Current Research in Microbiology and Biotechnology
Vol. 5, No. 2 (2017): 989-991
Research Article
Open Access

ISSN: 2320-2246

Phenotypic detection of Carbapenemase producing gram-negative bacteria that dissemination in hospitals of Baghdad city

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Received: 02 February 2016 Accepted: 16 February 2017 Online: 05 March 2017

ABSTRACT

The emergence and global spread of carbapenemase resistance in gram negative bacteria are considered as a great concern to health services worldwide. This study aimed to detect metallo-β-lactamase enzymes in gram negative bacteria that are emerging as a worldwide public health concern. Totally, 103 isolates of gram negative bacteria were included in the study. The results of identification showed different gram negative strains include (*Pseudomonas aeruginosa* 38.8%, *Acinetobacter baumannii* 5.6%, *Escherichia coli* 17.4%, *Klebsiella pneumonia* 23.3%, *Serratia sp* 0.9%, *Salmonella* spp.6.7%, *Citrobacter spp.*2.9%, *Proteus spp*. 2.9%). All these isolates were subjected for antimicrobial susceptibility by using antibiotics: piperacillin (100 µg), piperacillin/ tazobactam (100 µg/10 µg), ceftazidime (30 µg), cefepime (30 µg), cefoperazone (30 µg), ceftriaxone (30 µg), cefotaxime (30 µg), amikacin (30 µg), levofloxacin (5 µg), ciprofloxacin (5 µg) ,ticarcillin/clavulinic acid (75/10 µg), and imipenem (10 µg). The results of antibiotic sensitivity showed that isolates were 64 (62.1%) resistant to ceftazidime, 54 (52.4%) to piperacilin/tazobactam, 68 (66%) to piperacillin, 58 (56.3%) to ticarcillin/clavulanic acid, 75 (72.8%) to cefoperazone, 67(65%) to amikacin, 74 (71.8%) to cefepime, 65 (63.1%) to levofloxacin, 72 (69.9%) to ciprofloxacin and 26 (25.2%) to imipenem. The positive results of imipenem-EDTA combined disc test exhibited enhancement about ≥ 7 mm in inhibition zone size in the combined disc test. Our study found dissemination of carbapenemase-producing strains in our hospitals highlights the emerging therapeutic challenge.

Keywords: Gram negative bacteria, Metallo-β-lactamase, EDTA.

1. INTRODUCTION

Carbapenems (class of beta-lactam antibiotics) are capable of killing most bacteria by inhibiting the synthesis of one layer of their cell wall. The carbapenem antibiotics were developed to aid in overcome antibiotic resistance that resulted by bacterial beta-lactamase enzymes. In recent years, the emergence of various carbapenemases in members of the family Enterobacteriaceae has become established as a major public health threat [1]. Carbapenemase-producing bacterial isolates have a severe clinical problem, as resistance to beta-lactams that accompanied by co-resistance to additional drug classes like aminoglycosides or quinolones [2, 3]. During the last decade, the carbapenemase is remarkable in P*seudomonas spp*., *Acinetobacter baumanni*i and Enterobacteriaceae. However, infections caused by carbapenemes - producing bacteria have limited treatment options and have been associated with high mortality and morbidity rates[4]. Resistance to carbapenems is mediated mostly by two main mechanisms include (i) the production of a β-lactamase enzymes capable of hydrolyzing almost all cephalosporins and β-lactam except carbapenems combined with decreased in permeability related to porin loss or alteration ,(ii)The production of a carbapenem-hydrolyzing β-lactamase enzymes. There are three classes of carbapenemases produced by carpanamase - producing bacteria included the

Amber class A, B and D carbapenemases. Class A carbapenemes include some chromosomally-encoded "NmcA, Sme,IMI-1 and SFC-1", and also plasmid-mediated genes like *Klebsiella pneumoniae* carbapenemase (KPC),GES and IMI-2 . While , Class B consists of Verona in coded metallo β-lactamase types, New Delhi metallo β- lactamase (NDM) and IMP types [5, 6]. Finally, Class D includes OXA type of enzymes with OXA-48 as the commonest one.

In addition, Metallo-ß-lactamase (MBL) is one of the latest and most important resistance mechanisms identified in Gram negative rods. The first MBL enzyme was identified from *Bacillus cereus* (BcII) in 1966 and exhibited interesting properties, including cephalosporinase activity and inhibition by EDTA .Studies have shown that MBL genes are found either intrinsically on chromosomes or acquired by horizontal gene transfer (acquired MBLs).

MBLs have become one of the major factors of resistance towards β-lactams over the past few decades. Further, MBLs exhibits broad-spectrum activity and hydrolyses virtually all classes of β-lactams with the exception of monobactams e.g. aztreonam. The active site in MBLs contains either 1 or 2 Zn2+ ions, coordinated by conserved amino acids and polarized water molecule(s) necessary for the hydrolysis of β-lactams [7]. All the MBLs share a common feature of being inhibited by EDTA and other metal chelating agents, due to the metal dependent catalytic mechanism.

Since therapeutical options are limited to very few antibiotics such as colistin, tigecycline, and fosfomycin, hospital- and community acquired infections caused by MBL- producers are became difficult to eradicate ,so this study was performed for detection of emergence and spread of MBL- producers within hospitals in Baghdad city.

2. MATERIALS AND METHODS

A total of 103 consecutive isolates of gram negative bacteria obtained from various clinical samples of admitted patients of Baghdad hospital. The isolates were identified by conventional methods. Antimicrobial susceptibility test was performed by the disc diffusion method according to the CLSI guidelines. [8]. The following antibiotics were used “piperacillin (100 µg), ticarcillin/clavulinic acid (75/10 µg), ceftazidime (30 µg), cefepime (30 µg), cefoperazone (30 µg), ceftriaxone (30 µg), cefotaxime (30 µg), amikacin (30 µg), levofloxacin (5 µg), ciprofloxacin (5 µg), piperacillin/ tazobactam (100 µg/10 µg), and imipenem (10 µg)" from Hi-Media Laboratories, BD Diagnostics Pvt Ltd, India.

The detection of MBL production performed by Imipenem(IMP)-EDTA combined disc test as described by [9]. Imipenem resistance isolate were inoculated on Mueller Hinton agar plates as recommended by the CLSI [8]. Two antibiotic disks were used: imipenem disk (10 µg) (Becton Dickinson) were placed on the plate, and the other one contain impenem (10 µg)and EDTA (930µg). The results of inhibition zones of imipenem and imipenem-EDTA antibiotic discs were compared after 16 to 18 hours of incubation at 37°C. In the combined disc test, the enhancement in inhibition zone around Imipenem - EDTA disc in about ≥ 7 mm in compare with Imipenem disc alone its considered as MBLs production .

The results of the percentage of gram negative bacteria for "103" isolates that included in the study were *"Escherichia coli*, *Klebsiella pneumonia*, *Salmonella spp, Serratia spp*., *Citrobacter spp., Proteus spp. Acinetobacter baumanni* and *Pseudomonas aeruginosa* 17.4%, 23.3 % ,6.7 %, 0.9 %, 2.9 %, 2.9 %, 5.8 % and 39.8 % respectively. Of the 103 isolates, 74 (71.8%) were resistant to cefepime, 64 (62.1%) to ceftazidime, 54 (52.4%) to piperacilin/tazobactam, 68 (66%) to piperacillin, 75 (72.8%) to cefoperazone, 58 (56.3%) to ticarcillin/clavulanic acid, 67(65%) to amikacin, 65 (63.1%) to levofloxacin, 72 (69.9%) to ciprofloxacin and 26 (25.2%) to imipenem " by the disc diffusion method.

All the imipenem resistance isolates were tested for MBLs enzyme production by imipenem –EDTA combined method ,all imipenem resistance isolates exhibited a ≥ 7 mm enhancement in the inhibition zone size around the disk with IMP-EDTA in compare with disk with IMP alone, this result was considered positive for MBL figure (1).

3. RESULTS AND DISCUSSION

Carbapenems are considered as the most effective agents of treatment of infections that caused by multidrug resistant gram negative bacteria because the stability of these antibacterial agents against β-lactamases producing bacteria and their ability to pass through bacterial outer membranes [10]. Resistance to carbapenems may due to production of MBLs enzymes, impermeability which resulted from the loss of the oprDporin and may due to the up regulation of an active efflux system present in these organisms. Carbapenem hydrolysing MBLs mechanism have been detected in many countries and have emerged as the most important mechanism of carbapenem resistance [11].

In current study, we utilized several unique methodological techniques in an endeavor to maximize the detection of such challenging organisms. imipenem/imipenem + EDTA combined disk method is considered a simple screening method that can be used. This technique is considered very useful and can be applied into the routine testing of any busy microbiology laboratory. In this test, the inhibition of MBLs enzymes are resulted from the presence of EDTA inhibitor agent that considered as metallo-chelator of zinc ion in active site of MBLs resulted in inactivated of MBLs enzymes [12]. In our study all imipenem resistance isolates were showed increase in inhibition

zone around imipenem - EDTA disk which considered a positive indicator that all 26 isolates were MBL producer [13].

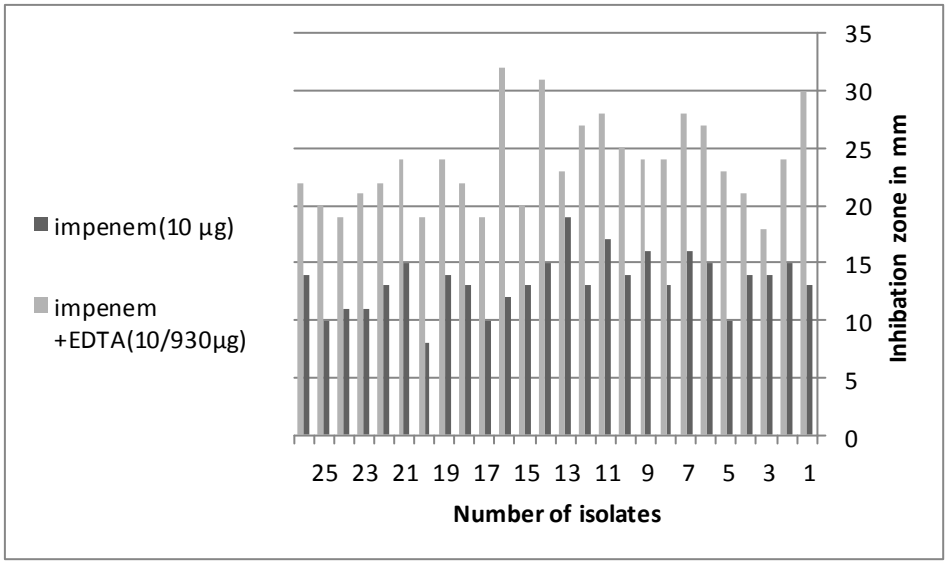

Figure 1: Difference between inhibition zone diameter with presence of impenem alone and impenem and EDTA agent.

4. CONCLUSION

In conclusion, this finding of MBL-producing gram negative bacteria highlights the emerging therapeutic challenge in our hospitals. The implementation of strict antimicrobial policies and infection control programs may help to prevent the rapid dissemination of these organisms. In addition, this study appears that imipenem-EDTA combined disc method effective for detection.

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