteria would seriously restrict therapeutic options. This challenging situation is difficult to manage in a resource-limited country. Conversely, the situation continues to become more complicated by the indiscriminate use of antibiotics in the population.

The occurrence of an MBL-positive isolate in a hospital setting poses a therapeutic problem, as well as serious concern for infection control management. The accurate identification and reporting of MBL-producing bacteria will aid infection control practitioners in preventing the spread of these multidrug-resistant isolates.

**Conclusion**

The study results demonstrate the serious therapeutic and epidemiological threat of the spread of metallo-beta-lactamase producers. Early detection and infection control practices are the best defense against these organisms; therefore systematic surveillance to detect MBL producers is necessary. Judicious use of carbapenems is essential to prevent the spread of these organisms.

**Acknowledgements**

Funding for this project was provided by Clinical Pathology Laboratory, Army Medical College, National University of Sciences and Technology.
References

Corresponding author
Dr Fatima Kaleem
Department of Microbiology
National University of Sciences and Technology
Army Medical College
Abid Majeed Road, Rawalpindi, Pakistan
Phone: (+92) 322-5087234
Email: fatima_kaleem@hotmail.com

Conflict of interests: No conflict of interests is declared.