# Resistance of *Pseudomonas aeruginosa* to multiple β-lactam antibiotics

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**Abstract:**
A total of 90 *Pseudomonas aeruginosa* (*P.aeruginosa*) strains clinical samples were collected from burn, ear, and foot diabetic samples in Al-Hilla, and Marjan teaching hospitals were identified to species level with a VITEK-2 system. The susceptibility to 11 antibiotics were tested using disc diffusion test resulting in 55.4% of 90 *P.aeruginosa* isolates were resistant to 11 antibiotics. Cefinase discs impregnated with nitrocefin method was used for the detection of β-lactamase production in antibiotics resistant 90 *P.aeruginosa* isolates. Results showed that 56% isolates were able to produce β-lactamase.

**Microbiology Classification QR** 75-99.5
**Key words:** malignancies, cardiopulmonary, renal failure.

molecular classes are found in *P.aeruginosa*, including ESBLs of classes A, B and D. Lipopolysaccharide plays a direct role in causing fever, shock, oliguria, leukocytosis and leucopenia, disseminated intravascular coagulation and adult respiratory distress syndrome [4].

**Materials and methods:**

**Bacterial isolates:**

Ninety *P.aeruginosa* isolates were obtained from clinical samples in Al-Hilla/Iraq during the period from February 2014 to April 2014. Clinical samples were collected from Al-Hilla and Marjan teaching hospitals in Al-Hilla city, in addition to some private clinic. Clinical isolates were as follows: burn, ear, and foot diabetic samples. These bacterial isolates were identified as *P.aeruginosa* based on their morphology, Gram-staining. Vitek 2 system was performed to identify species level of *P.aeruginosa* isolates.

**Antimicrobial Susceptibility Test:**

**1- Disc diffusion test (DD test):**

The antimicrobial susceptibility patterns of isolates to different antimicrobial agents was determined and interpreted according to [5]. Disk diffusion test was used against 11 antibiotics, the following antimicrobial

**Introduction:**

*Pseudomonas aeruginosa* is a leading cause of nosocomial infections throughout the world. Bacteremia and septic shock due to *P.aeruginosa* continue to be the major problems in hospitalized patients with underlying malignancies, cardiopulmonary disease, renal failure or diabetes. This organism has constitutive resistance to many drug classes and readily becomes resistant to all relevant treatments owing to its multiply acquired resistance mechanisms [1]. Enzyme production is the major mechanism of acquired resistance to β-lactam antibiotics in *P.aeruginosa*. Penicilloyl-serine transferases (usually referred to as β-lactamases) rupture the amide bond of the β-lactam ring, thus the obtained products lack antibacterial activity [2]. Molecular classification of β-lactamases is based on the nucleotide and amino acid sequences in these enzymes. To date, four classes are recognized (A–D), correlating with the functional classification defined by enzyme substrate and inhibitor profiles [3]. Classes A, C and D act through a serine-based mechanism, whereas class B or metallo β-lactamases (MBLs) need zinc for their action. A significant number of β-lactamases of all four

show no color change on the disc. For most bacterial strains a positive result

will develop within 5 min. However, positive reactions for some staphylococci

may take up to 1 h to develop.

**Results and Discussion:**

A total of 90 *P.aeruginosa* strains clinical samples were collected from burn, ear, and foot diabetic samples in Al-Hilla, and Marjan teaching hospital, Khuntayaporn *et al* [6] found that increased infection caused by multidrug resistant (MDR) *P. aeruginosa* has raised awareness of the resistance situation worldwide wherein 71.65% were collected from 2007–2009 found to be Carbapenem resistance - multidrug resistant *P.aeruginosa*. In this study, 11 antibiotics performed to all 90 *P.aeruginosa* isolates for testing their susceptility and to identify the most effective one against *P.aeruginosa*, The results revealed that 55.4 % of 90 *P.aeruginosa* isolates were resistant to 11 antibiotics. All 90 *P.aeruginosa* isolates showed high resistance 90% to oxacillin and less in 43.2% ampicillin-sulbactam as shown in (Figure1).

agents were obtained (from Oxoid, U.K) as standard reference disks as known potency for laboratory use: Oxacillin (10mg), Ampicillin-sulbactam (l0/10mg), Cefepime (30mg), Cefotaxime (30mg), Ceftazidime (30mg), Ceftriaxone (30mg), imipenem (10mg), gentamicin (10mg), amikacin (30 mg), tobramycin (10mg), and ciprofloxacin (5mg).

**2-Detection of β-lactemase production (Cefinase discs impregnated with nitrocefin):**

This test was performed for suspected *P.aeruginosa* isolates, as follow:

- An overnight bacterial culture on blood agar was prepared.
- Using a sterial loop, several colonies were streaking onto appropriate culture media plate.
- Using forceps moisten disc cefinase with one drop of purified water and

put on inoculated plate.

- Observe disc for color change, A positive reaction will show a yellow to red

color change on the area where the culture was applied. A negative result will

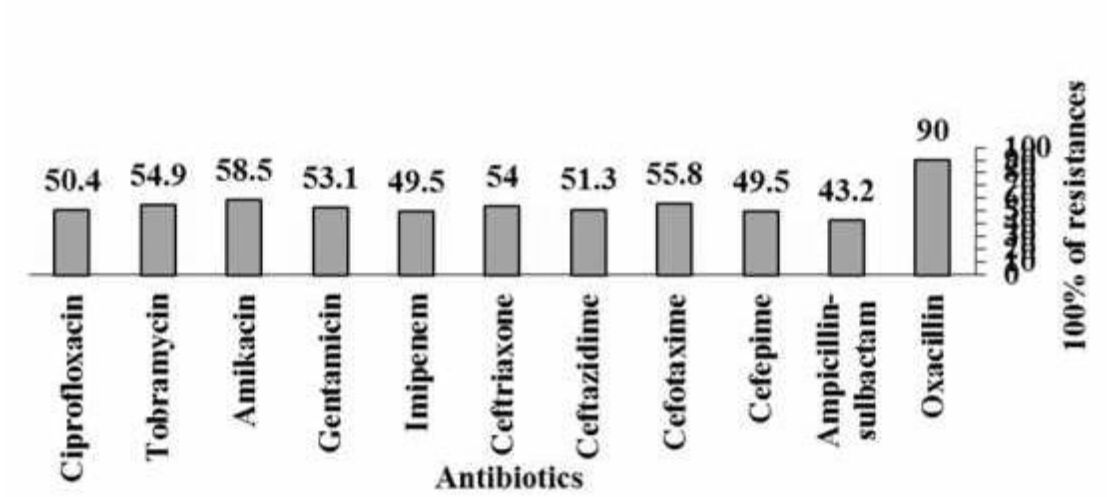


**Figure (1) Antibiotic resistance patteren of *Pseudomonas aeruginosa* isolates to different antibiotics by disc diffusion test**

(3rd generation), and cefepime (4th generation) showed that percentages of *P.aeruginosa* isolates resistant were to these antibiotics: 55.8%, 54%, 51.3%, 49.5%, respectively (Figure 1). The most important mechanism of resistance to cephalosporin is the production of plasmid mediated β-lactamase enzymes that inactivate the antibiotic by hydrolyzing the betalactam ring. *P.aeruginosa* also produces chromosomally mediated β-lactamases, majority of them are cephalosporinases, hydrolyzing cephalosporins more readily than penicillins [8].

In the present study the imipenem resistance was 49.5% (Figure 1). In 2006, McGowan [9] found that imipenem resistance in *P.aeruginosa* has increased steadily in recent years Resistance to penicillins (Beta-lactam Antibiotics) are due to production of β-lactamases whose genes are carried by plasmids, difficulty in penetration of the antibiotics to the target site. The outer membrane permeability is reduced. The main reason for high intrinsic resistance to beta-lactams is the low rate of passage of the antibiotic across the outer membrane. The F protein is probably the key determinant of outer membrane permeability, and The altered affinity of target penicillin binding proteins (PBP's). Resistance to beta-lactams can be acquired by modifications in one or more of the PBP's leading to a decrease in the affinity of the lethal targets for the drugs [7].

Results of Cephalosporins; cefotaxime, ceftriaxone, ceftazedim,

aminoglycoside modifying enzymes [7].

*P.aeruginosa* isolates represented resistance 50.4% against ciprofloxacin (Figure 1). Mechanism of bacterial resistance to quinolones includes chromosomal mutations that either alters DNA gyrase (resistance to quinolones alone) or reducing drug accumulation in association with changes in the bacterial outer membrane proteins. Fluoroquinolone resistance mutations in *P.aeruginosa* are also associated with decreased membrane permeability to quinolones and other antimicrobial agents. In other mutants, altered lipopolysaccharide structure and additional outer membrane proteins are credited for this multidrug resistance [11].

Cefinase discs impregnated with nitrocefin method was used for the detection of β-lactamase production in antibiotics resistant 90 *P.aeruginosa* isolates. Results showed 56% of the bacterial isolates were able to produce β-lactamase that converts yellow to red color within 5 minutes. Nitrocefin disks are impregnated with nitrocefin, a chromogenic cephalosporin. As the amide bond in a beta-lactam ring is hydrolyzed by a beta-lactamase, nitrocefin changes color from yellow to red. Bacteria which produce beta-and is often also associated with resistance to other antibiotics. From 1989 through 2006, the annual percentage of all *P.aeruginosa* isolates that demonstrated resistance to imipenem increased from 13% to 20% [10].

*P.aeruginosa* isolates exhibited resistance to gentamicin, amikacin, and tobramycin accounted for 53.1% , 58.5%, 54.9%, respectively (Figure 1). *P.aeruginosa* can modify aminoglycosides by acetylation, phosphorylation or adenylation. Virtually all strains possess a 6-phosphotransferase that modifies neomycin and kanamycin. The most common mechanisms of gentamicin resistance are N-acetylation and O-adenylation and to a much lesser extent, phosphorylation. The enzymes, 3acetyltransferase (AAC-3) and 2-adenyl transferase (ANT-2) are involved in enzymatic modification of gentamicin. AAC-3 and ANT-2 also modify tobramycin; and production of 6acetyltransferase (AAC-6) confers resistance to amikacin. A more common form of resistance is cross-resistance among all aminoglycosides, due to reduction in permeability of the cell surface to these antibiotics, which is not associated with plasmids or

on the nitrocefin disk [12].

lactamase in significant amounts produce this yellow to red color change

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## تعدد مقاومة الزوائف الزنجارية لمضادات البيتالاكتام

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**الخلاصة:**

تم عزل 90 (90%) من الزوائف الزنجارية (*P.aeruginosa*) كانت مأخوذة من عينات الحروق ، الأذن و أقدام مرضى السكري في مستشفى الحلة ومرجان التعليمي وقد تم تشخيص أنواع الزوائف الزنجارية تبعا" لنظام الفايتك 2. تم اختبار استجابة 90 عزلة من *P.aeruginosa* تجاه 11 من المضادات الحيوية بواسطة فحص انتشار الأقراص فتبين إنها مقاومة بنسبة 55.4% لـ 11 من المضادات الحيوية. تم استخدام طريقة اقراص النايتروسفين للكشف عن قابلية العزلات المقاومة للمضادات الحيوية (90) على إنتاج البيتالاكتاميز وأظهرت النتائج أن هذه العزلات كانت قادرة على إنتاج انزيمات البيتالاكتاميز وبنسبة (56٪).

**الكلمات الافتتاحية:**

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