Effect of Inhibitors β-Lactamase on Recovery Effectiveness of Some β-Lactam Antibiotics Against Pseudomonas Aeruginosa

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Abstract

Thirty-four samples with position *Pseudomonas aeruginosa* cultures isolated from burns, wounds urinary tract infection and Otities media were collected from Baquba General Hospital during September-December 2010. The sensitivity of these isolates were tested against (16) antibiotics. The results showed that the highest resistances were for Amoxicillin ,Ampicillin , CO-Trimoxazole and Nitroforautoin with 100% , while the lowest resistance was for Ofloxacin with 3% .The results of minimum inhibitory concentration (M.I.C) toward eleven antibiotics showed different range among isolates , some were able to resist high concentration of Ampicillin and Amoxicillin reach to 1024µg/ml , while others were inhibited by 2µg/ml of Ciprofloxacin . The isolates showed low sensitivity for combination Ampicillin- Sulbactam with 0%, while it shwed high sensitivity toward combination of Piperacillin-Tazobactam and Ceftazidime-Clavulanic acid 91.17, 100% respectively. The results of plasmid content was studied indicate that all isolates contain single large plasmid band, while the study of plasmid curing appear the plasmid loss at concentration 512 µg/ml of acridin orange.

**Key wards:** Antibiotics, *Pseudomonas aeruginosa*, β-Lactamase inhibitors, Plasmid curing.

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Introduction

*Pseudomonas aeruginosa* is widely distributed in nature and commonly presents in moist environments of hospitals. It can colonize normal humans, in whom it is a saprophyte[1]. *Pseudomonas aeruginosa* and other *Pseudomonades* are resistant to many antimicrobial agents and therefore become dominant and important when more susceptible bacteria of the normal flora are suppressed [2] *P Pseudomonas aeruginosa* which is considered important bacterial species responsible for numerous nosocomial infections causes burn and post-operative wounds infections. [3]

The extensive use of third and fourth generation cephalosporins as an important component of empirical therapy in intensive care units and high risk wards , resistance to these drugs has became a major problem all over the world [4] . Resistance has developed in bacteria by possessing extended spectrum beta – lactamase (ESBLs) capable of hydrolyzing these newer cephalosporins [5,6]. Beta – lactamase mediated resistance
may be overcome by combining beta – lactam antibiotics with beta – lactamase inhibitors which bind irreversibly to the beta – lactamases and render them inactive thus sparing the beta – lactam antibiotic [7].

In 2005 Using of beta-lactamase inhibitors in combination with beta-lactam antibiotics represents an effective measure to combat a specific resistance mechanism of beta-lactamase producing organisms [7]. In 2001 Three beta-lactamase inhibitors such as Clavulanic acid, Sulbactam and Tazobactam are in clinical use, and in combination with beta-lactam antibiotics, represent a successful strategy to combat a specific resistance mechanism [8,9,10].

The aim of study is to illustrate the comparative invitro activities of three beta-lactamase inhibitors such as Clavulanic acid, Sulbactam and tazobactam against beta-lactamase producing Pseudomonas aeruginosa causing different infections in Baquba Hospitals.

Materials and Methods
Activation of Pseudomonas aeruginosa
Thirty-four Pseudomonas aeruginosa isolated from various clinical samples(12 from urin , 9 from ear , 6 from wound , 7 from burn) collected from Baquba General Hospital over a period of 4 months (September 2010 to December 2010) were activated by brain heart infusion medium at37 C⁰, 24 hour and 120r.p.m.

Antimicrobial susceptibility test and determination of MIC
Sixteen antibiotics including, Beta lactam group, Quinolones group and aminoglycoside group were used to testing sensitivity of Pseudomonas aeruginosa . The minimum inhibitory concentration (MIC) was determined for each bacterial isolate by an agar dilution technique on Mueller – Hinton agar plates, the antimicrobial agents were obtained from standard laboratory powders and were used immediately after their solubilization, the agents were Ampicillin, Amoxicillin, Cephalexin, Carbencillin, Cefotaxime, Ceftriaxone, Ceftazidime, Pipercillin. Results of susceptibility testing were recorded according to the guidelines of the National Committee for Clinical Laboratory standards [11] after incubation at 37ºC for 18h . The MIC was determined by using beta-lactamase inhibitors including (Clavulanic acid, Sulbactam, Tazobactam ).

Plasmid profile (Plasmid DNA analysis)
Plasmid DNA of the four isolates ( PU5 (urin), PE20 (ear), PW27 (wound), and PB32 (burn) ) are extracted using the Pure Yield™ Plasmid Miniprep Kit (Promega U.S.A ). Plasmid DNA was analyzed by electrophoresis on 0.7% agarose gel containing 0.5µg of ethidium bromide per ml (12).

Curing of plasmid DNA
Curing was conducted by using different concentrations of Acridin orange (16 , 32, 64 , 128 , 256 , 512 , 1024 , 2000 , 2500 , 3000) µg/ml (12,13).

Statistical analysis
Statistical analysis was carried out using t – test.

Results and Discussion
Determination MIC and antimicrobial susceptibility test of Pseudomonas aeruginosa
The sensitivity of these isolation were tested against [16] antibiotics. The results showed that high resistance of Amoxicillin, Ampicillin, CO-Trimoxazole and Nitrofourantoin with 100%. This result agrees with local studies by Al-Saffar [14] and Abuduah et al. [15], who showed that resistance rates in Pseudomonas aeruginosa as 100%, fig.(1). The resistance of Carbencillin was 93% , while Pseudomonas aeruginosa resists Cefotaxime, Ceftriaxone and Ceftazidium with 88%,85%,and 72% respectively. The results showed that
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**Pseudomonas aeruginosa** resists piperacillin with 73%, while resistance of aminoglycoside group including gentamicine, amikacin and tobramycin was 60%, 45% and 28% respectively. The isolates resists Norfloxacin, Ciprofloxacin and Ofloxacin with 49%, 21%, 3% respectively. This resistance of different antibiotic due to the presence of multiple drug-resistant strains [16]. Antibiotic resistance has probably developed by the transfer of R plasmids from other drug-resistant enteric Gram-negative bacteria [17]; or because of its propensity to develop resistance during therapy [18].

The minimum inhibitory concentration (MIC) was determined for eleven antibiotics. The result showed that high resistance with 1024µg/ml Ampicillin, Amoxicillin, Cephalexin and Carbencillin table (1), this result was agreed with [19], who found the resistance was 512-1024 µg/ml against these four antibiotics by

![Percentage of antibiotics resistance.](image)

**Figure (1):** Percentage of antibiotics resistance.

**Table (1):** The minimum inhibitory concentration (MIC) of some antibiotics using against *P. aeruginosa*.

<table>
<thead>
<tr>
<th>Antibiotic</th>
<th>Break point</th>
<th>M.I.C (µg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ampicillin</td>
<td>≥ 32</td>
<td>512 – 1024</td>
</tr>
<tr>
<td>Amoxicillin</td>
<td>≥ 32</td>
<td>512 – 1024</td>
</tr>
<tr>
<td>Cephalexin</td>
<td>≥ 32</td>
<td>128 – 1024</td>
</tr>
<tr>
<td>Carbencillin</td>
<td>≥ 128</td>
<td>64 – 1024</td>
</tr>
<tr>
<td>Cefotaxime</td>
<td>≥ 32</td>
<td>16 – 1024</td>
</tr>
<tr>
<td>Ceftriaxone</td>
<td>≥ 32</td>
<td>16 – 1024</td>
</tr>
<tr>
<td>Ceftazidime</td>
<td>≥ 32</td>
<td>8 – 512</td>
</tr>
<tr>
<td>Piperacillin</td>
<td>≥ 128</td>
<td>32 – 512</td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td>≥ 4</td>
<td>1 – 64</td>
</tr>
<tr>
<td>Gentamicin</td>
<td>≥ 8</td>
<td>2 - 1024</td>
</tr>
</tbody>
</table>
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The lower value of resistance was toward Ciprofloxacin with 1-64 μg/ml. The results was agreed with local studies by (15), who showed that MIC value by P.aeruginosa was 1-16 μg/ml.

The minimum inhibitory concentration MIC was determined by using β-lactamase inhibitors including (Clavulanic acid, Sulbactam, Tazobactam). In this study antibiotic mixed with clavulanic acid at percentage 1:4 and use of three commercially available β-lactam / β-lactamase inhibitor combinations: piperacillin/tazobactam (Tazocin), ampicillin/sulbactam (Sulba) and amoxicillin/clavulanic acid (Augmentin). The values of (M.I.C) for β-Lactam antibiotics (Amoxacillin, Carbencillin, Cephalexin, Cefotaxime, Ceftriaxone, Ceftazidime, Pipracillin) were decreased at the presence of β-Lactamase inhibitors. Results showed that (100%) of Pseudomonas aeruginosa isolates were sensitive to Ampicillin – Sulbactam and Amoxicillin / Clavulanic acid with (0%, 26.47) respectively table (2) Fig (2), while these isolates showed sensitivity against (Carbenicillin / Clavulanic acid, Cephalexin / Clavulanic acid, Cefotaxim / Clavulanic acid and Ceftriaxone/ Clavulanic acid with (41.17,32.35,73.52,79.41)% respectively table (2) fig (3,4,5,6). The results indicate that isolates were sensitive toward Pipracillin / Clavulanic acid, Pipracillin – Tazobactam, and Ceftazidime – Clavulanic acid with (85.29%, 91.17%) and (100%) respectively table (3) fig (7,8,9). The results were agreed with (20;21;22), who found that use of these combination lead to increase sensitive of Pseudomonas aeruginosa. These results indicate that combination have synergistic effect. This effect explain by fact that inhibitors beta lactamse enzymes is weak antibiotics and contains a ring-like-lactam antibiotics makes beta- lactamase enzymes attack this ring and leave antibiotic free [23].

Table (2): The percentage of β-lactam / β-lactamase inhibitor combinations against Pseudomonas aeruginosa.

<table>
<thead>
<tr>
<th>Antibiotic</th>
<th>Inhibitor</th>
<th>Percentage of sensitive isolates (%)</th>
<th>Percentage of resistance isolates (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ampicillin</td>
<td>0</td>
<td>100</td>
<td></td>
</tr>
<tr>
<td>Ampicillin / Sulbactam</td>
<td>0</td>
<td>100</td>
<td></td>
</tr>
<tr>
<td>Amoxicillin</td>
<td>0</td>
<td>100</td>
<td></td>
</tr>
<tr>
<td>Amoxicillin / Clavulanic acid</td>
<td>26.47</td>
<td>73.52</td>
<td></td>
</tr>
<tr>
<td>Carbenicillin</td>
<td>5.88</td>
<td>94.11</td>
<td></td>
</tr>
<tr>
<td>Carbenicillin / Clavulanic acid</td>
<td>41.17</td>
<td>58.82</td>
<td></td>
</tr>
<tr>
<td>Cephalexin</td>
<td>0</td>
<td>100</td>
<td></td>
</tr>
<tr>
<td>Cephalexin / Clavulanic acid</td>
<td>32.35</td>
<td>67.64</td>
<td></td>
</tr>
<tr>
<td>Cefotaxim</td>
<td>17.64</td>
<td>82.35</td>
<td></td>
</tr>
<tr>
<td>Cefotaxim / Clavulanic acid</td>
<td>73.52</td>
<td>26.47</td>
<td></td>
</tr>
<tr>
<td>Ceftriaxone</td>
<td>23.52</td>
<td>76.47</td>
<td></td>
</tr>
<tr>
<td>Ceftriaxone/ Clavulanic acid</td>
<td>79.41</td>
<td>20.58</td>
<td></td>
</tr>
<tr>
<td>Ceftazidime</td>
<td>41.17</td>
<td>58.82</td>
<td></td>
</tr>
<tr>
<td>Ceftazidime/ Clavulanic acid</td>
<td>100</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Pipracillin</td>
<td>35.29</td>
<td>64.70</td>
<td></td>
</tr>
<tr>
<td>Pipracillin / Clavulanic acid</td>
<td>85.29</td>
<td>14.70</td>
<td></td>
</tr>
</tbody>
</table>
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| Pipracillin / Tozabactam | 91.17 | 8.82 |

**Figure (2):** Synergism effect of Amoxicillin / Clavulanic acid against *Pseudomonas aeruginosa* isolates (* *P<0.05,0.01*).

**Figure (3):** Synergism effect of Cephalexin / Clavulanic acid against *Pseudomonas aeruginosa* isolates(* *P<0.05,0.01*).
Figure (4): Synergism effect of Carbencillin / Clavulanic acid against Pseudomonas aeruginosa isolates (* * P<0.05,0.01 )

Figure (5): Synergism effect of Cefotaxime / Clavulanic acid against Pseudomonas aeruginosa isolates(* * P<0.05,0.01 ).
Figure (6): Synergism effect of Ceftriaxone / Clavulanic acid against *Pseudomonas aeruginosa* isolates(\*\* \(P < 0.05,0.01\)).

Figure (7): Synergism effect of Ceftazidime / Clavulanic acid against *Pseudomonas aeruginosa* isolates(\*\* \(P < 0.05,0.01\)).
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Figure (8): Synergism effect of Piperacillin / Clavulanic acid against *Pseudomonas aeruginosa* isolates(** P<0.05,0.01**).

Figure (9): Synergism effect of Piperacillin / Tazobactam against *Pseudomonas aeruginosa* isolates(** P<0.05,0.01**).

**Pseudomonas aeruginosa plasmid profile**

The plasmid –DNA content for four isolates was detected, findings showed that isolates have one ( large) plasmid band table (4) fig (10). This result was agreed with (24), who showed that *Pseudomonas aeruginosa* contain one mega plasmid.
Table (4): Plasmid content of *Pseudomonas aeruginosa* isolated from different clinical sources.

<table>
<thead>
<tr>
<th>Number of isolate</th>
<th>Site of infection</th>
<th>Number of Plasmid band</th>
</tr>
</thead>
<tbody>
<tr>
<td>PU5</td>
<td>Urin</td>
<td>1</td>
</tr>
<tr>
<td>PE20</td>
<td>Otities media</td>
<td>1</td>
</tr>
<tr>
<td>PW27</td>
<td>Wound</td>
<td>1</td>
</tr>
<tr>
<td>PB32</td>
<td>Burn</td>
<td>1</td>
</tr>
</tbody>
</table>

Figure (10): Agarose gel electrophoresis of plasmids from *Pseudomonas aeruginosa*.

1. Plasmid content of PU5 isolate
2. Plasmid content of PE20 isolate
3. Plasmid content of PW27 isolate
4. Plasmid content of PB32 isolate

**Plasmids curing**

Acridin orange was used in order to cure plasmids of *Pseudomonas aeruginosa*. The result showed the best concentration was 512 μg/ml, which able to cure plasmids from all isolates. The results was agreed (partially) with (21), who found the best concentration was 1024 μg (11).

Figure (11): Losing of plasmid band from curing *Pseudomonas aeruginosa* isolates.
Conclusions
The study shows that the combination of β-lactams / β-lactamase inhibitors is highly effective in treatment of *Pseudomonas aeruginosa* infections. Ceftazidime/Clavulanic acid has the best activity against nosocomial *Pseudomonas aeruginosa* followed by Piperacillin/Tazobactam.

References
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