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Identification Of Virulence Resistance Genes İn *Pseudomonas Aeruginosa* Strains İsolated From Blood Samples

Kan Örneklerinden İzole Edilen *Pseudomonas Aeruginosa* Suşlarında Virülans Direnç Genlerinin Belirlenmesi

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Abstract

Aim In this it is aimed to the determine the presence of virulence resistance genes (toxA, algD, plcN, lasB, plcH) in P. aeruginosa isolates isolated from blood samples.

Material and Method DNA extraction of the study isolates was done by boiling method. Optimization was done using positive control after DNA extraction. After optimization, the presence of virulence (toxA, algD, plcN, lasB, plcH) resistance genes was investigated by polymerase chain reaction (PCR) method.

Results As a result of PCR of the virulence gene regions (toxA, algD, lasB, plcN, plcH); Positive rates of KR 25 isolates were 80% (n=20), 100% (n=25), 100% (n=25), 100% (n=25), 96% (n=24) in KS 46 isolates. On the other hand, the positive rate distributions were found to be 93.47% (n=43), 100% (n=46), 100% (n=46), 100% (n=46), 97.82% (n=45).

Conclusion As a result of PCR of virulence gene regions (toxA, algD, lasB, plcN, plcH) of P. aeruginosa, it was determined that algD, lasB, plcN genes were found in all 25 carbapenem resistant (KR) and 46 carbapenem sensitive (KS) isolates.

Keywords P. aeruginosa, virulence, bactaremia

Özet

Amaç *Kan örneklerinden izole edilen P. aeruginosa izolatlarındaki virülans direnç genlerinin (toxA, algD, plcN, lasB, plcH) varlığının belirlenmesidir.*

Gereç ve Yöntem *Çalışma izolatlarının DNA ekstraksiyonu kaynatma yöntemiyle yapıldı. DNA ekstraksiyonundan sonra pozitif kontrol kullanılarak optimizasyon yapıldı. Optimizasyondan sonra virülans (toxA, algD, plcN, lasB, plcH) direnç genlerinin varlığının polimeraz zincir reaksiyonu (PZR) yöntemi ile araştırıldı.*

Sonuçlar *Yapılan virülans gen bölgelerinin (toxA, algD, lasB, plcN, plcH) PZR işlemi sonucunda; karbapenem dirençli (KR) 25 izolatta pozitif oranları sırasıyla % 80 (n=20), % 100 (n=25), % 100 (n=25), % 100 (n=25), %96 (n=24) olduğu, karbapenem duyarlı (KS) 46 izolatta ise pozitif oran dağılımlarının % 93,47 (n=43), % 100 (n=46), % 100 (n=46), % 100 (n=46), %97,82 (n=45) olduğu saptanmıştır.*

Sonuç *P. aeruginosa'ın virülans gen bölgelerinin (toxA, algD, lasB, plcN, plcH) PZR işlemi sonucunda KR 25 ve KS 46 izolatların hepsinde algD, lasB, plcN genlerinin bulunduğu belirlenmiştir.*

Anahtar Kelimeler *P. aeruginosa, Virülans, bakteriyemi*

INTRODUCTION

Pseudomonas aeruginosa is an aerobic, non-sporeforming and oxidase-positive gram-negative bacillus.[1] Since it is hydrophilic, it can be isolated from many environmental environments and antiseptic solutions.[2] It is among the serious causes of blood infections associated with high mortality.[3] It is a major cause of hospital-acquired infections, especially in immune suppressed patients.[4] Also in terms of general hygiene of the hospital, this bacterium is known to cause epidemics by contaminating water resources.[5,6] *P. aeruginosa* quickly develops resistance due to its structural features and the effect of intense antibiotic stress in the hospital environment.[7]

The virulence factors determine the disease-causing capacity of the bacteria. These factors are such as structural components, toxins, and enzymes of *P. aeruginosa*.[8] Both cellular and extracellular factors play a role in the virulence of *P. aeruginosa*.

In particular, surface components such as pili, flagella and lipopolysaccharide adhere to the host cell surface, leading to the host immune response. In many animal model studies, it has been shown that proteases, toxins (exotoxin A and exoenzyme S) and hemolysins (phospholipase and rhamnolipid) play a role in the virulence of P. aeruginosa[9]. Exotoxin A encoded by the ToxA gene and has a role in inhibition of protein synthesis by inhibiting elongation factor 2 of eukaryotic cells. Alginates and algD genes of P. aeruginosa produce mucoid colonies which protects the bacterium from the antimicrobials and immun cells.[10] LasB elastase, is encoded by the LasB gene, and destroys collagen and elastin, help bacterium to invade tissues..[11] In addition, two phospholipase Cs which are encoded by hemolytic phospholipase C (plcH) and nonhemolytic phospholipase C (plcN) hydrolyse phospholipids in lung.[12,13]

P. aeruginosa has intrinsic resistance to numerous antimicrobial agents and also easily acquires resistance to many antibiotics.[14] Carbapenems are drug of choice for treatment of serious infections caused by *P. aeruginosa*.[15,16] Carbapenem resistance are involved in various mechanisms; such as intrinsic RND (Resistance-Nodulation- Division) efflux pump systems and lack of outer membrane porin (OprD). However, *P. aeruginosa* is becoming increasingly resistant against carbapenems have shown that recent study results.[17,18]

Antibiotic resistance status differ from region to region, even among patients hospitalized in different wards in the same hospital. Therefore, it is necessary to monitor the antibiotic resistance profiles of the isolates isolated in the hospital at regular intervals in each hospital, and to update the treatment protocols by looking at the resistance rates to the antibiotics used in the treatment.[19]

The distribution of carbapenemases shows differences between geographical area and clinical origins, and studies about the coexistence of carbapenem resistance and multiple virulence factors of in *P. aeruginosa* are limited.[20]

This study is aimed to determine the data results obtained by studying the presence of virulence genes (toxA, algD, plcN, lasB, plcH) in carbapenem resistant and susceptible P. aeruginosa isolates isolated from blood samples sent to the Medical Microbiology laboratory of hospitalized patients in Ondokuz Mayıs University hospital.

MATERIAL and METHOD

P. aeruginosa isolates (n: 71) obtained from blood samples sent to the Medical Microbiology laboratory of patients hospitalized in Ondokuz Mayıs University Hospital Medical Microbiology Laboratory at 01/01/2020 - 16/09/2021 were included in our study. The study was a retrospective study.

The blood cultures were incubated on BacT/Alert (Biomeriux, France) blood culture system until they signaled positive or for a maximum of five days. The Gram stains were done directly from positive blood culture bottles.

According to the result from the staining, specimen from the positive bottles were subcultured onto relevant agar plates. TIdentification of the isolates was performed by matrix-assisted laser desorption/ ionization time-of-flight mass spectrometry (Vitek MS, Biomeriux, France). Antimicrobial susceptibilities were determined by Vitek2Compakt (Biomeriux, France) and evaluated according to the European Committee on Antimicrobial Susceptibility Testing. The isolates were stored at -20ºC until the studied.

Bacterial DNA extraction was done by boiling method that includes a heating step at 100˚C of colonies from Mueller-Hinton agar in a 500µl sterile distilled water for 15 min. followed by a centrifugation step of the cell suspension at 15000g for 20min, supernatant was used as template DNA. The DNA templets were stored at -20°C until the molecular study.

Determination of virulence genes

After DNA extraction, toxA was studied as uniplex PCR (polymerase chain reaction) and algD, lasB, plcN and plcH were studied as multiplex PCR. The primer sequences used in the study are given in Table 1.[21]

Table 1. toxA, algD, lasB, plcN, plcH primer sequences			
Forward Primer		Reverse Primer	
algD	5'-CGTCTGCCGCGAGATCGGCT-3'	5'-GACCTCGACGGTCTTGCGGA-3'	313
lasB	5'-GGAATGAACGAACGAAGCGTTCTC-CGAC-3'	5'-TTGGCGTCGACGAACACCTCG-3'	284
toxA	5'-CTGCGCGGGTCTATGTGCC-3'	5'-GATGCTGGACGGGTCGAG-3'	270
plcH	5'-GCACGTGGTCATCCTGATGC-3'	5'-TCCGTAGGCGTCGACGTAC-3'	608
plcN	5'-TCCGTTATCGCAACCAGCCCTACG-3'	5'-TCGCTGTCGAGCAGGTCGAAC-3'	481

Table 2. toxA, algD, lasB, plcN, plcH positivity rate in carbapenem susceptible P. aeruginosa isolates.					
	toxA	algD	lasB	plcN	plcH
Positive	43 (%93,4)	46 (%100)	46 (%100)	46 (%100)	45 (%97,8)
Negative	3	0	0	0	1
Total	46	46	46	46	46

Table 3. toxA, algD, lasB, plcN, plcH positivity rate in carbapenem resistant P. aeruginosa isolates					
	toxA	algD	lasB	plcN	plcH
Positive	20 (%80)	25 (%100)	25 (%100)	25 (%100)	24 (% 96)
Negative	5	0	0	0	1
Total	25	25	25	25	25

RESULTS

Twenty-five (35.2%) of the isolates included in the study were carbapenem resistant. The intensive care unit (22.5%) was the clinic where the most isolates sent. At the end of PCR of virulence gene regions (toxA, algD, lasB, plcN, plcH) of *P. aeruginosa,* algD, lasB and plcN virulence genes were detected in all isolates. ToxA and plcH virulence genes were found to be 88.7% and 97.2%, respectively. AlgD, lasB, and plcN gene regions were detected in all carbapenem-susceptible (CS) *P. aeruginosa* isolates, while toxA gene regions were detected in 93.4% and plcH gene regions in 97.8% of the isolates (Table 2). Similarly, algD, lasB, and plcN gene regions of carbapenem-resistant (CR) *P. aeruginosa* isolates were detected in all of them, while toxA gene regions were detected in 80% and plcH gene regions in 96% (Table 3).

DISCUSSION

Worldwide, *P. aeruginosa* isolates are an important pathogen responsible for 10-15% of hospital-acquired infections.[22,23] *P. aeruginosa* is among the most important pathogens infecting the lungs, urinary tract, blood circulation and soft tissue in intensive care units.[24] The main virulence factors of *P. aeruginosa* are exotoxin A, exoenzyme S, alginate, phospholipase and elastase.[25] Exotoxin A, alkaline protease, and elastase are documented as important virulence factors for systemic infections in immunocompromised patients.[26]

Ozer et al. stated that 68.8% of P. aeruginosa isolates were isolated from intensive care in their study.[27] In the European Prevalence Infection Intensive Care (EPIC) study, which included 1417 intensive care units from 17 different European countries, *P. aeruginosa* isolates isolated from blood samples were examined in terms of clinical services, and it was seen that they were most frequently isolated from the intensive care unit. *P. aeruginosa* isolates, which we isolated from blood samples in our study, were examined in terms of clinical services, it was seen that they were most frequently isolated from the intensive care unit. Intensive care units are wards where hospital infections are more common due to critically ill patients and invasive procedures in these units.[28]

Faraji et al., when comparing the positivity rates of resistance genes of P. aeruginosa isolate isolated from cystic fibrosis (CF) and burn patients; reported that toxA (63.1%), lasB (95.4%) and exoS (70.8%) genes were higher in patients with CF, and lasB (95.4%) was higher than other virulence genes. 21 In our study, it was determined that the presence of toxA and lasB was higher.

The distribution of algD, lasB, pilB, nan1 virulence regions in *P. aeruginosa* isolates showing multidrug resistance was examined and it was reported that lasB and algD gene regions were detected in all isolates, as in our study. It has been reported that there is a high correlation between chronic infections (urine, lower respiratory tract infection, urinary tract infection, blood and wound infections) and lasB and algD genes (100%).[29]

Wolska et al. in a study they conducted, the presence of six virulence genes (algae D, las B, tox A, plc H, plc N, exo S) was investigated in 49 *P. aeruginosa* isolates and they were detected algae D, las B, plc H in all isolates, while tox A and plc N on the other hand, it was determined in 91.8% of the isolates.[30] It was stated that the prevalence of virulence genes in *P. aeruginosa* among 143 isolates obtained from CF patients was 100% lasB, 100% plcB, and 96.5% plcH.31 The results of these studies are similar to the results of our study.

CR clinical *P. aeruginosa* ranks second based on most criteria for bacteria among 20 antidrug-resistant bacterial species by the World Health Organization reports.[17] Many studies reported that VIM gene is the most frequent MBL found in CR P. aeruginosa; however, IMP gene was the most common detected in a study conducted in Iran.[32,33,34] There have been limited data on of virulence genes in CR *P. aeruginosa* isolates. Bogiel et al. (2021), stated the toxA gene indicate highly common and between toxA genes

and algD genes; significant correlations between algD and lasB. Also altogether found in almost all of the examined strains were shown that a statistically significant correlation in the co-existence of lasB and both phospholipases genes (plcH, plcN).[35] In our study, KR isolates no CR gene was detected; however was detected that toxA, algD, lasB, plcN, plcH respectively 80%, 100%, 100%, 100%, 96%.

In the study by Ellapan et al. algD expression was detected in 93% of carbapenem resistant *P. aeruginosa* isolates, followed by algU (89%), rhlR (84%), lasR (81%) and exoS (76%). The lasB and plcH genes were detected in 94% and 92% of isolates, respectively.[32] And in a study by Park and Koo nine virulence factor genes (toxA, exoT, plcN, plcH, phzM, phzS, lasB, aprA, and algD) were identified in all of carbapenem resistant P. aeruginosa isolates.20 However, in those studies thay did not compare the presence of virulence genes in CR and CS *P. aeruginosa* isolates. In our study the coexistance of virulence genes in carbapenem resistant and susceptible isolates were found to be similar. One of the great challenges of modern medicine is the increase in antibiotic resistance in bacteria.[36] Because of their ability to develop rapid resistance to various virulence factors and antibiotics, they cause infections that are difficult to treat, especially in immunocompromised and hospitalized patients.[37]High prevalence of virulence factors and multiple resistance mechanism is worrisome in *P. aeruginosa* isolates. It is important to monitor the virulence and resistance mechanisms of *P. aeruginosa*, which is known to cause significant infections especially in intensive care patients.

CONCLUSION

In our study, algD, lasB, and plcN virulence genes were detected in both CS and CR *P. aeruginosa* isolates, and the presence of toxA and plcH was found to be slightly higher in CS isolates. The number of samples in our study was not high and blood samples were included because it caused serious infections. It would be useful to compare the presence of virulence genes in different sample types with more comprehensive studies.

Ethics approval

Ethical approve was taken from Ondokuz Mayıs University Medical Faculty Cinical Research Committee.

Conflict of interest

There is no conflict of interest.

Author contributions

Idea: IB, YTC; Study Design: IB, YTC; Literature review: IB; Laboratory work: IB, EBA; Editing: IB, YTC; Evaluation: YTC, AB; Review: YTC, AB

References

1. Palleroni NJ. Prokaryote taxonomy of the 20th century and the impact of studies on the genus Pseudomonas: a personal view. Microb. 2003;149(1):1-7.
2. Speert DP, Campbell ME, Davidson AGF, Wong LT. Pseudomonas aeruginosa colonization of the gastrointestinal tract in patients with cystic fibrosis. J Infect Dis. 1993;167(1):226-229.
3. Lister PD, Wolter DJ, Hanson ND. Antibacterial-resistant Pseudomonas aeruginosa: clinical impact and complex regulation of chromosomally encoded resistance mechanisms. Clin Microbiol Rev. 2009;22(4):582-610.
4. Blanc DS, Petignat C, Janin B, Bille J, Francioli P. Frequency and molecular diversity of Pseudomonas aeruginosa upon admission and during hospitalization: a prospective epidemiologic study. Clin Microb Infect. 1998;4(5):242-247.
5. Varin A, Valot B, Cholley P, et al. High prevalence and moderate diversity of Pseudomonas aeruginosa in the U-bends of high-risk units in hospital. Int J Hyg Environ Health. 2017;220(5):880-885.
6. Witney A, Gould K, Pope C, et al. Genome sequencing and characterization of an extensively drug-resistant sequence type 111 serotype O12 hospital outbreak strain of Pseudomonas aeruginosa. Clin Microb Infect. 2014;20(10):609-618.
7. Öztürk C, Albayrak HT, Altinöz A, Ankarali H. Pseudomonas aeruginosa Suşlarinda Antibiyotiklere Direnç ve Beta-Laktamaz Oranları. Ankem derg. 2010;24(3):117-23.
8. Murray TS, Egan M, Kazmierczak BI. Pseudomonas aeruginosa chronic colonization in cystic fibrosis patients. Cur Opn Ped. 2007;19(1):83-88.
9. Wang Y, Dai Y, Zhang Y, Hu Y, Yang B, Chen S. Effects of quorum sensing autoinducer degradation gene on virulence and biofilm formation of Pseudomonas aeruginosa. Science in China Series C: Life Sciences. 2007;50(3):385-391.
10. Ra'oof WaM. Distribution of algD, lasB, pilB and nan1 genes among MDR clinical isolates of Pseudomonas aeruginosa in respect to site of infection. Tikrit Med J. 2011;17(2):148-160.
11. Jaffar-Bandjee MC, Lazdunski A, Bally M, Carrère J, Chazalette JP, Galabert C. Production of elastase, exotoxin A, and alkaline protease in sputa during pulmonary exacerbation of cystic fibrosis in patients chronically infected by Pseudomonas aeruginosa. J Clin Microb. 1995;33(4):924-929.
12. König B, Vasil M, König W. Role of haemolytic and non-haemolytic phospholipase C from Pseudomonas aeruginosa in interleukin-8 release from human monocytes. J Med Microb. 1997;46(6):471-478.
13. Ostroff R, Vasil A, Vasil M. Molecular comparison of a nonhemolytic and a hemolytic phospholipase C from Pseudomonas aeruginosa. J Bacteriol. 1990;172(10):5915-5923.
14. Livermore DM. Multiple mechanisms of antimicrobial resistance in Pseudomonas aeruginosa: Our worst nightmare? Clin. Infect Dis. 2002;34(5):634–640.
15. Bradley JS, Garau J, Lode H, Rolston KV, Wilson SE, Quinn JP. Carbapenems in clinical practice: a guide to their use in serious infection. Int J Antimicrob Agents. 1999;11(2):93–100.
16. Khosravi AD, Mihani F. Detection of metallo-beta-lactamase-producing Pseudomonas aeruginosa strains isolated from burn patients in Ahwaz, Iran. Diagn Microbiol Infect Dis. 2008;60(1):125–8.
17. Tacconelli E, Carrara E, Savoldi A, et al. Discovery, research, and development of new antibiotics: the WHO priority list of antibiotic-resistant bacteria and tuberculosis. Lancet Infect Dis. 2018;18(3): 318-327.
18. Hu YY, Cao JM, Yang Q, et al. Risk Factors for carbapenem-resistant Pseudomonas aeruginosa, Zhejiang Province, China. Emerg Infect Dis. 2019;25(10):1861-1867.
19. Berrin U, Güngör S, Sezak N, Afşar İ, ŞERİFHAN-İLGÜN M, Demirci M. Yoğun bakım hastalarının kan kültürlerinden izole edilen Pseudomonas aeruginosa ve Acinetobacter baumannii izolatlarının antibiyotik direnç yüzdelerindeki değişim. Türk Hij Den Biy Derg. 2012;71(1):25-30.
20. Park Y, Koo SH. Epidemiology, Molecular Characteristics, and Virulence Factors of Carbapenem-Resistant Pseudomonas aeruginosa Isolated from Patients with Urinary Tract Infections. Infection and Drug Resistance, 2022;15:141.
21. Faraji F, Mahzounieh M, Ebrahimi A, Fallah F, Teymournejad O, Lajevardi B. Molecular detection of virulence genes in Pseudomonas aeruginosa isolated from children with Cystic Fibrosis and burn wounds in Iran. Microb Pathogen. 2016;99:1-4.
22. Maçin S, KITTANA FNA, YILMAZ YA. Çeşitli klinik örneklerden izole edilen pseudomonas aeruginosa suşlarının virulans faktörlerinin incelenmesi. Cukurova Med J. 2017;42(2):308-313.
23. Alvarez-Buylla A, Allen M, Betts D, et al. Multicentre study of the in vitro activity of ceftolozane/tazobactam and other commonly used antibiotics against Pseudomonas aeruginosa isolates from patients in the UK. J Antimicrob Chemother. 2020;2(2): doi:10.1093/jacamr/dlaa024
24. Trautmann M, Lepper PM, Haller M. Ecology of Pseudomonas aeruginosa in the intensive care unit and the evolving role of water outlets as a reservoir of the organism. Am J Infect Cont. 2005;33(5):S41-S49.
25. Alhede M, Bjarnsholt T, Givskov M, Alhede M. Pseudomonas aeruginosa biofilms: mechanisms of immune evasion. Adv Appl Microb. 2014;86:1-40.
26. Döring G. Significance of Pseudomonas aeruginosa virulence factors in acute and chronic Pseudomonas aeruginosa infections. Infect. 1987;15(1):47-50.
27. Özer B, Babayiğit C, Çolak S, Önlen C, Çimen F, Boyacıgil I, Akküçük Ş. Pnömoni Etkenleri ve Antimikrobiyal Direnç Durumları. Mustafa Kemal Üni Tıp Derg. 2016;7(27):45-53.
28. Sonmezer MC, Ertem G, Erdinc FS, Kaya Kılıc E, Tulek N, Adiloğlu A. Evaluation of risk factors for antibiotic resistance in patients with nosocomial infections caused by Pseudomonas aeruginosa. Can J Infect Dis Med Microb. 2016; doi.org/10.1155/2016/1321487.
29. Mitov I, Strateva T, Markova B. Prevalence of virulence genes among bulgarian nosocomial and cystic fibrosis isolates of Pseudomonas aeruginosa. Brazil J Microb 2010;41(3):588-595.
30. Wolska K, Szweda P. Genetic features of clinical Pseudomonas aeruginosa strains. Pol J Microbiol. 2009;58(3):255-260.
31. Pournajaf A, Razavi S, Irajian G, et al. Integron types, antimicrobial resistance genes, virulence gene profile, alginate production and biofilm formation in Iranian cystic fibrosis Pseudomonas aeruginosa isolates. Infez Med. 2018;26(3):226-236.
32. Ellappan K, Belgode Narasimha H, Kumar S. Coexistence of multidrug resistance mechanisms and virulence genes in carbapenem-resistant Pseudomonas aeruginosa strains from a tertiary care hospital in South India. J Glob Antimicrob Resist. 2018;12:37–43.
33. Al Dawodeyah HY, Obeidat N, Abu-Qatouseh LF, Shehabi AA. Antimicrobial resistance and putative virulence genes of Pseudomonas aeruginosa isolates from patients with respiratory tract infection. Germs. 2018;8(1):31–40.
34. Dogonchi AA, Ghaemi EA, Ardebili A, Yazdansetad S, Pournajaf A. Metallo-beta-lactamase-mediated resistance among clinical carbapenem-resistant Pseudomonas aeruginosa isolates in northern Iran: a potential threat to clinical therapeutics. Tzu-Chi Medical Journal. 2018;30(2):90–96.
35. Bogiel T, Prażyńska M, Kwiecińska-Piróg J, Mikucka A, Gospodarek-Komkowska E. Carbapenemresistant Pseudomonas aeruginosa strains distribution of the essential enzymatic virulence factors genes. Antibiotics. 2021;10(8): 1-10.
36. Andersson DI, Balaban NQ, Baquero F, et al. Antibiotic resistance: turning evolutionary principles into clinical reality. FEMS Microb Rev 2020;44(2):171-188.
37. Pseudomonas aeruginosa in healthcare settings. HAI/CDC [online]< https://www cdc gov/hai/organisms/pseudomonas html>[accessed 12 March 2022].