

## Correspondence

### Comparative evaluation of phenotypic tests for identification of metallo $\beta$ -lactamases producing clinical isolates of *Pseudomonas aeruginosa*

Sir,

Carbapenems have been the most successful  $\beta$ -lactam antibiotics used in the treatment of infections caused by  $\beta$ -lactam resistant Gram-negative bacteria. However, the clinical utility of these antimicrobials is under threat with the emergence of carbapenemases, particularly the Ambler class B metallo  $\beta$ -lactamases (MBLs). MBLs can hydrolyze most  $\beta$ -lactams except for monobactams and confer a broad-spectrum  $\beta$ -lactam resistance phenotype to the bacterial host, which is not reversible by conventional therapeutic  $\beta$ -lactamase inhibitors. The prevalence of MBLs has been increasing worldwide, notably among *Pseudomonas aeruginosa* and lately, amongst other Gram-negative bacteria as well<sup>1</sup>.

All the methods for detection of MBL producing bacterial isolates depend on the principle, that MBLs are affected by the removal of zinc from their active site. Still, no single screening method has been found to be perfect. Currently, there is no Clinical Laboratory Standards Institute (CLSI) recommended method available. Also, no standard method is recommended by any other international committee for the detection of MBL producers.

Most of the studies from different parts of the world compared some of the available tests, but no study has ever been undertaken which has compared all the available tests. Such studies are lacking in India also. The present study thus, envisaged comparative evaluation of the various available phenotypic methods for detection of MBLs in *P. aeruginosa* in order to identify the most sensitive method for Indian clinical isolates.

One hundred non repetitive clinical isolates of *P. aeruginosa* randomly collected between March 2002 and December 2005 were screened for susceptibility to imipenem (IPM) using the CLSI disc diffusion

method<sup>2</sup>. Of these 100 isolates, 41 had been collected from Safdarjung Hospital, New Delhi, and 59 were from Vallabhbai Patel Chest Institute, Delhi. All imipenem non susceptible isolates were tested for MBL production using a battery of phenotypic tests viz., modified Hodge test on Mueller-Hinton agar (MHT-MHA)<sup>3</sup>, modified Hodge test on MacConkey agar (MHT-MCA)<sup>4</sup>, imipenem-ethylenediaminetetraacetic acid + sodium mercapto acetic acid double disc synergy test (IPM-EDTA+SMA DDST)<sup>3</sup>, combined disc test (CDT)<sup>5</sup>, extended EDTA disc synergy test (eEDST)<sup>6</sup> and EDTA-IPM microbiological assay (EIM)<sup>6</sup>.

Of the 100 clinical isolates of *P. aeruginosa* screened, 21 were non susceptible to IPM. All these 21 isolates were subjected to the phenotypic tests listed above. MHT-MHA identified 15 (71.4%) isolates, whereas MHT-MCA identified 19 (90.5%) isolates as MBL producers. Each of the three techniques viz., DDST, EIM assay, and eEDST was able to identify 20 (95.2%) isolates as MBL producers. Similarly, CDT could detect 20 (95.2%) isolates as positive. In eEDST, IPM disc synergy could identify 20 isolates; meropenem (MEM) disc synergy identified 18 while ceftazidime (CAZ) disc synergy could detect only 12 isolates as MBL producers.

Of the 21 IPM non susceptible isolates as many as, 20 could be identified as MBL positive by four techniques viz., EIM assay, DDST, CDT and eEDST-IPM. However, only 15 of 21 isolates were positive by all the tests employed in the study (Table). One isolate was negative by all the tests.

Varying prevalence rates of MBLs producing *P. aeruginosa* have been reported worldwide. As many as, 43.9 per cent of Brazilian and 39.1 per cent of Italian imipenem resistant isolates of *P. aeruginosa*

**Table.** Comparison of different phenotypic tests for detection of MBL producing isolates

Phenotypic test	Number of MBL positive isolates (n=21)
Modified Hodge test on Mueller Hinton Agar	15
Modified Hodge test on MacConkey Agar	19
Combined disc test	8
Ceftazidime + EDTA	8
Imipenem + EDTA	20
Double disc synergy test	20
EDTA-Imipenem microbiological assay	20
Extended EDTA disc synergy test	12
Ceftazidime	12
Imipenem	20
Meropenem	18

n, number of screen positive isolates tested

were reported to be MBL producers<sup>7</sup>. MBL positive *P. aeruginosa* constituted nearly 20 per cent of all nosocomial isolates in Korea<sup>8</sup>. In India, a few studies have been carried out in different parts of the country in the recent past. The prevalence rates reported by these workers have ranged between 4.5 to 14 per cent<sup>9-11</sup>. In our study, however, 20 per cent of the clinical isolates of *P. aeruginosa* were confirmed as MBL producers. The higher incidence recorded in our study could possibly be ascribed to use of multiple and sensitive techniques employed. It also indicated the alarming rise in incidence of MBL positive *P. aeruginosa*.

Conventionally, MHT is performed using MHA; however, Lee *et al*<sup>4</sup> reported better results using MCA instead of MHA. Our study corroborated the results reported by Lee *et al*<sup>4</sup> as the MHT-MCA detected MBLs in 19 (90.5%) isolates; while the conventional MHT-MHA detected only 15 (71.4%) isolates to be MBL positive.

A study incorporating the comparative evaluation of variations of CDT *viz.*, CAZ + EDTA and IPM + EDTA showed the former to be more sensitive in detecting MBLs in *P. aeruginosa*<sup>12</sup>. A study from India, reported detecting MBLs in 6 of 8 isolates of *P. aeruginosa* using CAZ + EDTA and in 5 of 8 using IPM + EDTA<sup>10</sup>. However, we could detect MBLs in 38.1 per cent of the isolates using CAZ + EDTA, while, the IPM + EDTA combination detected MBLs in as many as 95.2 per cent of the isolates.

Comparison of MHT, CDT, DDST and E-test found DDST and CDT to be more sensitive for *P. aeruginosa*<sup>13</sup>. A study from India, found CDT and MBL E- test to be equally sensitive in detecting

MBL producers<sup>14</sup>. IPM+EDTA DDST was reported to be more sensitive in detecting MBL producing *P. aeruginosa* than MHT by another group of workers<sup>15</sup>. We found CDT (IMP+EDTA), EIM assay, DDST and eEDST (IMP) to be equally efficient for detection of MBLs in Indian clinical isolates of *P. aeruginosa*. One isolate, which was negative by all the tests, could possibly have a non-enzymatic mechanism of imipenem resistance.

### Acknowledgment

Authors thank Dr Rajni Gaiind, Department of Microbiology, Safdarjung Hospital and Associated Vardhman Mahavir Medical College, New Delhi, for providing isolates of *P. aeruginosa*.

**Sakshi P. Singh, Malini Shariff**

**Tanushree Barua & S.S. Thukral\***

Department of Microbiology, Vallabhshai Patel Chest Institute, University of Delhi, Delhi 110 007, India

\*Present address:

Department of Microbiology, College of Medicine Sultan Qaboos University, Muscat, Oman

\*For correspondence:

sharant@hotmail.com, sharant@squ.edu.om

### References

- Walsh TR, Toleman MA, Poirel L, Nordman P. Metallo  $\beta$ -lactamases: the quiet before the storm? *Clin Microbiol Rev* 2005; 18 : 306-25.
- Clinical and Laboratory Standards Institute. *Performance standards for antimicrobial susceptibility testing, 7<sup>th</sup> Informational Supplement (M100-S17)*. Wayne, PA: Clinical Laboratory Standards; 2007.
- Lee K, Lim YS, Yong D, Yum DH, Chong Y. Evaluation of the Hodge test and imipenem EDTA double disc synergy test for differentiating metallo  $\beta$ -lactamase producing isolates of *Pseudomonas* spp. and *Acinetobacter* spp. *J Clin Microbiol* 2003; 41 : 4623-9.
- Lee K, Yum JH, Dongeun Y, Lee HM, Kin HD, Docquier J-D, *et al*. Novel acquired metallo  $\beta$ -lactamase gene *bla*<sub>SIM-1</sub> in a class I integron from *Acinetobacter baumannii* clinical isolates from Korea. *Antimicrob Agents Chemother* 2005; 49 : 4485-91.
- Yong D, Lee K, Yum JH, Shin HB, Rossolini GM, Chong Y. Imipenem EDTA disc method for differentiation of metallo  $\beta$ -lactamase producing clinical isolates of *Pseudomonas* spp. and *Acinetobacter* spp. *J Clin Microbiol* 2002; 40 : 3798-801.
- Marchiaro P, Mussi MA, Ballerini V, Pasteran F, Viale AM, Vila AJ, *et al*. Sensitive EDTA based microbiological assay for the detection of metallo  $\beta$  lactamase in non fermentative Gram negative bacteria. *J Clin Microbiol* 2005; 43 : 5648-52.
- Toleman MA, Biedenbach D, Bennet DMC, Jones RN, Walsh TR. Italian metallo  $\beta$ -lactamases: a national problem? Report from the SENTRY Antimicrobial Surveillance Program. *J Antimicrob Chemother* 2005; 55 : 61-70.

8. Lee K, Lee WG, Uh Y, Ha GY, Cho J, Chong Y. VIM- and IMP- type metallo  $\beta$ -lactamase producing *Pseudomonas* spp. and *Acinetobacter* spp. in Korean hospitals. *Emerg Infect Dis* 2003; 9 : 868-71.
9. Navaneeth BV, Sridaran D, Sahav D, Belwadi MRS. A preliminary study on metallo  $\beta$ -lactamase producing *Pseudomonas aeruginosa* in hospitalized patients. *Indian J Med Res* 2002; 116 : 264-7.
10. Hemalatha V, Sekar U, Kamat V. Detection of metallo  $\beta$ -lactamase producing *Pseudomonas aeruginosa* in hospitalized patients. *Indian J Med Res* 2005; 122 : 148-52.
11. Mendiratta DK, Deotale V, Narang P. Metallo  $\beta$ -lactamase producing *Pseudomonas aeruginosa* in a hospital from a rural area. *Indian J Med Res* 2005; 121 : 701-3.
12. Picao RC, Andrade SS, Nicoletti AG, Campana EH, Moraes GC, Mendes RE, *et al*. Metallo  $\beta$ -lactamase detection: Comparative evaluation of double disc synergy versus combined disc tests for IMP-, GIM-, SIM-, SPM-, or VIM-producing isolates. *J Clin Microbiol* 2008; 46 : 2028-37.
13. Fam N, Diab M, Gomma H, El-Defrawy I. Phenotypic detection of metallo  $\beta$ -lactamases and extended spectrum  $\beta$ -lactamases among Gram negative bacterial clinical isolates. In: 16<sup>th</sup> European Congress of Clinical Microbiology and Infectious Diseases. Abstract no. p- 1452; 2006.
14. Behera B, Mathur P, Das A, Kapil A, Sharma V. An evaluation of four different phenotypic techniques for detection of metallo  $\beta$ -lactamase producing *Pseudomonas aeruginosa*. *Indian J Med Microbiol* 2008; 26 : 233-7.
15. Jesudason MV, Kandathil AJ, Balaji V. Comparison of two methods to detect carbapenemases and metallo  $\beta$ -lactamase production in clinical isolates. *Indian J Med Res* 2005; 121 : 780-3.