Correspondence

Metallo-β-lactamase producing *Pseudomonas aeruginosa* in a hospital from a rural area

Sir,

*Pseudomonas aeruginosa* exhibits resistance to a variety of antimicrobials including beta lactams. Carbapenems are often used as antibiotics for treatment of infections caused by β-lactam resistant *P. aeruginosa*\(^1\). However a group of carbapenem hydrolyzing beta-lactamases known as metallo beta-lactamases (MBLs), belonging to Ambler’s class B\(^2\) or to Bush *et al* group 3\(^3\) classification of ESBLs (extended spectrum beta-lactamases), have been reported in several countries, particularly in clinical isolates of *P. aeruginosa*\(^4,5\). The MBLs are inhibited *in vitro* by CuCl\(_2\), FeCl\(_3\), EDTA, sodium mercaptoacetic acid (SMA), 2 mercaptopropionic acid (2MPA) and 2-mercaptoethanol (2ME)\(^7\) but not by clavulanic acid, sulbactam or tazobactam\(^8,9\). Aztreonam is the treatment of choice for infections with organisms producing MBLs\(^3\). In order to identify the correct antibiotic for treatment and to prevent dissemination of such infection, it is important to detect these strains in the laboratory.

We studied 174 *P. aeruginosa* isolates obtained from various clinical specimens received in the Department of Microbiology, Mahatma Gandhi Institute of Medical Sciences, Sevagram between January and August 2004 and identified as per standard techniques. Antibiotic susceptibility to chloramphenicol (30 µg), gentamycin (10 µg), amikacin (10 µg), ceftriaxone (30 µg), ciprofloxacin (10 µg), ceftazidine (30 µg) and imipenem (10 µg) was performed by Kirby Bauer’s disk diffusion method as per National Committee for Clinical Laboratory Standards (NCCLS) guidelines (2002)\(^10\). *E. coli* ATCC 25922 and *P. aeruginosa* ATCC 27853 (courtesy Microbiology Department, Lady Hardinge Medical College, New Delhi) were used as negative controls. Isolates resistant to imipenem (IMP) and ceftazidime (CAZ) were suspected to be MBL producers and were confirmed by double disk synergy test (DDST)\(^7\).

In brief, a colony of the suspected isolate was suspended in Muller Hinton broth and diluted to obtain 10\(^6\) cfu/ml. Lawn culture was made on Muller Hinton agar and DDST was put. The combinations used in DDST were: CAZ-2ME, CAZ-EDTA, IMP-2ME and IMP-EDTA. All MBL positives were checked twice for reproducibility.

All MBL positive isolates were also subjected to DDST using ceftazidime-amoxycyclav and cefaperazone-sulbactam combination to determine if the MBLs were inhibited by clavulanic acid or sulbactam.

Of the 174 isolates studied, 15 (8.62%) were suspected to be MBL producers (resistant to both IMP and CAZ). In DDST, all 15 suspected isolates gave a clear large zone of inhibition around CAZ disc towards EDTA as well as 2ME (Table and Fig.). When IMP was used in place of CAZ, only 14 of the 15 isolates could be confirmed for MBL production and 2 of these gave small zone with EDTA while 3 gave small and 3 narrow zone of inhibition (Table; Fig.) in contrast to all 15 giving large zone when CAZ was used with these inhibitors.

MBLs are enzymes that are either chromosomal or plasmid encoded\(^4,5,11,12\). IMP-1 was the first MBL identified in *P. aeruginosa*\(^12,13\). Several other novel MBLs like VIM-1, VIM-2 and VIM-3\(^5,6\) have been identified recently from *P. aeruginosa*. 

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A preliminary study from an urban hospital in Bangalore\textsuperscript{14} reported 6 of the 50 isolates of \textit{P. aeruginosa} to be MBL positive. Our study revealed that MBL producing \textit{P. aeruginosa} is prevalent in this rural hospital also. All our MBL producer isolates were resistant to all antibiotics tested and the MBL production was not inhibited by beta lactam inhibitors (clavulanic acid and sulbactam) as has been reported earlier\textsuperscript{8,9,14,15}.

In our study DDST using CAZ with either 2ME or EDTA gave better results than IMP-2ME or IMP-EDTA. CAZ was found to be the most useful substrate for detection of IMP-1\textsuperscript{8} or both IMP-1 and VIM producers\textsuperscript{6} by DDST. However, IMP disk and a disk impregnated with both EDTA (750 $\mu$g) + SMA (2 $\mu$g)\textsuperscript{1} or a single disc containing both IMP and EDTA (750 $\mu$g)\textsuperscript{16} were found to be excellent in differentiating MBL producers.

Different methods such as PCR\textsuperscript{13}, E test\textsuperscript{17}, microdilution test\textsuperscript{19} and modified Hodge\textsuperscript{19} test have been used besides DDST to identify MBL producers. However, these test are either not cost-effective (PCR, E test) or time consuming (microdilution) for routine testing in clinical laboratories. PCR has become more difficult with the increase in number of types of MBLs\textsuperscript{16}. Hodge test performed on positive isolates in our laboratory did not give an appreciable distorted zone of inhibition and therefore was not found suitable when compared to DDST (unpublished data).

Considering the need to institute correct antibiotics to the patients infected with MBL producers and to prevent spread of MBL positive organisms, all clinical microbiology laboratories must routinely identify the MBL producers, and simple DDST test using CAZ-2ME or CAZ-EDTA could be used in developing countries.
References


