Correspondence

Imipenem-resistance among multi-drug resistant clinical strains in urinary infections from Kolkata

Sir,

Imipenem (N-formimidyl thienamycin), a carbapenem, is a semisynthetic derivative of thienamycin produced by *Streptomyces cattleya*. It exerts its bactericidal action by inhibiting cell wall synthesis in aerobic and anaerobic Gram-positive and Gram-negative bacteria. Imipenem shows strong affinity for penicillin binding protein of *Escherichia coli* and selected strains of *Pseudomonas aeruginosa* resulting in rapid lysis and cell death without filament formation. Bacterial resistance to imipenem can occur due to production of carbapenemase or metallo-beta lactamase capable of hydrolyzing the carbapenem nucleus and also able to alter the porin channels in the bacterial cell wall, thereby reducing the permeability of the drug. Carbapenems have been used in clinical settings as a last resort for their broad-spectrum antibacterial activity and stability against various beta-lactamases produced by Gram-negative bacteria including extended-spectrum beta-lactamases (ESBLs). Imipenem (IPM) was approved for clinical use in 1987 in Japan, followed by panipenem and meropenem in 1993 and 1995, respectively. Carbapenem resistant strains emerged in Japan by 1999.

The metallo-beta-lactamase (MBLs) are carbapenem-hydrolyzing enzyme, MBLs differ from other beta-lactamases in having a serine moiety at the active site and are inhibited by thiol compounds like 2-mercaptoethanol, 2-mercaptopropionic acids and mercaptoacetic acid. MBL enzymes require divalent cations for activation (especially Zn$^{2+}$), are resistant to inhibition by clavulanic acid, sulbactam and tazobactam, are inhibited by chelating agents, and do not hydrolyze monobactam like aztreonam.

Scanty data are available on imipenem resistance from India especially from Kolkata. Hence we conducted this study to determine imipenem-resistance and the production of MBL by clinical isolates from different hospitals in Kolkata.

During February 2002 to April 2003, 284 non-repeat clinical isolates were collected from patients from six hospitals of Kolkata. Of these, 90 (31.7%) were isolated from pus, 132 (46.5%) from urine, 57 (20.1%) from sputum and 5 (1.8%) from other sources. The antibiotic resistant patterns of these isolates were obtained. Five isolates were found resistant to IPM and those which did not follow National Committee for Clinical Laboratory Standards (NCCLS) criteria for ESBL production were chosen for Hodge test and double disc synergy test (DDST) using EDTA (0.5 M solution; Merck) and thiol 2-mercaptoethanol (Sigma Chemical Co., USA) along with imipenem disc, the presence of even a small synergistic inhibition zone was interpreted as positive for MBL production.

Of the 284 clinical isolates tested, 123 (43.3 %) were multidrug resistant (MDR), i.e., they were resistant to at least seven antibiotics (ampicillin, amoxicillin, cephalaxin, ciprofloxacin, cotrimaxazole, erythromycin, gentamycin) and also...
to third-generation cephalosporins (3GC). Of these MDR isolates, 5 (1.8%) were found to be resistant to all commercially available beta-lactamase inhibitors, i.e., clavulanic acid, sulbactam and tazobactam, and to imipenem. Although MBL are supposed to be susceptible to aztreonam, we found four of the MBL producers (except Proteus vulgaris RT-37) to be resistant to it. This variation in the four strains, may be due to, simultaneous presence of the combined mechanisms of resistance of the isolates, i.e., enzyme production, impermeability and efflux. IMP-1 beta-lactamase producing K. pneumoniae from Singapore, were resistant to all carbapenems, penicillins and cephalosporins, and these resistance cannot be overcome with any beta-lactamase inhibitors. Aztreonam is stable against these enzymes, but producers are often resistant by other mechanisms.

Of the five imipenem-resistant strains, one was P. aeruginosa and four were P. vulgaris. These isolates were then tested by Hodge test to distinguish the MBL producers from the non-producers, and all five gave positive results with 10 µl of 50 mM zinc sulphate (140 µg/disc). Lee et al recommended putting one disc of IPM at the centre of the plate but we placed two discs of IPM on two sides of the plate. The next day zinc ions (zinc sulphate) were applied to one and one was left as such, and this demonstrated the effect of Hodge test clearly. To further confirm the production of MBL by these organisms, DDST was performed using imipenem, ceftazidime along with 2-mercaptoethanol and EDTA as inhibitors for MBL. Among the MBL inhibitors used, 2-mercaptoethanol gave the clearest synergistic effect, i.e., zone of inhibition. EDTA gave a growth inhibitory zone between the two discs, but its appearance and reproducibility were relatively less in three of the five strains (P. aeruginosa CMC-21, P. vulgaris RT-37 and P. vulgaris CMC-172).

In our study all the imipenem resistant isolates of P. aeruginosa and P. vulgaris were isolated from urine samples, however their clinical significance as yet is not clear. Takeyama et al also reported P. aeruginosa isolates producing MBL in patients with urinary tract infection.

Ours is probably the first report of P. vulgaris producing MBL in India. The only report of Proteus spp. producing MBL is from Japan in 1999. P. aeruginosa producing MBL have been reported by from Bangalore, in a rural area in India, and in non-fermenting Gram-negative bacilli. A study from Chennai on P. aeruginosa detected 16 per cent resistance to imipenem; of these 87.5 per cent were found to be metallo-beta-lactamase producers. In 1984, we reported all isolates from our laboratory to be susceptible to N-formimidoyl theinamycin. In 1989, Gram-negative organisms recovered from neutropenic patients in India showed susceptibility to imipenem. Chitnis et al found 11 per cent of Gram-negative bacilli from urine, pus, body fluid and blood from hospitalized patients to be susceptible only to meropenem, while Gupta et al reported P. aeruginosa showing resistance for imipenem and meropenem.

Our study indicates the urgent need for action to prevent further spread of MBL-producing organisms by developing methods for rapid detection of MBL and containment of infection.

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