Presence of exoY, exoS, exoU and exoT genes, antibiotic resistance and biofilm production among Pseudomonas aeruginosa isolates in Northwest Iran

Vorkommen von exoY-, exoS-, exoU- und exoT-Genen, Antibiotikaresistenz und Biofilmbildung bei Pseudomonas aeruginosa-Isolaten im Nordwestiran

Abstract

Background: Pseudomonas aeruginosa, as Gram-negative rod bacilli, has an important role in human infection. In the present study we aimed to investigate the presence of exo genes and biofilm production among Pseudomonas aeruginosa isolates in Northwest Iran.

Material and methods: 160 isolates of P. aeruginosa were collected and identified by biochemical tests and were characterized for antibiotic resistance. Biofilm production was evaluated by microtiter plate assay and the presence of exo genes was evaluated by allele-specific PCR (polymerase chain reaction). Chi-square test was used for statistical analysis.

Results: The most effective antibiotics against isolates were colistin and polymyxin B. 87% of the isolates were biofilm producers of which 69% were strongly biofilm producers. 55% of the isolates carried exoY, 52% of the isolates carried exoU, and 26.3% and 5% carried exoS and exoT, respectively.

Conclusion: Our findings showed different distribution of exo genes in clinical isolates of P. aeruginosa in Northwest Iran. ExoS and exoU were more prevalent in non-biofilm producers and exoY was more prevalent in biofilm producer isolates. These results might indicate the importance of exoY in biofilm production of Pseudomonas aeruginosa.

Keywords: Pseudomonas aeruginosa, infection, biofilm, exo genes, type III secretion system

Zusammenfassung

Hintergrund: Pseudomonas aeruginosa, ein Gram-negatives Stäbchenbakterium, besitzt eine wichtige Rolle als Krankheitserreger. Daher untersuchten wir Pseudomonas aeruginosa-Isolate im Nordwestiran auf das Vorkommen von exo-Genen und Biofilmbildern.

Material und Methode: 160 P. aeruginosa-Isolate wurden biochemisch identifiziert und die Antibiotikaresistenz charakterisiert. Die Fähigkeit zur Biofilmbildung wurde im Mikrotiterplatten-Assay, das Vorkommen von exo-Genen mit Allel-spezifischer PCR (Polymerase-Kettenreaktion) analysiert. Zur statistischen Analyse wurde der Chi-Quadrat-Test eingesetzt.

Ergebnisse: Als effektivste Antibiotika erwiesen sich Colistin und Polymyxin B. 87% der Isolate waren Biofilmbildner, davon 69% mit massiver Biofilmbildung. In 55% der Isolate wurden exoY, in 52% exoU, in 26,3% exoS und in 5% exoT nachgewiesen.

Schlussfolgerung: Die Analyse ergab eine unterschiedliche Verteilung der exo-Gene bei klinischen Isolaten von P. aeruginosa im Nordwestiran. ExoS und exoU kamen häufiger bei nicht biofilmbildenden Isolaten, exoY häufiger bei Biofilmbildnern vor. Die Ergebnisse können ein Hinweis...
auf die Bedeutung von exoY bei Biofilm bildenden Pseudomonas aeruginosa-Isonaten sein.

Schlüsselwörter: Pseudomonas aeruginosa, Infektion, Biofilmbildung, exo-Gene, Typ III-Sekretionssystem

Introduction

Pseudomonas aeruginosa is an important causative agent of human infection, especially in a host with compromised defense mechanisms [1]. This bacterium has minimal nutritional requirements, tolerates a wide variety of physical conditions [2], [3] and forms biofilm on the biotic or abiotic surface [4]. In human hospitals, Pseudomonas is a leading cause of nosocomial infections via colonization of catheters, skin wounds, ventilator-associated pneumonia, and is also a cause of respiratory infections in individuals with cystic fibrosis (CF) [5]. Colonization by Pseudomonas spp. occurs when the fibronectin coat surrounding host cells is destroyed due to trauma or infection [6].

The virulence factors can be chemical or proteinaceous, and either cell-associated or secreted. Proteinaceous virulence factors are often secreted through one of the five protein secretion systems so far described as P. aeruginosa: type I, II, III, V [7] and the recently discovered type VI [8]. Especially the type III secretion system (TTSS), which injects effector proteins directly into the eukaryotic host cell cytoplasm, has been associated with high virulence. Infection with a type III secreting isolate has been shown to correlate with severe disease [9], and type III secretion (TTS) in lower respiratory and systemic infections is associated with an increased mortality rate. P. aeruginosa has an impressive array of cell-associated and secreted virulence factors that contribute to its pathogenesis. Key among these is type IV pili, the major bacterial adhesion factor, and the type III secretion system with its secreted exotoxins [10]. Upon host cell contact, the type III secretion system allows bacteria to directly inject toxins into the host cell, where they subvert host cell defense and signaling systems [11]. Four type III-secreted effectors have been identified in P. aeruginosa, although few if any strains secrete all four of them [12].

ExoU is a potent cytotoxin whose host cell targets and mechanism of action are not yet known [13]. ExoT is a bifunctional protein, possessing an N-terminal GTPase-activating domain with GAP (G-protein-activating protein) activity toward Rho, Rac, and Cdc42, and a C-terminal ADP-ribosyltransferase domain [14]. ExoS and exoT are highly homologous bifunctional proteins with an amino terminal GAP domain and a carboxy-terminal ADP-ribosylation domain [15], [16]. In the present study we aimed to investigate the presence of exo genes and biofilm production among Pseudomonas aeruginosa isolates in Northwest Iran.

Methods and materials

Bacterial isolates and identification of Pseudomonas aeruginosa

A total of 160 P. aeruginosa isolates were collected from wounds, respiratory tract, urinary tract, blood stream and sputum of patients admitted to Imam Reza, Shaheed Madani, and Sina hospitals in Tabriz during September 2013 to July 2014. The isolates were confirmed as P. aeruginosa by colony morphology, motility, pigment production, growth at 42°C and 4°C, Gram staining, and conventional biochemical tests [17].

Antibiotic susceptibility tests

Antimicrobial susceptibility of the isolates against 11 antibiotics was performed by the Kirby-Bauer disk diffusion method on Muller-Hinton agar in order to determine the resistance pattern according to the CLSI (Clinical and Laboratory Standards Institute) guideline [18]. The susceptibility and resistance of P. aeruginosa to the following antibiotic disks were tested: amikacin, cefepime, ceftazidime, tobramycin, gentamicin, imipenem, colistin, ciprofloxacin, pipercillin, gatifloxacin and polymyxin B (antibiotic selection was according to CLSI recommendation and local use of antibiotic in this region). The interpretation of sensitivity was done according to the CLSI breakpoint. P. aeruginosa (ATCC 27853) was used for quality control.

Protocol preparation of bacterial DNA

DNA extraction was done according to the tissue buffer boiling method. First, 20 µl of tissue buffer (0.25% SDS + 0.05 M NaOH) was mixed with a single colony of a bacterial isolate and the mixture was incubated for 10 minutes in 95°C. After incubation the mixture was centrifuged for 1 minute in 13,000 g and finally 180 µl of Milli-Q water was added and the extracted DNA was frozen in −20°C for long time storage [19], [20].

Detection of virulence genes encoding type III secretion systems

The virulence genes exoY, exoS, exoU and exoT genes, antibiotic...
Table 1: Primers used in the present study for evaluating presence of *exo* genes in *P. aeruginosa* isolates

| Target gene | Primer sequence (5′–3′) | Ref. |
|-------------|--------------------------|------|
| *exoY*      | F − TCCAAGCTATGCATCGACGGTCTAC<br>R − CGTATGCATCCAGGGGGTGTATCTGACC | [37] |
| *exoS*      | F − ATCCCTAGGCCTACATCC<br>R − ACGACCGCTATCTCCAC | [23] |
| *exoT*      | F − AGAACCGCGTCCTTTCCGTGGCTAGTT<br>R − CAGCTCGTCGGCCTTGGCAAGT | [38] |
| *exoU*      | F − GATTCATCACAGGCTCG<br>R − CTAGCAATGGCACTAATCG | [23] |

0.2 µl dNTP, 2 µl of 10 × PCR buffer, 0.5 µl of Taq DNA polymerase (5 U/µl) (Fermentase) and 13.9 µl of molecular grade water. The PCR condition was carried out as follows: initial denaturation step (at 94 °C for 10 min), followed by 30 to 40 cycles repetitions of denaturation (40 s at 94 °C), annealing (50 s a 57 to 68 °C), and extension (55 s at 72 °C) with a final extension at 72 °C for 10 min [21], [22], [23]. PCR products were analyzed by electrophoresis in 1% of agarose gels for 70–80 min at 100 V. Finally the PCR products were stained with ethidium bromide (0.5 mg/ml) and analyzed by a UV transilluminator.

**Biofilm formation**

Quantitative determination of biofilm forming capacity was determined by a colorimetric microtiter plate assay [24]. Briefly, bacterial colonies were grown overnight at 37 °C in Trypticase Soy Broth (TSB) (Merck Darmstadt, Germany) for 24 h. The bacterial suspensions were diluted (1:100) in a new TSB medium and 150 µl of this dilution was used to inoculate the sterile flat-bottomed 96-well polystyrene microtiter plates. Subsequent to an incubation period of 24 h at 37 °C without shaking, the wells were gently washed three times with 200 µl of PBS (phosphate buffered saline). For the fixation of the biofilms, 100 µl of 99% methanol was added and, after 15 min, the solutions were removed and the plate was air-dried. In the next step, 150 µl of crystal violet 1% (CV) was added to all wells for 20 min. After removing the dye, the bound CV was released with adding 150 µl of 33% acetic acid. The optical density (OD) of each well was measured at 590 nm using a microtitre plate reader. All the assays were repeated for three times. As a control, uninoculated medium was used to determine background OD. The cut-off OD (ODc) was defined as three standard deviations above the mean OD of the negative control [25]. All isolates were classified into three groups on the base of OD (ODc) value: OD ≤ ODc = non biofilm producer (−), ODc < OD ≤ 2 * ODc = weak biofilm producer (+), 2* ODc < OD ≤ 4 * ODc = moderate biofilm producer (++), 4* ODc < OD = strong biofilm producer (+++) [26]. All the tests were done triplicate [27].

**Statistical methods**

The prevalence of the virulence gene, with respect to the site of infection, was compared by the chi-square test. The correlation between the prevalence of the virulence gene and the antibiotic resistance patterns were tested by the t-test.

**Results**

The resistance pattern to the 11 antimicrobials tested is shown in Table 2. According to the results, isolates had the lowest rate resistant to polymyxin B and colistin. Biofilm data showed that 87% of isolates were biofilm producers in which 69% of them were strongly biofilm producers and the rate of moderate and weakly biofilm producers were 11% and 7%, respectively. The type III secretion-toxin encoding gene patterns are shown in Table 3. 55% of samples carried *exoY*, 52% of samples carried *exoU*, and 26.3% and 5% carried *exoS* and *exoT*, respectively. 12% of the isolates carried both *exoY* and *exoU* while 32% showed a concomitant existence of *exoS* and *exoY* and 4% carried both *exoS* and *exoU* genes. Coexistence of *exoS*, *exoY*, and *exoU* was seen in 4% of the isolates.

**Discussion**

*Pseudomonas aeruginosa* is a common nosocomial pathogen, notorious for its multidrug resistance (MDR) and life threatening infections in critically ill patients. Lately, carbapenems are being used as the last resort antimicrobial treatment for serious infections due to MDR *P. aeruginosa* [28]. In the current study 2.5% of the isolates were resistant to colistin and polymyxin B, which shows that these 2 antibiotics could be in first line drug therapy regimen and the last choice of therapy for these infections. Emergence of resistance to these two antibiotics can treat therapy strategies and there will be no other choice of therapy [29]. *P. aeruginosa* secretes four known effector proteins via the type III secretion system: *exoS*, *exoT*, *exoU*, and *exoY* [30]. These proteins modulate host cell functions which
Table 2: Antimicrobial resistance properties in *Pseudomonas aeruginosa* isolated from clinical infections

| Antibiotic | Wound (%) | Burn (%) | Urine (%) | Sputum (%) | Respiratory (%) | CSF (%) | Blood (%) | Total (%) |
|------------|-----------|----------|-----------|------------|----------------|--------|-----------|-----------|
| AMK        | 72        | 82       | 44.30     | 62         | 59.14          | 100    | 71.23     | 70.81     |
| CEP        | 66        | 42       | 48.15     | 82         | 40.09          | 100    | 78.92     | 65.3      |
| TOB        | 66        | 82       | 50.07     | 82         | 78.19          | 100    | 71.23     | 75.64     |
| CAZ        | 88        | 62       | 57.76     | 100        | 73.42          | 100    | 71.23     | 74.63     |
| GEM        | 78        | 82       | 46.23     | 82         | 73.42          | 100    | 71.23     | 76.12     |
| IMP        | 58        | 82       | 27        | 82         | 68.66          | 100    | 71.23     | 69.84     |
| COL        | 2         | 0        | 0         | 0          | 2              | 0      | 0         | 2         |
| CIP        | 70        | 42       | 50.07     | 0          | 68.66          | 100    | 71.53     | 57.55     |
| PRL        | 72        | 62       | 54.92     | 82         | 68.66          | 100    | 71.23     | 72.97     |
| GAT        | 76        | 62       | 74.07     | 100        | 92.47          | 100    | 71.23     | 82.252    |
| PB         | 3.6       | 0        | 2.7       | 0          | 2.5            | 0      | 3         | 3         |

n = 160, NO%, abbreviations: AMK = amikacin (30 µg/disk); CEP = ciprofloxacin (5 µg/disk); TOB = tobramycin (30 µg/disk); CAZ = ceftazidime (30 µg/disk); GEM = gentamicin (30 µg/disk); IMP = imipenem (30 µg/disk); COL = colistin (10 µg/disk); CIP = ciprofloxacin (5 µg/disk); PRL = piperacillin (100 µg); GAT = gatifloxacin; PB = polymyxin B (300 U/disk)

Table 3: Different distribution of *exo* genes in isolates with different origins

| Virulence gene | Wound n = 50 No (%) | Urine n = 55 No (%) | Blood n = 15 No (%) | Respiratory n = 23 No (%) | Burn n = 7 No (%) | Sputum n = 5 No (%) | CSF n = 5 No (%) | Total n = 160 No (%) |
|----------------|----------------------|---------------------|---------------------|--------------------------|------------------|--------------------|------------------|---------------------|
| *exoY*         | 28 (56)              | 29 (52.72)          | 10 (66.66)          | 10 (51.71)               | 7 (100)          | 1 (20)             | 2 (40)           | 55%                 |
| *exoT*         | 0                    | 3 (5.45)            | 0                   | 2 (8.69)                 | 2 (40)           | 0                  | 1 (20)           | 5%                  |
| *exoU*         | 50 (1)               | 18 (32.72)          | 2 (13.33)           | 9 (39.13)                | 2 (40)           | 2 (40)             | 0                | 52%                 |
| *exoS*         | 13 (26)              | 13 (23.63)          | 5 (33.33)           | 7 (30.43)                | 1 (20)           | 1 (20)             | 2 (40)           | 26.3%               |

are important in cytoskeletal organization and signal transduction [31]. ExoS and exoT are bifunctional toxins exhibiting ADP-ribosyltransferase and GTPase-activating activity [32]. ExoT shows a lower ADP-ribosyltransferase activity than exoS [32]. ExoY has adenylate cyclase activity whilst exoU exhibits phospholipase activity and disrupts eukaryotic membranes following its delivery into the cytoplasm. It has been shown that exoS and exoU were the major cytotoxins in both in vitro and in vivo assays [33]. The majority of *P. aeruginosa* strains carry exoT and exoY genes; however, the presence of exoS and exoU differ noticeably between the isolates and appear to be mutually exclusive [31]. Different frequencies of cytotoxin encoding genes, however, have been reported in different studies [34]. This may reflects the fact that the genes, encoding the cytotoxins exoS and exoU, are present as variable traits in *P. aeruginosa* and their presence depends on the disease site or background [35]. Unlike other studies, that show high prevalence of exoS and exoT, in this study we observe lower prevalence of exoS and exoT (26.3% and 5%, respectively) (P<0.05) [36]. ExoY had the most prevalence (55%) but is found less than in other studies done in Bulgaria (85.8%) and the USA (89%) [21], [37]. In a similar study done in Iran the rate of exoU and exoS was lower. Jabalameli et al. [38] report a rate of exoU as 64.5%, Fazeli et al. [22] in a study on isolates from Iranian hospital infections, report the rate of exoS as 67.64% and Dadmanesh et al. [39] published a rate of exoS and exoT as 73.91% and 69.21% respectively. This lower rate of exoS and exoU prevalence in our study can be due to less clonal diversity of isolates. Further studies on epidemiological issues can help us understand the pathogenesis of the isolates better. No significant association between MDR resistance and prevalence of the virulence gene carriage was observed (P=0.490).

Biofilm production has been considered to be an important determinant of pathogenicity in *P. aeruginosa* infections [32]. The formation of biofilms facilitate chronic bacterial infections and reduces the efficacy of antimicrobial therapy [23], [32], [40]. In the current study 87% of isolates were biofilm producers and among them 69% of isolates were strong biofilm producers. 50% of the isolates that encode exoY (most prevalent in the current study) were the biofilm producer, but only 2.5% of isolates that encode both exoS and exoU (the major cytotoxins in both in vitro and in vivo assays) were biofilm producers. Interestingly, all non-biofilm producer isolates had at least one of the exoS or exoU genes. These results can indicate the importance of exoS and exoU in non-biofilm producer isolates. Also, the exoY gene was highly prevalent in biofilm producer isolates. There was no association between the origin of isolates and presence of exo genes (Table 3).

The antibiotic resistant profile of isolates showed increasing resistance, especially in wound and CSF (cerebrospi-
nal fluid) isolates. *P. aeruginosa* isolates from CSF were resistant to all antibiotics, except for colistin. This indicates the importance of antibiotic stewardship development and control of infection in hospital settings.

In conclusion, findings of the present study showed different distribution of *exo* genes in clinical isolates of *P. aeruginosa* in Northwest Iran. When comparing the presence of *exo* genes and biofilm formation, it was found that *exoS* and *exoU* were more prevalent in non-biofilm producers and *exoY* was more prevalent in biofilm producer isolates. These results indicate the importance of *exoY* in biofilm production of *Pseudomonas aeruginosa*.

**Notes**

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**Competing interests**

The authors declare that they have no competing interests.

**References**

1. Cross AS. Evolving epidemiology of *Pseudomonas aeruginosa* infections. Eur J Clin Microbiol. 1985 Apr;4(2):156-9. DOI: 10.1007/BF02013589
2. Livermore DM. Multiple mechanisms of antimicrobial resistance in *Pseudomonas aeruginosa*: our worst nightmare? Clin Infect Dis. 2002 Mar;34(5):634-40. DOI: 10.1086/338782
3. Nakajima A, Sugimoto Y, Yoneyama H, Nakae T. High-level fluoroquinolone resistance in *Pseudomonas aeruginosa* due to interplay of the MexAB-OpmN efflux pump and the DNA gyrase mutation. Microbiol Immunol. 2002;46(8):391-5. DOI: 10.1111/j.1348-0421.2002.tb02711.x
4. Chiang P, Burrows LL. Biofilm formation by hyperpiliated mutants of *Pseudomonas aeruginosa*. J Bacteriol. 2003 Apr;185(7):2374-8. DOI: 10.1128/JB.185.7.2374-2378.2003
5. Pier GB. *Pseudomonas aeruginosa*: a key problem in cystic fibrosis. ASM News-Am Soc Microbiol. 1998;64(6):339-47.
6. Koenig A. Gram-negative bacterial infections. In: Greene CE, editor. Infectious diseases of the dog and cat. 4th ed. St. Louis, Missouri: Elsevier, Saunders; 2012. p. 349-59.
7. Ma Q, Zhai Y, Schneider JC, Ramseier TM, Saier MH Jr. Protein secretion systems of *Pseudomonas aeruginosa* and *P. fluorescens*. Biochim Biophys Acta. 2003 Apr 1;1611(1-2):223-33. DOI: 10.1016/S0005-2736(03)00099-2
8. Filloux A, Hachani A, Bleves S. The bacterial type VI secretion machine: yet another player for protein transport across membranes. Microbiology. 2008 Jun;154( Pt 6):1570-83. DOI: 10.1099/mic.0.2008/016840-0
9. Hauser AR, Cobb E, Bodi M, Mariscal D, Vallés J, Engel JN, Rello J. Type III protein secretion is associated with poor clinical outcomes in patients with ventilator-associated pneumonia caused by *Pseudomonas aeruginosa*. Crit Care Med. 2002 Mar;30(3):521-8. DOI: 10.1097/00003246-200203000-00005
10. McMorran B, Town L, Costelloe E, Palmer J, Engel J, Hume D, Wainwright B. Effect of *ExoT* from the type III secretion system is an important modulator of gene expression in lung epithelial cells in response to *Pseudomonas aeruginosa* infection. Infect Immun. 2003 Oct;71(10):6035-44. DOI: 10.1128/IAI.71.10.6035-6044.2003
11. Hueck CJ. Type III protein secretion systems in bacterial pathogens of animals and plants. Microbiol Mol Biol Rev. 1998 Jun;62(2):379-433.
12. Engel JN. Molecular pathogenesis of acute *Pseudomonas aeruginosa* infections. In: Hauser AR, Rello J, eds. Severe infections caused by *Pseudomonas aeruginosa*. New York: Springer; 2003. p. 201-29.
13. Finck-Barbançon V, Frank DW. Multiple domains are required for the toxic activity of *Pseudomonas aeruginosa* ExoU. J Bacteriol. 2001 Jul;183(14):4330-44. DOI: 10.1128/JB.183.14.4330-4344.2001
14. Kazmierczak BI, Engel JN. *Pseudomonas aeruginosa* ExoT acts in vivo as a GTPase-activating protein for RhoA, Rac1, and Cdc42. Infect Immun. 2002 Apr;70(4):2198-205. DOI: 10.1128/IAI.70.4.2198-2205.2002
15. Barbieri JT. *Pseudomonas aeruginosa* exoenzyme S, a bifunctional type-III secreted cytotoxin. Int J Med Microbiol. 2000 Oct;290(4-5):381-7. DOI: 10.1016/S1438-4221(00)80047-8
16. Goehringer UM, Schmidt G, Pederson KJ,Aktories K, Barbieri JT. The N-terminal domain of *Pseudomonas aeruginosa* exoenzyme S is a GTPase-activating protein for Rho GTPases. J Biol Chem. 1999 Dec 17;274(51):36369-72. DOI: 10.1074/jbc.274.51.36369
17. Najafi K, Kafii HS, Shokriani S, Azimi S, Asgharzadeh M, Yousefi M, Aghazadeh M. Virulence Genes and Antibiotic Resistance Profile of *Pseudomonas aeruginosa* Isolates in Northwest of Iran. J Pure Appl Microbiol. 2015;9:383-9.
18. Mitov I, Strateva T, Markova B. Prevalence of virulence genes among bulgarian nosocomial and cystic fibrosis isolates of *Pseudomonas aeruginosa*. Braz J Microbiol. 2010 Jul;41(3):588-95. DOI: 10.1590/S1517-83822010000300008
19. Asgharzadeh M, Daraei H. Mannose-binding lectin gene and promoter polymorphism and susceptibility to renal dysfunction in systemic lupus erythematosus. In: 1st Tabriz International Life Science Conference and 12th Iran Biophysical Chemistry Conference; 2013 May 22-24; Tabriz university of medical sciences, Iran.
20. Asgharzadeh M, Khakpour M, Salehi TZ, Kafii HS. Use of mycobacterial interspersed repetitive unit-variable-number tandem repeat typing to study *Mycobacterium tuberculosis* isolates from East Azerbaijan province of Iran. Pak J Biol Sci. 2007 Nov 1;10(21):3769-77. DOI: 10.3923/pjbs.2007.3769.3777
21. Strateva T, Markova B, Ivanova D, Mitov I. Distribution of the type III effector proteins-encoding genes among nosocomial *Pseudomonas aeruginosa* isolates from Bulgaria. Ann Microbiol. 2010;60:503-9. DOI: 10.1007/s13213-010-0079-3
22. Fazeli N, Momtaz H. Virulence Gene Profiles of Multidrug-Resistant *Pseudomonas aeruginosa* Isolated From Iranian Hospital Infections. Iran Red Crescent Med J. 2014 Oct 5;16(10):e15722. DOI: 10.5812/ircmj.15722

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23. Finnan S, Morrissey JP, O’Gara F, Boyd EF. Genome diversity of Pseudomonas aeruginosa isolates from cystic fibrosis patients and the hospital environment. J Clin Microbiol. 2004 Dec;42(12):5783-92. DOI: 10.1128/JCM.42.12.5783-5792.2004

24. Kafil HS, Mobarez AM. Assessment of biofilm formation by enterococci isolates from urinary tract infections with different virulence profiles. J King Saud Univ Sci. 2015;27:312-7. DOI: 10.1016/j.jksus.2014.12.007

25. Kafil HS, Mobarez AM, Moghadam MF, Hashemi S, Yousefi M. Gentamicin induces efaA expression and biofilm formation in Enterococcus faecalis. Microb Pathog. 2016 Mar;92:30-5. DOI: 10.1016/j.micpath.2015.12.008

26. Stepansonic V, Vukovic D, Dakic I, Savic B, Svabic-Vlahovic M. A modified microtitre-plate test for quantification of staphylococcal biofilm formation. J Microbiol Methods. 2000 Apr;40(2):175-9. DOI: 10.1016/S0167-7012(00)00122-6

27. Fattahi S, Kafil HS, Nahai MR, Asgharzadeh M, Nori A. Relationship of biofilm formation and different virulence genes in uropathogenic Escherichia coli isolates from Northwest Iran. GMS Hyg Infect Control. 2015 Jul 13:10.Doc11. DOI: 10.3205/gmk000254

28. Behera B, Das A, Mathur P, Kapil A. High prevalence of carbapenem resistant Pseudomonas aeruginosa at a tertiary care centre of north India. Are we under-reporting? Indian J Med Res. 2008 Sep;128(3):324-5.

29. Roy-Burman A, Savel RH, Racine S, Swanson BL, Revadigar NS, Fujimoto J, Sawa T, Frank DW, Wiener-Kronish JP. Type III protein secretion is associated with death in lower respiratory and systemic Pseudomonas aeruginosa infections. J Infect Dis. 2001 Jun;183(12):1767-74. DOI: 10.1086/320737

30. Lee VT, Smith RS, Tümmler B, Lory S. Activities of Pseudomonas aeruginosa effectors secreted by the Type III secretion system in vitro and during infection. Infect Immun. 2005 Mar;73(3):1695-705. DOI: 10.1128/IAI.73.3.1695-1705.2005

31. Wareham DW, Curtis MA. A genotypic and phenotypic comparison of type III secretion profiles of Pseudomonas aeruginosa cystic fibrosis and bacteremia isolates. Int J Med Microbiol. 2007 Jul;297(4):227-34. DOI: 10.1016/j.ijmm.2007.02.004

32. Hall-Stoodley L, Stoodley P. Evolving concepts in biofilm infections. Cell Microbiol. 2009 Jul;11(7):1034-43. DOI: 10.1111/j.1462-5882.2009.01323.x

33. Garey KW, Vo QP, Larocco MT, Gentry LO, Tam VH. Prevalence of type III secretion protein exoenzymes and antimicrobial susceptibility patterns from bloodstream isolates of patients with Pseudomonas aeruginosa bacteremia. J Chemother. 2008 Dec;20(6):714-20. DOI: 10.1179/joc.2008.6.714

34. Cho MH, Stapleton F, Willcox MD, Zhu H. Comparison of virulence factors in Pseudomonas aeruginosa strains isolated from contact lens- and non-contact lens-related keratitis. J Med Microbiol. 2008 Dec;57(12):1539-46. DOI: 10.1099/jmm.0.2008/003723-0

35. Berthelot P, Attree I, Pletié P, Chabert J, de Bentzmann S, Pozzetto B, Gratard F. Groupe d’Etudes des Septicémies à Pseudomonas aeruginosa. Genotypic and phenotypic analysis of type III secretion system in a cohort of Pseudomonas aeruginosa bacteremia isolates: evidence for a possible association between O serotypes and exo genes. J Infect Dis. 2003 Aug;188(4):512-8. DOI: 10.1086/377000

36. 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 Oct;147(10):2659-69. DOI: 10.1099/00221287-147-10.2659

37. Jabalameli F, Mirsaleheian A, Khoramian B, Aligholi M, Khoramrooz SS, Asadollahi P, Taheri-Kalani M, Emameini M. Evaluation of biofilm production and characterization of genes encoding type III secretion system among Pseudomonas aeruginosa isolated from burn patients. Burns. 2012 Dec;38(8):1192-7. DOI: 10.1016/j.burns.2012.07.030

38. Dadmanesh M, Pilehvarzadeh M, Eramabadi M, Eramabadi P, Moghadam MB, Mashayekhi F. Community acquired Pseudomonas aeruginosa urinary tract infections in children hospitalized in a Baqijatallah hospital, Tehran, Iran: Virulence profile and antibiotic resistance properties. Biosci Biotech Res Asia. 2014;11(2):417-26. DOI: 10.13005/bbra/1290

39. Parsek MR, Singh PK. Bacterial biofilms: an emerging link to disease pathogenesis. Annu Rev Microbiol. 2003;57:677-701. DOI: 10.1146/annurev.micro.57.030502.090720

Corresponding author:
Mohammad Aghazadeh, PhD
Department of Medical Microbiology and Virology, Faculty of Medicine, Tabriz University of Medical Sciences, Tabriz, Iran, Phone: +98-9143134820
Aghazadehm@tbzmed.ac.ir

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