THE PREVALENCE OF METALLO-B-LACTAMASE GENES IN IMIPENEM AND MEROPENEM RESISTANT PSEUDOMONAS AERUGINOSA ISOLATED FROM PATIENTS IN EL-OBIED HOSPITALS – SUDAN


doi: 10.2478/ijaset-2021-0002

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Abstract - Acquired Metallo-β-lactamases (MBLs) are emerging worldwide as powerful resistance determinants. So far, six MBL enzyme types have been described in clinical isolates of P. aeruginosa-VM, IMP, SPM, GIM, NDM and AIM type. This study aimed to determine the prevalence of Metallo-β-Lactamase genes in imipenem and meropenem-resistant Pseudomonas aeruginosa isolated from Elobied Hospitals. All collected specimens (721) were cultured and identified using API 12A/12E. Hundred (100) isolated Pseudomonas aeruginosa were subjected to antimicrobial susceptibility testing (Kirby Bauer), for selected imipenem and meropenem. PCR was performed for detection of blaVIM-1, NDM-1 and VIM-like genes in imipenem and meropenem-resistant P. aeruginosa strains. Nineteen (19%) and 14 (14%) isolates were found resistant to imipenem and meropenem, respectively, 1/19 (5.26%) of isolated resistant P. aeruginosa carry the NDM-1 gene, and 1/14 (7.1%) of the VIM gene which encoding resistant to imipenem and meropenem, respectively, while no detection to blaVIM-1 gene in our study. The study concluded that evidence of the presence of the NDM-1 and VIM genes, while none of the isolated P. aeruginosa strains carry blaVIM-1. Further studies and a regular monitoring system for early detection of MBL-producing organisms are recommended.

Keywords - MBLs, P. aeruginosa, NDM-1, VIM-1, VIM, imipenem and meropenem.

I. INTRODUCTION

Pseudomonas aeruginosa and Acinetobacter baumannii, are among the most important causes of serious hospital-acquired and community-onset bacterial infections in humans, and resistance in these bacteria has become a growing problem. They often colonize hospital food, sinks, taps, mops, and respiratory equipment and tolerates a variety of physical conditions so it can remain in both community and hospital settings. The increasing prevalence of chronic and hospital-acquired infections produced by multidrug-resistant (MDR) or extensively drug-resistant (XDR) Pseudomonas aeruginosa strains is associated with significant morbidity and mortality. The multidrug-resistant (MDR) phenotype in P. aeruginosa could be mediated by several mechanisms including multidrug efflux systems, enzyme production, and outer membrane protein (porin) loss and target mutations.

The prevalence of MBL-producing Gram-negative bacilli has increased in some hospitals, particularly among clinical isolates of P. aeruginosa. Metallo-beta-lactamases are a group of β-lactamase enzymes that have one or two zinc (Zn) in their active site to cleave the amide bond of the β-lactam ring to inactive β-lactam antibiotics. Acquired Metallo-β-lactamases (MBLs) are emerging worldwide as powerful resistance determinants in Gram-negative bacteria. So far, six MBL enzyme types have been described in clinical isolates of P. aeruginosa-VM, IMP, SPM, GIM, and AIM type. The three main clusters of the VIM MBL, represented by blaVIM-1, VIM-2, and VIM-7. The rapid spread of MBLs, particularly in P. aeruginosa, is an emerging threat and a matter of concern worldwide. Since the first report of acquired Metallo-β-lactamases (MBL) in Japan in 1994, genes encoding enzymes have spread rapidly among Pseudomonas spp.

Carbapenems are considered the last-line drugs for the treatment of infections caused by multi-resistant. In Iran 2013, most of the isolates was resistant to meropenem, cefotaxime, and imipenem (IPM). PCR amplification showed that 23/41 (56%) carried blaVIM and 10/41 (24.3%) possessed blaIMP gene. Also, 31/44 (70.5%) isolates contained class 1 integron gene.

In Egypt 2017, a total of 114 P. aeruginosa isolates were recruiting. Antimicrobial susceptibility testing revealed that 50 isolates (43.8%) exhibited multidrug-resistant (MDR) phenotype, of them 14 isolates (12.2%) were imipenem (IPM)-resistant. Of these 14 isolates, 13 isolates (11.4%) exhibited the Metallo-β-lactamase (MBL) phenotype. MBLs encoding genes, VIM and IMP, were identified by PCR. The results revealed that four isolates harbored the VIM gene alone, one isolate harbored IMP gene alone, and four isolates harbored both genes.
NDM-1-producers are now alarmingly on the increase worldwide and pose a potential risk for therapeutic failure with the empirical treatments currently in place. The first identification of a bla<sub>NDM</sub> gene in a clinical isolate originated from Egypt, 2014. (14)

II. MATERIALS AND METHODS

2.1 Bacterial isolation and identification:
A Cross-sectional descriptive hospital-based study was conducted in Elobied city, North Kordofan State from August 2016 to March 2017. Seven hundred and twenty-one (721) specimens were collected from patients and different hospitals settings. All specimens (wound and ear swabs, urine samples, ascetic fluid, pus, blood, pleural fluid and hospitals settings) were cultured, and P. aeruginosa were identified on the bases of conventional biochemical tests and API 12A/12E (Oxoid Company, Australia).

2.2 Antimicrobial susceptibility testing:
The antimicrobial susceptibility testing was done using disk diffusion method (Kirby Bauer). The selected antimicrobial disks were imipenem (10) mcg and meropenem (10) mcg (Oxoid Company. UK). P. aeruginosa ATCC 27853 were used as the control for susceptibility testing. 24 hours. (15)

2.3 Molecular characterization of MBL genes:
DNA was extracted from P. aeruginosa colonies using a simple boiling, (6-16) and also using (Analytik Jena, Germany) according to manufacturer’s instructions. PCR was carried out for detection of (blaVIM-1, NDM-1 and VIM like) genes on a thermal cycler (Eppendorf, Germany), using VIM-1 primers (Forward: 5´ TTA TGG AGC AGC AAC CGA TGT 3´ and Reverse: 5´ CAA AAG TCC CGC TCC AAC GA 3´).Table1.NDM-1 primers (Forward: 5´ xGTA GTG CTG CTA GTG CTC TGGC 3´ and Reverse: 5´ xGGG CAG TCG CTT CCA AGCGT 3´).VIM-like primers (Forward: 5´ xSGR TRS RTG GRCR CATAS CRCS 3´ and Reverse: 5´ xGTA GTG CTC AGT GTC GGCA 3´).The primer sets were listed in Table 2. (6, 17) The PCR products were separated on 2.0% agarose gel, stained with 1% ethidium bromide, electrophoresed, and visualized under ultraviolet (UV) light. (18)

Table1: Nucleotide sequences of primer used for detection of metallo-beta-lactamase genes ;

<table>
<thead>
<tr>
<th>Primer name</th>
<th>Sequence</th>
</tr>
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<tbody>
<tr>
<td>blaVIM-1</td>
<td>5´ TTA TGG AGC AGC AAC CGA TGT 3´</td>
</tr>
<tr>
<td></td>
<td>5´ CAA AAG TCC CGC TCC AAC GA 3´</td>
</tr>
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</table>

<table>
<thead>
<tr>
<th>PCR Condition</th>
<th>Denaturing</th>
<th>Anneal</th>
<th>Extension</th>
<th>Size(bp)</th>
</tr>
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<tbody>
<tr>
<td>94°C, 60 s</td>
<td>55°C, 60 s</td>
<td>72°C,2 min</td>
<td>35°C, 830</td>
<td></td>
</tr>
</tbody>
</table>

Table2: Primer sets for amplification of carbapenem resistance genes ;

<table>
<thead>
<tr>
<th>Primer name</th>
<th>Sequence</th>
</tr>
</thead>
<tbody>
<tr>
<td>NDM-1-F</td>
<td>5´ GTA GTG CTG CTA GTG GGC 3´</td>
</tr>
<tr>
<td>NDM-1-R</td>
<td>5´ GGG CAG TCG CTT CCA AGCGT 3´</td>
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<table>
<thead>
<tr>
<th>Size(bp)</th>
<th>475</th>
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</thead>
<tbody>
<tr>
<td>Size</td>
<td>360</td>
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</table>

III. ETHICAL CONSIDERATION

The study was approved by ethical committee of Ministry of Health North Kordofan state. Individuals who were included in this study were notified about the objectives of this study and its importance.

IV. RESULT

Out of 721 clinical specimens and hospitals settings 100 isolates were identified as P. aeruginosa strains. The majority (56%), of isolated P. aeruginosa strains, was from urine, (18%) wounds, (8%) pus, (5%) pleural fluid, (5%) blood, (4%) ear swabs, and minority (3%) from sputum. The only (1%) of isolates were related to the hospital settings Fig [1]. Antimicrobial susceptibility testing (Kirby Bauer) was revealed that 19(19%) and 14(14%) of isolated P. aeruginosa were resistant to imipenem and meropenem respectively. Fig [2]

Molecular examination for MBL expression genes among 19(19%) imipenem and 14(14%) meropenem resistant P. aeruginosa, revealed that 1/19(5.26 %) of isolated resistant P. aeruginosa carry the NDM-1 gene which encoding resistant to imipenem and 1/14 (7.1 %) carry the VIM gene which encoding resistant to meropenem, while no detection to blaVIM-1 gene in our study. All isolated strains carry these genes were isolated from urine. Fig [3] &Fig [4].

![Fig 1: The frequency of isolation of P. aeruginosa from clinical specimens in Elobied Hospitals.](image)

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In the current study, the evidential microbiological diagnosis was made by isolation of 100 P. aeruginosa isolates, from 721 different clinical samples. The numbers of isolates similar to that isolated in studies conducted in 2013, Besancon, France, they collect 109 strains as well as other reported during 2014 in Iran.

Although the carbapenems are considered the last-line drugs for the treatment of infections caused by multi-resistant, the present study demonstrated emerges of a considerable level of Pseudomonas aeruginosa resistant to imipenem and meropenem. These findings in accordance with previous studies are similar to that conducted in Iran 2013; most of the isolates were resistant to meropenem, cefotaxime, and imipenem, as well as to that conducted in 2016 Bhopal, India, as a total of 20 (13.3%) isolates of P. aeruginosa showed resistance to imipenem and in Egypt 2017, 14 isolates (12.2%) were imipenem (IPM)-resistant. These findings were limited therapeutic options for management of the infections to these strains.

The present study demonstrated the presence of the VIM and NDM-1 genes encoding resistant to imipenem and meropenem detected from urine specimens, this finding for VIM is similar to recent study conducted in Egypt 2017 were P. aeruginosa MBL encoding genes, VIM and IMP, were identified by PCR, that results revealed four isolates harbored the VIM gene alone, another result for NDM-1 gene monitored in study conducted in Egypt while in southern India, 2014, NDM-1 was detected in four isolates only. Carbapenem resistance due to acquired Metallo-β-lactamases (MBLs) is considered to be more serious than other resistance mechanisms because MBLs can hydrolyze all β-lactam antibiotics. Each MBLs gene encoding determinants of resistance to carbapenems, conferring multidrug resistance to P. aeruginosa are transferable to other Gram-negative species, increasing the antimicrobial resistance rate and complicating the treatment of infected patients.

The blaVIM-1 gene was first reported in P. aeruginosa in Italy. As far as recent studies have shown, this enzyme has spread significantly. This gene has been reported in different areas in the world, but fortunately, our study showed that none of the isolated P. aeruginosa strains in El-Obied hospitals carry blaVIM-1 which imposed some difficulties in treating bacterial infections. This result was similar to that obtained in Iran 2015.

The study concluded that 19% and 14% of our isolates revealed resistance to imipenem and meropenem respectively and the evidence of the presence of the NAD-1 and VIM genes, but none of the isolated P. aeruginosa strains carry blaVIM-1 in study area. Further studies with considerable sample size and a regular monitoring system for early detection of MBL-producing organisms are recommended.

REFERENCES


