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# Molecular detection of *exoS* and *exoU* genes of *Pseudomonas aeruginosa* and its relation with antibiotic resistance.

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## Abstract

**Background.** *Pseudomonas aeruginosa* is one of the most clinically important bacteria. It can cause serious infections because it has many virulence factors, which are the main reasons for the emergence of antibiotic resistance. The most important one is the type III secretion system (T3SS), which includes exoS and exoU toxins which play a main role in bacterial invasion. **Aim.** The aim of this study was to identify the *exoS* and *exoU* genes that encoded for exoS and exoU toxins found in *P. aeruginosa* isolates and express their relationship with antibiotic resistance. **Methods.** The study included forty strains of *P.aeruginosa* isolated from different clinical samples of burns,wounds, and sputum from patients in Baghdad hospitals. The isolates were identified by microbiological methods and the VITEK-2 system. *ExoS* gene, which have size (230 bp) and *exoU* gene which have a size (1572 bp) were determined in forty *P. aeruginosa* by conventional PCR technique. **Results.** the result showed prevalence of *exoS* gene were (70.5%), (100%), and (90%) in isolates from burns, wounds, and sputum, respectively. Meanwhile, the presence of the *exoU* gene was determined in (29.4%), (30.7%), and (10%) from burns, wounds, and sputum, respectively. The antibacterial susceptibility test for each isolate was performed toward (14) antibiotics; the result revealed the resistance were (100%) to Amoxicillin, Piperacillin, and Cefixime; (97.5%) Cefazolin; (97%) Amoxicillin-Clavulanic acid; (92.5%)Tigeocyclin;(72.5%) Imipenem;(67.5%) Levofloxacin; (65%) Ceftazidime; (62%) Pepracillin\Tazobactam; (60%) Ciprofloxacin; (57.5%) Cefepime; (52.5%) Gentamicin and (40%) Amikacin. **Conclusion.** this study investigated that clinical isolates with high antibiotic resistance contained genes for toxin production (exoS and exoU), which means the greatest potential virulence factors of *P.aeruginosa* increase with the presence of *exoS* and *exoU* genes.

**Keywords:** *pseudomonas aeruginosa*, antibacterial susceptibility, ExoU,ExoS ,VITEK, PCR
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## Introduction

*Pseudomonas aeruginosa* is an opportunistic pathogen that causes a wide range of infections such as endocarditis, septicemia, brain membrane inflammation (meningitis), wounds, burns, otitis media, keratitis, and respiratory infections such as pneumonia, especially in patients with cystic fibrosis (1). These infections are very difficult to eradicate due to the presence of numerous virulence factors such as pili, flagella, elastase, proteases, biofilm formation, iron chelators, lipases, exopolysaccharides, antibiotic resistance, and a variety of many toxins, including *exoA* and T3SS, in addition to chemical compounds and pyocyanin (2).

The Type three secretion system is important for bacterial invasion, tissue lysis, and unfavorable clinical outcomes (3). This virulence factor plays an essential

role in the pathogenesis of *P. aeruginosa*, T3SS is unique in that it through a needle-shaped complex. The effector proteins which secreted are injected directly into cytosol of the host secretes and transports four different exotoxins (*ExoT, ExoY,ExoS, and ExoU*) from bacteria to their host cells cells to begin their damaging effects (4). Some lines of studies were recommended that *ExoS* may play a distinctive role in the pathogenicity of *P. aeruginosa.* The involvement of exotoxins was to be unselective in the selection of substrate proteins; however, it has now been discovered that it selectively ADP-ribosylates many low molecular- weight GTP- binding proteins. In addition to its enhanced resemblance to cholera toxin, *exoS* requires a protein from eukaryotic cells for enzymatic action; they also needed a protein from eukaryotic cells for enzymatic activity (5). *ExoU* toxin is cytolytic to various mammalian cell types, including neutrophils, macrophages, epithelial cells, and fibroblasts. In animal models of a cute pneumonia, disruption of the *exoU* gene caused decreased virulence, while transformation with an *exoU*-expressing plasmid amplified the virulence of strains that did not naturally secrete *exoU* (6). The object of the present study was to characterize the presence of the *exo*S and *exo*U genes in clinically isolated *P.aeruginos*a strains in Baghdad hospitals and detect their relation with the antibiotic resistance of these isolates.

## Materials and Methods Specimen Collection

One hundred clinical specimens were collected from burn swabs, wound swabs and sputum from different Baghdad hospitals during the period from October 2023 to March 2024.

### Isolation and identification of *P. aeruginosa*

All the specimens were cultured on MacConkey agar medium and incubated aerobically at 37ºC for 24 hours. For the primary isolation, the suspected non-lactose fermented colonies grown on MacConkey agar medium were cultured on the cetrimide agar medium at 37ºC for 24 to 48 hours. All suspected isolates obtained on cetrimide agar medium were cultured on brain heart infusion agar, and the isolates were further characterized by performing gram staining, and some conventional biochemical tests, like oxidase, catalase production, methyl red test, MR-VP test, Indole test, citrate test and Urease test (5). To confirm the diagnosis of isolates, VITEK-2 system was used.

### Antibacterial susceptibility test

Antibacterial susceptibility testing was accomplished on the VITEK 2 automated system for the following antimicrobials: Amoxicillin, , Piperacillin, piperacillin/ Tazobactam, Ceftazidime, Tigecycline, Cefepime, Amikacin, Imipenem, Gentamicin, , Ciprofloxacin, Levofloxacin, Amoxicillin/Clavulanic acid, Cefazolin and Cefixime

### Isolation of DNA

Each isolate was grown on nutrient agar and incubated at 37 °C for 18-24 hours. Colonies were suspended in 10 mL of nutrient broth and cultured overnight at 37°C with shaking (200 rpm). The cells were then harvested at a concentration of 13000 rpm. The HiPurA Bacterial Genomic DNA (M/s Himedia, Mumbai /catalog no. MB505) was isolated from the pure culture of clinical isolates of

*Pseudomonas aeruginosa*. Chromosomal DNA was harvested in accordance with the manufacturer's instructions, and the extracted DNA was tested for concentration

and purity before being stored at -20 °C. DNA bands were detected using 1% agarose gel electrophoresis (7). Detection of *exoS and exoU* genes in *Pseudomonas aeruginosa* Conventional PCR assay was done to identify T3SS virulence genes (*exoS* and *exoU*) by using definite primers for the genes were amplified with the extracted bacterial DNA as the template. Amplification was achieved in an Eppendorf Mastercycler in a 25-µl reaction using the thermal program and specific

primer details described in Table1.The PCR reaction mixture was done as follows: 5µl of template DNA, 12.5µl Taq Green PCR Master Mix, 1µl from each primer, and 5.5µl of nuclease free water (6). Augmented PCR products were separated by electrophoresis on a (1.5%) agarose gel stained with ethidium bromide at (100V) for (45) minutes. A (100bp) DNA ladder was used as a size marker.The gel images were taken under ultraviolet light using a system (BIO-RAD, USA). The expected bands were determined and documented (8). The primers of the genes summarized in table (1), and the thermal program used for the amplification of genes summarized in table (2).

**TABLE (1): The sequences of the used primers and the size of PCR products (pb).**

| Target gene | | Primer sequence (5'-3') | (pb) | References |
|---|---|---|---|---|
| *ExoS* | Forward | ATGTCAGCGGATACGAC | 230 bp | (9) |
| | Reverse | CAGGCGTACATCGTTCCT | | |
| *ExoU* | Forward | AGCGTTAGTGACGGCG | 1572 bp | (10) |
| | Reverse | GCGCATGGCATCGAGTAACTG | | |

**Table (2): thermal program used for the amplification of gene**

| Genes | Reaction conditions | | | References |
|---|---|---|---|---|
| | PCR Steps | Temp.&time | cycles | |
| *ExoS* | Initial denaturation | 94°C-10 min | 1 | (9) |
| | Denaturation<br>Annealing<br>Extension | 94°C-30min<br>58°C-45min<br>72°C-45min | 25 | |
| | Final Extension | 72°C-10 min | 1 | |
| *ExoU* | Initial denaturation | 94°C-3 min | 1 | (10) |
| | Denaturation<br>Annealing<br>Extension | 94°C-30 sec<br>55°C-30 sec<br>72°C-2 min | 40 | |
| | Final Extension | 72°C-10 min | 1 | |

## Detection of exotoxin S and U

Three isolates of *P. aeruginosa* containing *exoS* and *exoU* genes were cultured overnight in LB. Bacteria were ments at 600 nm. Bacteria were sedimented by centrifugation at 3,220 g for 15 minutes at 4 degrees Celsius. The culture subcultured 1:1,000 in LB with 5mM EGTA and cultivated for 24 hours at 37°C with aeration.Bacterial densities were estimated using optical density measure-

supernatant was collected, and proteins were precipitated with trichloroacetic acid and washed with acetone (11). Proteins

were resuspended at culture density and separated using sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDSPAGE)(12).

**Statistical Analysis**

The Statistical Packages of Social Sciences-SPSS (13) program was used to detect the effect of different factors in study parameters. AChi-square test was used to significantly compare between percentages (0.05 and 0.01 probability) in this study.

**Results**

**Isolation and identification of *Pseudomonas aeruginosa***

On MacConkey agar medium, *P. aeruginosa* colonies appeared round, small, convex, rough colony with irregular edges, whitish, or creamy in color (lactose non-fermenting), and had a fruity odor (14). The *P. aeruginosa* isolates were identified successfully on cetrimide agar medium; this medium is a selective medium for the identification of *P. aeruginosa* (15). For the representation of *P. aeruginosa*, diverse biochemical tests were done.The bacterial isolates were positive for catalase, oxidase, and citrate tests, while the negative results were for the indole test, methyl red test , MR-VP test, and urease test. Gram stain showed single, rod cells, and gram-negative. Then the suspected isolates were accomplished by using the VITEK-2 system. Forty isolates were classified as *P. aeruginosa* with a percentage of burn swab showed the highest prevalence among isolates 17(42.5%) followed by wound swab isolates 13(32.5%) and lowest prevalence for sputum isolates 10(25%) based on VITEK-2 Compact system.

**Antibacterial susceptibility test**

Ciprofloxacin, Levofloxacin, piperacillin/ Tazobactam, and Cefepim 7(53.8%) for each, followed by Gentamicin 5(53.8%), the lowest resistance for the

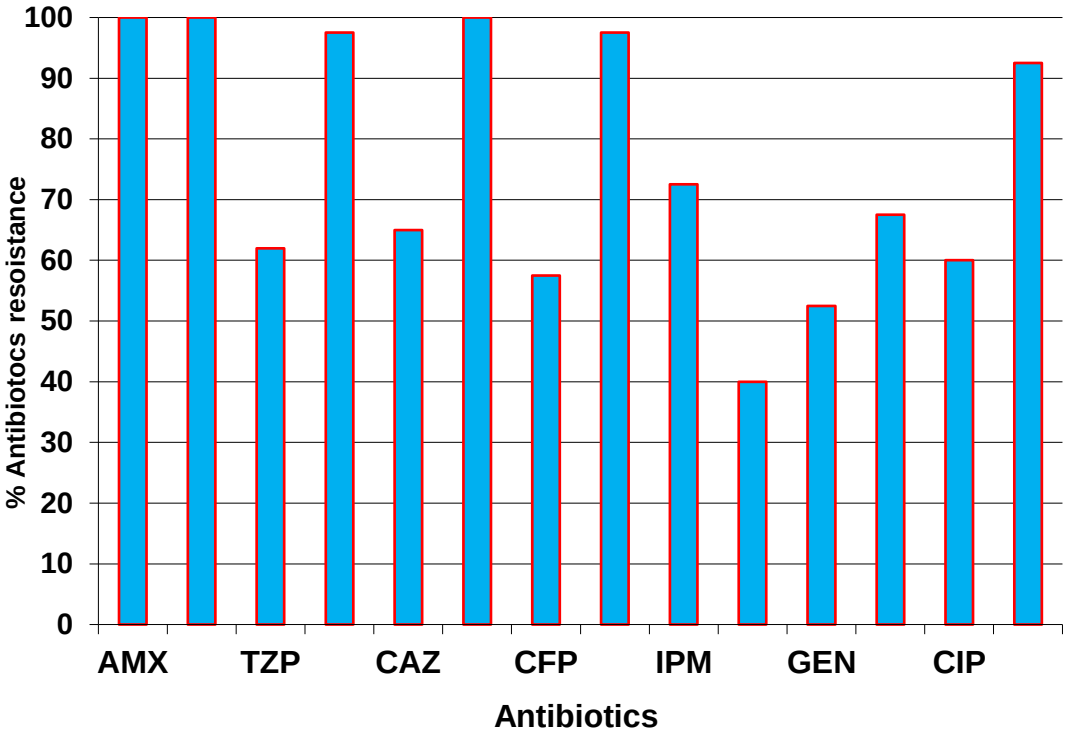
Bacteria have a variety of antibiotic resistance mechanisms, including efflux systems, decreased permeability, the creation of antibiotic-inactivating enzymes, and target alterations. *P. aeruginosa* exhibits most of the these known resistance mechanisms via both intrinsic chromosomally encoded and genetically imported resistance determinants that influence the major antibiotic classes (16). (16) In this study, antibacterial susceptibility testing was performed on the VITEK 2 automated system for the following antibiotics: Amoxicillin, piperacillin, piperacillin/ Tazobactam, Ceftazidime, Tigeocyclin, Cefepime, Amikacin, Imipenem, Gentamicin, Ciprofloxacin, Levofloxacin, Amoxicillin /Clavulanic acid, Cefazolin and Cefxime. Antibiotic susceptibility test results demonstrated that *P. aeruginosa* isolates from burn swab revealed highest resistance against Amoxicillin, Amoxicillin /Clavulanic acid, piperacillin, Cefazolin and Cefixim 17(100%) for each of them, followed by piperacillin/Tazobactam, Levofloxacin, Ceftazidime and Tigeocyclin 14 (82.3%) for each, followed by Cefepime 13(76.4), followed by Ciprofloxacin, Gentamicin, and Imipenem 12(70.5%) for each. Finally, the lowest antibiotic resistance was Amikacin 9(52.9%). While *P. aeruginosa* isolates from wound swabs showed the highest resistance against Amoxicillin, Piperacillin, Cefazolin, Tigeocyclin and Cefixim 13(100%) for each, followed by Amoxicillin /Clavulanic acid 12(92.3%), followed by Imipenem 10 (76.9%), then Ceftazidim 9(69.2%), followed by

antibiotic was Amikacin 3(23%). Finally, *P.aeruginosa* from sputum showed highest resistance against Amoxicillin, Amoxicillin- Clavulanic acid, Piperacillin,

Cefixime and Tigeocyclin 10(100%) for each, followed by Cefazolin 9(90%), then Levofloxacin 6(60%), followed by Imipenem and Ciprofloxacin 5(50%) for each , then and there Piperacillin/Tazobactam, Amikacin and Gentamicin 4(40%) for each ,the lowest antibiotic resistance were 3(30%) for Cefepime and Ceftazidime. The results of antibiotic resistance summarized in table (3) and figure(1).

**Table (3): Percentages of antibiotic resistance rate of *P. aeruginosa* isolates against antimicrobial agents according to the sources.**

| Antimicrobial agent | Burnswab isolates Total number=(17) | | Wound swab isolates Total number=(13) | | Sputum isolates Total number=(10) | | Total (40) | |
|---|---|---|---|---|---|---|---|---|
| β-lactam agents | No. | % | No. | % | No. | % | No. | % |
| Amoxicillin (AMX) | 17 | (100) | 13 | (100) | 10 | (100) | 40 | (100) |
| Piperacillin (PRL) | 17 | (100) | 13 | (100) | 10 | (100) | 40 | (100) |
| Piperacillin/tazobactam (TZP) | 14 | (82.3) | 7 | (53.8) | 4 | (40) | 25 | (62) |
| Amoxicillin-Clavulanic acid (AMC) | 17 | (100) | 12 | (92.3) | 10 | (100) | 39 | (97.5) |
| Cephalosporins | | | | | | | | |
| Ceftazidime (CAZ) | 14 | (82.3) | 9 | (69.2) | 3 | (30) | 26 | (65) |
| Cefexime (CFX) | 17 | (100) | 13 | (100) | 10 | (100) | 40 | (100) |
| Cefepime (CFP) | 13 | (76.4) | 7 | (53.8) | 3 | (30) | 23 | (57.5) |
| Cefazolin(CFZ) | 17 | (100)s | 13 | (100) | 9 | (90 | 39 | (97.5) |
| Carbapenems | | | | | | | | |
| Imipenem (IPM) | 14 | (82.3) | 10 | (76.9) | 5 | (50) | 29 | (72.5) |
| Aminoglycosides | | | | | | | | |
| Amikacin (AK) | 9 | (52.9) | 3 | (23) | 4 | (40) | 16 | (40) |
| Gentamicin (GEN) | 12 | (70.5) | 5 | (38.4) | 4 | (40) | 21 | (52.5) |
| Fluoroquinolones | | | | | | | | |
| Levofloxacin (LEV) | 14 | (82.3) | 7 | (53.8) | 6 | (60) | 27 | (67.5 ) |
| Ciprofloxacin (CIP) | 12 | (70.5) | 7 | (53.8) | 5 | (50) | 24 | (60) |
| Tetracyclines | | | | | | | | |
| Tigeocyclin (TIG) | 14 | (82.3) | 1 | (100) | 10 | (100) | 37 | (92.5 ) |




**Figure(1):Percentage of antibiotics rate of *Pseudomonas aeruginosa* isolates against antimicrobial according to the sources (Amoxicillin (AMX), Piperacillin (PRL), Piperacillin/tazobactam (TZP), Amoxicillin-Clavulanic acid (AMC), Ceftazidime (CAZ), Cefexime (CFX), Cefepime (CFP), Cefazolin(CFZ), Imipenem (IPM),Amikacin(AK),Gentamicin (GEN),Levofloxacin (LEV), Ciprofloxacin (CIP), Tigeocyclin (TIG)**

From figure (1) ,we noticed the resistance of a total of isolates from three sources (burns, wound swabs, and sputum) for each antibiotic, showing that the highest resistance was 100% for Amoxicillin, Piperacillin, and Cefixime, followed by Cefazolin, Amoxicillin-Clavulanic Acid, and Tigeocyclin (97.5%),(97%) and (92.5), respectively. Followed by Imipenem, Levofloxacin, Ceftazidime, Piperacillin/tazobactam, Ciprofloxacin, and Cefepime (72.5%),(67.5%),( 65%),(62%),(60) and (57.5%), respectively. The lowest resistance was (52.5%) for Gentamicin and (40%) for Amikacin.

**Detection of *exoS and exoU* genes in *Pseudomonas aeruginosa***

Conventional PCR amplification was performed for *pseudomonas* in order to consolidate the presence of extracellular surface protein, *exo*U, and *exo*S genes coding for different virulence factors by using specific primers for each gene. The product of PCR was detected by using gel electrophoresis, as shown in figures (1) and (2).The *exoS* 12 (70.5%), 13(100%) and 9(90%) genes were detected for *P. aeruginosa* in clinical isolates from burn swabs, wound swabs, and sputum, respectively. Menwhile, *exoU* 5(29.4%), 4(30.7%), and 1(10%) genes were detected for *P. aeruginosa* in clinical isolates from burn swabs, wound swabs, and sputum, respectively among the isolates of *P.aeruginosa*. The prevalence of type III secretion toxin-encoding gene patterns is shown in table (4).

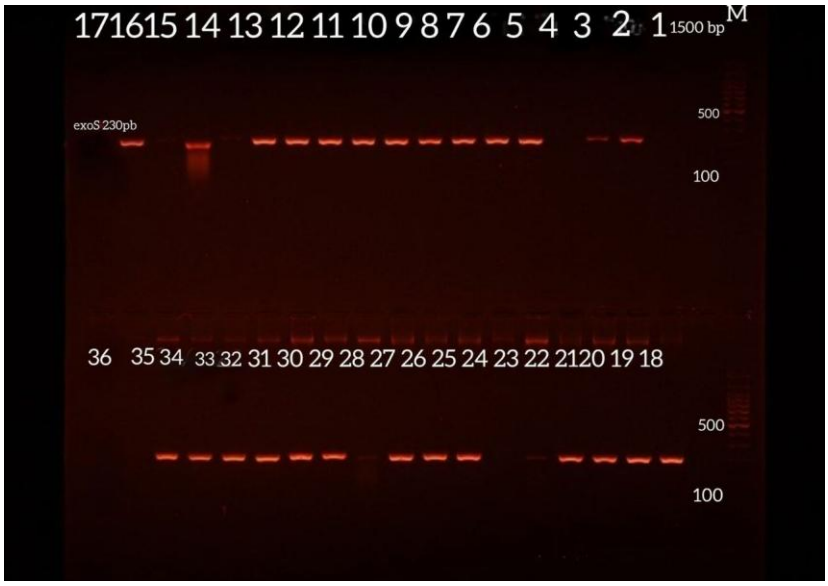



**Figure (2): Agarose gel electrophoresis (1.5% agarose, 100 Vol /45 min) of conventional PCR amplification products of *Pseudomonas aeruginosa exo*S gene (230bp).M:marker(100bp ladder). marker(100 bp ladder). Lane(1,4,23,35,36) is negative for exoS gene.All other lanes showed positive for *exoS* gene.**

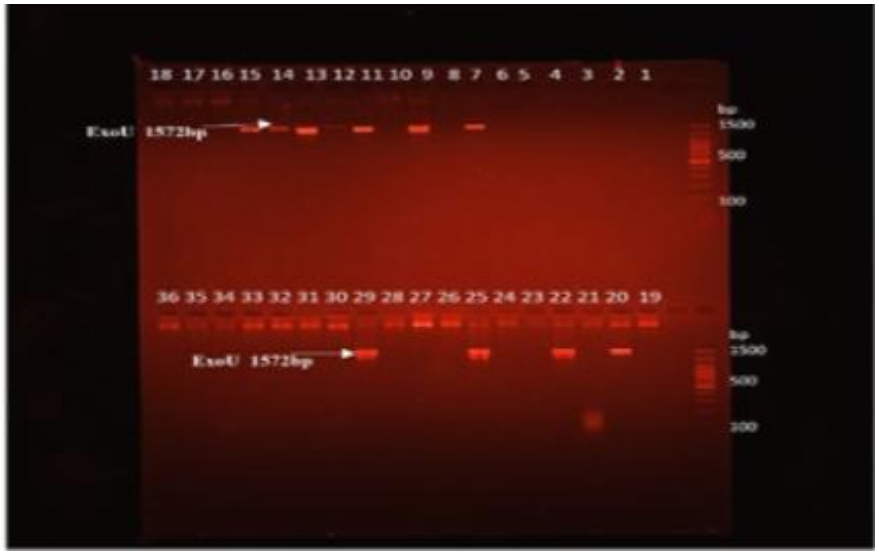



**Figure (3): Agarose gel electrophoresis (1.5%agarose, 100Vol / 45 min) of conventional PCR amplification products of *Pseudomonas aeruginosa exo* U gene (1572bp).M: marker (100bp ladder).Lanes (7,9,11,12,13,14,15,20,22 ,25,29) are positive for *exoU*gene. Other lanes showed negative for *xoU*gene.**

**Table (4): The prevalence of *exoU* and *exoS* genes in *Pseudomonas aeruginosa* isolates according to their source.**

| Isolates source | *exoS* gene | | *exoU*gene | |
|---|---|---|---|---|
| | No. | % | No. | % |
| Burn swab Total No.(17) | 12 | 70.5 | 5 | 29.4 |
| Wound swabs Total No.(13) | 13 | 100 | 4 | 30.7 |
| Sputum Total No.(10) | 9 | 90 | 1 | 10 |
| Total No.(40) | 34 | 85 | 10 | 25 |

Secretion system (T3SS), an extremely sophisticated virulence factor, is a main determinant of two pathogenic types (cyto-toxicity or invasiveness). *ExoS* has GTPase-activating protein activities and ADP-ribosyl transferase activities. *ExoU* is a potent phospholipase (17). The result for these two types of T3SS genes is shown in the table above. The table shows that the *exoS* gene found in almost all isolates (85%). While, only 25% of the isolates carried the *exoU* gene.

## Detection of exotoxin S and U

Sodium dodecylsulfate (SDS) polyacrylamide gel electrophoresis showed isolate number (12) contains both exotoxin's S and U, while isolate number (7,26) contains exoU. This result proved our molecular study. Figure (4) shows the bands of the toxins. Correlation between presence of the *exoS* gene and the *exoU* gene in *P. aeruginosa* and relation with antibiotic resistance. The result of the antimicrobial susceptibility test to forty different antibiotics showed Amoxicillin,Cefazolin, Cefixime, Piperacillin and Tigeocyclin; 12 (92.3%) isolates from burn swabs that have *exoS* shown the result of antibiotic resistance were 12(100%) to Amoxicillin, Amoxicillin-Clavulanicacid, Piperacillin, Piperacillin\ Tazobactam, Cefazolin, Ceftazidime, Cefixime, Imipenem and Tigeocyclin; 9 (75%) to Gentamicin ; 6 (50%) to Amikacin; 11(91.6%) to Levofloxacin; 10(83.3%) to Ciproflxacin and Cefepime, and In addition, antibiotic susceptibility results revealed that the isolates of *P.aeruginosa* that have *exoU* were mostly resistant as follows: 5 (100%) to Amoxicillin, Amoxicillin-Clavulanicacid, Piperacillinm, Piperacillin\Tazobactamm,Gentamicin,Cefepime, Cefazolin, Cefixime, Ceftazidime, Tigeocyclin, Levofloxacin and Imipenem; and lastly, 4 (80%) to Amikacin and Ciprofloxacin,. The isolates of P. *aeruginosa* from wound swabs, of which 100% of them have *exoS*, antimicrobial susceptibility testing revealed percentages of antibiotic resistance were 13(100%) to

to Amoxicillin-Clavulanicacid ; 5 (38.4%) to Gentamicin; (23%) to

Amikacin, 7(53.8%) to Ciprofloxacin, Levofloxacin, Cefepime and Piperacillin/ nTazobactam; 9(69.2%) to Ceftazidime; and finally 12(76.9%) to Imipenem.The isolates of wound swabs that contain both toxin genes *exoS* and *exoU* showed resistance for almost all antibiotics, 4 (100%) for Amoxicillin, Amoxicillin/ Clavulanic acid, Piperacillin, Piperacillin /Tazobactam, Cefixime, Cefazolin and Tigeocyclin ; 2(50%) for Ceftazidime, Cefepime, Ciprofloxacin and Levofloxacin; 1(20%) to Amikacin.

Finally, the result of the antibiotic susceptibility test for the isolates from sputum that have *exoS* showed Amoxicillin 9 (100%), Amoxicillin-Clavulanic acid, Piperacillin, Cefixime and Tigeocyclin; 3 (33.3%) to Gentamicin, Ceftazidime, Cefepime and Pipercillin/Tazobactam; 4 (44.4%) to Amikacin; 5 (55.5%) to Ciprofloxacin, and Imipenem; 6 (66.6%) to Levofloxacin and 8 (88.8%) to Cefazolin,and. The Most prevalent pattern was MDR 6(66.6%). 3(33.3%) of the isolates were XDR and 1(11.1%) of the isolates were PDR.

The Result of antibiotic resistance for the only isolate associated with *exoS* and *exoU* was (100%) for all types of antibiotics. But the isolate was sensitive to Tigeocyclin,this isolate showed a PDR pattern for the antibiotics. A significant difference was observed between the presence of the *exoU* gene and antibiotic resistance. Based on table (4), there is a low significant association between the presence of the *exoU* and *exoS* genes and resistance to Pipercillin/ Tazobactam, Amikacin, Gentamicin, and Ciprofloxacin. High a significant have been shown between a presence of *exoS* and *exoU* genes and resistance to (Ceftazidime, Cefepime, Imipenem, and Levofloxacin). No significant association was identified between the prevalence of virulence factor genes and the resistance to (Amoxicillin,Piperacillin, Cefixime, Amoxicillinl Clavulanic acid, Ceftazidime, and Tigeocycline).

All this information is summarized below in Table (5).

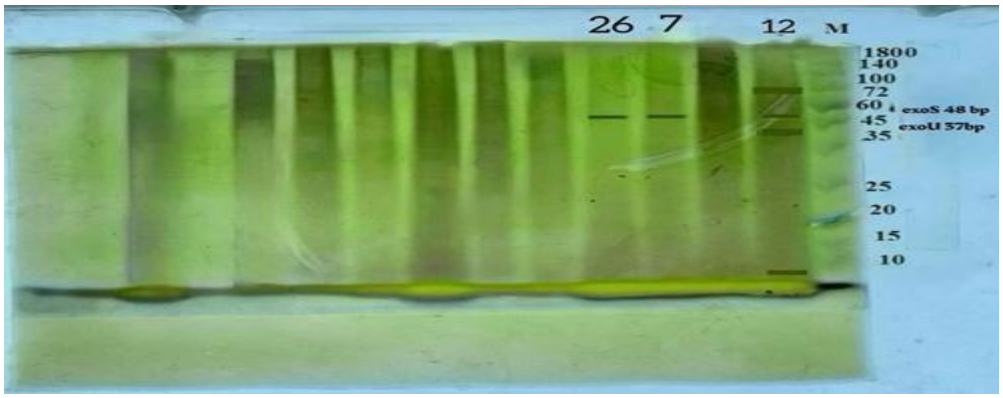



**Figure (4): SDS-PAGE profile of exoS and exoU of *P. aeruginosa*, (LaneM) showed protein markers, Lane (12) showed exoU at the molecular weight (37bp) and exoS at the molecular weight (48bp), Lane (7,26) showed exoS at the molecular weight(48bp)**

**Table (5): Correlation between presence of *exoS* gene and *exoU* gene in *P. aeruginosa* and relation with antibiotic resistance.**

| Source | (17) Burn swab isolates | (13) Wound swab isolates | | | (10) Sputum isolates | | *p*-value |
|---|---|---|---|---|---|---|---|
| Resistance | (12) ( *ExoS*) | (5) (*ExoU*) | (13) (*ExoS*) | (4) (*ExoU*) + (*ExoS*) | (9) ( *ExoS*) | (1) (*ExoS*)+ (ExoU) | |
| | No. % | No. % | No. % | No. % | No. % | No. % | |
| Antibacterial agent β-lactam agents | | | | | | | |
| Amoxicillin (AMX) | 12 (100) | 5 (100) | 13 (100) | 4 (100) | 9 (100) | 1 (100) | 1.00 NS |
| Piperacillin (PRL) | 12 (100) | 5 (100) | 13 (100) | 4 (100) | 9 (100) | 1 (100) | 1.00 NS |
| Piperacillin/tazobac-tam (PTZ) | 12 (100) | 5 (100) | 7 (53.8) | 4 (100) | 3 (33.3) | 1 (100) | 0.0296* |
| Amoxicillin- Clavulanic acid (AMC), | 12 (100) | 5 (100) | 12 (92.3) | 4 (100) | 9 (100) | 1 (100) | 0.582 NS |
| Cephalosporins | | | | | | | |
| Ceftazidime (CAZ) | 12 (100) | 5 (100) | 9 (69.2) | 2 (50) | 3 (33.3) | 1 (100) | 0.0062 ** |
| Cefexime(CFX) | 12 (100) | 5 (100) | 13 (100) | 4 ( 100) | 9 (100) | 1 (100) | 1.00 NS |
| Cefipem(CFP) | 10 (83.3) | 5 (100) | 7 (53.8) | 2 (50) | 3 (33.3) | 1 (100) | 0.0078 ** |
| Cefazolin(CFZ) | 12 (100) | 5 (100) | 13 (100) | 4 (100) | 8 (88.8) | 1 (100) | 1.00 NS |
| Carbapenems | | | | | | | |
| Imipenem (IPM) | 12 ( 100) | 5 (100) | 10 (76.9) | 2 (50) | 5 (55.5) | 1 (100) | 0.0095 ** |
| Aminoglycoside | | | | | | | |
| Amikacin (AK) | 6 (50) | 4 (80) | 3 (23) | 1 (20) | 4 ( 44.4) | 1 ( 100) | 0.0377 * |
| Gentamicin (GEN) | 9 (75) | 5 (100) | 5 (38.4) | 0 ( 0) | 3 (33.3) | 1 (100) | 0.0481 * |
| Quinolones | | | | | | | |
| Levofloxacin (LEV) | 11 (91.6) | 5 (100) | 7 (53.8) | 2 (50) | 6 (66.6) | 1 (100) | 0.0074 ** |
| Ciprofloxacin (CIP) | 10 (83.3) | 4 (80) | 7 (53.8 | 2 (50) | 5 (55.5) | 1 (100) | 0.0261 * |
| Tigeocyclin(TIG) | 12 (100) | 5 (100) | 13 (100) | 4 (100) | 9 ( 100) | 1 (100) | 1.00 NS |
| Antibiotic patterns | Burn swab No. % | Wound swab No. % | | | Sputum No. % | | |
| MDR | 6 | (35.2) | 8 | (61.5) | 6 | (60) | 0.652 NS |
| XDR | 4 | (23.5) | 2 | (15.3) | 3 | (30) | 0.489 NS |
| PDR | 12 | (70.5) | 3 | (23) | 1 | (10) | 0.0065 ** |

* (P≤0.05), ** (P≤0.01), NS: Non-Significant.

The increasing prevalence of chronic and hospital-acquired infections produced by MDR or extensively drug resistant (XDR).The presence of MDR and XDR *P. aeruginosa* isolates leads to various problems, about the treatment of infections. Therefore, continuous investigation to prevent the further extent of MDR *P. aeruginosa* isolates and inhibition of colonization in burn departments should be in employment. Pseudomonal infections in patients of burn are highly resistant. Therefore, it is important to develop management strategies against these highly resistant infections. For this, it is necessary to plan such a type of research study that catch the correct amount of antibiotics for the treatment of the patient (18). In the present study, correlation was applied to find the association between detected virulence genes and antibiotic resistance patterns that is, MDR, XDR, and PDR phenotypes of *Pseudomonas aeruginosa*. The table shows that the isolates from burn swabs revealed 6 (35%) of them were MDR,4 (23%) XDR, and the most prevalent pattern was PDR 12 (70.5%).

According to the present study, the isolates from wound swab showed MDR 8 (61%), XDR 2 (35%) and PDR 3 (23%). The isolates from sputum showed MDR 6 (60%), XDR 3 (30%) and PDR 1(10%). Statical analysis showed a high significant association between the presence of *exoS* and *exoU* genes and PDR rate. While our result was no significant association between the presence of exoS and *exoU* genes with MDR and XDR rates.

## Discussion

These results of *Pseudomonas aeruginosa* of the current study reveal the importance of the infection by Pseudomonas isolates, and these results agreed with (19). Our result of *P.aeruginosa* isolates to Piperacillin was concordant with a study conducted in Baghdad by (20,21) that revealed that *P.aeruginosa* isolated from burn and wound swabs were 100% resistant for Piperacillin.On the other hand, our result was disagree with the study conducted by (22,23) showed that *P.aeruginosa* isolated from different sources were 32% and 66.67%, respectively. The study result of resistance against Cefazolin was highly like the result of a study conducted by (24,25) revealed resistance against Cefazolin exceeded (90.0%).

On the other hand, our result is dissimilar to the result of a study conducted by (26) which showed resistance against Cefazolin (39.5%). Also, the result of resistance of isolates in the current to Cefexime agreed with a study by (27) when their resistance to Cefexime was (95%). But this result disagreed with the result of the study by (25) when their results were (84.3%) resistant to Cefexime. The result of resistance against Amoxicillin/Clavulanic acid was close to the result of a study conducted by (6) showed 100% of the isolates resistance against Amoxicillin/Clavulanic acid.Whereas, a similar study by (28) disagreed with our study result, when showed only 25% of the isolates were resistant to Amoxicillin/Clavulanic acid. Also, the result of resistance isolates of *P. aeruginosa* to

Imipenem agreed with (27) when the result was (86.8%), and disagreed with the study conducted by (29) was (39.4%).

Also, in the current study, the result of resistance isolates Amoxicillin was similar to the result of a study conducted by (30) was (97%). The result of the present study showed resistance against Ceftazidime, Ciprofloxacin and Amikacin were similar to the result of the study by (26) (68%),(66.3%) and (48.8%) respectively. While, disagree with the result of the same study by (6) observed that (82%) and (66%) of the isolates were resistant against Ceftazidim and Amikacin. The result of resistance to Ciprofloxacin disagreed with a study by (31) when the results were (27.27%). Also, the result of the study conducted by (6) consistent with our result of resistance to Piperacillin/ Tazobactam when the result was (63%). While, a study conducted by (32) showed resistance to Piperacillin/ Tazobactam (32.9%). The results of resistance of *Pseudomonas* spp. isolate to Gentamycin were similar to those obtained by local research by (33) when their *P.aeruginosa* isolates were (58%).

On the other hand, a study conducted by (21) revealed different results when their *P.aeruginosa* isolated from different clinical sources were 20% resistant to Gentamicin. Our result of resistance against Levofloxacin was agreed with the result of a study conducted by (6). While disagreeing with a study by (34) showing 40% of the isolates were resistant to Levofloxacin. The result of resistance against Cefipeme was close to the result of a study conducted by (32) showed (55.8%) of the isolates resistance against Cefipeme. Whereas, a similar study by (35) showed a higher percentage of results when 100% of the isolates were resistant to Cefepime. Finally, our results for Tigeocyclin resistance were consistent with the result of study conducted by (36) when their result was 93.3%. while a different result reaveled by study conducted by (37) when their results were (25%). Our result for *exoS* gene is similar to the result of a study conducted by (4,35,38) who reported that (90.47%) (100%) and (97.7%) respectively, of *P. aeruginosa* isolated from different clinical sources contain *exoS* gene. Meanwhile, disagree with a study conducted by (39,40,6) when their results for frequency of *exoS* gene in the isolates were 50%, 75%, and 38.46%, respectively. The prevalence of *exoU* in the isolates in the present study is consistent with the result of a study by(38) when the results were (25%), but different from the results obtained by (7,41,6) when their results were (60.31%),(57.69%) and (41%), respectively.

Based on table (4), the findings of a low significant association between the presence of the *exoU* and *exoS* genes and resistance to Pipercillin/ Tazobactam, Amikacin, Gentamicin, and Ciprofloxacin were supported by the studies done by (41). Our result is different from the result of a study conducted by (42) when he reported that there was no significant resistance to Ciprofloxacin. The result of a high a significant between a presence of *exoS* and *exoU* genes and resistance to (Ceftazidime, Cefepime, Imipenem, and Levofloxacin) was agreed with a study by (43) who showed a high significant relationship between the presence of virulence genes and resistance to Imipenem(P=0.13),and no significant of

resistance to Ceftazidime when the P-value were > 0.05.Also, our result disagreed with study conducted by (44) reported that the presence of the virulence genes was not significantly related to the resistance against the tested antibiotics. No significant association which identified between the prevalence of virulence factor genes and the resistance to (Amoxicillin,Piperacillin, Cefixime, Amoxicillinl Clavulanic acid,Ceftazidime, and Tigeocycline) was the same as the result of the study by (16). Our result is different from result of a study conducted by (45) when their result showed a statistical significance between the presence of virulence genes and resistance to Piperacillin (P= 0.01). From the table (5), the result of prevalence resistance patterns was consistent with a previous report conducted by (46) when their results of antimicrobial resistance pattern of *p.aeruginosa* isolated from burn infection were XDR (29.6%), but inconsistent with the same study in relation to MDR (11.1%). Another study recorded a different rate of MDR disagree with our result (47,48) were MDR (21%; 80%), respectively. Meanwhile, MDR similar to MDR of study by (49) was (38%).

According to the present study, the isolates from wound swab showed rates of MDR, XDR, and PDR disagreed rates associated with XDR/MDR *P. aeruginosa* isolates have been reported by (50). MDR (35%), XDR (26.5%). But, MDR result close to the result of the study on isolates of wound swab by (51,41) were (57.4%; 59%), respectively. The isolates from sputum showed rates of MDR, XDR, and PDR agreed with the rates of a study by (52,53) with the same study by (54,40,55) MDR were (8.4%;4.32%; 50%). The result of XDR consistent with the result of the study by (56) was(38%).

The result of statical analysis was consistent with a study by (57,32,58) when ($P \leq 0.01$). But inconsistent with a study by (10) when he revealed a strong relationship between the distribution of *exoS* and *exoU* genotypes and MDR and XDR rates.

## Conclusion

From this study, we concluded the importance of *pseudomonas aeruginosa* toxins as virulence factors and the sources of isolation effect on the presence of these virulence genes and antibiotic resistance; also, there was a relation between virulence factor genes and the range of antibiotic resistance. This study proved that the presence of *exoS* and *exoU* genes increased the resistance of the isolates against antibiotics.

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