

## Detection of Multidrug resistant *Pseudomonas aeruginosa* Isolates producing IMP-1 Metallo- $\beta$ -Lactamase in some Baghdad hospitals

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### Abstract

This Study describe the Molecular characterization of a Multiresistant *Pseudomonas aeruginosa* isolates in three hospitals in Baghdad ( Central Child hospital, Central Medicine City hospital and Al-Noman hospital). Analysis included antimicrobial susceptibility profile and Plasmid profile

Bacterial isolates were tested against (11) antimicrobial agents: Imipenem, Ciprofloxacin, Aztreonam, Cefuroxime, Amoxicillin, Cefotaxime, Nalidixic acid, Trimethoprim, Tetracycline, Mezlocillin and Gentamicin. Results showed that all the isolates were resistant to Cefuroxime, Amoxycillin, and Tetracycline, and all the isolates have shown multiple resistance for antibiotics. The majority of isolates remained susceptible to Imipenem (22%).

Our results showed also that 9 isolates ( 18 %) had the ability to produce Metallo  $\beta$ -lactamase enzymes (MBLs). On the other hand, DNA analysis ( Plasmid profile) showed that 80 % of the Bacterial isolates contained plasmid of different molecular weights.

### Introduction

*Pseudomonas aeruginosa* causes nosocomial infections as a result of its ubiquitous nature, ability to survive in moist environments, and resistance to many antibiotics and antiseptics. A serious problem is the emergence of multidrug-resistant *P. aeruginosa* strains resistant to  $\beta$ -lactams, aminoglycosides, and quinolones (1). Although intrinsically sensitive to  $\beta$ -lactams (e.g., Ceftazidime [CAZ] and Imipenem [IPM]), aminoglycosides (e.g., Amikacin [AMK] and Tobramycin), and fluoroquinolones (e.g., Ciprofloxacin [CIP] and Ofloxacin [OFX]), *P. aeruginosa* resistant to these antibiotics has emerged and is widespread (2).

Carbapenems are the drugs of choice for the treatment of infections caused by multiresistant gram-negative bacilli (3). An increasing prevalence of carbapenem resistance mediated by acquired metallo- $\beta$ -lactamases (MBLs) is being reported, particularly for *Pseudomonas aeruginosa* clinical isolates in several countries (4). In Korea, approximately 10 and 50% of imipenem resistance strains in *P. aeruginosa* (5) and *Acinetobacter* spp. (6), respectively, are due to MBL production. The resistance may spread rapidly to various species of gram-negative bacilli, as the MBL genes reside in mobile gene cassettes inserted in integrons. Since 1988, transferable carbapenem resistance has been found in several *P. aeruginosa* strains isolated in Toyama Prefecture, Japan (7).

Carbapenemases are members of the molecular class A, B, and D  $\beta$ -lactamases. Class A and D enzymes have a serine-based hydrolytic mechanism, while class B enzymes are metallo- $\beta$ -lactamases that contain zinc in the active site. The class A carbapenemase group includes members of the SME, IMI, NMC, GES, and KPC families. Of these, the KPC carbapenemases are the most prevalent, found mostly on plasmids in *Klebsiella pneumoniae*. The class D carbapenemases consist of OXA-type  $\beta$ -lactamases frequently detected in *Acinetobacter baumannii*. The metallo- $\beta$ -lactamases belong to the IMP, VIM, SPM, GIM, and SIM families and have been detected primarily in *Pseudomonas aeruginosa*; however, there are increasing numbers of reports worldwide of this group of  $\beta$ -lactamases in the *Enterobacteriaceae* (8). The rapid detection of MBL-

positive gram-negative bacilli is necessary to aid infection control and to prevent their dissemination (9). A Polymerase Chain Reaction (PCR) method was simple to use in detecting MBL-producing isolates initially (10).

### The aim of this work

was to detection of Multidrug resistant (MDR) *Pseudomonas aeruginosa* Isolates producing IMP-1Metallo- $\beta$ -Lactamase in some Baghdad hospitals, and studying the plasmids of genetic for IMP-1Metallo- $\beta$ -Lactamase production isolates.

### Materials and Methods

**1) Clinical isolates:** Fifty Clinical *Pseudomonas aeruginosa* isolates were collected from different infections sources from three hospitals in Baghdad City (Central Child, Central Medicine City and Al-Noman hospitals). Isolates were identified according to Greenwood *et al.* (11) by classical microbiological methods and API 20-E system.

### 2) Antimicrobial susceptibility:

**A)** The disk diffusion test was used to determine antimicrobial susceptibility of bacterial isolates on Mueller-Hinton agar using the following antibiotics: Imipenem (IPM), Ciprofloxacin (CIP), Aztreonam (ATM), Cefuroxime (CXM), Amoxicillin (AML), Cefotaxime (CTX), Nalidixic acid (NA), Trimethoprim (W), Tetracycline (TE), Mezlocillin (MEZ) and Gentamicin (G).

To determine the antibiotic sensitivity for the selected isolate, 10 ml of nutrient broth was inoculated with 100  $\mu$ l of isolate and incubated at 37 °C to mid log phase, 100  $\mu$ l of the inoculum was streaked on nutrient agar plates, the selected antibiotic disc were placed on the inoculated plates and inoculated over night at 37°C. After incubation, the zone of inhibition of growth were measured using mm units according to the NCCLS (12).

**B)** Values of MICs for Imipenem and Cefotaxime were determined by the serial-dilution method according to the NCCLS (12).

**3)** Isolated organisms were streaked onto plates of Mueller-Hinton agar. A 0.5M EDTA solution was prepared. Two imipenem disks (10- $\mu$ g) were placed on the plate, and appropriate amounts (10  $\mu$ l) of an EDTA

solution were added to one of them to obtain the desired concentration. The inhibition zones of the imipenem and imipenem-EDTA disks were compared after 18 h of incubation at 37°C (10).

4) **plasmid extraction:** Plasmid DNA was extracted according to (13) by Alkaline method. Electrophoresis was conducted at 5 V/cm in TBE buffer. plasmid DNA bands were observed under U.V. light (Transilluminator) with wave length of 340 nm.

### Results and Discussion

*P. aeruginosa* is known to colonize the hospital environment, particularly moist sites, sources such as tap water, sink, antiseptic solutions, respiratory equipment, and bronchoscopes are the most commonly incriminated

nosocomial reservoirs of *P. aeruginosa*. Recent studies also reported health care workers as a transient reservoir and possible vehicle of nosocomial outbreaks (14).

The gram-negative bacilli used in this study were 50 isolates of *P. aeruginosa*. All of the isolates were multiresistant, isolates were highly resistant (100%) to Cefuroxime, Amoxicillin and Tetracycline; The resistance percentages were as follows: for Trimethoprim, 84%; Gentamicin, 80%; Ciprofloxacin, 76%; Nalidixic acid, 72%. The majority of isolates remained susceptible to Imipenem (78%) (Fig 1).

The high frequency of multiple resistance among *P. aeruginosa* strains makes its eradication difficult, and mortality associated with *P. aeruginosa* infection is high compared to other bacteria (15).

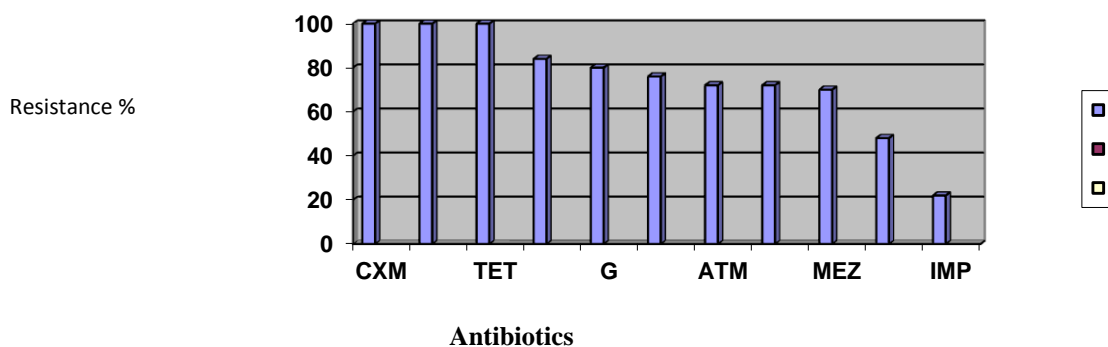


Fig (1) : Antibiotic resistance of *P. aeruginosa* isolates .

Cefuroxime (CXM), Amoxicillin (AML), Tetracycline (TE), Trimethoprim (W) Gentamicin (G) Ciprofloxacin (CIP), Aztreonam (ATM), Nalidixic acid (NA), Mezlocillin (MEZ), Cefotaxime (CTX), and Imipenem (IMP).

The results showed also that 9 isolates (18%) had the ability to produce Metallo  $\beta$ -lactamase enzymes. On the other hand, DNA analysis (Plasmid profile) showed that 80% of the Bacterial isolates contained plasmids of different molecular weights (Fig-2).

It is necessary to perform MBL detection tests with suspicious isolates because these enzymes cannot be identified by routine susceptibility tests. Suspicious isolates are those with significantly reduced susceptibility to carbapenems, e.g., *E. coli* and *Klebsiella* isolates with imipenem MICs of 1  $\mu\text{g/ml}$  or higher; *Enterobacter*, *Citrobacter freundii*, and *S. marcescens* isolates with imipenem MICs of 4  $\mu\text{g/ml}$  or higher; and *P. aeruginosa* isolates with imipenem MICs of 8  $\mu\text{g/ml}$  or higher. Tests involving chelating agents such as EDTA and MPA are useful because the chelators are specific inhibitors of MBLs but not of other  $\beta$ -lactamases (16). A single chelating agent may sometimes not adequately inhibit all MBLs in certain pathogens, making it necessary to use a mixture of chelating agents for the reliable detection of MBLs.

Metallo- $\beta$ -lactamases (MBLs) -producing gram-negative bacteria have been recognized to be among the most important nosocomial pathogens, and further proliferation of these strains in clinical settings will pose a serious global problem in the future. For this reason, aggressive surveillance of MBLs producers with respect to the classification of the genetic determinant for MBLs as well as the integron will be extremely (7).

*Pseudomonas* was found to produce metallo- $\beta$ -lactamase activity and to harbor a 50-kb plasmid, named pVA758, carrying a new *bla*<sub>IMP</sub> determinant, named *bla*<sub>IMP-12</sub>. Plasmid pVA758 was not self-transferable by conjugation to either *Escherichia coli* or *Pseudomonas aeruginosa* but could be introduced by electroporation and maintained in the latter host, where it conferred resistance or decreased susceptibility to various  $\beta$ -lactams (17).

*P. aeruginosa* is known to colonize the hospital environment, particularly moist sites. Sources such as tap water, sink, antiseptic solutions, respiratory equipment, and bronchoscopes are the most commonly incriminated nosocomial reservoirs of *P. aeruginosa* (18). Recent studies also reported health care workers as a transient reservoir and possible vehicle of nosocomial outbreaks (19).

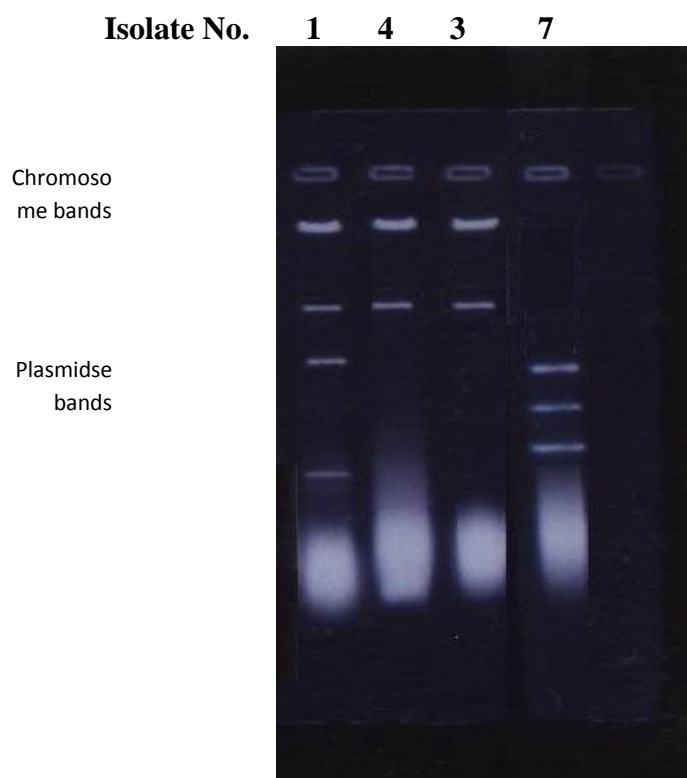


Figure ( 2 ) : Plasmid profile for *P. aeruginosa* isolates

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## التحري عن بكتريا *Pseudomonas aeruginosa* التي تحمل صفة المقاومة المتعددة لبعض المضادات الحيوية والمنتجة لأنزيم البيبتالاكتاميز المعدني (IMP-1) في بعض مستشفيات بغداد

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### الملخص

أجريت دراسة جزيئية على عزلات بكتريا *Pseudomonas aeruginosa* التي تحمل مقاومة متعددة لبعض المضادات الحيوية والمعزولة من ثلاثة مستشفيات في مدينة بغداد ( مستشفى الطفل المركزي و مستشفى مدينة الطب المركزي ومستشفى النعمان ) ، أذ تم دراسة نسق المقاومة المتعددة للمضادات الحيوية والنسق البلازميدي للعزلات . بينت النتائج ان جميع عزلات بكتريا *Pseudomonas aeruginosa* قيد الدراسة ( 50 عزلة ) قد أبدت مقاومة متعددة للمضادات من مجاميع: البنسلينات والسيفالوسبورينات والكارباميم والأمينوكلايكوسايد والفلوروكوينولون. أبدت تسع عزلات ( 18 % ) قابليتها على إنتاج أنزيمات البيبتالاكتاميز المعدنية (MBLs) من جانب آخر أظهرت نتائج عزل الدنا البلازميدي أملاك 80% من العزلات البكتيرية حزم بلازميدية ذات اوزان جزيئية مختلفة.