Original Article | Iran J Med Microbiol. 2022; 16(6): 520-527

10.30699/ijmm.16.6.520

Iranian Journal of Medical Microbiology | ISSN:2345-4342

Type III Secretion System (Exoenzymes) as a Virulence Determinant in *Pseudomonas aeruginosa* Isolated from Burn Patients in Mansoura University Hospitals, Egypt

Rasha Mokhtar Elnagar[1*], Mohammed Elshaer[2], Omar Osama Shouman[3], Samah Sabry El-Kazzaz[1]

1. *Department of Medical Microbiology and Immunology, Faculty of Medicine, Mansoura University, Mansoura, Egypt*
2. *Department of Clinical Pathology, Faculty of Medicine, Mansoura University, Mansoura, Egypt*
3. *Department of Plastic and Reconstructive Surgery, Faculty of Medicine, Mansoura University, Mansoura, Egypt*

ABSTRACT

Background and Aim: *Pseudomonas aeruginosa (P. aeruginosa)* is an important causative organism of burn infection. Several virulence factors are implicated in *P. aeruginosa* colonization and invasion, making *P. aeruginosa* infection's outcome worse. Type III secretion system (T3SS) effector proteins are among these virulence factors. The present study evaluated the frequency of genes encoding T3SS effectors as a virulence determinant in *P. aeruginosa* isolates from burn patients.

Materials and Methods: Wound swabs were collected from burn patients admitted to the Plastic and Reconstructive Surgery Center, Mansoura University, Egypt, and identified by different microbiological testing methods. The modified Kirby Bauer's disc diffusion method was used to test the antibiotic susceptibility of P. aeruginosa isolates against different antibiotics. Prevalence and the presence of exo genes that encode type T3SS proteins (*exoS*, *exoT*, *exoU,* and *exoY*) in *P. aeruginosa* isolates were evaluated by the multiplex PCR. Chi-square and Fisher's test were used for statistical analysis.

Results: A total of 45 *P. aeruginosa* isolates were identified from 101 burn patients, including 27 males and 18 females, with a mean age of 15.78±2.65 years old. *P. aeruginosa* isolates were mostly susceptible to piperacillin/tazobactam and imipenem (73.33 and 62.22%), respectively; while the lowest susceptibility rates were in ceftazidime (4.44%), Tobramycin (4.44%), and ceftriaxone (6.67%). The *exoY* and *exoT* genes were detected in 100% of the *P. aeruginosa* isolates, while 62.22% and 42.22% of clinical isolates harbored *exoS* and *exoU* genes, respectively.

Conclusion: This study established a correlation between T3SS proteins, particularly *exoS* and *exoU* genes and antimicrobial resistance in *P. aeruginosa* isolates from burn infection.

Keywords: Type III secretion system, *Pseudomonas aeruginosa*, Antimicrobial resistance, Burn infection

Received: 2022/05/16; **Accepted**: 2022/08/08; **Published Online:** 2022/09/09

Corresponding Information: **Rasha Mokhtar Elnagar**, *Department of Medical Microbiology and Immunology, Faculty of Medicine, Mansoura University, Mansoura, Egypt* Email: drrasha_m@mans.edu.eg

1. Introduction

Pseudomonas aeruginosa is an opportunistic Gram-negative bacterium implicated in serious Healthcare-Associated Infections (HAIs), especially in burn and chronic pulmonary infections **(1)**. Many sources within healthcare facilities are considered reservoirs of *P. aeruginosa* isolates, including taps, showers, drains and sink traps. Also, Cross infections of *P. aeruginosa* may occur from other patients, hands of healthcare workers and contaminated medical devices **(2)**.

Several virulence factors are implicated in *P. aeruginosa* colonization, invasion, and dissemination. These factors include lipopolysaccharides, adhesion factor (pili type IV), flagella, phospholipase C, exotoxins "Exo A", exoenzymes (exo), sialidase

adherence factor, as well as exo-proteases that been involved in tissue destruction as (alkaline protease "Apr A", proteases like elastase, staphylolysin and protease IV). In addition to the ability of *P. aeruginosa* to produce biofilms **(3)**. With the presence of these virulence factors, morbidity and mortality due to *P. aeruginosa* infection markedly increased **(4)**.

The virulence determinant involved in *P. aeruginosa* pathogenicity and antimicrobial resistance that results in the poor clinical outcome of *P. aeruginosa* infection is type III secretion system (T3SS) effector proteins **(5)**.

So far, four effectors' proteins belonging to the T3SS have been reported, including two exoenzymes with ADP-ribosyltransferase (ADPRT) and GTPase activities as exoenzyme S (ExoS) and exoenzyme T (ExoT), and other two exoenzymes with cytolytic and adenylate cyclase activity as exoenzyme U (ExoU) and exoenzyme Y (ExoY) respectively. In addition, ExoU has a phospholipase A2-like activity **(6)**. These exoenzymes help *P. aeruginosa* evade the host immune system and cell apoptosis by modulating the host inflammatory response; also, it may damage physical barriers such as actin cytoskeleton and endothelial barriers. This results in major tissue destruction, worsens *P. aeruginosa* burn infection, and hinders wound healing **(7)**.

Previous studies reported that all clinical *P. aeruginosa* isolates possess ExoY and ExoT effector proteins, while few isolates can express either ExoS or ExoU proteins **(8)**.

Due to the lack of studies that evaluate the frequency of T3SS proteins among resistant *P. aeruginosa* isolates in Egypt, particularly those isolated from burn infection, this work aims to assess the proportion of genes encoding T3SS effector proteins among burn patients with *P. aeruginosa* wound infection.

2. Materials and Methods

Study Design

This descriptive cross-sectional study was conducted at the Bacteriology Lab, Microbiology Diagnostics, and Infection Control Unit (MDICU), Department of Medical Microbiology and Immunology, Faculty of Medicine, Mansoura University, Dakahliya, Egypt, during the period from May 2020 to October 2021.

Sample Collection and Bacterial Isolation

Burn wound specimens were collected from patients hospitalized in the Burn unit of Plastic and Reconstructive Surgery Center, Mansoura University, Egypt. The wound swabs specimens were taken from all patients showing signs and symptoms of burn infection, using a sterile cotton swab moistened with sterile physiological saline. They were placed into Stuart's transport medium tubes (Oxoid, UK) and transported to the laboratory within 2h after collection; if a delay in transporting the samples to the laboratory is expected, they were kept in the refrigerator at 4°C **(9)**.

Swabs were streaked on suitable culture media as nutrient agar, blood agar, and MacConkey's agar plates (Oxoid, UK) and incubated for 24-48 h at 37˚C. Colonies of *P. aeruginosa* were identified by Gram staining, growth at 42°C, exopigment production on a nutrient agar plate, and different biochemical reactions as oxidase test using oxidase detection strips (Oxoid, UK), Kligler iron agar (KIA) test, Lysine iron agar (LIA) test, Motility Indole Ornithine (MIO) test, and Citrate utilization test (Oxoid, UK) **(10)**. Isolates of *P. aeruginosa* were kept at -80°C in tryptic soy broth (TSB) (Oxoid, UK) containing 30% glycerol for further study; reference strain *P. aeruginosa* (ATCC 27853) obtained from NAMRU-3 Institute (Naval Medical Research Unit Three), Cairo, Egypt was used as control.

Antimicrobial Susceptibility Testing

Modified Kirby Bauer's disc diffusion technique was used to detect the susceptibility of a group of antibiotics on Mueller-Hinton agar plates (Oxoid, UK) according to the guidelines adopted by the clinical and laboratory standards institute (CLSI) **(11)**. The following antibiotics were used: Ampicillin/sulbactam (SAM) (10/10 µg), Piperacillin/tazobactam (TPZ) (100/10 µg), Cefotaxime (CTX) (30 µg), Ceftazidime (CAZ) (30 µg), Ceftriaxone (CRO) (30 µg), Cefepime (FEP) (30 µg), Azteronam (ATM) (30 µg), Gentamicin (CN) (10 µg), Amikacin (AK) (30 µg), Tobramycin (10 µg), Ciprofloxacin (CIP) (5 µg), and Imipenem (IMP) (10 µg) (Oxoid, UK). Multi-drug resistant (MDR) was defined as non-susceptible (including intermediate or resistant) to at least one agent in ≥ three antimicrobial categories based on previous definitions **(12)**.

Molecular Detection of T3SS Effector Proteins Genes

The DNA was extracted from the overnight *P. aeruginosa* cultures using (QIA amp® DNA mini kit, Qiagen Inc.) according to the instructions by the manufacturer. Using the multiplex PCR technique, detection of the genes encoding for T3SS effector proteins (*exoS*, *exoT*, *exoU* and *exoY*) was done with a set of specific oligonucleotide primers obtained from Sigma, Aldrich, Germany (Table 1) **(13)**.

Table 1. List of primers used in the present study and length of the PCR products

Target genes	Sequences	Amplicon length (bp)	Reference
exoS	F5'-GCG AGG TCA GCA GAG TAT CG-3' R5'-TTC GGC GTC ACT GTG GAT GC-3	118	(13)
exoT	F5'-AAT CGC CGT CCA ACT GCA TGC G-3' R5'-TGT TCG CCG AGG TAC TGC TC-3'	152	
exoU	F5'-CCGTTG TGG TGCCGT TGA AG-3' R5'-CCA GAT GTT CAC CGA CTC GC-3'	134	
exoY	F5'-CGG ATT CTA TGG CAG GGA GG-3' R5'-GCC CTT GAT GCA CTC GAC CA-3'	289	

DNA amplification was performed in a 50 µL reaction mix containing 25 µL of 2X GoTaq Green Master Mix (Willowfort, UK), 5 µL of genomic DNA, 2 µL from each of the forward and reverse primer of four genes (40 nmol), and DNase free water. The following PCR cycling conditions were used: initial denaturation at 95ºC for 3min; followed by 36 cycles of denaturation at 95ºC for 1min, annealing at 58ºC for 40 s, and extension at 72ºC for 1 min; finally, a single extension step at 72ºC for 10 min. The amplified PCR products were visualized by 2% agarose gel electrophoresis stained with ethidium bromide based on fragment size as compared with the 100bp DNA marker (Lonza Rockland. Inc, USA) using UV transilluminator (FBTIV-88, Fisher, USA) **(14)**.

Data Analysis

Data were tabulated and statistically analyzed using Microsoft Excel 2010 and Statistical Package of Social Science (SPSS) version 23 (SPSS Inc., Chicago, IL, USA) software. Categorical data were presented as numbers and percentages. Quantitative variables were expressed as the mean ± standard deviation (SD). Categorical data were analyzed using the Chi-square and Fisher's test. The *P* value of 0.05 or less was considered statistically significant.

Ethical approval statements

This study obtained approval from the Mansoura University Institutional Review Board (R.21.09.1459). Informed written consent was obtained from each patient. All methods were done according to the Helsinki declarations.

3. Results

Forty-five *P. aeruginosa* isolates were obtained from non-repetitive one hundred and one patients' wound specimens included in this study. Of these 45 *P. aeruginosa* isolates, 27 (60.00%) were recovered from males and 18 (40.00%) from females with a mean age of 15.78±2.65 years old, ranging from 1 to 65 years old. About 31 (68.89%) of *P. aeruginosa* infected patients had a white blood cell count of more than 11000/mL. Meanwhile, 6 (13.33%) patients had a superficial second-degree burn, 25 (55.56%) patients had a deep second-degree burn, and 14 (31.11%) patients had a third-degree burn (Table 2).

Results of the antimicrobial susceptibility of tested *P. aeruginosa* isolates revealed that piperacillin/tazobactam 33 (73.33%) and imipenem 28 (62.22%) were the most susceptible agents; in comparison with the lowest susceptibility rates in ceftazidime 2 (4.44%), tobramycin 2 (4.44%), and ceftriaxone 3 (6.67%). Meanwhile, the sensitivity of ampicillin/sulbactam, ciprofloxacin, and amikacin was recorded in 10 (22.22%), 9 (20.00%), and 9 (20.00%) isolates, respectively. Of the 45 tested *P. aeruginosa* isolates, 23 (51.11%) isolates were MDR. The antibiotic susceptibility profiles for *P. aeruginosa* strains are illustrated in Table 3.

Table 2. Demographic and clinical characteristics of *Pseudomonas aeruginosa* infected patients

Demographic and clinical characteristics	Total no. of *P. aeruginosa* infected patients =45	
	No	%
Male	27	60.00
Female	18	40.00
Age	15.78 ± 2.65 years, range (1 – 65)	
Site of burn:		
Upper limb	28	62.22
Lower limb	5	11.11
Head	3	6.67
Face	3	6.67
Chest	4	8.89

Demographic and clinical characteristics	Total no. of *P. aeruginosa* infected patients =45	
	No	%
Abdomen	2	4.44
Burn degree:		
Superficial second	6	13.33
Deep second	25	55.56
Third	14	31.11
White blood cell count >11000 /mL	31	68.89
Cause of burn:		
Hot water	33	73.33
Flame	9	20.00
Chemicals	3	6.67

Table 3. Antibiotic susceptibility profile for *Pseudomonas aeruginosa* isolates

Class	Antimicrobial agent	Total *P. aeruginosa* isolates (no = 45)					
		Sensitive		Intermediate		Resistant	
		No.	%	No.	%	No.	%
β-lactam+inhibitors	Pipracillin/tazobctam	33	73.33	5	11.11	7	15.56
	Ampicillin/sulbactam	10	22.22	10	22.22	25	55.56
Carbapenems	Imipenem	28	62.22	3	6.67	14	31.11
Monobactam	Azteronam	11	24.44	7	15.56	27	60.00
Aminoglycosides	Amikacin	9	20.00	13	28.89	23	51.11
	Gentamicin	5	11.11	8	17.78	32	71.11
	Tobramycin	2	4.44	9	20.00	34	75.56
Fluoroquinolones	Ciprofloxacin	9	20.00	6	13.33	30	66.67
Cephalosporins	Cefepime	7	15.56	5	11.11	33	73.33
	Ceftazidime	2	4.44	4	8.89	39	86.67
	Cefotaxime	5	11.11	0	0.00	40	88.89
	Ceftriaxone	3	6.67	0	0.00	42	93.33

All tested *P. aeruginosa* isolates expressed the *exoY* and *exoT* genes (100%), while 28 (62.22%) and 19 (42.22%) of the clinical isolates harbored *exoS* and *exoU* genes, respectively. The displaying of *exoS* and *exoU* genes was significantly associated with higher level of antibiotic resistance to fluoroquinolones, aminoglycosides, cephalosporins and MDR rate; meanwhile, there was no significant correlation between resistance to β-lactam+inhibitors, carbapenems, and monobactam and presence of *exoS* and *exoU* genes. On analyzing the resistance pattern to specific antimicrobial agents, the isolates harboring *exoS* and *exoU* genes were associated with increased levels of resistance to ciprofloxacin (P=0.001, 0.002); cefepime (P=0.001, 0.002); ceftazidime (P=0.063, 0.014); ceftriaxone (P=0.021, 0.008); cefotaxime (P=0.002, 0.043); as well as amikacin (P=0.006); gentamicin (P=0.002, 0.043) and tobramycin (P=0.021, 0.043). Harboring *exoS* and *exoU* genotypes was also associated with MDR (P= 0.001, 0.024) strains (Table 4).

Table 4. Correlation of *exoS* and *exoU* virulence genes with antibiotic resistance pattern

Class	Antimicrobial agent	Total *P. aeruginosa* isolates (no = 45)					
		exoS + (n= 28)	*exoS* – (n= 17)	*P* value	*exoU* + (n= 19)	*exoU* – (n= 26)	*P* value
β-lactam+inhibitors	Pipracillin/tazobctam (n=7)	6	1	0.195	5	2	0.670
	Ampicillin/sulbactam (n=25)	23	2	0.911	16	9	0.248
Carbapenems	Imipenem (n=14)	13	1	0.616	11	3	0.787
Monobactam	Azteronam (n=27)	25	2	0.187	20	7	0.572
Aminoglycosides	Amikacin (n=23)	23	0	0.006*	21	2	0.006*
	Gentamicin (n=32)	31	1	0.002*	26	6	0.043*
	Tobramycin (n=34)	32	2	0.021*	29	5	0.043*
Fluoroquinolones	Ciprofloxacin (n=30)	29	1	0.001**	27	3	0.002*
Cephalosporins	Cefepime (n=33)	32	1	0.001**	28	5	0.002*
	Ceftazidime (n=39)	37	2	0.063	29	10	0.014*
	Cefotaxime (n=40)	36	4	0.002*	29	11	0.043*
	Ceftriaxone (n=42)	38	4	0.021*	30	12	0.008*
MDR (n=23)		21	2	0.001**	19	4	0.024*

MDR: Multi drug-resistant; *significant at the $P \leq 0.05$; **highly significant at the $P \leq 0.01$.

4. Discussion

Pseudomonas aeruginosa is a significant organism with a complex structure that enhances the excretion of virulence factors in the cytoplasm of target cells by a type III secretion system mediated by cell contact. These virulence determinants are usually associated with higher mortality outcomes in patients infected with those isolates, particularly burn patients **(15)**.

In the current research, *P. aeruginosa* was detected in 45 patients among the studied 101 patients with burn infection with an infection rate of 44.6%. This means that *P. aeruginosa* represents nearly fifty percent of the infectious bacteria that could cause wound infection in our locality. The isolation rate of *P. aeruginosa* was previously found to be higher among samples that were taken from cases with respiratory system and urinary tract infections than those with burn infections. This was recorded in an Egyptian study conducted in 2012 **(16)**. On the other hand, the present results were parallel to that observed in another study by Saleh et al., as 55% of their isolates were obtained from burn specimens **(17)**.

The increasing rate of burn infection by *P. aeruginosa* was recorded in previous studies **(18, 19)**. This encourages the need to test those resistant isolates for the presence of different virulence factors, mostly the type III secretion system examined in the present research.

Most of the examined isolates observed an elevated pattern of antibiotic resistance. 51.11% of them were recorded as MDR. A higher frequency of antimicrobial resistance was observed in *P. aeruginosa* isolated from burn in previous research performed on patients in Iran (93.1%) **(19)**. This prototype of resistance to antimicrobials was a common observation, particularly for burn-recovered strains of *P. aeruginosa* in several studies **(7, 20, 21)**. Contrary to the current results, a lower percentage of MDR *P. aeruginosa* was observed in a late study conducted in 2020 (40% only) **(13)**. The higher antibiotic resistance in the studied strains, particularly in our locality and the previously mentioned areas, is usually supported by the heavy use of antibiotics which is usually prescribed without correct antibiotic sensitivity testing, together with the natural barriers of *pseudomonas* bacteria itself.

Fortunately, piperacillin/tazobactam was the most effective antibiotic against the examined *P. aeruginosa* isolates (73.33% sensitivity) followed by imipenem (62.22%), contrary to the present results, Jarees et al. **(13)** observed that 100% of the examined *P. aeruginosa* were found to be resistant to piperacillin/tazobactam. However, only 6% of them were found to be resistant to amikacin and ciprofloxacin, which also matches the previously documented results of Khodayary et al. **(6)**. This is found to be against the results observed in the present research as only 20% of the examined isolates recorded sensitivity for amikacin and ciprofloxacin.

The apparent disparity in the pattern of sensitivity of *P. aeruginosa* isolates among different studies is

usually supported by the variation in the antibiotic policy among different localities that allows the gaining of variable determinants of antimicrobial resistance in *P. aeruginosa* species.

The major target of the present research was to examine the burn *P. aeruginosa* isolates for presence of protein encoding T3SS, one hundred percent of the tested *P. aeruginosa* were found to harbor *exoY* and *exoT* genes, this seems to be expected in our locality as *P. aeruginosa* isolated from different infection sites particularly burn is usually exhibited high level of resistance, both genes were more frequently detected than *exoU* and *exoS*. Lower frequency of *exoY* and *exoT* genes among *P. aeruginosa* isolates was documented in previous study (60 and 85% respectively) **(17)**.

The present results run in parallel with that of Adwan et al. and also, they confirmed the presence of *exoT* genes in 100% of their isolates; however, *exoY* gene was detected only in 72.2% of *P. aeruginosa* isolates, and neither *exoS* nor *exoU* was detected in *P. aeruginosa* isolated in that research **(7)**.

Also, another study agreed with the present study's results and reported the high frequency of the *exoT* and *exoY* genes (100%) of the isolates **(22)**.

ExoY and *exoT* genes seems to be the most frequently existing genes of T3SS in *P. aeruginosa* as observed in the present study and other studies that confirm the same results **(6, 23)**.

The current data showed that *exoS* was found to be more prevalent than *exoU*, this agreed to other results which documented that *exoS* is more prevalent compared to *exoU* **(24-27)** and differed with another study that concluded that *exoU* genotype was more prevalent than *exoS* genotypes in clinical *P. aeruginosa* isolates **(28)**. On the other hand, the co-occurrence of *exoU* and *exoS* genes was significantly associated with higher antibiotic resistance to fluoroquinolones, aminoglycosides, and cephalosporins. On the other hand, although it is not significant, the *P. aeruginosa* isolates displaying *exoU* and *exoS* genes were more non-susceptible to β-lactam+inhibitors, carbapenems and monobactam. These results were aligned with a study by Horna et al., as they also report the significant association of resistance to fluoroquinolones and aminoglycosides with the expression of *exoU* and *exoS* genotypes **(22)**. However, they differed that there no significant correlation between *exoU* and *exoS* genotypes and resistance to cephalosporins and carbapenems. Other studies established the significant interrelation between decreased susceptibility to fluoroquinolones and aminoglycosides in *P. aeruginosa* isolates displaying *exoU* genotype **(6, 29)**, contrasting with another study that concluded *exoS* genotype was mostly associated with a higher proportion of MDR among *P. aeruginosa* strains from burn patients **(30)**.

The present research faced some limitations like the small sample size; the current data should be confirmed by a higher number of *P. aeruginosa* isolated from infection sites other than burn.

5. Conclusion

This work established the high proportion of T3SS gene expression among non-susceptible *P. aeruginosa* isolates recovered from patients with burn wound infection. Also, the presence of *exoS* and *exoU* genotypes may indicate increased morbidity and mortality due to *P. aeruginosa* infection in those patients.

Acknowledgment

We would like to thank all the patients who participated in this study.

Authors' contributions

Rasha Mokhtar Elnagar and Samah Sabry El-Kazzaz designed and carried out the study. Rasha Mokhtar Elnagar performed all microbiological and molecular laboratory work, analyzed, and interpreted all data, and wrote the manuscript. Samah Sabry El-Kazzaz contributed to a critical review of the manuscript. Mohammed Elshaer and Omar Osama Shouman collected clinical samples and patients' data. All authors revised and approved the final version of the manuscript.

Funding

The authors received no financial support for this article.

Conflict of Interest

The authors declare no conflict of interest.

Reference

1. Hasannejad-Bibalan M, Jafari A, Sabati H, Goswami R, Jafaryparvar Z, Sedaghat F, et al. Risk of type III secretion systems in burn patients with Pseudomonas aeruginosa wound infection: A systematic review and meta-analysis. Burns. 2021;47(3):538-44. [DOI:10.1016/j.burns.2020.04.024] [PMID]
2. Quick J, Cumley N, Wearn CM, Niebel M, Constantinidou C, Thomas CM, et al. Seeking the source of Pseudomonas aeruginosa infections in

a recently opened hospital: an observational study using whole-genome sequencing. BMJ open. 2014;4(11):e006278. [PMID] [PMCID] [DOI:10.1136/bmjopen-2014-006278]

3. Benie CKD, Dadié A, Guessennd N, N'gbesso-Kouadio NA, Kouame NzD, N'golo DC, et al. Characterization of virulence potential of Pseudomonas aeruginosa isolated from bovine meat, fresh fish, and smoked fish. Eur J Microbiol Immunol. 2017;7(1):55-64. [PMID] [PMCID] [DOI:10.1556/1886.2016.00039]
4. Hassuna NA, Mandour SA, Mohamed ES. Virulence constitution of multi-drug-resistant Pseudomonas aeruginosa in Upper Egypt. Infect Drug Resist. 2020;13:587. [DOI:10.2147/IDR.S233694] [PMID] [PMCID]
5. Emami A, Kazempour A, Pirbonyeh N, Keshavarzi A, Zardosht M. Hospitalization length survey and relation with distribution of LasA protease and type III secretion system encoding-genes in multi-drug resistant Pseudomonas aeruginosa isolates from burn wounds in southwest of Iran. Gene Rep. 2017;9:81-5. [DOI:10.1016/j.genrep.2017.09.006]
6. Khodayary R, Nikokar I, Mobayen MR, Afrasiabi F, Araghian A, Elmi A, et al. High incidence of type III secretion system associated virulence factors (exoenzymes) in Pseudomonas aeruginosa isolated from Iranian burn patients. BMC Res Notes. 2019;12(1):1-6. [DOI:10.1186/s13104-019-4071-0] [PMID] [PMCID]
7. Adwan G. Detection of Type III secretion toxins encoding-genes of Pseudomonas aeruginosa isolates in the West Bank-Palestine. J Adv Biol Biotechnol. 2017;11(3):1-10. [DOI:10.9734/JABB/2017/31319]
8. Gawish AA, Mohamed NA, El-Shennawy GA, Mohamed HA. An investigation of type 3 secretion toxins encoding-genes of Pseudomonas aeruginosa isolates in a University Hospital in Egypt. J Microbiol Infect Dis. 2013; 3(03):116-22. [DOI:10.5799/ahinjs.02.2013.03.0093]
9. Kallstrom G. Are quantitative bacterial wound cultures useful? J clin microbiol. 2014;52(8): 2753-6. [DOI:10.1128/JCM.00522-14] [PMID] [PMCID]
10. Mahon CR, Lehman DC, Manuselis G. Textbook of diagnostic microbiology-e-book: Elsevier Health Sciences; 2018.
11. Institute CaLS. Performance standards for antimicrobial susceptibility testing: Twenty-seven informational supplements; document (M100-S27): Wayne, PA: CLSI; 2017.
12. Magiorakos AP, Srinivasan A, Carey RB, Carmeli Y, Falagas ME, Giske CG, et al. Multidrug-resistant, extensively drug-resistant and pandrug-resistant bacteria: an international expert proposal for interim standard definitions for acquired resistance. Clin Microbiol Infect. 2012;18(3):268-81. [DOI:10.1111/j.1469-0691.2011.03570.x] [PMID]
13. Jarjees KK. Molecular detection of type III secretory toxins in Pseudomonas aeruginosa isolates. Cell Mol Biol. 2020;66(5):9-14. [DOI:10.14715/cmb/2020.66.5.2] [PMID]
14. Ajayi T, Allmond LR, Sawa T, Wiener-Kronish JP. Single-nucleotide-polymorphism mapping of the Pseudomonas aeruginosa type III secretion toxins for development of a diagnostic multiplex PCR system. J Clinical Microbiology. 2003;41(8): 3526-31. [DOI:10.1128/JCM.41.8.3526-3531.2003] [PMID] [PMCID]
15. Roy-Burman A, Savel RH, Racine S, Swanson BL, Revadigar NS, Fujimoto J, et al. Type III protein secretion is associated with death in lower respiratory and systemic Pseudomonas aeruginosa infections. J infect dis. 2001;183(12): 1767-74. [DOI:10.1086/320737] [PMID]
16. Eid D, En W, Barwa R, El-Sokkary MA. Phenotypic and genotypic characterization of some virulence factors in Pseudomonas aeruginosa strains isolated from different clinical sources in Mansoura University Hospitals. New Egypt J Microbiol. 2012;32(2):151-67.
17. Saleh RH, Naher HS, Al-Saadi MAK. Molecular Investigation of Type III Secretion System Toxins-Encoding Genes in Clinical Isolates of Pseudomonas aeruginosa. Med J Babylon. 2012;9(4):857-66.
18. Church D, Elsayed S, Reid O, Winston B, Lindsay R. Burn Wound Infections. Clin Microbiol Rev. 2006;19(2):403-34. [PMID] [PMCID] [DOI:10.1128/CMR.19.2.403-434.2006]
19. Ghanbarzadeh Corehtash Z, Khorshidi A, Firoozeh F, Akbari H, Mahmoudi Aznaveh A. Biofilm Formation and Virulence Factors Among Pseudomonas aeruginosa Isolated From Burn Patients. Jundishapur J Microbiol. 2015;8(10): e22345. [DOI:10.5812/jjm.22345] [PMID] [PMCID]
20. Shahcheraghi F, Feizabadi MM, Yamin V, Abiri R, Abedian Z. Serovar determination, drug resistance patterns and plasmid profiles of Pseudomonas aeruginosa isolated from burn

patients at two hospitals of Tehran (IRAN). Burns. 2003;29(6):547-51. [DOI:10.1016/S0305-4179(03)00142-6]

21. Nikbin VS, Abdi-Ali A, Feizabadi MM, Gharavi S. Pulsed field gel electrophoresis & plasmid profile of Pseudomonas aeruginosa at two hospitals in Tehran, Iran. Indian J Med Res. 2007;126(2):146-52.
22. Horna G, Amaro C, Palacios A, Guerra H, Ruiz J. High frequency of the exoU+/exoS+ genotype associated with multidrug-resistant "high-risk clones" of Pseudomonas aeruginosa clinical isolates from Peruvian hospitals. Sci Rep. 2019;9(1):1-13. [DOI:10.1038/s41598-019-47303-4] [PMID] [PMCID]
23. Finck-Barbançon V, Goranson J, Zhu L, Sawa T, Wiener-Kronish JP, Fleiszig SM, et al. ExoU expression by Pseudomonas aeruginosa correlates with acute cytotoxicity and epithelial injury. Mol Microbiol. 1997;25(3):547-57. [PMID] [DOI:10.1046/j.1365-2958.1997.4891851.x]
24. Feltman H, Schulert G, Khan S, Jain M, Peterson L, Hauser AR. Prevalence of type III secretion genes in clinical and environmental isolates of Pseudomonas aeruginosa. Microbiology. 2001; 147(Pt 10):2659-69. [DOI:10.1099/00221287-147-10-2659] [PMID]
25. Georgescu M, Gheorghe I, Curutiu C, Lazar V, Bleotu C, Chifiriuc M. Virulence and resistance features of Pseudomonas aeruginosa strains isolated from chronic leg ulcers. BMC Infect Dis. 2016;16(1):92. [DOI:10.1186/s12879-016-1396-3] [PMID] [PMCID]
26. Mitov I, Strateva T, Markova B. Prevalence of virulence genes among bulgarian nosocomial and cystic fibrosis isolates of pseudomonas aeruginosa. Braz J Microbiol. 2010;41(3):588-95. [DOI:10.1590/S1517-83822010000300008] [PMID] [PMCID]
27. Fazeli N, Momtaz H. Virulence Gene Profiles of Multidrug-Resistant Pseudomonas aeruginosa Isolated From Iranian Hospital Infections. Iran Red Crescent Med J. 2014;16(10). [DOI:10.5812/ircmj.15722] [PMID] [PMCID]
28. Azimi S, Kafil HS, Baghi HB, Shokrian S, Najaf K, Asgharzadeh M, et al. Presence of exoY, exoS, exoU and exoT genes, antibiotic resistance and biofilm production among Pseudomonas aeruginosa isolates in Northwest Iran. GMS Hyg Infect Control. 2016;11.
29. Park M-H, Kim SY, Roh EY, Lee HS. Difference of Type 3 secretion system (T3SS) effector gene genotypes (exoU and exoS) and its implication to antibiotics resistances in isolates of Pseudomonas aeruginosa from chronic otitis media. Auris Nasus Larynx. 2017;44(3):258-65. [DOI:10.1016/j.anl.2016.07.005] [PMID]
30. Khosravi AD, Shafie F, Montazeri EA, Rostami S. The frequency of genes encoding exotoxin A and exoenzyme S in Pseudomonas aeruginosa strains isolated from burn patients. Burns. 2016;42(5): 1116-20. [DOI:10.1016/j.burns.2016.02.012] [PMID]