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Triple-disk assay for phenotypic detection of predominant carbapenemases

Sir,

Carbapenems form an integral part of treatment regimen for serious and multi drug resistant Gram-negative bacterial infections. However, there are reports on increasing prevalence of carbapenem resistance in clinical isolates of *Enterobacteriaceae* mainly due to the production of metallo-β-carbapenemases (MBL),[1] *Klebsiella pneumonia* carbapenemase (KPC)[2] and ampC β-lactamases (AmpC)[3]. Thus, there arises an urgent need for establishment of a sensitive phenotypic assay that can facilitate simultaneous detection of MBL, KPC and AmpC. Recent studies have reported the use of β-lactum inhibitors coupled with meropenem disks for simple and accurate identification of carbapenemase producing organisms; for example, 3-aminophenylboronic acid (APBA), dipicolinic acid (DPA), and simultaneous use of APBA and cloxacillin (CLX) for detection of KPC, MBL and AmpC with porin loss respectively[4]. Thus, the purpose of this study was to determine the most predominant carbapenemase using the triple-disk assay in carbapenem resistant clinical isolates of *Enterobacteriaceae*.

A total of 19 consecutive meropenem resistant clinical isolates received in the Microbiology Department of P.D. Hinduja National Hospital and Research Centre, Mumbai, India, from March-July, 2010 were considered for this analysis. Meropenem resistance was determined using disk diffusion method as per the Clinical and Laboratory Standards Institute (CLSI) guidelines[5]. These resistant isolates were further evaluated for detection of the carbapenemases using the triple-disk assay[4]: a bacterial lawn was prepared using 0.5 McFarland inoculum on Muellar-Hinton agar plates. Four disks were placed on each plate: meropenem (10 µg, Rosco Diagnostica A/S, Taastrup, Denmark), meropenem (10 µg) + APBA (600 µg), meropenem (10 µg) + DPA (1000 µg) and meropenem (10 µg) + CLX (750 µg). An increase of ±5 mm in zone diameter around disks containing β-lactamase inhibitors, as compared with the disk with meropenem alone, was considered to be a positive result for APBA, DPA and CLX.

The 19 meropenem resistant isolates represented four different bacterial populations *i.e. K. pneumoniae* (68.4%, n=13), *Escherichia coli* (15.7%, n=3), *Citrobacter* species (10.5%, n=2) and *Enterobacter* species (5.2%, n=1). The triple-disk assay revealed 94.7% (n=18) organisms to be MBL producers, of which one isolate also coproduced AmpC; and one isolate was reported negative for production of cabapenemases suggesting the existence of an alternative mechanism responsible for conferring resistance. All 18 (100%) MBL producing isolates were molecularly proven to be positive for the presence of bla_{NDM-1} gene (data not shown). This triple-disk assay was found to be useful in detecting carbapenemases in *Enterobacteriaceae*, with MBL being the most predominant mechanism of resistance. Similar findings have been reported in an another study, wherein almost 100 per cent sensitivity and specificity have been reported on the use of triple-disk assay for detection of MBL and KPC[4]. More importantly, different carbapenemases produced are also known to cause variable levels of resistance to carbapenems and also other non-β-lactum drugs, hence this would facilitate initiation of a further optimized treatment regimen[6].

In conclusion, triple-disk assay could be considered as a simple phenotypic assay for identification of carbapenemases.

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