Sir,

A number of Gram negative bacilli especially belonging to family *Enterobacteriaceae* are rapidly acquiring resistance to the routinely used antimicrobial agents due to beta-lactamases production. This has led to an increase in the usage of newer drugs including carbapenems. Further, a number of bacteria belonging to the nonfermenter group - *Pseudomonas aeruginosa* and *Acinetobacter* species are being reported to be even resistant to this group of carbapenems due to metallo beta lactamases (MBL) production. Nonfermenter group of organisms is a group of aerobic, non-spore-forming Gram-negative bacilli that either do not utilize carbohydrates as a source of energy or degrade them through metabolic pathways other than fermentation. There are various reports on increasing prevalence of MBL worldwide including India.

Clinical and Laboratory Standards Institute (CLSI) has not laid down any specific guidelines for detection of MBL production in various organisms. Though there are several screening methods recommended for detection of MBL production, no single test, when used alone is specific for these enzymes. Polymerase chain reaction (PCR) is the most reliable diagnostic test but main difficulty is the prevalence of increasing types of MBLs requiring multiple primers. We thus evaluated two types of double disc diffusion tests (DDSTs): imipenem- ethylene diamine tetra acetic acid (IPM-EDTA) method and ceftazidime- mercapto propionic acid (CAZ-MPA) method to detect MBL production in *P. aeruginosa* and *Acinetobacter* species.

The present study was undertaken on the 100 isolates of *P. aeruginosa* and *Acinetobacter* species in the Department of Microbiology, Government Medical College Hospital, Chandigarh, North India during the time period of July 2005 to December 2006. The selection criteria for MBL detection was reduced susceptibility to ceftazidime (inhibition zone diameter <18 mm) and/or to imipenem (inhibition zone diameter <16 mm). MBL detection was done by Lee *et al.* modification of imipenem- EDTA (IPM-EDTA) disc method of Yong *et al.* by adding zinc sulphate to the medium to increase the sensitivity of the test, and ceftazidime- MPA (CAZ-MPA) disc method of Arakawa *et al.* All the MBL positive isolates were repeatedly checked for reproducibility and for susceptibility with aztreonam.

Of the total 100 isolates, 33 were *P. aeruginosa* and 67 belonged to *Acinetobacter* species. All these isolates were ceftazidime resistant and when tested by CAZ-MPA method, MBL production was seen in 97 (97%) of them. Imipenem resistance was seen in only 72 isolates and by IPM-EDTA method, 62 (86.11%) of these were positive for MBL production. All were sensitive to aztreonam.

Of the 33 *P. aeruginosa* isolates tested, 32 (96.97%) were found to be positive for MBL production by CAZ-MPA method. Of these, 25 isolates were also imipenem resistant and IPM-EDTA method was found to be positive for 21 (84%) of them.

Of the 67 isolates of *Acinetobacter* species tested by CAZ-MPA method 65 (97.01%) were positive for MBL production by CAZ-MPA method. Of these, 25 isolates were also imipenem resistant and IPM-EDTA method was found to be positive for 21 (84%) of them.

Of the 67 isolates of *Acinetobacter* species tested by CAZ-MPA method 65 (97.01%) were positive for MBL production. Only 47 isolates were imipenem resistant and when tested by IPM-EDTA method, 41 were positive (87.23%). Of the 100 isolates tested, 97 per cent isolates showed MBL production considering either method. Of the 72 imipenem resistant isolates, 62 were positive by IPM-EDTA disc method, remaining 10 which did not show any increase in growth inhibition zone after addition of EDTA, may be belonging to IMP-1 category, as these were ceftazidime resistant also and positive by CAZ-MPA method. The reason for detection by CAZ-MPA method than IPM-EDTA method could be that IMP-1 isolates usually show high level resistance to...
CAZ. Furthermore, 2-MPA has also been found to block IMP-1 activity strongly even at low concentrations giving clear-cut growth inhibition zones.

Use of imipenem resistant strains alone can reduce the screening work greatly and imipenem with EDTA is found to give larger inhibition zones especially for VIM-2 isolates. Moreover, some of the resistant strains may also have another ceftazidime resistance mechanism than MBL production. In such cases, DDST with imipenem can show better results than ceftazidime disc. However, there are chances that some strains may be missed out by using imipenem alone. The previous studies show that for some of the nonfermenter organisms, minimum inhibitory concentration (MIC) to imipenem may not be very high (MIC <4 µg/ml) and such organisms may appear susceptible to carbapenems by using current CLSI methods. The rate of resistance of Acinetobacter species to ceftazidime is also found to be more than that to imipenem at some places. Therefore, use of both CAZ-MPA method and IPM-EDTA method on ceftazidime resistant strains and imipenem resistant strains is being recommended.

Our study emphasizes the use of two different substrates with different inhibitors as each method individually has its own merits and demerits. This methodology can be adapted for MBL screening in routine diagnostic microbiology laboratories.

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References