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The Prevalence of Metallo-β-Lactamase IMP-7 Gene in Imipenem and Meropenem Resistant Pseudomonas aeruginosa Among Patients With Different Diseases in El-Obied Hospitals – Sudan

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Abstract: *Rapid spread of MBLs is an emerging threat and a matter of concern worldwide. So far, six MBL enzyme types have been described in clinical isolates of P. aeruginosa. In this article, we investigated the prevalence of Metallo-β-Lactamase IMP-7 genes in imipenem and meropenem-resistant Pseudomonas aeruginosa isolated from Elobied Hospitals. A hundred (100) isolated Pseudomonas aeruginosa were cultured and identified using API thin subjected to antimicrobial susceptibility testing (Kirby Bauer), for selected imipenem and meropenem. PCR was performed for detection of IMP-7 gene in imipenem and meropenem resistant P. aeruginosa strains. 19% and 14% isolates were found resistant to imipenem and meropenem respectively, 16 % of isolated resistant P. aeruginosa carry the IMP-7gene which encoding resistant to imipenem and meropenem in our study. The study concluded that evidence of the presence of the IMP-7gene.*

Keywords— MBLs, P. aeruginosa, Prevalence, IMP-7, Imipenem and Meropenem

1. INTRODUCTION

Pseudomonas aeruginosa, an opportunistic pathogen, is an important cause of infection in patients with impaired immune systems, 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, 2] Pseudomonas aeruginosa is one of the 'ESKAPE' pathogens(Enterococcus faecium, Staphylococcus aureus, Klebsiella pneumoniae, Acinetobacter baumannii, P. aeruginosa and Enterobacter spp.) known to be responsible for a majority of antimicrobial-resistant hospital-associated infections. They often colonize hospital equipment and tolerate a variety of physical conditions. [3, 4] The multidrug-resistant (MDR) phenotype in *P. aeruginosa* could be mediated by several mechanisms. Furthermore, MBLs encoding genes are usually located on integrons, the mobile genetic elements that also carry genes encoding for resistance to aminoglycoside and other antibiotics resulting in multidrug resistance.[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) inactive β-lactam antibiotics. [6, 7, 8]Acquired Metallo-β-lactamases (MBLs) are emerging worldwide as powerful resistance determinants in Gram-negative bacteria.[9] The most widespread carbapenemases in *Pseudomonas spp*. are metallo-β-lactamases of VIM- (Verona imipenemase) and IMP- (Imipenemase) types.[10]The rapid spread of MBLs, particularly in *P. aeruginosa*, is an emerging threat and a matter of concern worldwide. [11] Since the first report of acquired Metallo-β lactamases (MBL) in Japan in 1994, genes encoding enzymes have spread rapidly among *Pseudomonas* spp., [7]

Most of the isolates resample to that in Egypt 2017 was resistant to carbapenems tested, including imipenem and meropenem. 13 isolates (11.4%) exhibited the metallo-β-lactamase (MBL) phenotype. MBLs encoding genes, *VIM* and *IMP*, were identified by PCR. [12]

The goal of this study was to identify the presence of bacterial genes involved in multiple resistances to antimicrobials in P. aeruginosa using the PCR method.

2. MATERIALS AND METHODS

2.1 Bacterial isolates

A hundred (100) specimens were obtained from patients and different hospitals settings during the study period from August 2016 to March 2017. All specimens 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

Susceptibility testing of the isolates was performed by disk diffusion (Kirby Bauer) method. The selected antimicrobial disks were imipenem (10) mcg and meropenem (10) mcg

(Oxoid Company. UK). *P. aeruginosa* ATCC 27853 was used as a quality control in the susceptibility testing. [13]

2.3 Detection of MBL encoding genes by PCR

DNA was extracted from *P. aeruginosa* colonies using a simple boiling, [6-14] and also using (Analytik Jena, German) according to manufacturer's instructions**.** PCR was carried out for detection of *(bla IMP-7)* gene on a thermal cycler (Eppendorf, Germany), using *IMP-7* primers (Forward: 5×AAG GCA GTA TCT CCT CTC ATT TC 3 × and Reverse: 5 × ACT CTA TGT TCA GGT AGC CAA ACC 3×). The primer sets were listed in Table 1. [7] The PCR products were separated on 2.0% agarose gel, stained with 1% ethidium bromide, electrophoresed, and visualized under ultraviolet (UV) light. [7]

Table (1) Primer used for PCR amplification of Pseudomonas aeruginosa :[7]

Primer Name	Sequence (5´- 3´)
IMP-7	AAGGCAGTATCTCCTCTCATTTC/ ACTCTATGTTCAGGTAGCCAAACC

PCR				Condition
Denaturing	Anneal	Extension	Cycles	Size (bp)
94°C, 60 s	62°C, 60 s	72°C,10 min	30°C	243

3. RESULTS

All 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.

Of the total isolates, only (19%) and (14%) Antimicrobial susceptibility testing (Kirby Bauer) was resistant to imipenem and meropenem respectively.

Of the 100 P. aeruginosa clinical isolates included in this study, PCR assay detected IMP-7 type of MBL among 16 (16%) of the isolates. Fig [1].

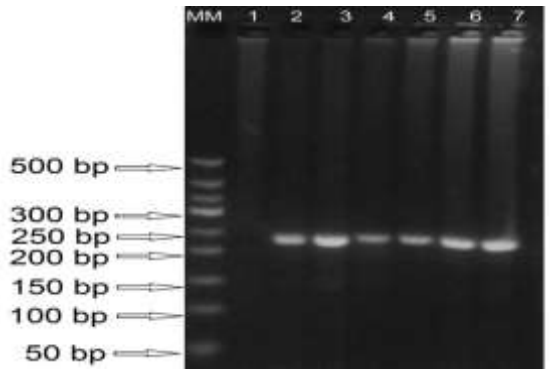

Fig [1]: Agrose gel electrophoresis of PCR products after amplification of (IMP-7) gene. MM: molecular weight marker (Ruler 50bp DNA ladder); 1 – Negative control; 2 – Positive control; 3-7 different strains.

4. DISCUSSION

Pseudomonas aeruginosa is one of the most important nosocomial pathogens. [7] The prevalence of MBL-producing has increased in some hospitals, particularly among clinical isolates of *P. aeruginosa.* [7, 8, 6]

Our study showed that the evidential microbiological diagnosis was made 100 *P.aeruginosa* isolates. The numbers of isolates similar to that isolated in studies conducted in France and in Iran. [8, 17]

With increasing reports of MBLs in carbapenem susceptible isolates, we intended to determine MBL frequency in IMP- resistant and susceptible P.aeruginosa isolates by phenotypic and molecular testing as well as search for screening criteria for MBLs to select them out in IMP- susceptible P. aeruginosa isolates as has been urgently required Although the carbapenems are considered the last-line drugs for the treatment of infections caused by multi-resistant, [12, 18] IMP-types MBLs were reported from several countries, [7] 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, most of the isolates were resistant to meropenem, cefotaxime, and imipenem, [19] as well as to that conducted in India, as a total of 20 (13.3%) isolates of *P. aeruginosa* showed resistance to imipenem. [20]

Among the studied P. aeruginosa isolates, the presence of the *IMP-7* had been detected in 16 and 16% by phenotypic tests and PCR assay respectively. Previous reports have supported the present study findings, in Khartoum, Sudan showed that Fifty seven isolates were recognized to have IMP-7 and IMP-10, only twenty six isolates produce IMP-7 and the remainder thirty one isolates had IMP-10. Five isolates of them were giving same reactives in tow genes IMP-7 & IMP-10.[7] The prolonged use of imipenem/meropenem for the treatment of nosocomial infections can favor the development of resistance to carbepenems as well as other antibiotics. [18]

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 *IMP-7* genes.

In conclusion, the prevalence of isolates possessing MBL activity among the population study represents an emerging threat of complete resistance to carbapenems among P. aeruginosa in Sudan. *IMP-7* type of MBL was detected in IMP-susceptible P. aeruginosa isolates; a finding which may underscores the necessity of screening all imipenem and

meropenem -resistant isolates for MBL production and implementation of infection control programs to prevent spread of such organisms. New antimicrobial agents capable of treating patients with MDR *P. aeruginosa* infections are urgently required.

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