Indian J Med Res 149, February 2019, pp 216-221
DOI: 10.4103/ijmr.IJMR_1_18

Quick Response Code:

Phenotypic & genotypic profile of antimicrobial resistance in *Pseudomonas* species in hospitalized patients

Vijeta Bajpai[1], Aishwarya Govindaswamy[1], Surbhi Khurana[1], Priyam Batra[1], Anjana Aravinda[1], Omika Katoch[1], Fahmi Hasan[1], Rajesh Malhotra[2] & Purva Mathur[1]

[1]Department of Microbiology, Jai Prakash Narayan Apex Trauma Centre & [2]Department of Orthopaedics, All India Institute of Medical Sciences, New Delhi, India

Received January 1, 2018

***Background & objectives*: Nosocomial infections caused by multidrug-resistant, *Pseudomonas* species have become a major clinical and public health concern. The aim of this study was to characterize phenotypic and genotypic profile of antimicrobial resistance (AMR) in *Pseudomonas* spp. isolated from hospitalized patients.**

***Methods*: A total of 126 consecutive, non-duplicate isolates of *Pseudomonas* spp. isolated from various clinical samples were included in the study over a period of two years. Identification and antimicrobial sensitivity was performed using automated culture system according to the Clinical and Laboratory Standards Institute (CLSI) recommendations. Phenotypic detection of extended-spectrum β-lactamases (ESBLs), Amp-C β-lactamase (AmpC) and metallo-β-lactamases (MBLs) were done by various combinations of disc-diffusion and *E*-test methods, followed by polymerase chain reaction-based detection of β-lactamase-encoding genes.**

***Results*: Among 126 clinical isolates, 121 (96.1%) isolates were identified as *Pseudomonas aeruginosa*. Most of the isolates were recovered from pus sample, 35 (27.8%) followed by urine, 25 (19.84%); endotracheal aspirate, 24 (19.04%); blood, 14 (11.11%) and sputum, four (3.17%). The highest rate of resistance was against ticarcillin-clavulanic acid, 113 (89.7%) followed by meropenem, 92 (72.5%) and ceftazidime, 91 (72.3%). Overall, ESBLs, AmpC and carbapenemase production was detected in 109 (96.4%), 64 (50.8%) and 105 (94.6%) isolates by phenotypic methods. The most prevalent ESBL gene was bla_{TEM} in 72 (57.1%) and the least prevalent was bla_{SHV} in 19 (15.1%) isolates. AmpC gene was seen less compared to ESBL gene. The most prevalent carbapenemases gene was bla_{NDM-1} 41 (46.06%) followed by bla_{VIM} and bla_{OXA-1}.**

***Interpretation & conclusions*: Our findings suggested that a high rate of ESBLs and carbapenemases production was observed in *Pseudomonas* spp. Therefore, phenotypic and genotypic detection of AMR needs to be combined for better characterization of resistance patterns in *Pseudomonas* spp.**

Key words Amp-C β-lactamase - carbapenemases - extended-spectrum β-lactamases - *Pseudomonas aeruginosa*

Pseudomonas aeruginosa is a Gram-negative opportunistic pathogen responsible for a wide range of nosocomial infections, including surgical-site infections, septicaemia, urinary tract infections and lower respiratory tract infections[1-4]. Various antimicrobial agents, anti-*Pseudomonas* penicillin, cephalosporins and carbapenems have been used for the treatment of infections caused by *P. aeruginosa*[5]. Selection of the most appropriate antibiotic for the treatment of these nosocomial infections is a serious therapeutic challenge because of the ability of *P. aeruginosa* to develop resistance to multiple classes of antimicrobial agents, even during the course of treating an infection.

Antimicrobial resistance (AMR) in *P. aeruginosa* is usually the result of combination of different imported (mobile genetic elements) and chromosomally encoded resistance mechanisms[6]. Imported resistance to the β-lactams involves the production of inactivating β-lactamases, *i.e.* extended-spectrum β-lactamases (ESBLs) and metallo-β-lactamases (MBLs). ESBLs mediate resistance to extended-spectrum cephalosporins such as cefotaxime (CTX), ceftriaxone (CXN) and ceftazidime (CAZ)[7,8]. The most common imported β-lactamases found among *P. aeruginosa* isolates belong to the molecular class A serine β-lactamases (PSE, CARB and TEM families)[9]. MBLs are carbapenem-hydrolysing enzymes and belong to four major families (IMP, VIM, SPM and GIM) identified from nearly all regions of the globe[10]. In India, the prevalence of MBLs ranges from 7.5 to 71 per cent[11]. New Delhi metallo-β-lactamase-1 (NDM-1) is a transferable molecular Class B β-lactamase that was first reported in 2008 in Sweden[12]. NDM-1-producing strains exhibit multidrug-resistant profile because these also harbour other genes that encode for resistance to aminoglycosides and fluoroquinolones. Till date, there are only a few reports of NDM-1 in *P. aeruginosa* all over the world including India[11-13]. Apart from imported resistance, other encoded resistance involves the production of Amp-C β-lactamase (AmpC) which hydrolyzes cefoxitin (CTN) as well as other extended-spectrum cephalosporins and aztreonam (AZT) and is poorly inhibited by clavulanic acid (CLAV)[14]. Detection of ESBL-, AmpC- and MBL-producing *Pseudomonas species* (spp.) isolates are of crucial importance for optimizing the treatment of patients and also prevention of spread of resistant strains. Therefore, the aim of the present study was to investigate phenotypic and genotypic profile of ESBL, MBL and AmpC production among *Pseudomonas* spp. obtained from various clinical specimens from hospitalized patients.

Material & Methods

This study was conducted at the microbiology laboratory of the Jai Prakash Narayan Apex Trauma Center (JPNATC) of All India Institute of Medical Sciences (AIIMS), New Delhi, India, over a period of two years (2013-2015). All consecutive, non-duplicate isolates of *Pseudomonas* spp. isolated from various clinical samples of patients were included in the study. The isolates were collected consecutively for two years from 2013 to 2015 to obtain temporally representative data.

The study was conducted after due approval from the Institutional Ethics Committee. Written informed consent was obtained from all patients. All the isolates were identified by standard microbiological methods and the automated system, VITEK-2 identification system (BioMérieux, France). The antimicrobial susceptibility testing of all isolates was done by the disc-diffusion method using on Mueller-Hinton agar (Becton Dickinson) according to the Clinical and Laboratory Standards Institute (CLSI) guidelines[15]. The antimicrobial susceptibility was validated by testing of the reference strain *P. aeruginosa* ATCC 27853 against all tested antimicrobial agents. The following antimicrobials from Becton Dickinson were tested: CAZ (30 μg), CAZ/CLAV (30/10 μg), CTX (30 μg), CTX/CLAV (30/10 μg), CXN (30 μg), CXN/CLAV (30/10 μg), cefepime (CPM, 30 μg), CPM/CLAV (30/10 μg), CTN (30 μg), AZT (30 μg), ticarcillin/CLAV (TICAR/CLAV, 75/10 μg), imipenem (IMI, 10 μg), meropenem (MERO, 10 μg) and doripenem (DORI, 10 μg). Apart from this, the minimum inhibitory concentrations (MICs) were determined for all isolates by the VITEK-2 system (BioMérieux, France).

Phenotypic testing for ESBLs production: ESBL production was detected using cephalosporin/clavulanate combination by disc method, *E*-test (BioMérieux, France) and VITEK ESBL card test (BioMérieux, France) according to previously standardized guidelines[16,17]. In the disc method, CTX (30 μg), CAZ (30 μg) and CPM (30 μg) discs with and without clavulanate (10 μg) were used. A ≥5 mm increase in zone diameter of CTX/CAZ/CPM in the presence of CLAV compared to when the antibiotic tested alone was a positive test for an ESBL. In the *E*-test; ≥8-fold reduction in cephalosporins' MICs in

the presence of clavulanate was taken as confirmatory of ESBL[16,17]. *P. aeruginosa* ATCC 27853 was used as a control strain for a positive ESBL.

Phenotypic testing for Amp-C β-lactamase (AmpC) production: A 30 µg CTN disc diameter or its MIC value was used for screening of AmpC production, and all positive isolates were selected for confirmation. Isolates were further tested by the three-dimensional extract test (TDET), AmpC disc test (Sigma-Aldrich, USA), boronic acid (BA) disc (Sigma) and disc approximation method, according to previously standardized guidelines[18,19]. *P. aeruginosa* ATCC 10145 (Oxoid, UK) strain was used as control strain.

Phenotypic testing for carbapenemases production: Initial screening for carbapenemase production was done by detection of carbapenem-intermediate or -resistant isolates. Phenotypic confirmation of carbapenemases was done by modified Hodge TEST (MHT) according to the study done by Lee *et al*[19]. Detection of MBLs' production was confirmed by following method according to previous published studies[19-22]. MBL *E*-test was done using combination of carbapenem/carbapenem-ethylene-diaminete-triacetic-acid (EDTA) (Sigma) and carbapenem/carbapenem-mercapto-propionic-acid inhibition (s) *E*-test (Sigma)[20]. Zone enhancements were done with EDTA-impregnated IMI and CAZ discs[21]. 2-MPA test was done using of CAZ (30 µg), IMI (10 µg) and CPM (30 µg)[22], and BA disc test method[23]. *P aeruginosa* strains producing IMP-I, VIM-l and VIM-2 MBLs were used as control strains.

Genotypic detection of ESBLs, AmpC & MBLs genes: Genotypic analysis was performed to detect ESBL gene (bla_{TEM}, bla_{SHV}, bla_{CTX-M}, bla_{PER} and bla_{VEB}) and plasmid-mediated AmpC gene (MOXM, CITM, DHAM, ACCM, EBCM and FOXM) and carbapenemase-encoding genes (bla_{IMP}, bla_{VIM}, bla_{OXA}, bla_{KPC} and bla_{NDM}) β-lactamases genes[16,24]. The primers details have been described previously[24]. Genomic DNA was extracted with QIAamp DNA Mini Kit (Qiagen, Hilden, Germany). The amplification was performed using Genepro Thermal Cycler (Hangzhou Bioer Technology, Bioer, China) in 25 µl volume, containing 20 pmol of primers, 1 mM dNTPs, 1 unit Taq polymerase, 1.5 mM $MgCl_2$; 2.5 µl of 10× PCR buffer and 100 ng DNA templates as previously described[24]. The amplified products were separated in 1.5 per cent agarose gel containing 2.5 µl of 10 mg/ml ethidium bromide. A 100 bp ladder was used to measure the molecular weights of amplified products. The gel images were taken under ultraviolet light using gel documentation system (Bio-Rad, USA). A 100 bp DNA ladder was used as a marker. PCR mixtures with the addition of water instead of template DNA served as the negative control. *Klebsiella pneumoniae* subsp. *pneumoniae* (ATCC 700603) (ESBL producer, SHV positive), *K. pneumoniae* (ATCC BAA-1144) (low-level AmpC producer) and *Enterobacter cloacae* subsp. *cloacae* (ATCC BAA-1143) (high-level AmpC producer), *K. pneumoniae* (ATCC BAA1705) (KPC positive), *E. cloacae* (ATCC BAA2468) (NDM-1 positive) and clinical Gram-negative bacteria strains isolated in the laboratory, served as positive controls for PCR-based detection of β-lactamase genes.

Statistical analysis: The correlation analysis of concordance was done between phenotypic methods and genotypic methods and molecular methods were considered the gold standard. The results for all parameters were calculated as a percentage as applicable. Chi-square test was performed to determine the significant difference between variables. Data were analyzed using IBM SPSS Statistics v.20 (Armonk, NY: IBM Corp).

Results

A total of 126 non-duplicate isolates of *Pseudomonas* spp. isolated from equal number of consecutive patients were analysed. The mean age of patients was 33.29±16.27 years, predominantly male patients, 107 (85.4%); 121 (96.1%) isolates were identified as *P. aeruginosa* followed by two (1.6%) *Pseudomonas putida*, one each of *Pseudomonas mendocina*, *Pseudomonas luteola* and *Pseudomonas stutzeri*. Most of the isolates were recovered from pus sample, 35 (27.8%) followed by urine, 25 (19.84%); endotracheal aspirate, 24 (19.04%); bronchoalveolar lavage, 21 (16.67%); blood, 14 (11.11%) and sputum, four (3.17%). The isolates were resistant to multiple class of antibiotics and had the highest resistance to TICAR/CLAV, 113 (89.7%) followed by MERO, 92 (72.5%); CAZ, 91 (72.3%); AZT, 87 (69%); CPM, 86 (68.5%); IMI, 78 (62.6%) and DORI, 77 (61.6%).

Phenotypic detection of ESBLs, AmpC & carbapenemase production: Overall prevalence of ESBLs, AmpC and carbapenemase production was detected in 109 (96.4%), 64 (50.8%) and 105 (94.6%) *Pseudomonas* isolates. Highest detection of ESBL

Table I. Detection of extended-spectrum β-lactamases (ESBL) in *Pseudomonas* spp. by phenotypic methods (n=109)

Combination disc methods	D-D test	Sensitivity (95% CI)	*E*-test	Sensitivity (95% CI)	VITEK 2	Sensitivity (95% CI)
CAZ/CAZ - clavulanate (%)	28 (25.2)	57 (0.49-0.64)	76 (65.2)	69.7 (0.60-0.77)	51 (45)	65.4 (0.57-0.72)
CPM/CPM - clavulanate (%)	-		98 (88.3)	89.9 (0.82-0.94)	-	

D-D, disc diffusion; CAZ, ceftazidime; CPM, cefepime; CI, confidence interval

production was observed in CPM/CPM-clavulanate combined *E*-test and CAZ/CAZ-clavulanate combined *E*-test in 98 (88.3%) and 76 (65.2%) isolates with sensitivity 89.9 per cent [confidence interval (CI) 0.82-0.94] and 69.7 per cent (CI, 0.60-0.77), respectively ($P<0.001$). Maximum detection of AmpC production was observed in 36 (32.7%) isolates by disc approximation method with sensitivity (CI) 69.5 per cent (0.58-0.78) followed by BA disc test, TDET and AmpC disc test in 18 (16%), seven (6.93%) and three (2.99%) isolates, respectively. Carbapenemases production was observed in 105 (94.6%) isolates by IMI/IMI EDTA *E*-test with highest sensitivity 95.4 per cent (0.89-0.98) followed by CAZ/2-MPA inhibition test and MHT in 102 (90.3%) and 20 (20.2%) isolates. Description of different phenotypic methods is given in Tables I-III.

Genotypic detection of β-lactamase gene: All three categories of β-lactamase genes (ESBL, AmpC and carbapenemases) were found to be present in *Pseudomonas* spp. The details of β-lactamase genes in various *Pseudomonas* spp. isolates are provided in Table IV.

ESBL, AmpC & carbapenemase-encoding gene: The most prevalent ESBL gene in bla_{TEM} in 72 (57.1%) and the least prevalent gene was bla_{SHV} in 19 (15.1%) isolates. AmpC gene detection was less in comparison to ESBL and carbapenemases gene. bla_{FOX-1} to bla_{FOX-5B} was the most common AmpC gene among all the *Pseudomonas* isolates. bla_{NDM-1} 41 (46.06%) was the most prevalent carbapenemases gene detected after ESBLs, followed by bla_{VIM} and bla_{OXA-46}.

Table II. Detection of AmpC in *Pseudomonas* spp. by phenotypic methods

Phenotypic method	n=126 (%)	Sensitivity% (CI)
TDET	7 (6.93)	10 (0.04-0.20)
AmpC disc test	3 (2.99)	4 (0.012-0.13)
Boronic acid disc test	18 (16)	28 (0.17-0.40)
Disc approximation method	36 (32.7)	69.5 (0.58-0.78)

AmpC, Amp-C β-lactamase; TDET, three-dimensional extract test; CI, confidence interval

Table III. Detection of carbapenemases in *Pseudomonas* spp. by phenotypic methods

Phenotypic method	n (%)	Sensitivity% (CI)
MHT	20 (20.4)	18 (0.11-0.26)
IMI-IMI/EDTA E-test	105 (94.6)	95.4 (0.89-0.98)
MERO-MERO/EDTA E-test	51 (69.8)	67 (0.58-0.74)
CAZ + 2 MPA	102 (90.3)	92.7 (0.85-0.96)
CPM + 2 MPA	96 (85.7)	87 (0.79-0.92)
IMI-IMI + 2 MPA	77 (69.4)	71 (0.60-0.78)
MERO-MERO + 2 MPA	3 (2.99)	0.27 (0.07-0.8)
EDTA impregnated IMI disc	79 (68.7)	71 (0.62-0.79)
EDTA impregnated CAZ disc	77 (65.25)	70 (0.60-0.78)
IMI + BA disc	60 (54.1)	54 (0.44-0.63)
MERO + BA disc	41 (36.6)	37 (0.28-0.47)
CTN + BA disc	23 (21.5)	20 (0.13-0.29)
CPM + BA disc	57 (50.4)	51 (0.42-0.61)
CEF + BA disc	47 (43.5)	42 (0.33-0.52)
CAZ + BA disc	34 (30.1)	69 (0.59-0.77)
CTX + BA disc	46 (44.6)	41 (0.32-0.51)

MHT, modified Hodge test; IMI, imipenem; MERO, meropenem; MPA, mercaptonoic acid inhibition test; EDTA, ethylenediaminetetraacetic acid; CAZ, ceftazidime; BA, boronic acid; CTN, cefoxitin; CPM, cefepime; CEF, cefoxitin; CTX, cefotaxime; CI, confidence interval

Discussion

AMR in *Pseudomonas* spp. is a serious global problem and is one of the leading causes of treatment failure. Characterization of AMR profile of *Pseudomonas* spp. is important from an epidemiological, clinical, laboratory and infection control point of view. In our study, a high rate of ESBLs and carbapenemase-producing *Pseudomonas* spp. was detected by a wide panel of phenotypic methods and genotypic methods. CPM-clavulanate ESBL *E*-test was the most suitable method to test

Table IV. Frequency of β-lactamases genes among clinical isolates of *Pseudomonas* spp. (n=126)

β-lactamase gene	Distribution n (%)
ESBL gene	
bla_{TEM}	72 (57.1)
bla_{CTXM}	32 (28.0)
bla_{PER}	27 (21.4)
bla_{VEB}	24 (19.0)
bla_{SHV}	19 (15.1)
AmpC gene	
bla_{MOXM}	3 (2.3)
bla_{CITM}	9 (7.1)
bla_{DHAM}	6 (4.8)
bla_{ACCM}	0 (0)
bla_{EBCM}	0 (0)
bla_{FOXM}	11 (8.7)
Carbapenemases gene	
bla_{KPC}	14 (11.6)
bla_{NDM-1}	41 (46.06)
bla_{VIM}	19 (33.9)
bla_{IMP}	3 (5)
bla_{OXA-46}	23 (23.7)

ESBL, extended-spectrum β-lactamases; AmpC, Amp-C β-lactamase

for ESBL production, especially in organism such as *Pseudomonas* spp. which is co-producing AmpC. This finding was in concordance to another study done in *Enterobacteriaceae*[25].

The carbapenem drugs are thought to be the most active drugs against *P. aeruginosa.* The current study highlighted the increased rate of carbapenems resistance (72.5%) in *Pseudomonas* spp. similar to other published studies[26,27]. Although MHT is standardized method for carbapenemase detection in *Enterobacteriaceae*, in this study, IMI/IMI EDTA-impregnated *E*-test detected highest rate with maximum sensitivity of carbapenemases in comparison to MHT. AmpC production was higher among the isolates using the disc approximation test, compared to other methods. On the other hand, only 6.93 per cent of the isolates showed AmpC production by TDET. Thus, most suitable method for ESBL, AmpC and carbapenemases detection was found to be CPM-clavulanate ESBL *E*-test, disc approximation test and IMI/IMI EDTA-impregnated *E*-test [sensitivity 89.9 per cent (CI 0.82-0.94), 69.5 per cent (CI 0.58-0.78), 95.4 per cent (CI 0.89-0.98), respectively]. Although our findings showed a spectrum of AMR pattern in *Pseudomonas* spp., detected by various phenotypic and genotypic methods, results could not be generalized because power analysis was not performed in data which was a major limitation of our study.

In conclusion, our findings suggest that phenotypic or genotypic detection of AMR must be combined for better characterization regarding the resistance patterns of *Pseudomonas* spp. so that clinicians can select an appropriate antibiotic for the timely treatment of infectious diseases while helping to prevent the dissemination of resistance.

***Financial support & sponsorship*:** The study was funded through a grant received from the Indian Council of Medical Research, New Delhi (Grant No. 5/3/3/26/2011-ECD-I).

***Conflicts of Interest*:** None.

References

1. Emori TG, Gaynes RP. An overview of nosocomial infections, including the role of the microbiology laboratory. *Clin Microbiol Rev* 1993; *6* : 428-42.
2. Richards MJ, Edwards JR, Culver DH, Gaynes RP. Nosocomial infections in pediatric Intensive Care Units in the United States. National nosocomial infections surveillance system. *Pediatrics* 1999; *103* : e39.
3. Gaynes R, Edwards JR; National Nosocomial Infections Surveillance System. Overview of nosocomial infections caused by gram-negative bacilli. *Clin Infect Dis* 2005; *41* : 848-54.
4. Kollef MH, Shorr A, Tabak YP, Gupta V, Liu LZ, Johannes RS, *et al.* Epidemiology and outcomes of health-care-associated pneumonia: Results from a large US database of culture-positive pneumonia. *Chest* 2005; *128* : 3854-62.
5. Obritsch MD, Fish DN, MacLaren R, Jung R. Nosocomial infections due to multidrug-resistant *Pseudomonas aeruginosa*: Epidemiology and treatment options. *Pharmacotherapy* 2005; *25* : 1353-64.
6. Lister PD, Wolter DJ, Hanson ND. Antibacterial-resistant *Pseudomonas aeruginosa*: Clinical impact and complex regulation of chromosomally encoded resistance mechanisms. *Clin Microbiol Rev* 2009; *22* : 582-610.
7. Livermore DM, Woodford N. The beta-lactamase threat in *Enterobacteriaceae*, *Pseudomonas* and *Acinetobacter*. *Trends Microbiol* 2006; *14* : 413-20.
8. Livermore DM. Multiple mechanisms of antimicrobial resistance in *Pseudomonas aeruginosa*: Our worst nightmare? *Clin Infect Dis* 2002; *34* : 634-40.
9. Bert F, Branger C, Lambert-Zechovsky N. Identification of PSE and OXA beta-lactamase genes in *Pseudomonas aeruginosa* using PCR-restriction fragment length polymorphism. *J Antimicrob Chemother* 2002; *50* : 11-8.

10. Queenan AM, Bush K. Carbapenemases: The versatile beta-lactamases. *Clin Microbiol Rev* 2007; *20* : 440-58.
11. Flateau C, Janvier F, Delacour H, Males S, Ficko C, Andriamanantena D, *et al.* Recurrent pyelonephritis due to NDM-1 metallo-beta-lactamase producing *Pseudomonas aeruginosa* in a patient returning from Serbia, France, 2012. *Euro Surveill* 2012; *17* . pii: 20311.
12. Shanthi M, Sekar U, Kamalanathan A, Sekar B. Detection of New Delhi metallo beta lactamase-1 (NDM-1) carbapenemase in *Pseudomonas aeruginosa* in a single centre in Southern India. *Indian J Med Res* 2014; *140* : 546-50.
13. Jovcic B, Lepsanovic Z, Suljagic V, Rackov G, Begovic J, Topisirovic L, *et al.* Emergence of NDM-1 metallo-β-lactamase in *Pseudomonas aeruginosa* clinical isolates from Serbia. *Antimicrob Agents Chemother* 2011; *55* : 3929-31.
14. Thomson KS. Extended-spectrum-beta-lactamase, AmpC, and carbapenemase issues. *J Clin Microbiol* 2010; *48* : 1019-25.
15. Clinical and Laboratory Standards Institute. *Performance standards for antimicrobial susceptibility testing.* 27th ed. CLSI Document M100. Wayne, PA: CLSI; 2017. .
16. Laudy AE, Róg P, Smolińska-Król K, Ćmiel M, Słoczyńska A, Patzer J, *et al.* Prevalence of ESBL-producing *Pseudomonas aeruginosa* isolates in Warsaw, Poland, detected by various phenotypic and genotypic methods. *PLoS One* 2017; *12* : e0180121.
17. European Committee on Antimicrobial Susceptibility Testing. EUCAST guidelines for detection of resistance mechanisms and specific resistances of clinical and/or epidemiological importance. Version 1.0. EUCAST; 2013.
18. Chika E, Charles E, Ifeanyichukwu I, Chigozie U, Chika E, Carissa D, *et al*. Phenotypic detection of AmpC beta-lactamase among anal *Pseudomonas aeruginosa* isolates in a Nigerian abattoir. *Arch Clin Microbiol* 2016; *7* : 1-5.
19. Lee K, Lim YS, Yong D, Yum JH, Chong Y. Evaluation of the Hodge test and the imipenem-EDTA double-disk synergy test for differentiating metallo-beta-lactamase-producing isolates of *Pseudomonas* spp. and *Acinetobacter* spp. *J Clin Microbiol* 2003; *41* : 4623-9.
20. Walsh TR, Bolmström A, Qwärnström A, Gales A. Evaluation of a new Etest for detecting metallo-beta-lactamases in routine clinical testing. *J Clin Microbiol* 2002; *40* : 2755-9.
21. Yong D, Lee K, Yum JH, Shin HB, Rossolini GM, Chong Y, *et al.* Imipenem-EDTA disk method for differentiation of metallo-beta-lactamase-producing clinical isolates of *Pseudomonas* spp. and *Acinetobacter* spp. *J Clin Microbiol* 2002; *40* : 3798-801.
22. Hemalatha V, Sekar U, Kamat V. Detection of metallo betalactamase producing *Pseudomonas aeruginosa* in hospitalized patients. *Indian J Med Res* 2005; *122* : 148-52.
23. Tsakris A, Kristo I, Poulou A, Themeli-Digalaki K, Ikonomidis A, Petropoulou D, *et al.* Evaluation of boronic acid disk tests for differentiating KPC-possessing *Klebsiella pneumoniae* isolates in the clinical laboratory. *J Clin Microbiol* 2009; *47* : 362-7.
24. Khurana S, Mathur P, Kapil A, Valsan C, Behera B. Molecular epidemiology of beta-lactamase producing nosocomial Gram-negative pathogens from North and South Indian hospitals. *J Med Microbiol* 2017; *66* : 999-1004.
25. Mohanty S, Gaind R, Ranjan R, Deb M. Use of the cefepime-clavulanate ESBL Etest for detection of extended-spectrum beta-lactamases in AmpC co-producing bacteria. *J Infect Dev Ctries* 2009; *4* : 24-9.
26. Behera B, Das A, Mathur P, Kapil A. High prevalence of carbapenem resistant *Pseudomonas aeruginosa* at a tertiary care centre of North India. Are we under-reporting? *Indian J Med Res* 2008; *128* : 324-5.
27. Chaudhary M, Payasi A. Rising antimicrobial resistance of *Pseudomonas aeruginosa* isolated from clinical specimens in India. *J Proteomics Bioinform* 2013; *6* : 5-9.

For correspondence: Dr Purva Mathur, Department of Microbiology, 2nd Floor, Jai Prakash Narayan Apex Trauma Centre, All India Institute of Medical Sciences, New Delhi 110 029, India
e-mail: purvamathur@yahoo.co.in