Detection of Virulence Factors and Antibiotics Susceptibility of *Pseudomonas aeruginosa* Isolated from Burns and Wounds Infections in Ba'quba City.

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**Abstract:** This study was conducted for the period from 1/11/2015 to 15/6/2016 in Baquba city in Iraq. 74 samples were collected from burns with ten isolate (32.2%), wounds infections with six isolate (13.9%) from Baquba General Hospital. The results showed that 15 isolate (93.75%) were able production Lecithenase. Furthermore, 13 (81.25%) isolate were able production Haemolysin, and 14 (87.5%) were able production proteinase. The sensitivity of these isolates were tested against (12) antibiotics. The results showed that the highest resistances were for Amoxicillin, Ampicillin, Cefotaxime, Trimethoprime with 100%, while the lowest resistance was for Ciprofloxacin 25%, while it showed high sensitivity toward combination Emipenem with 100%. As well as, 11 isolate (68.75%) were able to production β-lactamase. Furthermore, six isolate (37.5%) were able to production metalloβ-Lactamases. Finally, four isolate (25%) were able to production Extended Spectrumβ-Lactamases enzyme.

**Conclusion:** *P. aeruginosa* isolates highest resistances were Amoxicillin, Ampicillin, Cefotaxime, Trimethoprime with 100%, While the lowest resistance were for Ciprofloxacin 25%, while it showed high sensitivity toward combination Emipenem with 100%. Additionally, some of isolates production, β-lactamase, metallo β-Lactamases and Extended Spectrumβ-Lactamases enzyme.

**Keywords:** *Pseudomonas aeruginosa*, β-lactamases, metallo β-Lactamases, Extended Spectrumβ-Lactamases enzyme, Antibiotics.

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I. Introduction

*Pseudomonas aeruginosa* is a Gram-negative rod bacterium, which has a remarkable ability to adapt and thrive in a variety of environments: water (1), burn, Wounds, endocarditis and otitis externa (2). *P. aeruginosa* has been recognized as a frequent inhabitant of chronic non-healing wounds (3), and is one of the foremost opportunistic bacteria isolated from wounds which cause high morbidity and mortality despite antimicrobial therapy (4). *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. *P. aeruginosa* produces a number of virulence factors which after colonization can cause extensive tissue damage, bloodstream invasion, and dissemination (5).

Antibiotic resistance in *P. aeruginosa* may be mediated via several distinct mechanisms including modification of site-targeted drugs or outer membranes, β-lactamase production, and efflux pumps. The increase in antibiotic resistance is mostly due to extensive abuse of antibiotics such as ciprofloxacin, β-lactamase and aminoglycosides in the burn centers as well as non-availability and high costs of other effective drugs (6).

Pseudomonas aeruginosa producing metallo-β-lactamase (MBL) was first reported from Japan in 1991 and since then has been described from various parts of the world including Asia, Europe, Australia, South America, and North America (7). Metallo-β-lactamases belong to Ambler class B and have the ability to hydrolyze a wide variety of β-lactam agents, such as penicillins, cephalosporins (8)

Phenotyping methods are based on the ability of metal chelators, such as EDTA and thiol-based compounds, to inhibit the activity of MBL (9). Double Disk Synergy Test (DDST) and Combined Disc Test (CDT) are most commonly used to detect the MBL producing *P. aeruginosa* then Hodge test. Modified Hodge test detects only carbapenemase activity, which prevents the use of EDTA and therefore, does not confirm the metal dependence of the carbapenemase (10). Modified Hodge test, DDST and the CDT are easy, reliable, simple to perform and cheaper (11).

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 become amajor
problem all over the world. Resistance has developed in bacteria by possessing extended spectrum beta-lactamase (ESBLs) capable of hydrolyzing these newer cephalosporins. 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 sparring the beta-lactam antibiotic. 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. 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.

This study was aimed to isolate bacterial isolates from burn and wound infections, and test their antibiotic susceptibility pattern against available antibiotics and ability of production beta-lactamases, metallo-beta-lactamases, Extended Spectrum beta-lactamases enzyme in Baquba Hospitals.

### II. Materials And Methods

#### Samples collection:
74 clinical samples were collected from patients and carriers in Baquba General Hospital over period from 1/1/2015 to 15/5/2016. The samples.

<table>
<thead>
<tr>
<th>Sample type</th>
<th>Sample No.</th>
<th>Isolate No. P. aeruginosa</th>
<th>Percentage %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Burns sample</td>
<td>31</td>
<td>10</td>
<td>32.2%</td>
</tr>
<tr>
<td>Wounds sample</td>
<td>43</td>
<td>6</td>
<td>13.9%</td>
</tr>
<tr>
<td>Average</td>
<td>74</td>
<td>16</td>
<td>21.6%</td>
</tr>
</tbody>
</table>

#### Isolation and Identification of P. aeruginosa:
Samples were cultivated on MacConkey’s agar, blood agar and Nutrient agar to determine the characteristic features and morphology of the colonies. Gram's stain was done for each sample followed by biochemical tests according to the method described by. The isolates were kept on nutrient agar slants in screw-capped test tubes for not more longer than 20 days, the isolates were grown in nutrient broth containing 15% glycerol and stored in deep freeze for longer time maintenance. The isolates were identified according to the Bergey’s Manual. As the following: gram stain and biochemical tests Standard (Oxidase test, Catalase test, Triple sugar iron agar test (TSI),Indol, Methyl red, Citrate utilization and Diagnostic kits: API 20E system used for confirmation of suspected some isolates of P. aeruginosa were used for detecting P. aeruginosa strains.

#### Detect of Virulance Factors of Pseudomonas aeruginosa:
The ability P. aeruginosa to produce some of virulence factors (enzymes and toxins) were recognized and tested were applied on 16 isolates that identified such as:
1. Gelatin liquefaction test
2. Hemolysin production
3. Capsule production
4. Binding to Congo red
5. Protease production
6. DNase production
7. Urease activity
8. Lecithinase production
9. Lipase production (Tween 80 hydrolysis test)

#### Antimicrobial susceptibility test:
The sensitivity and resistance of P. aeruginosa to antimicrobials was tested by the disc diffusion method on Mueller-Hinton agar using antibiotic discs according to Clinical and Laboratory Standards Institute (CLSI) guidelines. Twelve antibiotics were tested: Ampicillin (25Mg), Penicillin (25Mg), Cefotaxime (30Mg), Erythromycin (15Mg), Ceftazidime (30Mg), Amoxicillin/Clavulanic acid (30Mg), Gentamicin (10Mg), Ciprofloxacin (5Mg), Pipercillin (30Mg), Amikacin (30Mg), Trimethoprim (10Mg) and Imipenem (10Mg). Interpretation of inhibition zones was carried out based on the manufactures and CLSI guidelines. Then the plates are incubated overnight at 37°C, and the zone of inhibition of bacterial growth is used as a measure of susceptibility, where large zones of inhibition indicate that the organism is susceptible, while small or no zone of inhibition indicate resistance. An interpretation of intermediate is given for zones which fall between the accepted cutoffs for the other interpretations.

#### Detection of beta-lactamase, Metallo-beta-lactamase (MBL) and extended spectrum beta-lactamase (ESBL) Production.
The iodometric method for detection of beta-lactamase was described by (WHO) 1978. Detection of Metallo-beta-lactamase by using Imipenem EDTA combined disk test, using two Imipenem disk (10µg) with 3cm between them, and then the (10µg) EDTA solution to one of the drives of Imipenem then incubated at a
temperature 37°C for 18-24 hour, after observing areas of inhibition zone, increase of inhibition zone above 7mm on the disk Imipenem with EDTA compared with the Imipenem disk alone, the result is positive. Disk Approximation method was performed for detection of ESβLs for all isolates which were positive to β-lactamase production (26). 

III. Results And Discussion:

Isolation and Identification:

Isolates that grow on pseudomonas ceterimide agar, that suspected to be P. aeruginosa were further identified according to their morphological, characteristics and biochemical tests. To do this, each colony that grew on pseudomonas agar was reincubated on nutrient agar. Certain morphological colonial appearance e.g. mucoidal growth, smooth shaped with flat edges and elevated center, whitish or creamy color, fruity odor and pyocyanin production were undertaken as a pseudomonas indication (29). Microscopical examination of each isolated has shown that they were all having single cells, non spore forming, Gram-negative an rod-shaped bacteria (30).

Biochemical tests were done to identify P. aeruginosa. These results are all of the isolates gave a positive result for catalase, oxidase, gelatinase, urease and pyocyanin pigment production. Those isolates have utilized glucose and citrate as carbene sources. β- hemolysis produced on blood agar, the isolates hemolyze human red blood cells (RBC_3) which indicates that they are of phospholipase C (which produced by P. aeruginosa). These biochemical results are in accord with (31,32, 33), who have dealt with the study isolation and diagnosis of P. aeruginosa from clinical infections of bacterial contaminated.

Except for methyl red test which showed variable results, Tryptophane (Indole), VP tests and H_2S production results were all gave negative results, non-lactose fermentation on macConky's agar. On TSI agar test, 100% gave an alkaline (slant) and red (no change) bottom, due to inability to ferment lactose and sucrose. In addition, ability of this bacterium to grow at 42°C and 4°C on pseudomonas agar containing ceterimide. These biochemical results were further confirmed by diagnostic key mentioned by Atlas (34), and also by applying API 20E system. However, the present work results were agreed with those observed by (Al- Hilli ; Al- Shaibani, and Al- Gherawi). (35,36,37)

Antibiotic sensitivity tests:

In the present study, Standard disc diffusion test has used for detection of susceptibility of pathogenic Pseudomonas to antibiotics. Ten different antibiotic discs belong to various groups were applied. These included β-lactams, aminoglycosides, cephalosporins, penicillins. Decision whether the isolate is resistant or sensitive to antibiotic was based on the observation and computing the diameter of inhibition zone and then compared them with that of standard value which stated by NCCLS. (38) However, the results of this work indicated that the isolates of P. aeruginosa have demonstrated a higher resistance to Penicillin, Amoxicillin, Erythromycin, Amoxicillin/Clavulanic acid, Trimethoprim that constituted a percentages of 100% this results are agreed with Abdullah et.al.. (39) The development of antibiotic resistance is considered a major therapeutic problem that can be explained by some hypothesis such as, the influence of excessive and/or inappropriate antibiotic used (40). P. aeruginosa and other Pseudomonads are resistant to many antimicrobial agents and therefore become dominant and important when more susceptible bacteria of the normal flora are suppressed (30). The results of this work also showed that Cefotaxime, Ceftazidine was effective on P. aeruginosa isolates with the percentage of resistance 87.5% and 75%. This result is nearly, in accordance with Qasim (41), percentage of resistance (92%), but this result not agreed with Al-Gherawi (66.7%). (37)

Detection of β-lactamase, Metallo-β-lactamase and ESβLs Production.

Detection of β -Lactamase Enzymes Production The production of β-lactamase enzymes by isolates were determined by rapid iodometric method, the isolates 11 (68.7%) were β-lactamase producer Table.(2). This result was in accordance with the study of Al-Mashhadani (42), in which all P. aeruginosa isolates recovered from different samples were (100%) β-lactamase producers (42). It was found that the major mechanism of resistance in gram negative bacteria causing clinically significant infection is the expression of β-Lactamases, of which there are several classes including plasmid encoded and chromosomally encoded enzymes (43). In Present study, MBL productions was six isolate (37.5%) in isolates of Pseudomonas which lower than values reported in other studies in Iran (44), Malaysia (45) and Pakistan (46). The multidrug resistant phenotype in P. aeruginosa could be mediated by several mechanisms including multidrug efflux systems, enzyme production, outer membrane protein (porin) loss and target mutations. Inappropriate empirical therapy has been associated with increased mortality in P. aeruginosa infections; Our study reported 25% ESBL production among P. aeruginosa isolates. The studies conducted by others depicted low rates, 3.7% by Woodford et al. (47), 4.2% by Lim et al. respectively,(48), of ESBL production in P. aeruginosa. The results of various studies showed high rate 20.3% by Aggarwal et al. (49), 39.41% by Mirsalehian et al. (44) and 35.85% by Ullah et al. (46) of ESBLs in the samples of P. aeruginosa isolates examined.
Table (2): The percentages of P aeruginosa isolates producing β-lactamase.

<table>
<thead>
<tr>
<th>β-lactamase production</th>
<th>Number of isolates</th>
<th>Ratio %</th>
</tr>
</thead>
<tbody>
<tr>
<td>β-lactamase</td>
<td>11</td>
<td>68.75%</td>
</tr>
<tr>
<td>Metalloβ-lactamase</td>
<td>6</td>
<td>37.5%</td>
</tr>
<tr>
<td>ESBLs</td>
<td>4</td>
<td>25%</td>
</tr>
</tbody>
</table>

References


Detection of Virulence Factors and Antibiotics Susceptibility of Pseudomonas aeruginosa Isolated


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