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

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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-VIM, 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 %) carried the *VIM* gene which encoding resistant to imipenem and meropenem respectively, while no detection to *blaVIM*-1gene 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.(1) They often colonize hospital food, sinks, taps, mops, and respiratory equipment (2)and tolerates a variety of physical conditions so it can remain in both community and hospital settings.(3) 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. (4)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. (5)

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. (6, 7, 8)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-VIM, IMP, SPM, GIM, and AIM* type.(9)The three main clusters of the *VIM MBL*, represented by *blaVIM-1*, *VIM-2*, and *VIM-7*.(10)The rapid spread of MBLs, particularly in *P. aeruginosa*, is an emerging threat and a matter of concern worldwide. (4) Since the first report of acquired Metallo-β lactamases (MBL) in Japan in 1994, genes encoding enzymes have spread rapidly among *Pseudomonas* spp., (7)

Carbapenems are considered the last-line drugs for the treatment of infections caused by multi-resistant. (11) 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. (12)

In Egypt 2017, a total of 114 *P. aeruginosa* isolates were recruited. 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. (13)

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_{nd} 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, German) 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 ×GTA GTG CTC AGT GTC GGCA3 × and Reverse: 5 × GGG CAG TCG CTT CCA ACGGT 3×).*VIM-like* primers (Forward: 5 ×SGR TRS RTG GRCR CATAS CRCS 3 × and Reverse: 5 × TCS SCR GRA CCR SAG CRC ACR 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 metalo-beta-lactamase genes :[6]

Primer name	Sequence
blaVIM-1	5´ TTA TGG AGC AGC AAC CGA TGT 3´
	5´ CAA AAG TCC CGC TCC AAC GA 3´
PCR Condition	Denaturing Anneal Extension Cycles Size(bp)
	94°C, 60 s 55°C, 60 s 72°C,2 min 35°C 830

Table2: Primer sets for amplification of carbapenem resistance genes :[17]

Primer name	Sequence
NDM-1-F	5´ GTA GTG CTC AGT GTC GGCA3´
NDM-1-R	5´ GGG CAG TCG CTT CCA ACGGT 3´
Size(bp)	
475	
VIM-F	5´ SGR TRS RTG GRCR CATAS CRCS 3´
VIM-R	5´ TCS SCR GRA CCR SAG CRC ACR 3´
Size	(bp)
360	

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-1gene in our study. All isolated strains carry these genes were isolated from urine. Fig [3] &Fig [4].

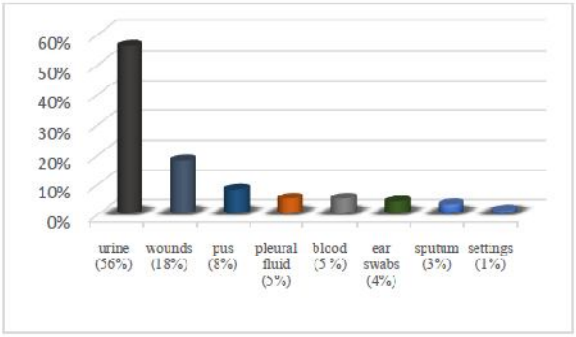


Fig [1]: The frequency of isolation of *P. aeruginosa* from clinical specimens in Elobied Hospitals.

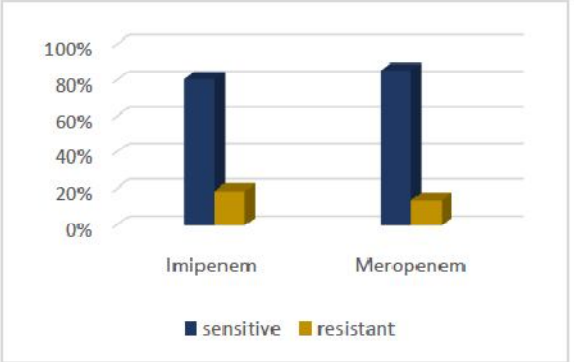


Fig [2]: Antimicrobials susceptibility patterns of isolated bacteria in Elobied Hospitals.

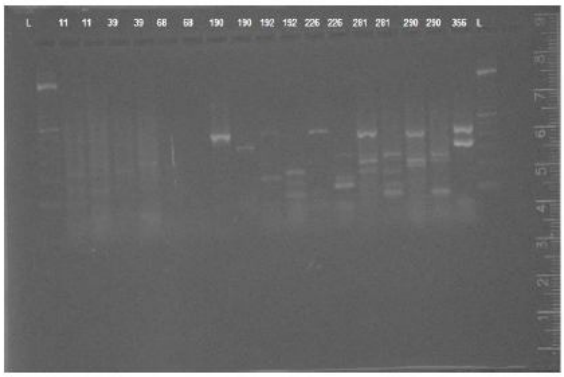


Fig [3]: Agrose gel electrophoresis of PCR products after amplification of (NDM-1)gene.

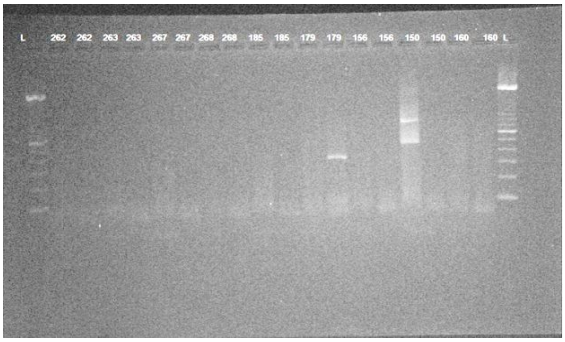


Fig [4]: Agrose gel electrophoresis of PCR products after amplification of (VIM Like) gene.

DISCUSSION

The rapid spread of MBLs among major Gram-negative pathogens, particularly *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., [7] the prevalence of MBL-producing has increased in some hospitals, particularly among clinical isolates of *P. aeruginosa*. [7, 8, 6]

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 [19] as well as other reported during 2014 in Iran. [8]

Although the carbapenems are considered the last-line drugs for the treatment of infections caused by multi-resistant, [20] 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, [12]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[21] and in Egypt 2017, 14 isolates (12.2%) were imipenem (IPM)-resistant.[20] 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* MBLs encoding genes, *VIM* and *IMP*, were identified by PCR, that results revealed four isolates harbored the *VIM* gene alone, [20] another result for *NDM-1* gene monitored in study conducted in Egypt 2014 [14] while in southern India, 2014, *NDM-1* was detected in four isolates only.[22]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. [6] 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.

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