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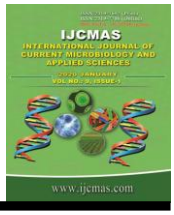

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Detection of MBL among Carbapenem resistant *Enterobacteriaceae* in a tertiary hospital using combined disc test

Martin M. Varghese*, Rama Sikka, Kausalya Raghuraman and Aparna Yadav

Department of Microbiology Pt. B. D. Sharma Post graduate Institute of Medical Sciences, Rohtak, Haryana, India

**Corresponding author*

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A B S T R A C T

MBL producing members of family *Enterobacteriaceae* have been responsible for numerous outbreaks of infection throughout the world. A total of 108 non repetitive carbapenem resistant strains isolated from various clinical samples, identified by standard microbiological techniques and antimicrobial susceptibility pattern was determined. MBL detection was done by combined disc test using ceftazidime-EDTA, imipenem-EDTA and meropenem-EDTA. Ceftazidime-EDTA detected 90 (97.8%) isolates, imipenem-EDTA detected 14 (15.2%) isolates and meropenem-EDTA detected 13 (14.1%) isolates showing MBL production. The combined disc test using inhibitors like EDTA was found to be sensitive in detecting MBL producers, although the results vary with different antibiotic discs used. Detection of MBL among Carbapenem resistant *Enterobacteriaceae* in a tertiary hospital using combined disc test.

Introduction

Gram-negative bacteria belonging to the family *Enterobacteriaceae* are the most habitually occurring bacterial isolates recovered from clinical specimens (Procop *et al.*, 2017). In addition, these organisms are among the major causative agents of nosocomial infections. Detecting these strains is not only important in treating the patients from whom the isolate is recovered but also has important implications for surveillance of nosocomial infections (Nordmann *et al.*,2011). Resistance to carbapenems among *Enterobacteriaceae* has primarily been attributed to the production of carbapenemases and to some extent to the decrease in the bacterial permeability and efflux pump mechanisms (Shinde *et al.*, 2017). Metallo- β -lactamases (MBL) are a unique class of enzymes that can degrade all but monobactams and are exceptional for their adequate carbapenemase activity.

In addition, these enzymes are not susceptible to β-lactamase inhibitors. MBLsare now regarded as a therapeutic challenge and the comprehension of their frightening properties is necessary in the fight to overcome them (Bebrone 2007).

Among the various methods for carbapenemase detection, it is the molecular methods which are considered gold standard, but the cost and inconvenience make it impractical for daily testing in many clinical laboratories, especially in developing countries like India (Nordmann *et al.,* 2009).

The need of the hour is the availability of a reliable, rapid and robust method for detecting carbapenemase production. Therefore, the purpose of this study is to identify rapid and cost-effective methods for detection of MBL production among *Enterobacteriaceae*.

Materials and Methods

This prospective study was carried out in the Department of Microbiology, Pt.B.D.Sharma PGIMS Rohtak. Study was done on isolates of the family *Enterobacteriaceae* obtained from clinical samples (urine, blood, pus, sputum, throat swabs, stool, various body fluids, etc.) received from various indoor and outdoor patients. A total of 108 multidrug resistant *Enterobacteriaceae* strains resistant to either one of the carbapenems (imipenem, meropenem and ertapenem) were included in the study(Jan 2018 to Dec2018).

The antimicrobial susceptibility testing was performed as per Clinical and Laboratory Standard Institute (CLSI) 2018 guidelines. All the antimicrobial discs were procured from HiMedia Laboratories, Mumbai, India. American Type Culture Collection (ATCC) strain viz. *E. coli* ATCC 25922 was used as control strain.

Combined Disc Tests (CDT)

Ceftazidime-EDTA test

The test organism was inoculated on MHA plate as per CLSI guidelines. A 0.5 M EDTA solution was prepared by dissolving 18.61gm of disodium EDTA.$2H_2O$ in 100 ml of distilled water and adjusting the pH 8 by using NaOH. Two 30µg ceftazidime discs were placed on the surface of agar plate and 10 µl of 0.5 M EDTA solution was added to one disc. The inhibition zone of ceftazidime and ceftazidime-EDTA disc was compared after 16-18 hours of incubation at 35ºC. A positive test was indicated if zone enhancement with EDTA impregnated ceftazidime disc was ≥7mm than the ceftazidime disc alone (Yong *et al.,* 2002).

Imipenem-EDTA test

Test organism was inoculated on MHA plate as per CLSI guidelines. A 0.5 M EDTA solution was prepared by dissolving 18.61gm of disodium EDTA.$2H_2O$ in 100 ml of distilled water and adjusting the pH 8 by using NaOH. Two 10µg imipenem discs were placed on the surface of agar plate and 10 µl of 0.5 M EDTA solution was added to one disc. The inhibition zone of imipenem and imipenem-EDTA disc was compared after 16-18 hours of incubation at 35ºC. A positive test was indicated if zone enhancement with EDTA impregnated imipenem disc was ≥7mm than the imipenem disc alone (Yong *et al.,* 2002).

Meropenem-EDTA test

The test organism was inoculated on MHA plate as per CLSI guidelines. A 0.5 M EDTA solution was prepared by dissolving 18.61gm of disodium EDTA.$2H_2O$ in 100 ml of distilled water and adjusting the pH 8 by using NaOH. Two 10µg meropenem discs

were placed on the surface of agar plate and 10 µl of 0.5 M EDTA solution was added to one disc. The inhibition zone of meropenem and meropenem-EDTA disc was compared after 16-18 hours of incubation at 35ºC. A positive test was indicated if zone enhancement with EDTA impregnated meropenem disc was ≥7mm than the meropenem disc alone (Yong *et al.*, 2002).

Statistical Analysis

The data collected was analysed with the help of software package (SPSS version 25.0). Frequency distribution and cross-tabulation was used to create summary tables and compare items within and across various categories.

Results and Discussion

A total of 2138 *Enterobacteriaceae*isolates were recovered from 27443 clinical samples, over a period of one year. Among these isolates, 108 were found to be resistant to either imipenem, meropenem or ertapenem. Among the 108 CRE, 39 (36.1%) were *Escherichia coli*, 36 (33.3%) were *Enterobacter spp.*, 26 (24.1%) were *Klebsiella pneumoniae*, 5 (4.6%) were *Citrobacter spp.* and 2 (1.9%) were *Klebsiella oxytoca*. MBL production was detected by CDT using ceftazidime-EDTA, imipenem-EDTA and meropenem-EDTA.On comparison of the various CDT (table 1), ceftazidime-EDTA detected 90 (97.8%) isolates, imipenem-EDTA detected 14 (15.2%) isolates and meropenem- EDTA detected 13 (14.1%) isolates showing MBL production. Sensitivity to carbapenems ranged from 0.9% to 13.9%, with ertapenem being most effective.All isolates were resistant to ampicillin and ceftazidime. Sensitivity to β-lactams/ β-lactam inhibitors ranged from 0.9% to 42.6%. Ticarcillin-clavulanic acid and piperacillin-tazobactam were the most effective drugs with 42.6% and 20.4% being susceptible respectively. Susceptibility to gentamicin and amikacin was 5.6% and 30.6% respectively. Among fluoroquinolones, sensitivity ranged from 7.4% to 14.8% with all strains being uniformly resistant to ciprofloxacin. Susceptibility of nitrofurantoin among urinary isolates was 72.5%.

The issue of carbapenem resistance is of utmost importance as it is often the antimicrobial agent of last resort against highly evolving and dangerous gram-negative bacteria. Often these isolates are multi drug resistant, extended drug resistant or pan drug resistant, which makes identifying, managing and treating such isolates difficult. The detection of antibiotic resistance among these isolates remains paramount. However, it remains problematic, especially in developing countries which bears much of the burden of these isolates. Carbapenems are often the antibiotics of last resort. At present, molecular methods remain the gold standard for the detection of these isolates, which is not economically feasible in most laboratory setups.

These methods also fail to identify novel methods or approaches adopted by strains to overcome and develop antibiotic resistance. On the other hand, there are no clear-cut phenotypic test able to detect all mechanisms of resistance (Aguirre-Quińenero *et al.*, 2017). In the present study employing 108 isolates, MBL detection by CDTwas observed in 92 isolates, out of which ceftazidime-EDTA method detected 90 isolates (97.8%), imipenem-EDTA method detected 14 isolates (15.2%) and meropenem-EDTA method 13 isolates (14.1%). It was observed that isolates which were positive by imipenem-EDTA method or meropenem-EDTA method were also positive by ceftazidime-EDTA disc method. Similar findings were reported in a study by Galani *et al.*, (2008) andPicao *et al.*, (2008).

Table.1 Detection of metallo-beta-lactamase production by combined disc test (n=108)

Combined disc synergy test (Total)	Ceftazidime-EDTA method		Imipenem-EDTA method		Meropenem-EDTA method	
	No	%	No	%	No	%
92	90	97.8	14	15.2	13	14.1

Table.2 Susceptibility of 108 carbapenem resistant isolates to various antimicrobial agents

Antimicrobial agents	Sensitive	
	No.	%
Ampicillin	00	00
Amoxycillin-clavulanic acid	01	0.9
Ampicillin/Sulbactam	06	5.6
Ticarcillin-clavulanic	46	42.6
Piperacillin/ Tazobactam	22	20.4
Ceftazidime	00	00
Cefepime	27	25
Imipenem	05	4.6
Meropenem	01	0.9
Ertapenem	15	13.9
Aztreonam	04	3.7
Doxycycline	00	00
Gentamicin	06	5.6
Amikacin	33	30.6
Ciprofloxacin	00	00
Ofloxacin	16	14.8
Norfloxacin	13	32.5
Levofloxacin	08	7.4
Trimethoprim/Sulfamethoxazole	16	14.8
Nitrofurantoin	29	72.5

* Nitrofurantoin and norfloxacin were tested against urinary isolates only(n=40)

Ceftazidime appears to produce a marked inhibitory effect with EDTA and therefore is a better substrate than imipenem or meropenem. Coincidental findings have also been reported by Galani *et al.*,(2008) and Umadevi *et al.*, (2011). The CDT using inhibitors like EDTA was found to be sensitive in detecting MBL producers although it suffers from poor specificity and the results vary with different antibiotic discs used. Another limitation of this test is that the inhibitor, i.e. EDTA itself can inhibit the growth of isolate, rather than synergizing the activity of the antibiotic disc used (Picão *et al.*, 2008).

The high prevalence of MBL emphasizes the

need for their early detection by simple screening methods, thereby providing adequate antimicrobial therapy as well as preventing the spread of such MDR strains.

The need of the hour is to develop a successful antimicrobial stewardship programme based on local epidemiological data and international guidelines that will optimize antimicrobial use among hospitalized patients, hereby improving patient outcomes in the longer run.

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