Int. j. adv. multidisc. res. stud. 2023; 3(4):1209-1214

Received: 09-07-2023
Accepted: 19-08-2023

International Journal of Advanced Multidisciplinary Research and Studies

ISSN: 2583-049X

Detection Genotyping Virulence Factor among *Pseudomonas Aeruginosa* Isolated from Burns Patients

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Abstract

A total of 153 samples (swabs) (48/153) isolates (31.37%), were taken from burned patients. were identified as *P. aeruginosa* using phenotypic techniques. The result were distributed between no growth (41\153) (26.83%) and other gram negative bacteria (64\153) (41.8%) *Proteus spp*. (5\153) (3.26%), *Klebsiella spp*. (27\153) (17.64%), *E. coli* (24\153) (15, 68%) and *Acintobacter spp*. (8\153) (5.22%). Clinical samples included (23\48) (47.9%) samples taken from male and (25\48) (52.1%) samples taken from females, as well as confirmation using method PCR for species-specific primer pairs to amplify 16S rRNA gene. In molecular study, 48 isolates were DNA extracted by boiling, measurement of the concentration of DNA samples was done by Nano-drop, chosen virulence factors genes (*AlgD* and *pslD*) which are responsible for biofilm formation the result was (70.84, 62.5) % respectively, gene (*pvc*) which is responsible for pyoverdine pigment the result was (83.34)% and gene (*Exo A*) responsible for exotoxin A enzyme the result was (72.92)%.

Keywords: Genotype, *Pseudomonas Aeruginosa*, Patients

Introduction

Burns are necrosis, and several things including chemicals, heat, nuclear radiation, electricity, and sunlight can harm the skin and tissue and cause burns. Burns infection is a major global public health issue that can significantly increase the mortality rate in developing nations [1]. Both gram-negative and gram-positive bacteria can cause burn infections. At the moment, *pseudomonas aeruginosa, Escherichia coli, coliform bacilli, klebsiella* spp., and *staphylococcus aureas* are the most prevalent pathogens isolated from burn patients [2]. *P. aeruginosa* is a common gram-negative bacteria from the pseudomonadaceae family that can endure under a variety of conditions. *P. aeruginosa* is an opportunistic pathogen that may infect a wide range of organisms, most notably humans [3]. This bacterium's ability to produce a variety of virulence factors, including secreted components like the pigments pyoverdin and pyocyanin, cytotoxic alkaline protease, elastase, exotoxin a, lipopolysaccharide, hemolysins, pili, flagella, and biofilm formation, contributes to its pathogenicity [4]. This bacteria produce many enzymes, some of which, such as proteinase (Alkaline protease and Elastase), are important in the development of acute lung infections and infections of burned wounds [5]. Also, produces a large number of soluble water pigments. The most significant pigment, pyoverdine (greenish-yellow), allows bacteria to proliferate and produce persistent illnesses even when iron is absent from the environment [6].

Materials and Methods

Isolation and Identification

Samples (153) were obtained from burn patients between September 2022 and January 2023. All samples came from Baghdad's government hospitals. Under the supervision of the responsible doctor, samples were collected from burn patients using a transporter media cotton swab. The burned area was cleaned thoroughly with sterile normal saline, and a sterile cotton swab was gently passed over the burned area. The samples were then transferred to the lab and incubated at 37 °C for 24 hours before being cultured. Identified *P. aeruginosa* isolates by diagnostic tests including biochemical tests, culture media like (cetrimide agar), and VITEK-2 Compact System as well as confirmation using method PCR for species-specific primer pairs to amplify *16S rRNA* gene.

Detection of Some Virulence Factors Genes in *P. aeruginosa*

Genotyping Detection of Biofilm Formation, Pyoverdine Pigment and Exotoxin A

Specific virulence genes were identified using the PCR technique. Include (*AlgD*, *pslD*, *pvc*, and *Exo A* gene).

Extraction of Bacterial DNA

After growing on macConky agar, the bacteria were removed, and 1-3 colonies were suspended in 500µl of distilled water in an eppendrof tube. The suspension was heated for 30 minutes at 100 °C, then quickly chilled in ice for 20 minutes, and then centrifuged for 20 minutes at 1000 rpm. The DNA suspension was kept at (-20) °C until it was utilized as a PCR template [7]. And determination of DNA purity and concentration by NANO-DROP.

PCR Amplifications

PCR amplification was used for the detection of virulence genes. The PCR primers used in this investigation are included in (Table 1) along with descriptions of their sequences.

Table 1: The primers used in the current study for gene detection

Primers	Sequence (5'-3')	Product size(bp)	References
Identification			
16S rRNA	F- GGGGGATCTTCGGACCTCA R- TCCTTAGAGTGCCCACCCG	956	[8]
Virulence factors			
PVC	F- CTATGAGAGCCATTATTCCG R- GTAGATCTGCTTGTACAGGTA	389	[9]
ExoA	F- CAG AAC TGG ACG GTG GAG C R- CCT GTT CCT TGT CGG GGA TG	535	[10]
AlgD	F- CTACATCGAGACCGTCTGCC R- CATCAACGAACCGAGCATC	593	[11]
PslD	F- TGTACACCGTGCTCAACGAC R- CTTCCGGCCCGATCTTCATC	369	[11]

Results and Discussion

Collection and Identification

A total of 153 samples (swabs) were collected by the sterile cotton swab from September 2022 to January 2023, the specimens Among 153, (48/153) isolates (31.37%) were diagnosed as *P. aeruginosa* based on phenotypic methods. The result was distributed between no growth (41\153)(26.83%) and other gram negative bacteria (64\153)(41.8%) Table 2 and Fig 1, confirming by PCR for species specific primer pairs to amplify 16S rRNA gene. The findings revealed that burns accounted for (48/153)(31.37%) of the *P. aeruginosa* isolates. This may be because this pathogen has a number of potentially virulent components that aid in its ability to colonize and infect mammalian tissues, including hemolysin, pyoverdine, and protease, which encourage adhesion to host cells, damage host tissue, and interfere with the immune system [12]. Clinical samples included (23\48) (47.9%) samples taken from male and (25\48) (52.1%) samples taken from females Fig 2. This study's findings are consistent with those of Kirkuk University [13], who found that females were more than men were (52.9% vs. 47.1%).The possible reasons for high percentage in curruent study to female may be due to the types of populations studied, females may have routine indoor work and are often at risk of infection from flame, oil and hot water, the age ranged between from (2 to 75 years). All samples were collected from Governmental hospitals in Baghdad. This study agree with [14] collected and diagnosed as *P. aeruginosa (*26\68) (38.23%) from burn patients. This study disagree with [15] showed higher rates of infection with *P. aeruginosa* (91.6%).

Table 2: Numbre and percentage the bacteria in this study

Bacteria	No. of isolates	Percent %
P. aeruginosa	48	31.37
Klebsiella spp.	27	17.64
E. coli	24	15.68
Acintobacter spp.	8	5.22
Proteus spp.	5	3.26
No growth	41	26.83
Total	153	100

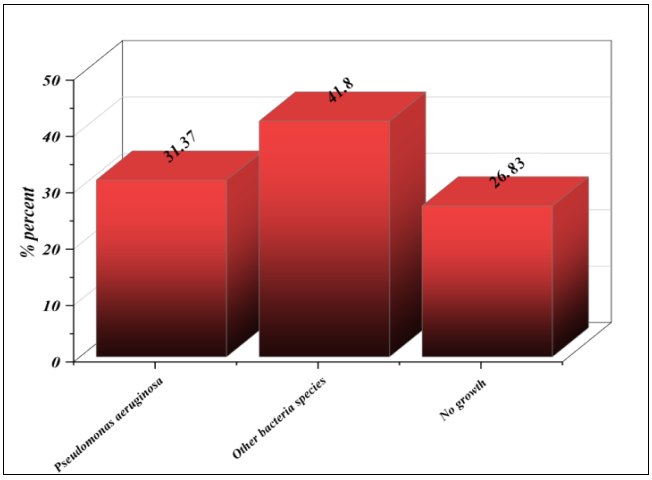

Fig 1: percentage of bacteria that isolated and identification from burns patients

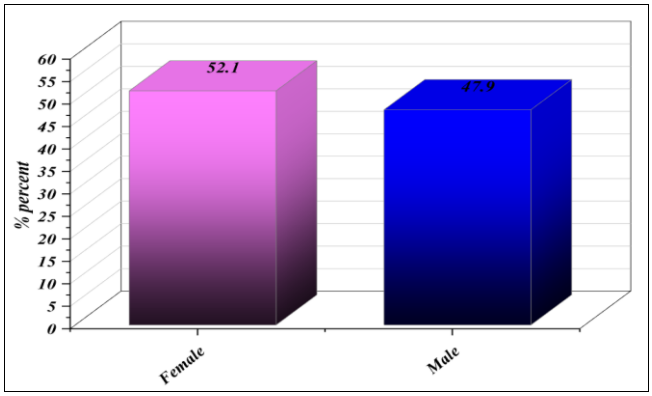

Fig 2: percentage of gender that isolated in this study

Identification of *P. Aeruginosa* Using PCR Technique

The species level identification of clinical isolates was confirmed by PCR by utilizing primers for the amplification of the *16S ribosomal RNA* gene specific for *p. aeruginosa.* The extracted DNA was subjected to polymerase chain reaction to amplify *16S rRNA* gene for (48) isolates in the present study, after PCR. As show Fig 3. (48) *P. aeruginosa* isolates were found to have been isolated from the burn, according to the study's findings, and all of them tested positive for the *16S rRNA* gene using PCR detection. The *16S rRNA* gene results were consistent with the findings of [16], who found 46 *P. aeruginosa* specimens from burn patients in the Kurdistan Region of Iraq. And in line with [17] research, *P. aeruginosa* isolated from burn patients in Basrah Governorate, Iraq, contained 100% of the current gene. Additionally, this research agrees with [9] discovery of the whole *p. aeruginosa* gene [18].

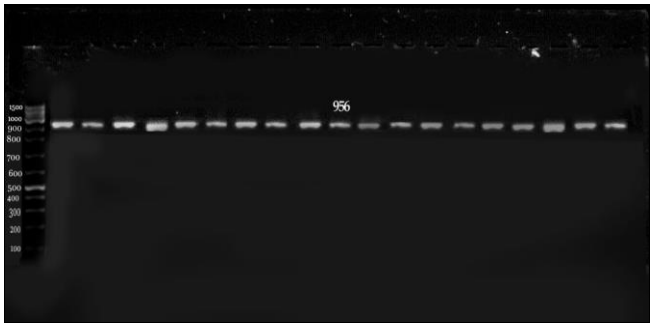

Fig 3: Electrophoresis diagram of PCR amplified products for extracted DNA of *p. aeruginosa* using *16SrRNA* gene primer show positive products at 956bp

Genotypic Detection Virulence Factor

The *AlgD* gene was identified by polymerase chain reaction using Master Mix, primer, and the reaction program mentioned in method show Table 1. *P. aeruginosa* isolates that produced alginate (34\48)(70.8) Fig 4 and isolates that did not create alginate (14\48)(29.2) were subjected to the polymerase chain reaction. The amplified gene *AlgD* isolates were electrophoresed, and the results revealed that all of the isolates had distinct bands at the same level. This study agree with [19]. Based on PCR amplification results, *AlgD* gene was present in 78.6% (88/112), also study of [20], found gene *AlgD* (85%) form total of 20 samples of *P. aeruginosa* obtained from a variety of locations in Baghdad hospitals isolated from burns wounds. High gene rate in this study because this gene encodes Alginate proteins, Alginate plays a very important role in the exopolysaccharides (EPS) matrix required for biofilm formation, especially in MRD bacteria that are isolated from burn patient. While *PslD* the reaction and electrophoresis results showed the most accurate band at 56°C. The polymerase chain reaction was used on (30\48) (62.5%) DNA samples from *P. aeruginosa* isolates that produced *pslD* Fig 5 and (18\48) (37.5%) samples that did not produce *pslD*. This study agree with [11] Based on PCR amplification results, *PslD* gene was present in (54.6%) *P. aeruginosa* isolates and [20] found gene *PslD* (75%) isolates *P. aeruginosa* form total of 20 samples obtained from a variety of locations in Baghdad hospitals isolated from burns wounds. The *PslD* protein is located in the periplasm/outer membrane and contributes to the export of

essential biofilm exopolysaccharides. For this reason, this gene is present in a high rate in this study [21].

The result of gel electrophoresis for amplification PCR for pyoviridine gene product showed that the presence of bands of the samples. That means the primers of this genes bind specifically to complement sequence within DNA template, responsible for the pigment formation of *P. aeruginosa*, allowing the pyoveridin gene to be used as *P. aeruginosa* detection gene. The prevalence of the pyoveridine gene in the target isolates positive results was (40\48) (83.34%) Fig 6 and negative results in the targt isolates for pyoverdine gene was (8\48) (16.66%) [9]. found (50%) produced pyoveridine gene in *p. aeruginosa* isolated from infected burn patients.

The results showed that *ExoA* gene was found in (35\48)(72.92%), isolates of *P. aeruginosa* as shown in Fig 7. (13\48) isolates of *P. aeruginosa* isolated don t have the *ExoA* gene The present study recorded (27,08%) of *P. aeruginosa* isolated, possess this gene. The study was agree with [22] indicated that the presence of the *ExoA* gene is (75%) of *P. aeruginosa* isolated from burn patients, and results was lower [23] who were detected the *ExoA* gene in (97%) of *P. aeruginosa* isolates. *ExoA* gene plays a significant role in the spread of *P. aeruginosa* within the burned and the emergence of endogenous septicemia. Moreover, this had a special role in regarding of wound healing [24].

Table 3: Number and percentage the molecular study

Gene	Positive	Percent %	Negative	Percent %
16S rRNA	**100**	**100**	**0**	**0**
AlgD	34	70.84	14	29.16
PslD	30	62.5	18	37.5
PVC	40	83.34	8	16.66
Exo A	35	72.92	13	27.08
BlaIMP	25	52.08	23	47.92
GyrA	35	72.92	113	27.08

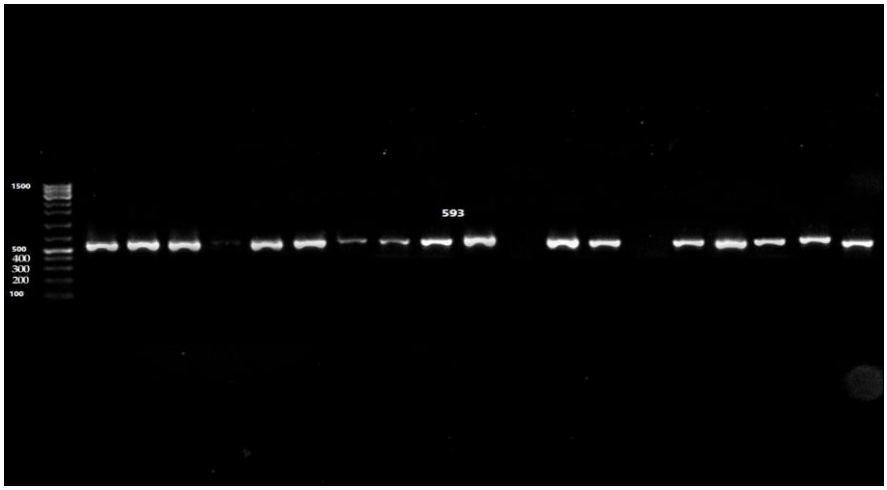

Fig 4: Electrophoresis diagram of PCR amplified products for extracted DNA of *p. aeruginosa* using specific *AlgD* gene primer show positive products at 593bp

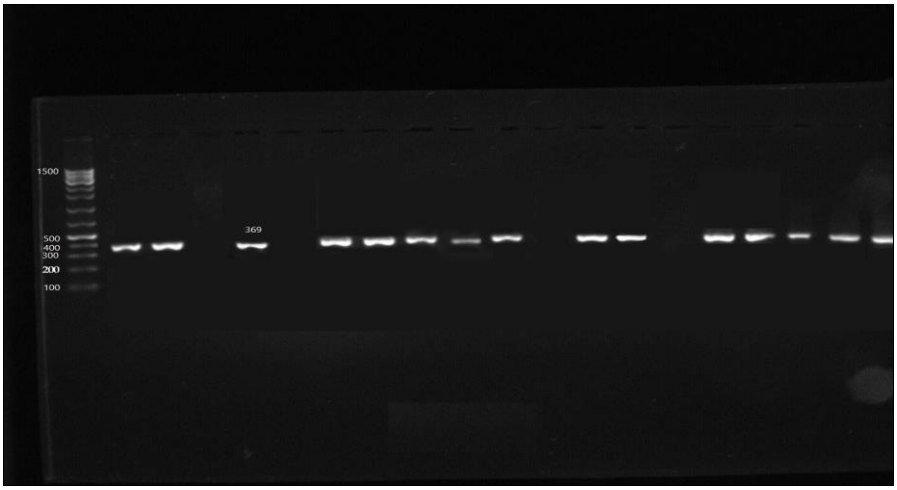

Fig 5: Electrophoresis diagram of PCR amplified products for extracted DNA of *p. aeruginosa* using specific *PslD* gene primer show positive products at 369bp

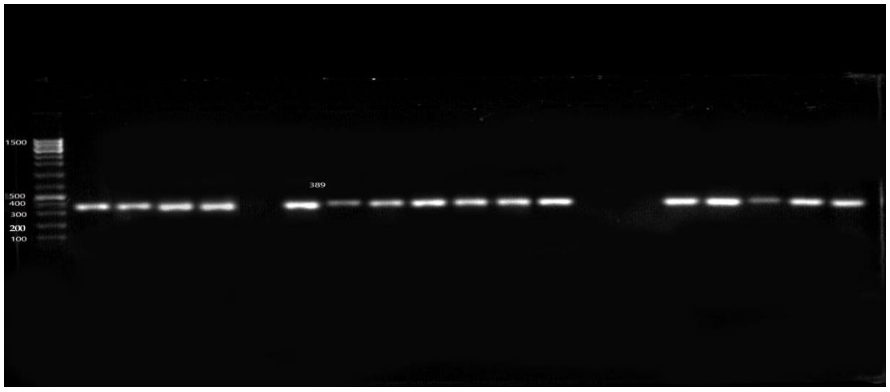

Fig 6: Electrophoresis diagram of PCR amplified products for extracted DNA of *p. aeruginosa* using specific *PVC* gene primer show positive products at 389bp

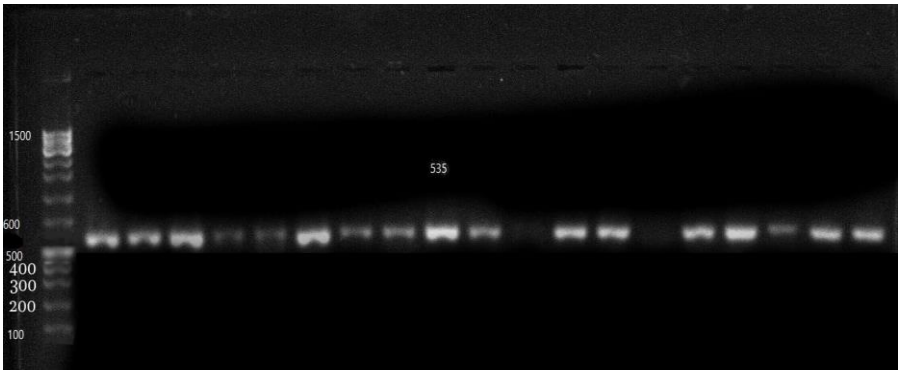

Fig 7: Electrophoresis diagram of PCR amplified products for extracted DNA of *p. aeruginosa* using specific *ExoA* gene primer show positive products at 535bp

References

1. Mabrouk MI, El-Hendawy HH, Basha AM, Saleh NM. Prevalence, antibiotic and oil resistance pattern of some bacterial isolates from burns. Journal of Applied Pharmaceutical Science, 2016, 123-130.
2. Hassan A, Nadhim F, Jawad N, Kandel APDM, Kadhim. Common bacterial types in burns in Burn ward of Nasiriyah, 2019.
3. Pachori P, Gothalwal R, Gandhi P. Emergence of antibiotic resistance Pseudomonas aeruginosa in intensive care unit; a critical review. Genes and diseases. 2019; 6(2):p109.
4. El-Mahdy R, El-Kannishy G. Virulence factors of carbapenemresistant *Pseudomonas aeruginosa* in hospital-acquired infections in Mansoura, Egypt. Infection and Drug Resistance. 2019; 12:3455-3461.
5. Seo J, Darwin AJ. The Pseudomonas aeruginosa periplasmic protease CtpA can affect systems that impact its ability to mount both acute and chronic infections. Infection and immunity. 2013; 81(12):4561-4570.
6. Moreau-Marquis S, Bomberger JM, Anderson GG, Swiatecka-Urban A, Ye S, O'Toole GA, Stanton BA. The {Delta} F508-CFTR mutation results in increased biofilm formation by *Pseudomonas aeruginosa* by increasing iron availability. Am J Physiol Lung Cell Mol Physiol. 2008; 295:L25-L37.
7. Yi H, Zhang L, Tuo Y, Han X, Du M. A novel method for rapid detection of class IIa bacteriocin-producing lactic acid bacteria. Food Control. 2010; 21(4):426-430.
8. Spilker T, Coenye T, Vandamme P, Lipuma JJ. PCR based assay for differentiation of Pseudomonas aeruginosa from other Pseudomonas species recovered from cystic fibrosis patients. J. Clin. Microbiol. 2004; 42(5):2074-2079.
9. AL-Jesmany, Eman Nassir Hussan, Oruba Khalid Abbas, Basima QASIM Hasan AlSaadi. Molecular technology for the detection of Pyoviridine gene in Pseudomonas aeruginosa isolated from burn cases. Mustansiriya Medical Journal. 2022; 21(1):p24.
10. Neamah AA. Molecular Detection of virulence factor genes in Pseudomonas aeruginosa isolated from human and animals in Diwaniya province. Kufa Journal for Veterinary Medical Sciences. 2017; 8(1):218-230.
11. Banar M, Emaneini M, Satarzadeh M, Abdellahi N, Beigverdi R, Leeuwen WBV, *et al*. Evaluation of mannosidase and trypsin enzymes effects on biofilm production of Pseudomonas aeruginosa isolated from burn wound infections. PloS one. 2016; 11(10):e0164622.
12. Baker P, Hill PJ, Snarr BD, Alnabelseya N, Pestrak MJ, Lee MJ, *et al*. Exopolysaccharide biosynthetic glycoside hydrolases can be utilized to disrupt and prevent Pseudomonas aeruginosa biofilms. Sci Adv. 2016; 2(5):1-10.
13. AL-Salihi SS, Hameed BH, Hameed BH. Antibiosis resistant of Pseudomonas aeruginosa isolated from different clinical specimens. Kirkuk University Journal for Scientific Studies. 2014; 9(2).
14. Mahdi, Raghad Jamil. Detection of some virulence factor of Pseudomonas aeruginosa isolated from Burn'Patients and their surrounding environment and the biological activity of some extracts on it. A thesis, College of Science, University of Basrah, 2020.
15. Abdullah ZH. Detection of Flagellin Gene (fli C) From Clinical and Environmental (watery) Pseudomonas

aeruginosa Isolates. (M. sc. thesis), 2012.
16. Merza NS, Hanoon RA, Khalid HM, Qader MK, Jubrael JMS. Molecular differentiation and determination of multi-drug resistant isolates of Pseudomonas species collected from burn patients in Kurdistan Region, Iraq, Zanco J. Med. Sci. Dec. 2018; 22(3).
17. Jalil MB. Isolation and Bacteriophages against MDR Pseudomonas aeruginosa Isolated from Burn Patients in Basrah Governorate-Iraq. Ph.D. Thesis, Biology, College of Science. University of Basrah, 2018, 128p
18. Abdullah RM, Mehdi AF. Identification of Pseudomonas aeruginosa From Clinical Specimen by Using 16S rDNA Gene. J Biotechnol Res Cent. 2016; 10(1):45-49.
19. Rajabi H, Salimizand H, Khodabandehloo M, Fayyazi A, Ramazanzadeh R. Prevalence of AlgD, *pslD*, pelF, Ppgl, and PAPI-1 Genes involved in biofilm formation in clinical Pseudomonas aeruginosa strains. BioMed research international, 2022.
20. Almzil, Noor Riyadh Hamoodah, Yaşar Kemal Yazgan, and Mohammed F, Al Marjani. Evaluation of Biofilm Formation in Pseudomonas Aeruginosa Isolated from Clinical Samples and the Presence of Biofilm-Related Genes (pelA, *pslD* AND AlgD). European Journal of Molecular & Clinical Medicine. 2022; 9(7).
21. Yakout, Marwa A, Ibrahim A, Abdelwahab. Diabetic Foot Ulcer Infections and Pseudomonas aeruginosa Biofilm Production during the COVID-19 Pandemic. Journal of Pure and Applied Microbiology. 2022; 16(1):138-147.
22. Corehtash ZG, Ahmad Khorshidi FF, Akbari H, Aznaveh AM. Biofilm formation and virulence factors among Pseudomonas aeruginosa isolated from burn patients. Jundishapur Journal of Microbiology. 2015; 8(10).
23. Khan AA, Cerniglia CE. Detection of Pseudomonas aeruginosa from clinical and environmental samples by amplification of the exotoxin a gene using PCR. Applied and Environmental Microbiology. 1994; 60(10):3739-3745.
24. El-Din AB, EL-Nagdy MA, Badr RAWIA, EL-Sabagh AM. Pseudomonas aeruginosa exotoxin A: Its role in burn wound infection and wound healing. Egypt Journal Plast Reconstr Surg. 2008; 32:59-65.