Original Article

Comparison Of Phenotypic And Genotypic Methods For The Detection Of Imipenem Resistant Enterobacteriaceae Isolates

[1]Dr. C.Anuradha,M.D, [2]Dr. S .Mathavi,M.D., [3]Dr. R.Indra Priyadharsini, M.D.,

[1]Assistant professor, Dept. of Microbiology, VMKV Medical College, Salem.
[2]Associate Professor, Dept. of Microbiology,VMKVMC, Salem. [3]Professor & Head, Dept. of Microbiology, VMKVMC, Salem.

ABSTRACT

INTRODUCTION : The rapid spread of metallobetalactamases (MBL) is a diagnostic challenge in clinical microbiology as there are concerns regarding the reliability of the various phenotypic detection methods.

AIMS AND OBJECTIVES : To assess the efficiency of the phenotypic assays namely the combined disc test (CDT), double disc synergy test (DDST) and the MBL E test as compared to PCR for the detection of MBLs in enterobacteriaceae.

MATERIALS AND METHODS: Two hundred and four non-repetitive enterobacteriaceae isolates from clinical samples from October 2013 to September 2014 were included in the study.Isolates that showed a resistant zone (≤ 19 mm) to imipenem were screened by means of combined disc test (CDT), double disc synergy test (DDST) and E test (imipenem/imipenem+EDTA E strips, Biomeriuex). Multiplex PCR was done to confirm the presence of MBL genes.

RESULTS : The study showed 49 isolates resistant to imipenem, of which 12 (24.48%) were positive for MBL production by both combined and double disc methods; 37 (75.52%) showed positivity by combined disc only and 12 (24.48%)isolates by double disc method only.Twelve isolates showed a MIC of ≥4µg/mL by E test .PCR detected genes in 11 isolates(22.4%) only.

CONCLUSION : Since phenotypic methods give variable results, it is not advisable to employ a single screening criterion for carbapenemase detection.Phenotypic methods though specific, do not differentiate between chromosomal and plasmid encoded genes and hence genotypic characterization (PCR) should be considered.

Key words : Phenotypic methods, genotypic methods , Imipenem , Enterobacteriaceae

INTRODUCTION:

The emergence and rapid spread of zinc dependant class B metallo beta lactamases (MBL) is a diagnostic challenge in routine clinical microbiology as there are concerns regarding the reliability of the various phenotypic detection methods.[1] Metallo beta lactamase producing isolates have been increasingly reported in many geographic regions. The production of these MBLs is one of the important mechanisms of bacterial drug resistance due to the ability of MBL producing isolates to spread and to hydrolyze most β-lactam agents. The genes responsible for MBL production are located on mobile genetic elements facilitating rapid spread to other bacteria.[1,2,3] This calls for an accurate detection of this resistance

Address for Correspondence :
Dr. C. Anuradha, M.D, Assistant Professor, Dept. of Microbiology, VMKV Medical College, Salem.
Ph : 9487521525 E mail : anudr@yahoo.com

phenotype by routine laboratories for effective therapeutic interventions and to implement proper infection control practices. Also infections caused by these carbapenemase-producing bacteria have spread rapidly all over the world which are difficult to treat.[4] Therefore, there is an urgent need for accurate and fast detection of carbapenemases in diagnostic laboratories. Current work flows involve an initial screening step for species and resistance pattern detection, followed by phenotypic and/or genotypic confirmation.[4,5]

AIM AND OBJECTIVES :

To assess the efficiency of the phenotypic assays namely the combined disc test (CDT) , double disc synergy test (DDST) and the MBL E test as compared to PCR for the detection of MBLs in enterobacteriaceae isolates.

To design an accurate MBL diagnostic method for effective therapeutic interventions and implementation of proper infection control procedures.

MATERIALS AND METHODS:

This study was done from October 2013 to September 2014. Various clinical samples like urine, blood, sputum, pus, endotracheal aspirates, bronchial secretions and wound swabs were collected and identified and speciated by standard methods.[6] 204 consecutive, non repeat Enterobacteriaceae isolates were included in the study. The isolates were subjected to antibiotic sensitivity testing by the Kirby-Bauer's disc diffusion method and the zone diameters were measured and interpretated as per CLSI guidelines.[7] The isolates that showed resistance to imipenem (zone diameter of ⩽ 19mm) were screened for MBL by the Combined disc (IMP-EDTA) and the Double disc synergy (DDST) tests.[2,3,7]

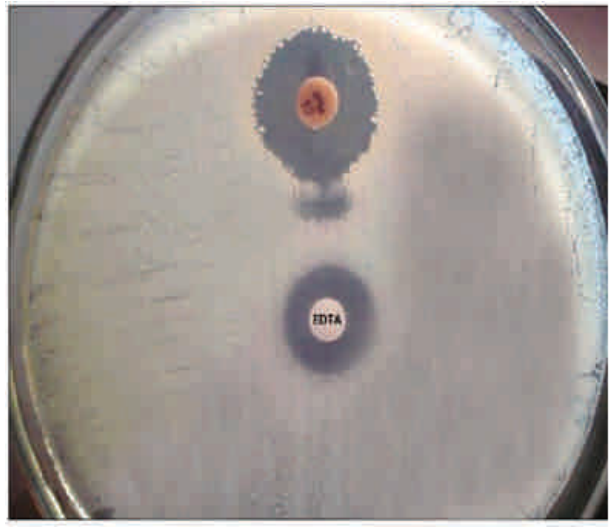
The IMP-EDTA combined disc test was performed as described by Yong et al.[2] [Figure-1]

Figure 1 : Combined disc method

The test organisms were inoculated on to plates with Mueller Hinton agar as recommended by the CLSI.[7] Two discs - 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 35°C.A zone diameter difference between the Imipenem and Imipenem / EDTA discs of ⩾7mm was considered as MBL positive.[2,7]

The IMP-EDTA double disc synergy test was performed as described by Lee et al and Arakawa et al.[3] [Figure-2]

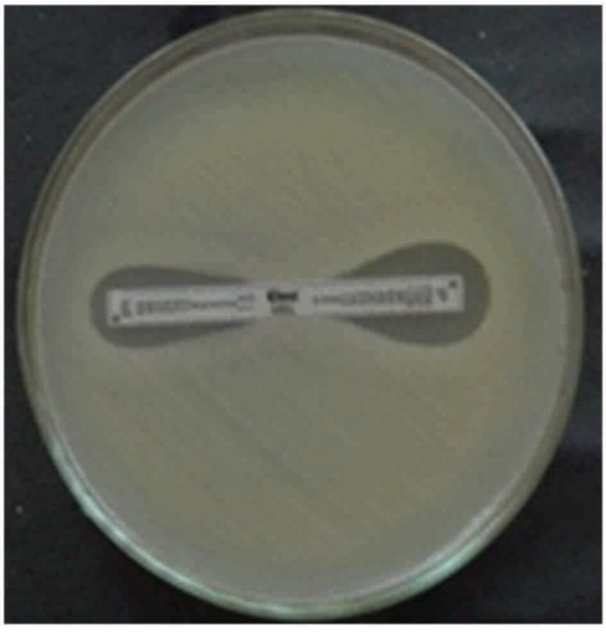
Figure 2 : Double Disc Synergy Test



The test organisms were inoculated on to plates with Mueller Hinton agar as recommended by the CLSI[1]. An imipenem (10 µg) disc was placed 20mm centre to centre[3] from a blank disc containing 10 µL of 0.5 M EDTA (750 µg). A zone of synergy between the imipenem and EDTA discs was interpreted as a positive result.[3,7]

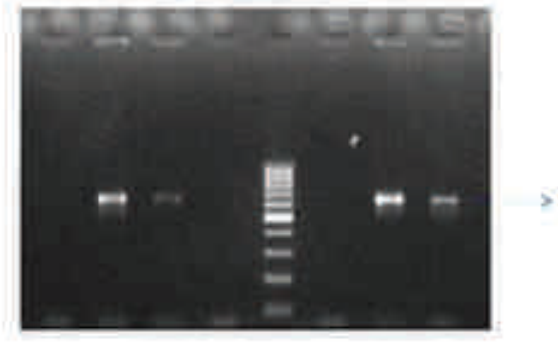
The minimum inhibitory concentration (MIC) was determined by using E strips for all 49 Imipenem resistant isolates (Biomeriuex).[1,7,8,9,10] [Figure-3] E-test MBL strip containing a double sided seven dilution range of Imipenem (4 to 256 mcg/ml) and Imipenem (1 to 64 mcg/ml) in combination with a fixed concentration of EDTA was used for MBL detection.After overnight incubation a MIC ratio of (Imipenem/Imipenem+EDTA) >8,or reduction of Imipenem MIC by >3 log2 dilutions in the presence of EDTA or an appearance of a phantom zone was interpreted as positive for MBL production[1,2]. The positive samples showed a minimum inhibitory concentration ratio of>8.

Figure 3: MBL E-Test



The genotypic detection was done for a total of 12 isolates i.e 11 isolates in resistant range (⩾4 µg/ml) and 1 isolate in intermediate range (2-3 µg/ml) by a Multiplex PCR targeting bla IMP, bla VIM, bla NDM, bla GIM , bla SIM to confirm the phenotypic results.[11,12,13,14] [Figure-4]

Figure 4: Multiplex PCR



The 37 isolates which had a MIC range of ⩽ 1µg/ml (sensitive) were not included for the genotypic detection.

RESULTS:

Of the 204 enterobacteriaceae isolates, 49 isolates that showed resistant zone diameters to imipenem [Chart 1] were screened for MBL by the CDT and DDST phenotypic methods [Chart 2]

Chart 1 : Imipenem Resistant Isolates

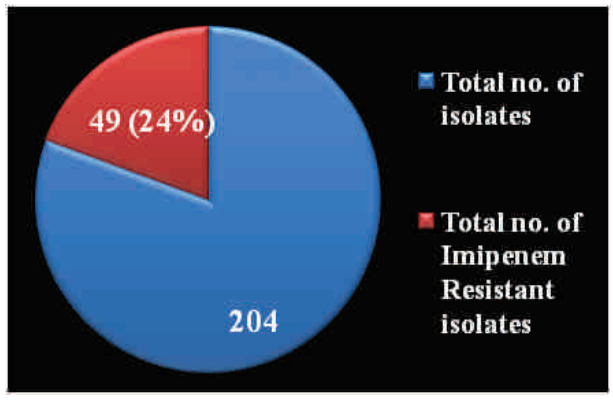
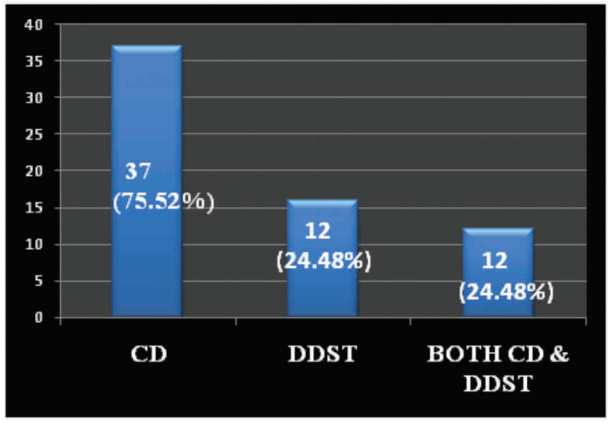
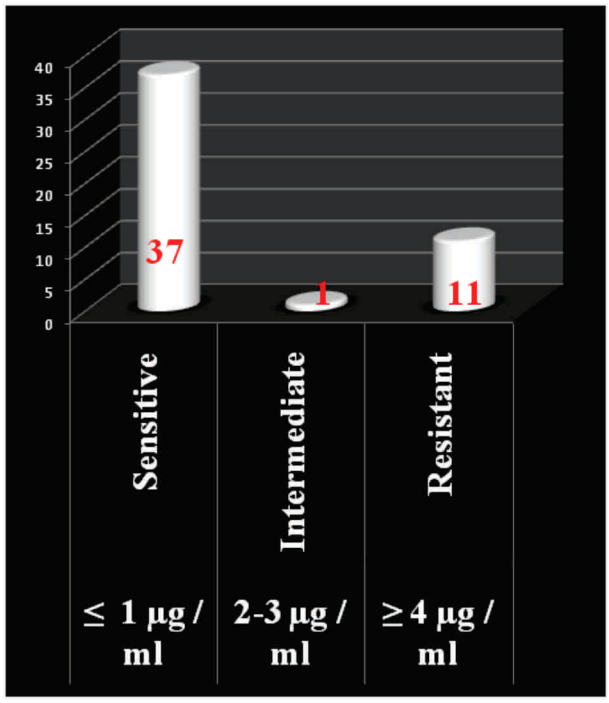


Chart 2 : Phenotypic MBL Screening



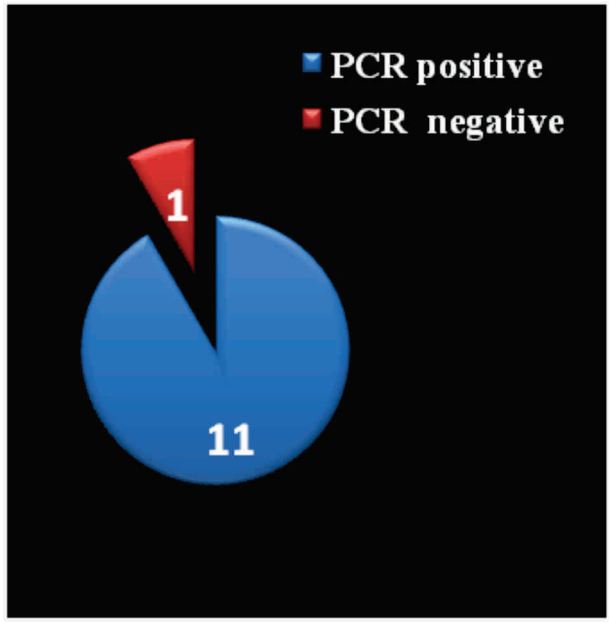
Twelve isolates (24.48%) were positive for MBL production by both combined and double disc methods; 37 (75.52%) isolates showed positivity by combined disc only and 12 (24.48%) isolates by double disc method only. The MIC was determined by E test for the isolates that were MBL positive by the phenotypic methods [Chart-3]

Chart 3 : Phenotypic Confirmation : MBL E-Test



Eleven isolates showed a MIC range of ⩾4µg/mL (resistant range),1 isolate showed a MIC range of 2-3 µg/mL (intermediate range) and remaining 37 isolates showed a MIC range of ⩽ 1µg/mL (sensitive range). Genotypic confirmation was done for the isolates in resistant and intermediate zone by PCR which detected genes in the 11 isolates in resistant zone (22.4%) only [Chart-4]

Chart 4 : Genotypic confirmation : PCR



DISCUSSION :

In this study, a total of 204 enterobacteriaceae isolates from various clinical samples were included. Of the total 204 isolates ,only 49 isolates showed a resistant zone diameter for imipenem. These 49 isolates were screened for MBL by the combined disc and the double disc synergy methods. It was found that MBL positivity was 75.52% (37 isolates) by CDT, 24.48 % (12 isolates) by DDST and 24.48 % (12 isolates) by both methods. The minimum inhibitory concentration by the MBL E-Test was done for all 49 Imipenem resistant isolates and it was found that 11 isolates were in resistant range (⩾ 4 µg/ml), 1 isolate in intermediate range (2-3 µg/ml) and 37 isolates in sensitive range (⩽ 1µg/ml) even though they showed resistant zone diameters for imipenem. Variable Carbapenem MICs may be due to the

following reasons like co-existence of ESBLs, Amp C over production with porin loss, bactericidal effect of EDTA through a membrane permeabilizing effect resulting in a larger zone diameter, all of which complicates phenotypic detection in routine tests.[11,15] Similar results were obtained by a study by Andrea Bartolini et al[19] and André Birgyin et al[20] which Amp C-positive samples showed a reduced susceptibility to imipenem, with MICs ⩾ 0.25 mg/L. These data confirmed the necessity to further test isolates with reduced susceptibility. So it is important to include a control disc containing only EDTA to assess its interference of the zone diameters along with MBL controls in phenotypic methods. Since phenotypic methods give variable results, it is not advisable to employ a single screening criterion for carbapenemase. Phenotypic methods though specific, do not differentiate between chromosomal and plasmid encoded genes and hence genotypic characterization (PCR) should be considered.[12,13]

The genotypic confirmation was done for a total of 12 isolates i.e 11 isolates in resistant range (⩾ 4 µg/ml) and 1 isolate in intermediate range (2-3 µg/ml) by a Multiplex PCR targeting bla IMP, bla VIM, bla NDM, bla GIM, bla SIM.[5,12,13,14] MBL gene was not detected in one isolate which was in the intermediate MIC range but the other 11 isolates which had a MIC of ⩾4 µg/ml in the resistant range had the MBL gene. bla VIM was detected in 8 isolates and bla IMP in 3 isolates. The application of molecular techniques associated with traditional phenotypic tests will be a faster and reliable method to detect carbapenemases, which helps in diagnostic, therapeutic and infection control measures. This study shows a very high rate of false positives occurring in MBL detection by the phenotypic methods. This could be due to a bactericidal effect of EDTA through a membrane permeabilizing effect resulting in a larger zone diameter rather than its metal chelating effect.[4,16,18] This calls for considerable caution in the interpretation of the zone diameters. It is important to include a control disc containing only EDTA to assess its interference of the zone diameters along with MBL controls. The frequent use of carbapenems has lead to an increase in carbapenem - resistant strains due to selective pressure.[17] 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.[5] To date there are no standard CLSI guidelines for MBL screening/detection.[16]

CONCLUSION :

The phenotypic appearance of MBL-carrying organisms varies depending on the bacterial host, with high incidence of carbapenem-susceptible Enterobacteriacea isolates. The emergence of these acquired carbapenemases, particularly Ambler class B metallo - P - lactamases (MBLs) makes the clinical use of carbapenems troublesome.

Such enzymes often confer high-level resistance to all beta-lactams except aztreonam. Five enzyme types have been identified (IMP, VIM, SPM, GIM and SIM types), involving various host organisms. A particular concern is that acquired MBL genes are located on integron structures that reside on mobile genetic elements such as plasmids or transposons, thus carring hidden MBL genes enabling widespread dissemination. Detection of MBL is imperative to halt the spread of Multi Drug Resistant (MDR) strains. The MIC to carbapenems may not be considerably raised in MBL-producing strains and it is important to implement simple screening procedures.More than one phenotypic screening coupled with PCR aids in preventing therapeutic failures and nosocomial outbreaks.[12,16]

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