ISSN: 0975-833X

Available online at http://www.journalcra.com

International Journal of Current Research
Vol. 9, Issue, 05, pp.51292-51295, May, 2017

INTERNATIONAL JOURNAL OF CURRENT RESEARCH

RESEARCH ARTICLE

CARBAPENEMASE PRODUCING PSEUDOMONAS AERUGINOSA: A CAUSE OF CONCERN

Dr. Sanchari Das and *Dr. Silpi Basak

Department of Microbiology, Jawaharlal Nehru Medical College, Wardha (MS), Pin-442004

ARTICLE INFO

Article History:
Received 20th February, 2017
Received in revised form
13th March, 2017
Accepted 26th April, 2017
Published online 31st May, 2017

Key words:

Pseudomonas aeruginosa,
Detection of Carbapenemase production:
MBL, KPC, Both MBL and KPC.

ABSTRACT

Background: *Pseudomonas aeruginosa* is one of the most important cause of Healthcare associated infections. Carbapenems are often used as a last resort for treating serious infections caused by multidrug resistant *Pseudomonas aeruginosa*. Carbapenamases are β-lactamases which cause carbapenem hydrolysis.

Aim: The present study was undertaken to detect the incidence of Carbapenemase producing *Pseudomonas aeruginosa* from clinical isolates.

Material and Methods: 150 clinical isolates of *Pseudomonas aeruginosa* were studied for antibiotic susceptibility profile by Kirby Bauer disk diffusion method as per CLSI guidelines, 2016. All *Pseudomonas aeruginosa* strains were screened for Carbapenemase activity by Classical Hodge test. Metallobetalactamases (MBL) production was detected and confirmed by Disc potentiation (DP) test using Imipenem and Imipenem plus EDTA and by E-test. Klebsiella pneumoniae Carbapenemases (KPC) production was detected by combine disc method using Imipenem and imipenem plus Phenyl boronic acid (PBA). Both MBL and KPC producing strains were detected by Imipenem disc and disc containing Imipenem plus PBA plus EDTA. Class D carbapenemase i.e, OXA β– lactamases were not included in our study.

Results: The highest sensitivity was observed to Colistin 149(99.3 %), followed by Imipenem 107(71.3%). 43(28.7%) *Pseudomonas aeruginosa* strains were Classical Hodge test positive, 6(4%) were positive for MBL only by DP test. 15(10 %) were positive for KPC only and 34 (22.6 %) were positive for both MBL and KPC. These 40 MBL producing strains were positive by MBL E test.

Conclusion: All *Pseudomonas aeruginosa* strains must be screened for carbapenamase production in Cinical Microbiology laboratory.

Citation: Dr. Sanchari Das and Dr. Silpi Basak, 2017. "Carbapenemase producing pseudomonas aeruginosa: A cause of concern", *International Journal of Current Research*, 9, (05), 51292-51295.

INTRODUCTION

Pseudomonas aeruginosa is an opportunistic pathogen responsible for wide range of Hospital acquired infections and noscomial outbreaks. They are responsible for 16 % of noscomial pneumonia, 12 % hospital acquired pneumonia, 8 % wound infections and 10 % blood stream infections. Carbapenemases are the most versatile family of β-lactamases. Carbapenems are resistant to hydrolysis by most of the β-lactamases (ESBLs and AmpC beta lactamases) and they are often used as antibiotics of the last resort in infections which are caused by multi-drug resistant Gram negative bacilli (Lee *et al*., 2009). Although known as " carbapenemases", many of these enzymes recognize almost all hydrolysable β-lactams, and most are resilent against inhibition by all commercially viable β-lactamase inhibitors (Livermore and Woodford, 2006; Nordmann and Poirel, 2002; Walther-Rasmussen and Hoiby, 2006). The most important carbapenemases are metallo β-lactamases (MBL) and they belong to Ambler class B. MBLs require divalent cations, usually zinc, as metal cofactors for their enzymatic activity and are inhibited by metal chelators such as ethylenediamine tetra acetic acid (EDTA) (Maltezou, 2009). Furthermore, MBLs are encoded either by genes that are part of the bacterial chromosome in some bacteria or by heterologous genes acquired by transfer of mobile genetic elements. Therefore, acquired MBL can spread among various species of bacteria such as *P. aeruginosa*, *Acinetobacter baumanii complex, Klebsiella pneumoniae, E.coli* etc. in a Health care set-up. *Pseudomonas aeruginosa* shows resistance to carbapenem due to decrease outer membrane permeability, increased efflux system, alteration of penicillin binding protiens and carbapenem hydrolyzing enzymes i.e. carbapenemases (Yong *et al*., 2002). *P. aeruginosa* producing MBL was first reported from Japan in 1991 (Watanabe *et al*., 1991) and since then, it has been reported from various parts of the world. KPCs have been reported in different parts of the world since the first identification of KPC-positive Klebsiella pneumoniae in the USA (Yigit *et al*., 2001; Nordmann *et al*., 2009). The increasing emergence and spread of KPCs leave fewer available therapeutic options due to their broad-spectrum

***Corresponding author:* Dr. Silpi Basak,**
Department of Microbiology, Jawaharlal Nehru Medical College, Wardha (MS), Pin-442004

hydrolytic activity and high mobility. The KPC-producing bacteria are mostly Enterobacteriaceae, but also found in *Pseudomonas aeruginosa* and *Acinetobacter spp.* (Nordmann *et al*., 2009) The first KPC-producing *P. aeruginosa* isolates were identified in Colombia (Villegas *et al*., 2007). Carbapenem resistant *P. aeruginosa* has become an important problem all over the world challenging the current therapeutic approaches. It has particularly become important considering the increase of resistance to all Cephalosporins, Cephamycins, Carbapenems and Monobactams. The infections which are caused by such Carbapenem resistant *P. aeruginosa* strains are believed to result in high mortality as well as high healthcare costs and a prolonged hospital stay. So, early detection and a regular monitoring of the incidence of Carbapenemases producing organisms in any healthcare setup has become the need of the hour.

Aim and objectives

The present study was undertaken to detect Carbapenemase producing *Pseudomonas aeruginosa* isolated from various clinical samples and to study the antibiotic susceptibility profile of these strains.

MATERIAL AND METHODS

This cross-sectional study was conducted in the Department of Microbiology. Various clinical specimens e.g. urine, blood, sputum, pus and wound swab, CSF, medical devices and other body fluids were received from patients attending Indoor patient Department (IPD) and Outdoor Patient Department (OPD) of our hospital. *Pseudomonas aeruginosa* was identified as per standard microbiological procedure (Mackie and McCartney, 2006). A total number of 150 *Pseudomonas aeruginosa* strains isolated from different clinical specimens, were included in the study. *Pseudomonas aeruginosa* ATCC 27853 was used as control strain. Antibiotic susceptibility test for Amikacin (AK-30 µg), Ciprofloxacin (CIP-10 µg), Netilin (NET-30 µg), Imipenem (IPM-10µg), Meropenem (MRP-10 µg) , Ceftazidime(CAZ-30µg), Pipericillin-Tazobactum (PIT-100/10 µg) and Colistin (Cl-10µg) were done by Kirby – Bauer disc diffusion method (Bauer *et al*., 1966) as per CLSI guidelines, 2016 (Wayne, 2016). Lawn culture of test strains (turbidity adjusted to 0.5 McFarland) was done on Mueller Hinton (MH) agar plate. Then with all aseptic precaution the antibiotic discs were put and the plates were incubated at 37^0C overnight and the results were noted. It has been observed that not only carbapenem resistant strains but even carbapenem sensitive strains can carry the genes for MBL and KPC. All 150 *P.aeruginosa* strains were studied for carbapenemase production by Classical Hodge test, for detection of MBL production by disc potentiation (DP) test and E test (BioMerieux) and for detection of KPC production by combined disc method.

Classical Hodge Test (Lee *et al*., 2001)

Lawn culture of the indicator strain, *Escherichia coli ATCC 25922*, (turbidity adjusted to 0.5 McFarland's standard), was done on a MH agar plate. The test strain was heavily streaked from the centre of the plate to its periphery and a 10µg Imipenem (IPM) disc was placed at the centre. The plate was incubated overnight. The presence of a distorted inhibition zone was interpreted as a positive result for the carbapenemase production.

Disc Potentiation test (Yong *et al*., 2002)

The turbidity of test strain was adjusted to 0.5 McFarland and lawn culture was put onto MH agar plate. Two discs of Imipenem (10 µg) were placed on inoculated MH agar plate wide apart. To one of the Imipenem (IPM) disc, 10µl of 0.5 M EDTA was added. After an overnight incubation at 37ºC, the zone of inhibition of ≥ 7 mm with the disc having IPM plus EDTA, compared to IPM alone was considered positive for MBL production.

Combined disk method (Tsakris *et al*., 2011)

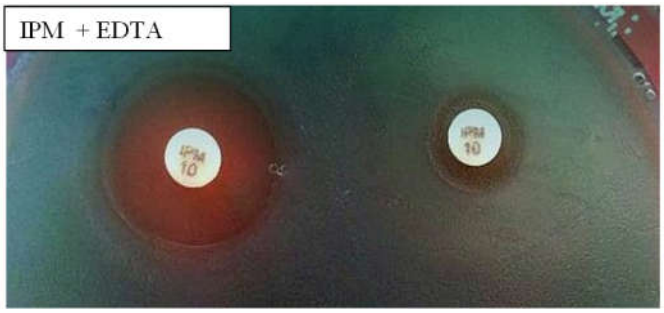
Detection of KPC producing Carbapenemases was done by Combined disk method using one disc containing 10 µg Imipenem with Phenyl boronic acid (PBA) (400µg/disc) and another disc of Imipenem (10µg). A lawn culture of test strain (turbidity adjusted to 0.5 McFarland).was done on a M H agar plate and two Imipenem discs (10 µg) were placed wide apart and 10 µl of PBA solution was added to one of the disc. The plates were incubated at 37ºC overnight. The zone of inhibition ≥ 5mm around Imipenem+PBA disc compared to Imipenem disc alone was considered positive for KPC production. Both MBL and KPC production was detected by- placing two Imipenem discs (10 µg) wide apart on a MH agar plate inoculated with test strain. To one of the disc, both 10µl of 0.5 M EDTA and 10 µl of PBA solution was added and incubated at 37 ºC overnight. The zone of inhibition around Imipenem+ EDTA+ PBA ≥ 5mm compared to Imipenem disc alone was considered positive for both respectively.

MBL E-Test

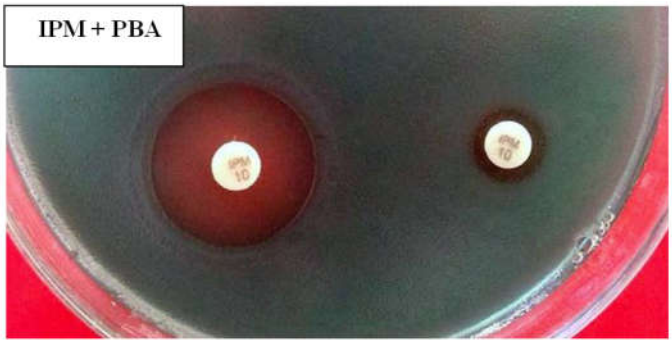
All 40 MBL producing *Pseudomonas aeruginosa* strains detected by Disc potentiation test was also confirmed by putting MBL E-test strip (bioMerieux). The Etest strip has concentration gradients of Imipenem (IP) (4-256 µg/ml) on one half and Imipenem+EDTA (IPI) (1-64 µg/ml) on another half. In this method lawn culture of test strain (turbidity adjusted to 0.5 McFarland) was done on a Mueller Hinton agar plate. With all aseptic precaution, the MBL E-test strip was placed onto the inoculated plate. After overnight incubation at 37^0C, the zone of inhibition was read from two halves of the strip. As per manufacturer's instruction, MIC ratio of Imipenem / Imipenem+EDTA (IM/IMI) ≥ 8 or deformation of ellipse or phantom zone present was considered as positive for MBL production.

RESULTS

Out of the 150 *P. aeruginosa* strains studied, 55(36.6 %) were Carbapenemase producers whereas 95 were Carbapenemase non-producers. Classical Hodge Test (CHT) identified 43(28.7%) isolates as carbapenemase producers. MBLs production was detected in 40 (26.7 %) isolates by Disk Potentiation test (Photograph 1). Out of these 40 strains, 6 (4%) strains produced only MBL and 34 (22.6%) strains produced both MBL and KPC. All these 40 (26.7 %) MBL producing *P. aeruginosa* strains were also E-Test positive (Photograph 3). KPC production was detected in 15 (10 %) isolates by Combined disk method (Photograph 2) and co-existance of both MBL and KPC in 34 (22.6 %) isolates as detected by using disc containing Imipenem plus PBA plus EDTA. The maximum number of Pseudomonas aeruginosa strains were sensitive to Colistin 149 (99.3 %), followed by Imipenem 107(71.3%).

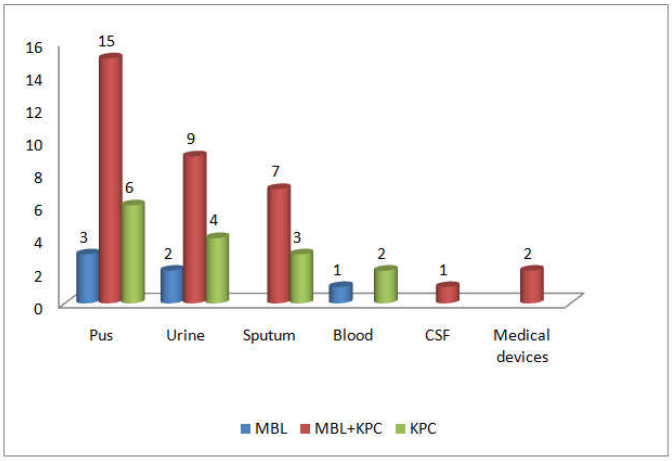


Photograph 1. Disk potentiation test for detection of MBL producing P. aeruginosa



Photograph 2 – Combined disk method for detection of KPC producing P. aeruginosa

Photograph 3 – E test for detection of MBL producing P. aeruginosa



Figuare 1. Isolation of MBL, KPC and both MBL+KPC producing P.aeruginosa strains from different clinical specimens

In the present study, out of total 55 Carbapenemase producig *Pseudomonas aeruginosa* strains the maximum number were isolated from pus and wound swab 24/55(43.6%), followed by urine 15/55(27.2%), sputum 10/55(18.1 %) and blood 3(5.4%), CSF 1(1.8 %) and medical devices 2(3.6%). Both MBL and KPC producing strains were also isolated in maximum number from Pus and wound swab 15/34 (44.1%).

Table 1. Isolation of Carbapenemase producing P. aeruginosa from different clinical specialities

Clinical specialities n= 150	MBL (6)		KPC (15)		MBL+KPC (34)	
	No.	%	No.	%	No.	%
Orthopedics (50)	1	16.6	6	40	9	26.4
Surgery (34)	2	33.3	2	13.3	7	20.5
Medicine (26)	3	50	1	6.6	8	23.5
Obs & Gynae (21)	-		6	40	4	11.7
ICUs (11)	-		-		3	8.8
Others* (8)	-		-		3	8.8

*Others include – Psychiatry ward (1), ENT ward(2).

Though the MBL producing *Pseudomonas aeruginosa* strains were isolated from most of the wards, the maximum number of strains were isolated from Medicine ward 11/40 (27.5. %) followed by Orthopedics ward 10/40 (25 %). Out of 11 P. aeruginosa strains isolated from different ICUs, 3/11(27.3%) strains produced both MBL+KPC.

DISCUSSION

Pseudomonas aeruginosa has a high resistance to antibiotics and is responsible for morbidity and mortality in hospitalized and immunocompromised patients (Vijay Mane *et al*., 2014). Infections caused by *Pseudomonas aeruginosa* show varying degrees of inherent resistance and hence their infections are difficult to treat. Acquired resistance is also reported by the production of newer β-lactamases such as ESBL, AmpC β-lactamase and metallobeta-lactamase enzymes (Manchanda and Singh, 2008). In the present study out of 150 P. aeruginosa strains 40 (26.6%) were MBL producers which correlated with other studies as Variya *et al*. have reported MBL producing strains as (25 %) (Variya *et al*., 2008). The incidence of MBL producing *P. aeruginosa strains* in other studies conducted by Navneeth *et al*. (12%) (2002), Hemlata *et al* (14%) (2005) and Senthamarai *et al.* (15.38%) (2014) respectively, from different parts of India and low level of carbapenem resistance were reported as 8.2% and 8.05% respectively by few workers (Mendiratta *et al*., 2005; Agrawal *et al*., 2008). Most of the studies in India only reported the incidence of MBL producing P. aeruginosa. Incidence of KPC and both MBL+KPC producing P.aeruginosa strains have not been reported so far. This is the first report of KPC and coexistence of both MBL+KPC producing P.aeruginosa strains from a tertiary care hospital in Central India. Detection of only MBL producing strains are just tip of the iceberg for Carbapenemase producing strains. Even in the present study the incidence of only KPC producing (10 %) and both MBL+KPC producing (22.6 %) P. aeruginosa strains were much higher than strains producing MBL only (4 %). When the strains producing both MBL and KPC, the MBL production may be masked and incidence of Carbapenemase producing strains are under reported. Still Oxa-D type carbapenem are not included in the study as it is very difficult to detect Oxa-D phenotypically. A crucial step towards a large scale monitoring of the emerging resistant strains is the development of simple screening tests which are designed to detect the β-lactamases. A good infection control practice and a careful introspection during prescribing beta-lactam drugs should be maintained for the formulation of a good

antimicrobial policy in a hospital. The drugs like Polymyxin B and Colistin should be kept as reserve drugs and they should be used only in patients who have carbapenem resistant infections, especially the strains producing Metalloβ-lactamases (MBLs). The phenotypic methods are easier to perform as they can detect the various beta lactamases, Hence, the phenotypic methods should be regularly performed in Clinical Microbiology laboratory where the molecular methods are not available. Strict infection control practices, the judicious use of antibiotics, an early detection of the MBL, KPC producing strains, all will together help in extending the effectiveness of the carbapenems, which are the last resort antibiotics for treating patients (Nutan Narayan Bhongle *et al*., 2012).

Limitations

Molecular methods like Polymerse Chain Reaction (PCR) was not done as PCR is very costly, time consuming and require expertise. Moreover PCR can not detect the new variants of MBLs or KPCs.

Conclusion

Phenotypic detection of carbapenamase production in Clinical Microbiology laboratory must be done for early detection of MBL or KPC producing *Pseudomonas aeruginosa* strains for effective patients' treatment and strict implementation of Infection Control Practices to prevent the dissemination of these strains in Health Care Set up

REFERENCES

Agrawal G, Lodhi R B, Kamalakar U P, Khadse R K, Jalgaokar S V. 2008. Study of metallobetalactamases production in clinical isolates of P. aeruginosa. *Indian J Med Microbiol*., 26 (4): 349 – 351.

Bauer A W, Kirby WMM, Sherris J C, Jurek M. 1966. Antibiotic susceptibility testing by standardised single disc method. *Am. J. Clin. Pathol*., 45: 493-496.

Hemalatha V, Sekar U, Kamat V. 2005. Detection of metallobetalactamases producing P.aeruginosa in hospitalized patients. *Indian J Med Res*., 122: 148– 152.

Lee K, Chong Y, Shin HB, Kim YA, Yong D, Yum JH. 2001. The modified Hodge and the EDTA-disk synergy tests are used to screen the metallo- β-lactamase-producing strains of *Pseudomonas* and *Acinetobacter* species. *Clin Microbiol Infect.*, 7: 88-91.

Lee K, Park AJ, Kim MY, Lee HJ, Cho J-H, Kang JO, *et al*. 2009. Metallo-β- lactamase producing Pseudomonas spp. in Korea: a high prevalence of the isolates with the VIM-2 type and the emergence of the isolates with the IMP-1 type. *Yonsei Med J.,* 50 (3):335-39.

Livermore, D. M. and N. Woodford. 2006. The β-lactamase threat in Enterobacteriaceae, Pseudomonas and Acinetobacter. *Trends Microbiol*., 14: 413-420.

Mackie and McCartney Practical Medical Microbiology. Tests for the identification of Bacteria.Elsevier Publication. 2006 ; 14:131-150.

Maltezou HC. 2009. Metallo-beta-lactamases in Gram-negative bacteria: introducing the era of pan-resistance? *Int J Antimicrob Agents,* 33 (5): 405 1–7.

Manchanda, V. and Singh, N.P. 2008. Occurrence and detection of AmpC beta-lactamases among Gram negative clinical isolates using a modified three-dimensional test at Guru TeghBahadur Hospital, Delhi, India. *J. Antimicrob. Chemother*., 51: 415–418.

Mendiratta D K, Deotale V, Narang P. 2005. Metallobetalactamases producing of P.aeruginosa in hospital from rural area. *Indian J Med Res*., 121:701 –703.

Navneeth B. Shridaran D, Sahay D, Belwadi MRS. 2002. A preliminary study on metallobetalactamases producing of P. aeruginosa in hospitalizedpatients. *Indian J Med Res*., 116: 264 – 267.

Nordmann P, Cuzon G, Naas T. 2009. The real threat of Klebsiella pneumoniae carbapenemase-producing bacteria. *Lancet Infect Dis*., 9: 228–36.

Nordmann, P. and L. Poirel. 2002. Emerging carbapenemases in Gram- negative aerobes. *Clin. Microbiol. Infect*., 8:321-331.

Nutan Narayan Bhongle *et al*. 2012. Prevalence of Metallo β-Lactamases in the Clinical Isolates of *Pseudomonas aeruginosa. Journal of Clinical and Diagnostic Research*, 6(7): 1200-1202.

Senthamarai S. *et al*. 2014. Resistance Pattern of *Pseudomonas aeruginosa* in a Tertiary Care Hospital of Kanchipuram. *Journal of Clinical and Diagnostic Research*, 8(5): DC30-DC32.

Tsakris A, Digalaki K T, Poulou a *et al*. 2011. Comparative evaluation of combined disk tests using different boronic acid compounds for detection of Klebseilla pneumonia carbapenemase – producing Enterobacteriaceae clinical isolates. *J Clin Microbiol*., 49(8): 2804-2809.

Variya A, Kulkarni N, Kulkarni M *et al*. 2008. The incidence of metallo beta lactamase producing Pseudomonas aeruginosa among ICU patients. *Indian J Med Res.*, 127: 398-402

Vijay Mane, A D Urekar, NitinGoelInsan. ESBL, MBL and AMP C detection in multidrug resistant pseudomonas aeruginosa and pan drug resistant pseudomonas aeruginosa isolated in tertiary care hospital. *Int. J. Curr. Microbiol. App. Sci*., 2014; 3 (11): 489-492.

Villegas MV, Lolans K, Correa A *et al*. 2007. First identification of Pseudomonas aeruginosa isolates producing a KPC-type carbapenem-hydrolyzing b-lactamase. *Antimicrob Agents Chemother*., 51: 1553–5.

Walther-Rasmussen, J. and N.Hoiby, 2006. OXA-type carbapenemases. *J. Antimicrob. Chemother*, 57: 373-383.

Watanabe M, Iyobe S, Inoue M, Mitsuhashi S. 1991. Transferable imipenem resistance in *Pseudomonas aeruginosa*. *Antimicrob Agents Chemother*, 35(1): 147-51.

Wayne PA. 2016. Performance standards for antimicrobial susceptibility testing; 26th informational supplement. *Clin Lab Standards Inst*. M100-S18.

Yigit H, Queenan AM, Anderson GJ *et al*. 2001. Novel carbapenem hydrolyzing b-lactamase, KPC-1, from a carbapenem-resistant strain of Klebsiella pneumoniae. *Antimicrob Agents Chemother*, 45:1151–61.

Yong D, Lee K, Yum J H, Shin H B, Rossolini G M, Chong Y. 2002. Imipenem-EDTA Disc method for differentiation of metallo betalactamases producing clinical isolates of Pseudomonas spp. And Acinetobacter spp. *J Clin Microbiol*., 40: 3798-3801.

****