DOI: 10.7860/JCDR/2020/16076.13697

Original Article

Microbiology Section

The Association between *lasB* and *nanI* Genes with Biofilm Formation in *Pseudomonas aeruginosa* Clinical Isolates

ZAHRA MAHDAVI[1], SAEED HEMATI[2], NOURKHODA SADEGHIFARD[3], FARID AZIZI JALILIAN[4], MOROVAT TAHERIKALANI[5], LIDA BIMANAND[6], IRAJ PAKZAD[7], SOBHAN GHAFOURIAN[8]

ABSTRACT

Introduction: Several acute and chronic infections in humans can be caused by *Pseudomonas aeruginosa (P.aeruginosa)*. Biofilm formation by these bacteria threatens health setting via increasing resistance against antimicrobial agents.

Aim: To investigate the association between *lasB* and *nanI* genes with biofilm formation in *P. aeruginosa* clinical isolates.

Materials and Methods: A total of 161 *P. aeruginosa* clinical isolates were collected. The Microtiter Plate (MTP) method was applied for biofilm formation assay. Gene amplification was conducted by PCR method with specific primers.

Results: Present study findings showed that the prevalence of *lasB* and the *nanI* were 80.7% (130/161) and 24.8% (40/161), respectively. The biofilm formation results demonstrated that 15.5% (25/161) of isolates were not able to produce biofilm. It was considerable that the prevalence of *lasB* in *P. aeruginosa* producing biofilm was higher.

Conclusion: *lasB* can be considered as an effective factor for biofilm formation in *P. aeruginosa* isolates.

Keywords: Elastase B, Neuraminidase, Quorum sensing

INTRODUCTION

Bacteria commonly are able to attach themselves and make biofilm structures. Biofilms formation could be observed on natural, medical or industrial settings and causes adverse effects on human's life or health [1]. Typically, in a mature biofilm, bacteria are more resistant to antimicrobial agents by different mechanisms [2].

P. aeruginosa is an important environmental organism which is capable enough to infect in human host [3]. It also causes acute and persistent infections in immunocompromised patients, especially Cystic Fibrosis (CF) individuals [4]. *P. aeruginosa* possesses many Virulence Factors (VFs) [5]. *LasB* or elastase B, encoded by *lasB* gene, is a bacterial metalloprotease which acts as a polysaccharide (alginate) secretion regulator, degrades elastin and surfactant protein D (SP-D). SP-D has been recognised to bacterial aggregation, alveolar macrophage function alteration and bacterial clearance regulation [6-9]. It has been shown that *lasB is* related to biofilm formation [6,10]. *P. aeruginosa* is a neuraminidase producer, which is encoded by *nan* gene. Some bacterial neuraminidase is responsible for airway colonisation, pathogenesis (i.e. respiratory tract infection), and biofilm formation [11,12]. Because of diverse issues by biofilm formation in bacteria, detection regulated forming genes in freely swimming cells may be a good strategy to encounter its' formation. Therefore, we investigated the prevalence of two previously introduced biofilm regulation genes (*lasB* and *nanI* genes) and their association with biofilm formation in clinical isolates of *P. aeruginosa.*

MATERIALS AND METHODS

Bacterial isolates and culture conditions: The present study was a cross-sectional investigation which was conducted on 161 *P. aeruginosa* isolates collected from clinical samples during March 2014 to February 2015 in Ilam hospitals, Iran. *P. aeruginosa* isolates were identified by standard biochemical tests.

Biofilm assays: The biofilm formation was evaluated by recommended protocol by Hemati et al., [5].

Gene amplification: The PCR was performed by specific primers listed in [Table/Fig-1] [13,14].

Primer	Sequence	Product length	Annealing temperature	References
lasB forward	5'-TTCTACCCGAAGGACTGATAC-3'			
lasB reverse	5'-AACACCCATGATCGCAAC-3'	153 bp	55	[13]
nanI forward	5'-CGCACTATACACAGGAACACG-3'			
nanI reverse	5'-GCCTAGCGGAAGGATCGTCGC-3'	620 bp	64	[14]

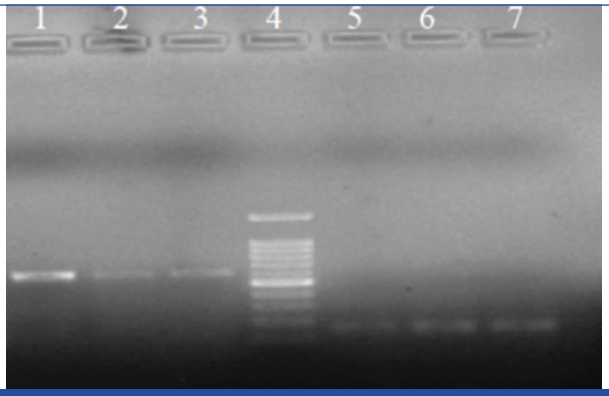
[Table/Fig-1]: Primers sequence and PCR condition [13,14].

STATISTICAL ANALYSIS

The SPSS software version 16.0 with χ^2 and Fisher's-Exact programs were applied for statistical analysis. The p-values <0.05 was considered as statistical significance.

RESULTS

PCR results: Present study demonstrated that 80.7% of isolates were positive for *lasB* (n=130) and 24.8% of isolates showed *nanI* (n=40) in their genome. Notably, it was not observed a significant association between the presences of *nanI* with *lasB* (p>0.05) [Table/Fig-2].



[Table/Fig-2]: Electrophoresis of PCR products for nanI (lanes 1-3) and lasB (lanes 5-7); ladder 100 bp (lane 4).

Biofilm formation results: The results showed that 84.5% (n=136) isolates were biofilm producer, while 15.5% (n=25) isolates had no ability to produce biofilm. Notably, the majority of *P. aeruginosa* isolates produced moderate (47.2%) and weak (24.8%) biofilm formation [Table/Fig-3]. Present study findings showed that there was a significant association between the presence of *lasB* and ability for biofilm formation (p=0.01), while it was not considered for *nanI* (p=0.102).

Samples	Biofilm formation producer				Total
	No producer	Weak producer	Moderate producer	Strong producer	
Urine	5 (3.1%)	21 (13.0%)	*34 (21.1%)	5 (3.1%)	65 (40.4%)
Tracheal	4 (2.5%)	8 (5.0%)	*11 (6.8%)	7 (4.3%)	30 (18.6%)
Wound	1 (0.6%)	1 (0.6%)	*9 (5.6%)	0 (0.0%)	11 (6.8%)
Purulent exudates	1 (0.6%)	1 (0.6%)	*2 (1.2%)	0 (0.0%)	4 (2.5%)
Burn	13 (8.1%)	8 (5.0%)	*20 (12.4%)	7 (4.3%)	48 (29.8%)
CSF	*1 (0.6%)	*1 (0.6%)	0 (0.0%)	0 (0.0%)	2 (1.2%)
Sputum	0 (0.0%)	0 (0.0%)	0 (0.0%)	*1 (0.6%)	1 (0.6%)
Total	25 (15.5%)	40 (24.8%)	76 (47.2%)	20 (12.4%)	161 (100.0%)

[Table/Fig-3]: Biofilm formation status in different samples.
*Majority form of biofilm formation producer

DISCUSSION

The pathogenicity of *P. aeruginosa* is multifactorial, including destroying host tissues, fighting host immune systems, biofilm formation [15,16]. *P. aeruginosa* is responsible for diverse human infections [17]. Here, most of the isolates were collected from urine samples (65, 40.4%). In previous published researches, maximum infection was reported from wound (45%), urinary tract (26.74%) and pulmonary tract (44%) infections [18-20].

These isolates also produces several proteolytic enzymes e.g., elastase B or pseudolysin (a Zn-metalloendopeptidase) endopeptidases enzymes that is encoded by *lasB* gene [21]. In the current study, *lasB* was presented in 80.7% of isolates. Although, Nikbin V et al., and Wolska K and Szweda P demonstrated that *lasB* presented in 100% and 96.8% of isolates, respectively [20,22]. Present study findings had also high frequency with lower quantity compared with mentioned results. The geographical difference and patient condition likely related to these variations.

Another important VFs in *P. aeruginosa* is neuraminidase, which encoded by *nanI* gene [20]. Its frequency (24.8%) was lower than *lasB* (80.7%). Mitov I et al., observed that 21.3% of isolates were positive for *nanI*, while *lasB* had 100% frequency [23]. The results of other studies were as follow: Nikbin et al.,: *nanI* (27.61%) and *lasB* (100%); Lanotte P et al.,: *nanI* (53%) and *lasB* (100%) [20,24]. Because *nanI* has the most prevalence in CF isolates [24], it can be probably the reason for low frequency in present study. Its frequency (89%) was also higher than elastase (84%) in CF isolates [25].

Present study finding demonstrated that 84.5% of isolates formed biofilm (12.4% strong, 47.2% moderate and 24.8% weak). Biofilm structures cause serious health problems by increasing bacterial resistance against host immune systems, biocides, antibiotics as well as various physicochemical agents [26]. Especially, biofilm formation in *P. aeruginosa* has been shown to play an important role in chronic infections in CF patients [27]. Wakimoto N et al., showed that 5.95%, 3.45% and 90.59% of isolates formed biofilm as strong, moderate and none or weak forms, respectively [28]. Hemati S et al., demonstrated that 19.28%, 21.4%, and 56.42% of isolates formed biofilm as weak, moderate and strong forms, while 12.85% were not able to produce biofilm formation [5]. Karimi S et al., reported that 29.3%, 38.6%, 23.3% of isolates formed strongly, moderately, and weakly biofilm respectively, while 8.6% of isolates were not able to biofilm forming [29]. Stepanovic S et al., showed that most *Salmonella* species and *Listeria monocytogenes* strains formed moderate and weak biofilm structures, respectively [30], while Borucki MK et al., reported that most *L. monocytogenes* strains formed strong biofilm formation [31]. However, the biofilm formation assay method, kind of medium, geographically difference of isolates can significantly be influenced on the biofilm forming in different studies. In addition, important limitation for in-vitro biofilm formation assay is that these methods are not able to exactly reflection in vivo situations [32].

It has been shown that *LasB* initiates biofilm formation pathway through secreted polysaccharides regulation and nucleoside diphosphate kinase (NDK) activation [33,34]. We also observed high tendency for biofilm formation among *lasB* positive isolates. Although it has been demonstrated that neuraminidase has relation in biofilm formation [35], no significant association between *nanI* (p=0.102) and biofilm formation was seen. Therefore, biofilm formation may occur by different other mechanisms [36].

Limitation(s)

Because of financial limitations, present study was conducted in a small number of molecular gene analysis that was limited to the *lasB* and *nanI* prevalence. The second is that to have better understanding of the relationship between biofilm formation and presence of *lasB* and *nanI* genes, it was better that biofilm formation compared in both bacterial wild-type and mutant-type.

CONCLUSION(S)

P. aeruginosa isolates have high ability for biofilim formation. Biofilim formation in these isolates depended on different mechanisms, and *lasB* considered as an important effective factor for biofilm formation.

Acknowledgement

The authors would like to thank Deputy of Research and Technology of Ilam University of Medical Sciences, Iran, for financial and equipment support. The results described in this paper were part of student thesis.

REFERENCES

[1] López D, Vlamakis H, Kolter R. Biofilms. Csh Perspect Biol. 2010;2(7):a000398.
[2] Jahid IK, Ha SD. A review of microbial biofilms of produce: Future challenge to food safety. FSB. 2012;21(2):299-316.
[3] McKnight SL, Iglewski BH, Pesci EC. The *Pseudomonas* quinolone signal regulates rhl quorum sensing in *Pseudomonas aeruginosa*. JB. 2000;182(10):2702-08.
[4] Schuster M, Greenberg EP. A network of networks: Quorum-sensing gene regulation in *Pseudomonas aeruginosa*. Int J Med. 2006;296(2):73-81.
[5] Hemati S, Jalilian FA, Pakzad I, Taherikalani M, Maleki A, Karimi S, et al. The correlation between the presence of quorum sensing, toxin-antitoxin system genes and MIC values with ability of biofilm formation in clinical isolates of *Pseudomonas aeruginosa*. Iran J Med Microbiol. 2014;6(3):133-39.
[6] Carson L, Cathcart GR, Ceri H, Walker B, Gilmore BF. Comparison of the binding specificity of two bacterial metalloproteases, *LasB* of *Pseudomonas aeruginosa* and *ZapA* of *Proteus mirabilis*, using N-alpha mercaptoamide template-based inhibitor analogues. Biochem Biophys Res Commun. 2012;422(2):316-20.
[7] Brumlik M, Storey D. Post-transcriptional control of *Pseudomonas aeruginosa lasB* expression involves the 5' untranslated region of the mRNA. Fems Microbiol Lett. 1998;159(2):233-39.
[8] Amaya S, Pereira JA, Borkosky SA, Valdez JC, Bardón A, Arena ME. Inhibition of quorum sensing in *Pseudomonas aeruginosa* by sesquiterpene lactones. Phytomedicine. 2012;19(13):1173-77.
[9] Bleves S, Viarre V, Salacha R, Michel GP, Filloux A, Voulhoux R. Protein secretion systems in *Pseudomonas aeruginosa*: A wealth of pathogenic weapons. Int J Med Microbiol. 2010;300(8):534-43.
[10] Kamath S, Chen M, Chakrabarty A. Secretion of nucleoside diphosphate kinase by mucoid *Pseudomonas aeruginosa* 8821: Involvement of a carboxy-terminal motif in secretion. JB. 2000;182(13):3826-31.
[11] Hsiao Y-S, Parker D, Ratner AJ, Prince A, Tong L. Crystal structures of respiratory pathogen neuraminidases. Biochem Biophys Res Commun. 2009;380(3):46771.
[12] Parker D, Soong G, Planet P, Brower J, Ratner AJ, Prince A. The NanA neuraminidase of *Streptococcus pneumoniae* is involved in biofilm formation. Infect Immun. 2009;77(9):3722-30.
[13] Soong G, Muir A, Gomez MI, Waks J, Reddy B, Planet P, et al. Bacterial neuraminidase facilitates mucosal infection by participating in biofilm production. J Clin Invest. 2006;116(8):2297.
[14] Petit SM-C, Lavenir R, Colinon-Dupuich C, Boukerb AM, Cholley P, Bertrand X, et al. Lagooning of wastewaters favors dissemination of clinically relevant *Pseudomonas aeruginosa*. Res Microbiol. 2013;164(8):856-66.

[15] Singh BN, Singh HB, Singh A, Singh BR, Mishra A, Nautiyal C. *Lagerstroemia speciosa* fruit extract modulates quorum sensing-controlled virulence factor production and biofilm formation in *Pseudomonas aeruginosa*. Microbiology. 2012;158(2):529-38.
[16] Oldak E, Trafny EA. Secretion of proteases by *Pseudomonas aeruginosa* biofilms exposed to ciprofloxacin. Antimicrob Agents Chemother. 2005;49(8):3281-88.
[17] Caselli D, Cesaro S, Ziino O, Zanazzo G, Manicone R, Livadiotti S, et al. Multidrug resistant *Pseudomonas aeruginosa* infection in children undergoing chemotherapy and hematopoietic stem cell transplantation. Haematologica. 2010;95(9):1612-15.
[18] Troillet N, Samore MH, Carmeli Y. Imipenem-resistant *Pseudomonas aeruginosa*: Risk factors and antibiotic susceptibility patterns. Clin Infect Dis. 1997;25(5):1094-98.
[19] Tavajjohi Z, Moniri R, Khorshidi A. Detection and characterization of multidrug resistance and extended-spectrum-beta-lactamase-producing (ESBLS) *Pseudomonas aeruginosa* isolates in teaching hospital. African J Microbiol Res. 2011;5(20):3223-28.
[20] Nikbin V, Aslani MM, Sharafi Z, Hashemipour M, Shahcheraghi F, Ebrahimipour G. Molecular identification and detection of virulence genes among *Pseudomonas aeruginosa* isolated from different infectious origins. Iran J Med Microbiol. 2012;4(3):118.
[21] Ghorbel-Bellaaj O, Hayet BK, Bayoudh A, Younes I, Hmidet N, Jellouli K, et al. *Pseudomonas aeruginosa* A2 elastase: Purification, characterization and biotechnological applications. Int J Biol Macromol. 2012;50(3):679-86.
[22] Wolska K, Szweda P. Genetic features of clinical *Pseudomonas aeruginosa* strains. Pol J Microbiol. 2009;58(3):255-60.
[23] 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.
[24] Lanotte P, Watt S, Mereghetti L, Dartiguelongue N, Rastegar-Lari A, Goudeau A, et al. Genetic features of *Pseudomonas aeruginosa* isolates from cystic fibrosis patients compared with those of isolates from other origins. J Med Microbiol. 2004;53(1):73-81.
[25] Lanotte P, Mereghetti L, Lejeune B, Massicot P, Quentin R. *Pseudomonas aeruginosa* and cystic fibrosis: Correlation between exoenzyme production and Patient's clinical state. Pediatr Pulmonol. 2003;36(5):405-12.
[26] Chavant P, Gaillard-Martinie B, Talon R, Hébraud M, Bernardi T. A new device for rapid evaluation of biofilm formation potential by bacteria. J Microbiol Meth. 2007;68(3):605-12.
[27] Musk DJ, Banko DA, Hergenrother PJ. Iron salts perturb biofilm formation and disrupt existing biofilms of *Pseudomonas aeruginosa*. Chem Biol. 2005;12(7):789-96.
[28] Wakimoto N, Nishi J, Sheikh J, Nataro JP, Sarantuya J, Iwashita M, et al. Quantitative biofilm assay using a microtiter plate to screen for enteroaggregative *Escherichia coli*. Am J Trop Med Hyg. 2004;71(5):687-90.
[29] Karimi S, Ghafourian S, Kalani MT, Jalilian FA, Hemati S, Sadeghifard N. Association between toxin-antitoxin systems and biofilm formation. Jundishapur J.Microbiol. 2015;8(1).
[30] Stepanovic S, cirkovic I, Ranin L. Biofilm formation by *Salmonella* spp. and *Listeria monocytogenes* on plastic surface. Lett Appl Microbiol. 2004;38(5):428-32.
[31] Borucki MK, Peppin JD, White D, Loge F, Call DR. Variation in biofilm formation among strains of *Listeria monocytogenes*. Appl Environ Microbiol. 2003;69(12):7336-42.
[32] Stepanovic S, Vukovic D, Hola V, Bonaventura GD, Djukic S, cirkovicl, et al. Quantification of biofilm in microtiter plates: Overview of testing conditions and practical recommendations for assessment of biofilm production by staphylococci. APMIS. 2007;115(8):891-99.
[33] Sharma G, Rao S, Bansal A, Dang S, Gupta S, Gabrani R. *Pseudomonas aeruginosa* biofilm: potential therapeutic targets. Biologicals. 2014;42(1):01-07.
[34] Cathcart GR, Gilmore BF, Greer B, Harriott P, Walker B. Inhibitor profiling of the *Pseudomonas aeruginosa* virulence factor LasB using N-alpha mercaptoamide template-based inhibitors. Bioorg Med Chem Lett. 2009;19(21):6230-32.
[35] Xu G, Ryan C, Kiefel MJ, Wilson JC, Taylor GL. Structural studies on the *Pseudomonas aeruginosa* sialidase-like enzyme PA2794 suggest substrate and mechanistic variations. J Mol Biol. 2009;386(3):828-40.
[36] Ghafourian S, Raftari M, Sadeghifard N, Sekawi Z. Toxin-antitoxin systems: Classification, biological function and application in biotechnology. Horizon Scientific Press. 2014.

PARTICULARS OF CONTRIBUTORS:

1. Postgraduate Student, Department of Microbiology, Faculty of Medicine, Clinical Microbiology Research Center, Ilam University of Medical Sciences, Ilam, Iran.
2. Postgraduate Student, Department of Microbiology, Faculty of Medicine, Clinical Microbiology Research Center, Ilam University of Medical Sciences, Ilam, Iran.
3. Professor, Department of Microbiology, Faculty of Medicine, Clinical Microbiology Research Center, Ilam University of Medical Sciences, Ilam, Iran.
4. Assistant Professor, Department of Microbiology, Faculty of Medicine, Clinical Microbiology Research Center, Ilam University of Medical Sciences, Ilam, Iran.
5. Professor, Department of Microbiology, Faculty of Medicine, Clinical Microbiology Research Center, Ilam University of Medical Sciences, Ilam, Iran.
6. Postgraduate Student, Department of Microbiology, Faculty of Medicine, Clinical Microbiology Research Center, Ilam University of Medical Sciences, Ilam, Iran.
7. Professor, Department of Microbiology, Faculty of Medicine, Clinical Microbiology Research Center, Ilam University of Medical Sciences, Ilam, Iran.
8. Assistant Professor, Department of Microbiology, Faculty of Medicine, Clinical Microbiology Research Center, Ilam University of Medical Sciences, Ilam, Iran.

NAME, ADDRESS, E-MAIL ID OF THE CORRESPONDING AUTHOR:

Dr. Iraj Pakzad,
PhD., Department of Microbiology, Faculty of Medicine, Ilam University of Medical Sciences, Ilam, Iran.
E-mail: Pakzad_i2006@yahoo.com

PLAGIARISM CHECKING METHODS: [Jain H et al.]

- Plagiarism X-checker: Aug 05, 2015
- Manual Googling: Aug 12, 2016
- iThenticate Software: Apr 18, 2020 (9%)

ETYMOLOGY: Author Origin

AUTHOR DECLARATION:

- Financial or Other Competing Interests: As declared above
- Was Ethics Committee Approval obtained for this study? Yes
- Was informed consent obtained from the subjects involved in the study? Yes
- For any images presented appropriate consent has been obtained from the subjects. NA

Date of Submission: **Aug 04, 2015**
Date of Peer Review: **Sep 05, 2015**
Date of Acceptance: **Aug 16, 2016**
Date of Publishing: **May 01, 2020**