DETECTION OF METALLO BETALACTAMASE PRODUCING ENTEROBACTERIACEAE ISOLATES FROM CRITICAL CARE PATIENTS

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ABSTRACT

Background : The production of Metallo beta lactamases(MBL) is one of the important mechanisms of bacterial drug resistance. The genes responsible for MBL production are horizontally transferable via plasmids or are chromosomally mediated facilitating rapid spread to other bacteria. This rapid spread of MBL genes is a matter of concern with regard to the antimicrobial chemotherapy. Aims and objectives : This study aims to provide early, rapid and effective phenotypic characterization of carbapenem resistant isolates from the family enterobacteriaceae for initiation of effective treatment especially in critically ill patients. Materials and methods : A total of 98 enterobacteriaceae isolates obtained from ICU samples from October 2010 to September 2011 were included in the study. All the samples were screened for imipenem resistance by Kirby-Bauer disc diffusion method. 49 isolates that were resistant to imipenem were screened for MBL by the combined disc(IMP-EDTA) and the double disc synergy (DDST) tests. The Minimum Inhibitory Concentration (MIC) was determined using E strips (Biomerieux). Results : Of the 49 imipenem resistant samples, 12 (24.48%) were positive for MBL production by both combined and double disc methods; 37 (75.51%) showed positivity by combined disc only and 16 (32.65%) isolates by double disc method only. The positive samples showed a minimum inhibitory concentration ratio of >8 (imipenem/imipenem+EDTA). Conclusion : The frequent use of carbapenems has lead to an increase in carbapenem-resistant strains due to selective pressure. It has to be made as a practice guideline to detect and control these resistant strains on a day to day basis in clinical microbiology as spread of MDR strains is by mobile genetic elements. To date there are no standard CLSI guidelines for MBL screening/detection. Most studies suggest more than one phenotypic method. In our study combined disc test is better than the disc synergy test for the early detection of metallobetalactamase producing enterobacteriaceae strains, thereby assisting in proper therapeutic interventions.

Key words : Enterobacteriaceae, metallobetalactamases, combined disc, double disc synergy tests.

INTRODUCTION

The emergence of drug resistance or antibiotic resistant organisms is a global health problem particularly in health care centres. Treatment options are limited for these multidrug resistant (MDR) strains leading to life threatening infections. The predominant mechanism for resistance to beta-lactam antibiotics in gram negative bacteria is the synthesis of beta lactamases which cleave the amide bond of the beta lactam ring rendering the drug ineffective. These beta lactamases are classified based on their functional and molecular properties. Metallobetalactamases (MBL) are class B betalactamases requiring one or two zinc ions for their activity. The genes responsible for MBL production are horizontally transferable via plasmids or are chromosomally mediated and can rapidly spread to other bacteria [1,2]. The appearance of MBL genes and their spread among bacterial pathogens is a matter of concern with regard to the future of antimicrobial chemotherapy.

AIMS & OBJECTIVES

This study aims to provide early, rapid and effective phenotypic characterization of carbapenem resistant isolates from the family enterobacteriaceae for initiation of effective treatment especially in critically ill patients.

MATERIALS AND METHODS

A total of 98 Enterobacteriaceae isolates collected from various samples of urine, blood, sputum, pus, endotracheal aspirates, bronchial secretions, wound swabs from ICU wards over a period from October 2010 to September 2011 were included in the study. Antibiotic
susceptibility testing was initially determined with penicillins, cephalosporins, aminoglycosides and carbapenems. Zone diameter was measured and interpreted as per CLSI[13] guidelines. The test isolates' opacity was matched and adjusted to 0.5 McFarlands standards and a lawn culture done on MHA plates (Kirby-Bauer disc diffusion method). 49 isolates that were imipenem resistant were screened for MBL by the Combined disc(IMP-EDTA) and the Double disc synergy(DDST) tests. Escherichia coli ATCC 25922 was used as the control strain. MIC was determined using E strips (Biomerieux).

**IMIPENEM(IMP)-EDTA combined disc test**
The IMP-EDTA combined disk test was performed as described by Yong et al[5]. Test organisms were inoculated on to plates with Mueller Hinton agar as recommended by the CLSI[13]. Two discs (HIMEDIA) - 10 μg imipenem and Imipenem/EDTA(10μg + 750μg) were placed on the plate. The inhibition zones of the imipenem and imipenem-EDTA disks were compared after 16 to 18 hours of incubation in air at 37°C. A zone diameter difference between the Imipenem and Imipenem/ EDTA discs of > 7mm was interpreted as positive for MBL production[6].

**IMIPENEM-EDTA Double disc synergy test(DDST)**
The IMP-EDTA double disk synergy test was performed as described by Lee et al[7] and Arakawa et al[8]. Test organisms were inoculated on to plates with Mueller Hinton agar as recommended by the CLSI[13]. An imipenem (10 μg) disc was placed 20mm edge to edge from a blank disc containing 10 μL of 0.5 M EDTA (750 μg).

A zone of synergy between the imipenem and EDTA discs after 16 to 18 hours of incubation in air at 37°C was interpreted as positive for MBL production.

**MBL E test**
MBL E test was performed according to the recommendations of the manufacturer (Biomerieux). Test organisms were inoculated on to plates with Mueller Hinton agar as recommended by the CLSI[13]. E test MBL strip (Biomerieux) containing a double sided seven dilution range of Imipenem (4 to 256 mcg/ml) and Imipenem (1 to 64 mcg/ml) in combination with a fixed concentration of EDTA was placed on the plate and incubated for 16 to 18 hours of incubation in air at 37°C. MIC ratio of Imipenem/Imipenem+EDTA of by >8, or reduction of Imipenem MIC by >3 log2 dilutions in the presence of EDTA or appearance of a phantom zone was interpreted as positive for MBL production[13,14]. The positive samples showed a MIC ratio of >8 in this study.

**TABLE.1 Distribution of clinical samples and isolates**

<table>
<thead>
<tr>
<th>S.NO</th>
<th>SAMPLE</th>
<th>E.Coli</th>
<th>Klebsiella spp</th>
<th>Proteus spp</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>PUS(wound swab including one burns wound swab)</td>
<td>8</td>
<td>7</td>
<td>1</td>
</tr>
<tr>
<td>2</td>
<td>URINE</td>
<td>9</td>
<td>3</td>
<td>0</td>
</tr>
<tr>
<td>3</td>
<td>TRACHEAL/ET TUBE</td>
<td>3</td>
<td>4</td>
<td>1</td>
</tr>
<tr>
<td>4</td>
<td>SPUTUM</td>
<td>3</td>
<td>3</td>
<td>0</td>
</tr>
<tr>
<td>5</td>
<td>FOLEY'S CATHER TIP</td>
<td>1</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>6</td>
<td>BLOOD</td>
<td>1</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>7</td>
<td>PLEURAL FLUID</td>
<td>0</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>TOTAL</td>
<td></td>
<td>25(51.02%)</td>
<td>21(42.85%)</td>
<td>3(6.12%)</td>
</tr>
</tbody>
</table>
The frequent use of carbapenems has led to an increase in carbapenem-resistant strains due to selection pressure[9]. This has also caused spread of MDR strains by mobile genetic elements in health care settings especially in critical care units. This study suggests that more than one phenotypic methods should be done for MBL screening for all the isolates because only 24.48% isolates showed positivity by both CD&D DST. The remaining were positive for MBL either by the DDST or by the CD test but not both. Of the two phenotypic detection methods employed here the combined disc test(75.51%) is better than disc synergy test(32.65%). There is variation in subjective interpretation of result in DDST[15]. Positive and negative results are more clearly seen in CDST[15].

In this study most of the MBL positive isolates were from pus and urine samples with E.coli and Klebsiella spp as the predominant isolates. The first isolation of carbapenemase enzyme in enterobacteriaceae was in 1991- MBL gene bla IMP-1 followed by bla VIM-1 and bla KPC. The NDM-1 (New Delhi metallo betalactamase) is a newer type of MBL first described in 2009 in Klebsiella pneumoniae[2]. Bacteria harbouring blaNDM-1 are found to be resistant to carbapenems, beta lactams, fluoroquinolones and aminoglycosides leaving only polymyxins as treatment of choice[3,4]. The blaNDM-1 found in India is located on a very mobile genetic element and has a more complex and a very unpredictable spread than the other genes coding for the carbapenemases[10].

It has to be made as a practice guideline to detect and control these MDR strains on a day to day basis in clinical microbiology. Many studies have shown Colistin and Tigecycline as the only treatment option for these resistant strains. But they are not effective against Proteus species due to the inherent resistance. To worsen there are upcoming reports of Tigecycline resistance in Klebsiella species[11,12]. This calls for a judicious use of carbapenems; phenotypic MBL screening methods in the diagnosis on a daily basis for all the samples—at least two methods; early detection and containment rather than treatment.

**REFERENCES**