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**INTRODUCTION**

Pseudomonas aeruginosa is an opportunistic pathogen responsible for wide range of Hospital acquired infections and nosocomial outbreaks. They are responsible for 16 % of nosocomial pneumonia, 12 % hospital acquired pneumonia, 8 % wound infections and 10 % blood stream infections. Carbapenemases are the most versatile family of β-lactamases. Carbapenemases 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 resilient 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 baumannii 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 proteins 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

**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. Carbapenemases 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 Imipem plus EDTA and by E-test. Klebsiella pneumoniae Carbapenemases (KPC) production was detected by combine disc method using Imipenem and imipen 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%) 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 carbapenemase production in Clinical Microbiology laboratory.

**ABSTRACT**

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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-10 µg), Meropenem (MRP-10 µg), Cefazidime (CAZ-30 µg), Piperacillin-Tazobactam (PIT-100/10 µg) and Colistin (Cl-10 µg) 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 on another half. The plates were incubated at 37°C overnight. The zone of inhibition ≥ 5mm around Imipenem+PBA disc compared to Imipenem disc alone was considered positive for KPC production. All 150 P. aeruginosa strains were also E-Test positive (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-existence of both MBL and KPC in 34 (22.6 %) isolates was 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%).

**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 37°C, 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 37°C 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°C 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 (IP) (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°C, the zone of inhibition was read from two halves of the strip. As per manufacturer’s instruction, MIC ratio of Imipenem / Imipenem+EDTA (IM/IM) ≥ 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-existence of both MBL and KPC in 34 (22.6 %) isolates was 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%).
In the present study, out of total 55 Carbapenemase producing 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

<table>
<thead>
<tr>
<th>Clinical specialities</th>
<th>MBL (6)</th>
<th>KPC (15)</th>
<th>MBL+KPC (34)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Orthopedics (50)</td>
<td>1</td>
<td>16.6</td>
<td>6</td>
</tr>
<tr>
<td>Surgery (34)</td>
<td>2</td>
<td>33.3</td>
<td>2</td>
</tr>
<tr>
<td>Medicine (26)</td>
<td>3</td>
<td>50</td>
<td>1</td>
</tr>
<tr>
<td>Obs &amp; Gynaec (21)</td>
<td>-</td>
<td>6</td>
<td>4</td>
</tr>
<tr>
<td>ICUs (11)</td>
<td>-</td>
<td>-</td>
<td>3</td>
</tr>
<tr>
<td>Others* (8)</td>
<td>-</td>
<td>-</td>
<td>3</td>
</tr>
</tbody>
</table>

*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 metallo-beta-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 NAVNEET 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 Metallo-β-lactamases (MBLs). The phenotypic methods are easier to perform as they can detect the various β-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 Polymerase 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 carbapenemase 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


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