



## Virulence Genes Profile of *Pseudomonas aeruginosa* Local Isolates from Burns and Wounds

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**Abstract:** In this study a total of 111 swabs were collected from 50 patients suffering from burns and wounds between the age 9 months -69 years from both gender, the specimens were collected from Educational Al-Yarmouk and AL Kadhimiya hospitals in Baghdad during the period between October-December\2014, All the samples were identified by biochemical tests, API 20 E and Vitak -2. From 111 specimens, 31 isolates were *Pseudomonas aeruginosa*, 33 isolates were *Escherichia coli* and 10 isolates were *Klebsiella* spp. Drug susceptibility tests for the 31 isolates of *Pseudomonas aeruginosa* were studied by the disk diffusion method against 5 antibiotics and the result showed the percentage of resistance: Carbenicillin 100%, Ticarcillin 61%, Pipracillin 32%, Cefprozil 93%, Colistin 84%. The DNA samples of the 31 isolates of *Pseudomonas.aeruginosa* were extracted by DNA extraction kit, the concentration for all 31 DNA samples were between 60- 110 ng/ul and the purity were between 1.8-2.0. PCR (Polymerase Chain Reactin) was used for screening the virulence factor genes, protein synthesis inhibition genes (*exo A*, *las B*, *exoU*) of the thirty one isolates of *Pseudomonas aeruginosa*. The result showed that 27 (87%) isolates were positive for *exo A* and *las B* genes, 4 (13%) were negative, from 25 burn isolates was 23 (92%) positive isolates and from 6 wound isolates was 4 (66%) positives isolates. For the *exoU* gene the result showed that 17 (55%) were positive, 14 (45%) were negative, From 25 burn isolates the presence of *exoU* was seen in 15 (60%), while from 6 wound isolates was 2 (33%).

**Key words:** *P. aeruginosa*, *exo A*, *las B*, *exoU*, Burn wound infection.

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### Introduction

Nosocomial Infections (NI) are common in burn cases due to the typical features of the disease: loss of the first line of defense against microbial

invasion, a vascularized tissue that provides a favorable environment for microbial growth; alterations in the specific and nonspecific components of the immune system; and therapeutic procedures (1). Burn injury, one of the

significant public health problems worldwide, is at high risk for nosocomial infections (2). Wound infection is one of health problems that are caused by the invasion of pathogenic organisms in different part of body and it threat life of large number of people in many countries (3). *P. aeruginosa* is found as major colonizer of the burn wound because it live on moist burn wound surface and usually gains access to burn patients through cross contamination. It persists as a major nosocomial infection threat to burn patients, arising of resistance against multiple antimicrobial drugs frequently complicates the treatment of *P. aeruginosa* infection. This may lead to serious infection and thus mortality rate in these patients become high (4). Infections of *P. aeruginosa* are difficult to eradicate because of their elevated intrinsic resistance as well as their capacity to acquire resistance to different antibiotics (5). Exotoxin A causes ADP-ribosylation of eukaryotic elongation factor 2, which results in inhibited protein synthesis (6). *las B* is a zinc metalloprotease has a wide range of substrates, including elements of connective tissue such as elastin, collagen, fibronectin and laminin, (7). *exo U* Possesses important cytotoxin and disrupts the membranes of the infected host cell (8). Genomic DNA was used as a template for PCR (Polymerase Chain Reaction) screening virulence genes of *P. aeruginosa*: genes codifying for proteases - *lasB*, *exoU*, *exoA* , to study correlating virulence patterns and infection clinical outcome could be useful for setting up efficient preventive and therapeutic procedures in hospitalized patients with positive *P. aeruginosa* cultures (9).

## Materials and Methods

### Identification and Susceptibility Test of *P. aeruginosa*

In this study 111swab were collected from 50 patients, between the age 9 months -69 years, (24 patient suffered from burn, and 26 patient suffered from wound). All samples were cultured on nutrient agar, MacConkey agar, Blood agar, Cetrimide agar, King A and king B medium. The identification confirmed by biochemical tests ( oxidase , catalase, motility, IMVIC) (10), in addition to Api 20 E , and Vitak 2 for the *P. aeruginosa* isolates . Antimicrobial susceptibility test were carried out on the identified isolates of *P. aeruginosa* using commercially prepared antibiotic disks on Mueller Hinton agar plates by the disk diffusion method against five antibiotics (Carbenicillin , Ticarcillin , Pipracillin , Cefprozil , Colistin ).

### DNA Extraction

DNA were extracted from thirty one *P. aeruginosa* isolates were extracted according to the instruction of the Promega kit , DNA concentration and purity were measured by Nano Drop 2000C Spectrophotometer and electrophoresed by the Gel Electrophoresis System for proofing that the Genomic DNA were intact and not sheared.

### Preparation and Optimization of the Primer

#### Preparation of Primer

Primers were prepared according to the instructions of Manufacturer Company (Alpha DNA, Canada). The primers choose (Table 1)

**Table (1) The primer sequence and product of virulence genes**

Genes	Primer sequence (5'-3')	Annealing temperature	product	Reference
<i>exo A</i>	F-ACCAGCTCAGCCACATGTC R-RCGCTGGCCCATTCGCTCCAGCGCT	55	400 bp	(11)
<i>las B</i>	F-TTCTACCCGAAGGACTGAT AC R- AACACCCATGATCGCAAC	55	150bp	(12)
<i>exo U</i>	F- CGTTGTGGTGCCGTTGAAG R- CAGATGTTACCCGACTCGC	55	150 bp	(13)

### Detection of the Virulence Factors Genes

1. For optimization of the primer , eppendorf tube was prepared containing 10 µl master mix ,0.7 µl forward primer, 0.7 µl reverse primer, 7.6 µl free nuclease water and 1ul DNA.

2. For optimization the programme of protein synthesis inhibition genes (*exoA*, *las B*, *exo U*) , gradient PCR was used , using different annealing temperature (53, 55, 60.3, 62.6, 65 °C) and the best annealing temperature was chosen (Table 2).

**Table (2): The PCR thermocycler programme for DNA amplification of *P.aeruginosa***

Steps	Temperature	Time	Cycles
Initial Denaturation	95 °C	4min	1 cycle
Denaturation	95 °C	30 sec	35 cycles
Annealing	55°C	30 sec	
Extension	72 °C	30 sec	
Final Extension	72 °C	7 min	1 cycle
Hold	4 °C		

### Results and Discussion

In this study from 111 specimens , 31 isolates of *Pseudomonas aeruginosa* (25 from burns and 6 from wounds), 33 isolates of *E.coli* ,10 isolates of *Klebsiella* spp ( Table 3).

In previews study by Ranjan, *et al.* (14) founded that the most common isolated organism from postoperative wounds was *P. aeruginosa* , and another study in Iraq by Alwan *et al.* (15) revealed that *P. aeruginosa* the most common isolate from burns and wounds .

**Table (3): Percentage of the isolated bacteria**

Type of bacteria	No.
<i>P.aeruginosa</i>	31(25burn 6 wound)
<i>E.coli</i>	33
<i>Klebsiella spp.</i>	10

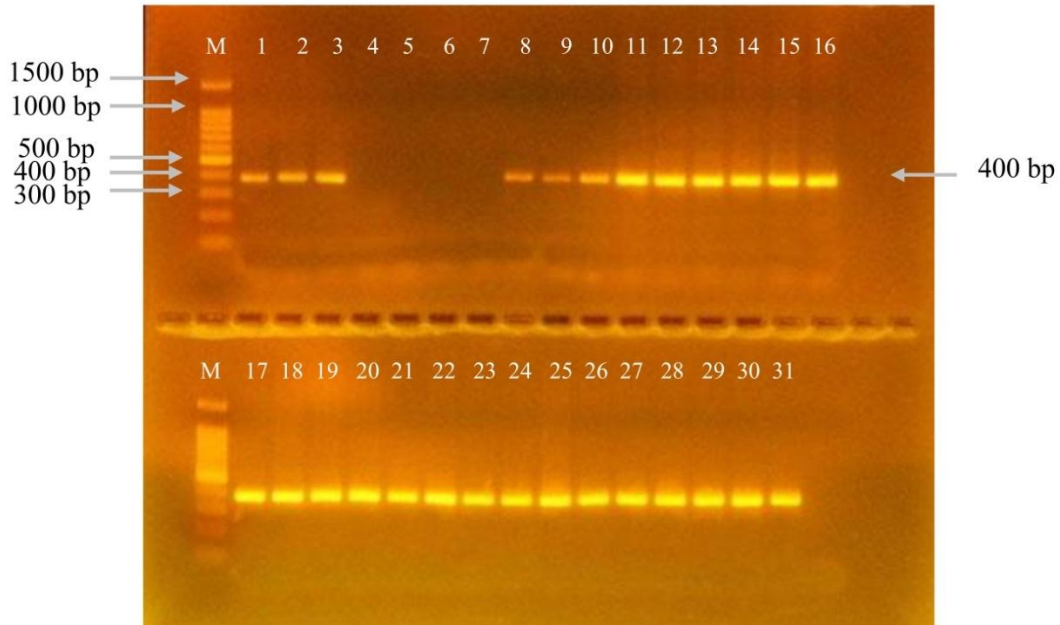
*Pseudomonas aeruginosa* colonies on nutrient agar appear bluish green in colour, on blood agar the colonies produce a clear zone due to  $\beta$ -hemolysis (16), on MacConkey agar medium, the bacterial colonies appeared smooth and pale because it does not ferment lactose (17). The identification of *P. aeruginosa* was confirmed by growing them on Cetrimide agar, King A and King B medium. These media were selective for *P. aeruginosa*, since it produce pyocyanin and fluorescence pigments. The positive isolates produce pyocyanin pigment (blue or green) on Cetrimide agar and King A and produce fluorescence pigment (yellow) on King B (18). The isolates of *P. aeruginosa* showed different percentage of resistance to each antibiotics of the penicillin group: Carbenicillin 100% (31/31), Ticarcillin 61% (19/31), Pipracillin 32% (10/31). The Cephalosporin group: Cefprozil 93% (29/31). The Polymyxin group: Colistin 84% (26/31). According to the percentage of Carbenicillin resistance. a study in Iraq by Abd *et al.*, (19) founded the same percentage. A study in Iraq by Alwan *et al.*, (15) founded close percentage 68.75% of Ticarcillin resistance to that of this study, while Al-Habib *et al.*, (20) founded different percentage which was 93.4%. The percentage of Pipracillin resistance was close to percentage of study of Kireçci *et al.*, (21) which was 24%, While study of Al-Habib *et al.*, (20)

founded different percentage which was 60%. The percentage of Cefprozil resistance was similar with percentage of Al-Habib *et al.*, (20) which was for Cefoxitin 96%, while Magneti *et al.*, (22) founded the percentage for Cefepime was 50%.

The percentage of Colistin resistance different from percentage of Yolbaş *et al.*, (23) which was 100%. The result revealed that the concentration for all DNA samples of the thirty one *P. aeruginosa* isolates were between 60-110 ng/ $\mu$ l and the purity was between 1.8-2. The PCR (Polymerase Chain Reaction) was used for screening the protein synthesis inhibition genes (*exo A*, *las B*, *exoU*) of the thirty one *P. aeruginosa* isolates, and the result showed that 27 (87%) isolates were PCR-positive for the *exo A* gene, and 4 (13%) isolates were PCR-negative (Figure 1), from 25 burn isolates was 23 (92%) positive isolates and from 6 wound isolates was 4 (66%) positive isolates. The percentage of *exo A* in the *P. aeruginosa* isolated from burns 85% was close to that obtained by Bahaa El-Din *et al.*, (24), which was 89.4%, while another study by Hossein *et al.*, (25) showed, the isolates obtained from Burns, wounds, ICC, CCU and ITU were 33% harbored this gene. Wolska and Szweida (26) found that all 100% isolates from wounds contain the gene *exo A* plays an important role in the spread of *P. aeruginosa* within the burned skin and the appearance of

endogenous septicemia. In addition, that had special role in retardation of wound healing and contraction. The conclusion

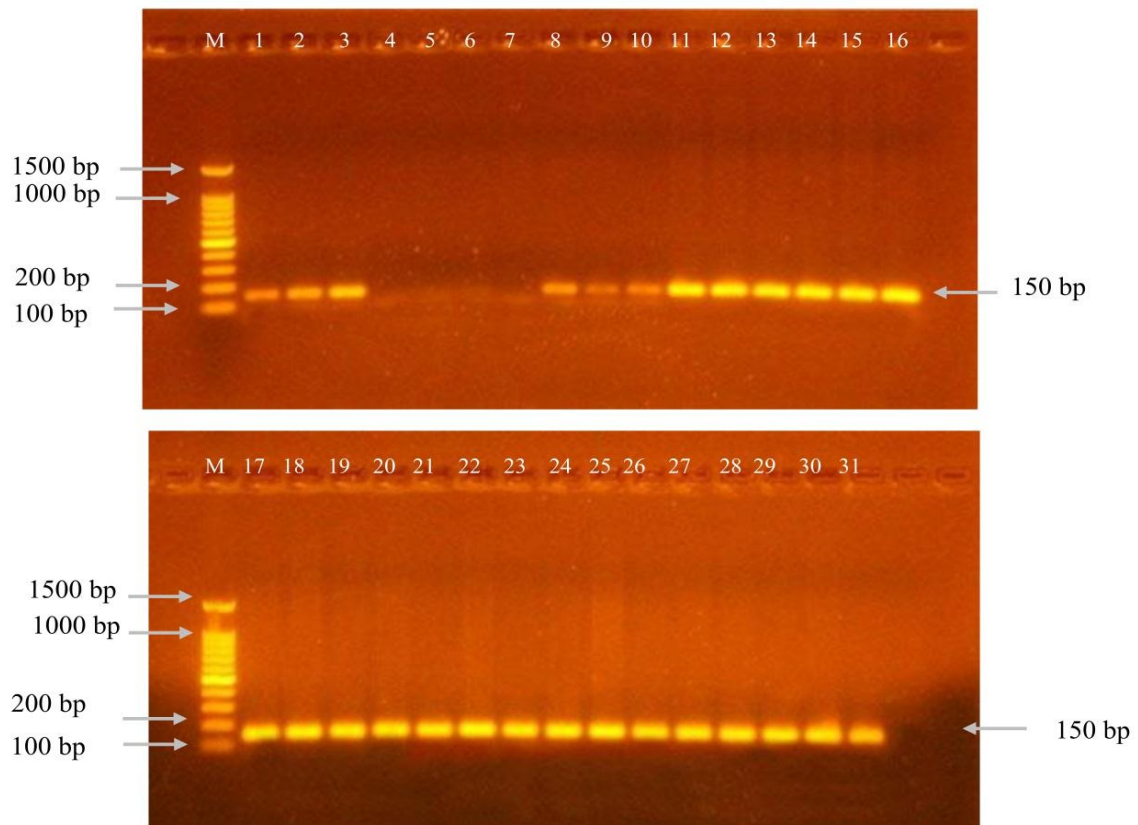
was that *exo A* contributed to the overall virulence of *P.aeruginosa* in those burned patients (24).



**Figure (1):** Gel electrophoresis of amplified PCR product of *exo A* gene(400bp) in monoplex PCR at 100 v for 90 min in 1 % agarose , TBE (1x) , stained with ethidium bromide . M: DNA ladder (100bp) , all the lanes were positive for the *exo A* gene except the lanes from (4-7) were negative

From the thirty one isolates of *P. aeruginosa* 27 (87 %) were PCR-positive for the *las B* gene, and 4 (13 %) isolates were PCR- negative (Figure 2). From 25 burn isolates was 23 (92%) positive isolates and from 6 wound isolates was 4 (66%) positives isolates. Khattab *et al.*, (27) found 100% of the isolates from burns harbored this gene.

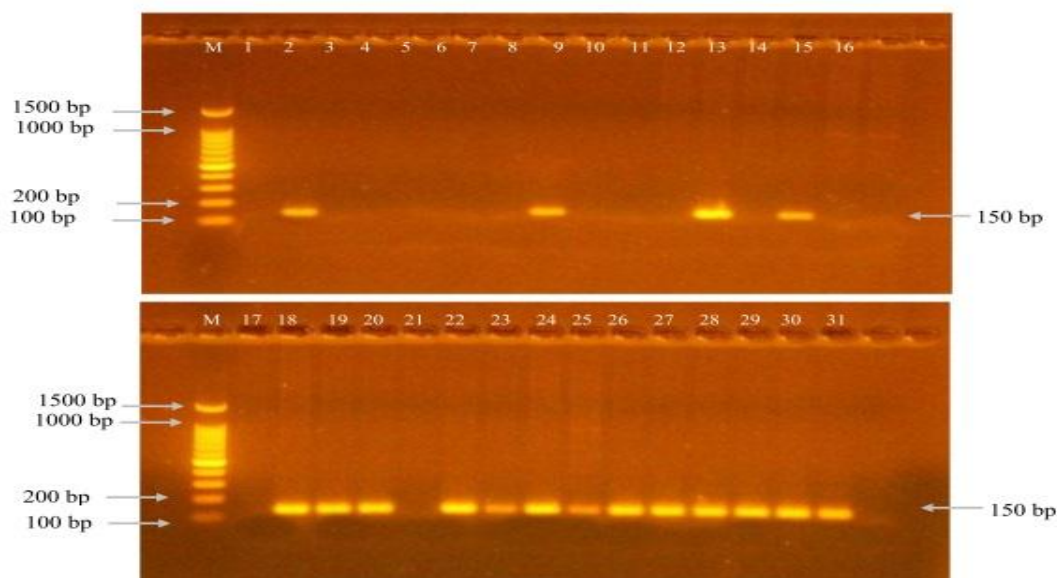
Nikbin *et al.*, (28) showed the isolates from wounds was 100 % harbored this gene. The PCR results for *las B* gene suggested that the production of elastase is important in all types of *Pseudomonas aeruginosa* infections, as reported in a study in Iraq by Ra'oof (29).



**Figure (2) : Gel electrophoresis of amplified PCR product of *las B* gene (150bp) . M: DNA ladder (100bp), all the lanes were positive for the *las B* gene except the lanes from (4-7) were negative**

From the thirty one isolates of *P. aeruginosa* 17 (55 %) isolates were PCR-positive for *exoU* gene, and 14 (45%) isolates were PCR- negative (Figure 3). From 25 burn isolates the presence of *exoU* was seen in 15 (60 %), while from 6 wound isolates was 2 (33 %). Gawish *et al.*, (30) showed that the isolated from burn was 66.7% harbored this gene. Kadhim and Ali (31) found that the isolated from burns wounds infections, ear infections, respiratory tract infection (RTI), urinary tract infection (UTI) and bacteremia was 55 % harbored this gene , while Bradbury

*et al* (32) showed that the isolated from the environment of intensive therapy wards was 18% harbored this gene. The isolate which harbor *exoU* gene are referred to as cytotoxic therefore the phenotypes of *P. aeruginosa*, is cytotoxic (33). These variations of *exoU* in different clinical isolates may be due to the isolates of the same source were from patients with different clinical conditions and different duration of hospitalization and different sources of infections, *exoU* have variable trait and present in different prevalence among *P. aeruginosa* strains (30).



**Figure (3): Gel electrophoresis of amplified PCR product of *exo U* gene (150bp) . M: DNA ladder (100bp) ,all the lanes were positive for the *exo U* , except the lanes (1,3,4,5,6,7,9,10,11, 13,15,16,17,21) were negative**

Results of present study showed that the burns infection harbored high positively rate of the virulence genes than the isolates from wounds infection as fellow 85% burn isolate ,15% wound isolate for *exo A* and *las B* genes , for *exoU* gene, 88% burn isolate ,12% wound isolate.

The low prevalence of this virulence factor genes in the isolates from wound infections may show the role of this genes in the wound infections is less important than the burn infections. Nikbin *et al.*, (28) reported in his study that the determination of different virulence genes of *P. aeruginosa* isolates are associated with different levels of intrinsic virulence and pathogenicity and the differences in the distributions of virulence factor genes in the populations strengthen the probability that some *P. aeruginosa* strains are better adapted to the specific conditions found in specific infectious sites .

There were differences in the virulence genes profiles of strains isolated from different clinical origins, correlating between virulence patterns and infection clinical outcome could be useful for therapeutic procedures in hospitalized patients with positive *P. aeruginosa* cultures (9). Significant correlations between some virulence genes and source of infections indicates implementation of infection control measures will help in controlling the dissemination of virulence genes among *P. aeruginosa* isolates as reported by the study in Iraq of Khattab *et al.*, (27). There are differences in the percentage of virulence infection between results of this study and others researchers, and the reasons for these variations in all studies may be due to the percentage of distribution of isolates which varied according to the place of clinical samples collection virulence, factors environmental factors, and nutrition requirements, as reported in the study of Ogunseilan (34).



## Conclusion

The burns infection revealed high percentage of *P. aeruginosa* than wounds infection, the most active compound against *P. aeruginosa* Pipracillin, and the detected of three virulence factors genes (exo A, las B, exoU) of *P. aeruginosa*, showed high positively rate of virulence in isolates from burns than that of wounds infection.

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