Original Article

Detection of Exopolysaccharides (*algD*, *pelF,* and *pslD*) Genes in Burn Wound *Pseudomonas aeruginosa* Isolates

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Abstract

Background: *Pseudomonas aeruginosa* biofilm is an essential component of virulence that plays a significant role in antimicrobial resistance and chronic burn wound infections. **Objective:** This study aimed to investigate the biofilm formation capacity of *P. aeruginosa* isolated from chronic burn wound from January to May 2022 by biochemical and molecular techniques. **Materials and Methods:** Quantification of biofilm was performed based on tube method for local isolates of *P. aeruginosa* after growing on brain heart Broth. The genes encoding exopolysaccharides (*algD*, *pelF*, and *pslD*) were targeted by using conventional polymerase chain reaction (PCR). **Results:** The results showed that 92.6% of isolates were biofilm former, interestingly 68% of isolates were considered as strong former comparing with other biofilm categories. Gel electrophoresis result of PCR products presented clear bands for *algD* and *psID* genes with percentages (96%) and (3.7%) respectively. However, there was no PCR product for *pelf* gene among all isolates. **Conclusion:** The prevalence of *algD*, the large operon necessary for alginate production, was high among *P. aeruginosa* biofilm producer in this study and it can be an essential agent in the pathogenicity of *P. aeruginosa* burn wound infections comparing with other biofilm genes (*pelF* and *pslD*) of exopolysaccharide structure.

Keywords: Biofilm, burn wounds, exopolysaccharides, *Pseudomonas aeruginosa*

Introduction

Burn wound infections are among the most serious issues that may arise after a burn injury, and they can result in significant clinical complications as well as a higher mortality rate and morbidity.[1,2] *Pseudomonas aeruginosa*, a G-ve bacillus, is one of the most common causes of burn infections.[1] The phenotypic adaptability and high level of genetic flexibility in these bacteria are responsible to increase the pathogenicity along with their ability to modify the expression of virulence factor during wound infection such as biofilm development.[2,3] In such a condition of wound infection, the period of treatment and healing might be delayed by two to four weeks due to these hospital-acquired illnesses.[4] In a 2013, a study by Roy *et al.*[5] demonstrated that biofilm acts as a mediator between the bacteria and their environment, providing protection against antibiotics and immune system. The ability to generate signaling molecules that mediate biofilm behavior and the production of genes implicated in antimicrobial resistance are two examples of the adaptive mechanisms provided by biofilms, that promote the expansion of bacterial growth.[6] The major issue in controlling *P. aeruginosa* burn infections is the establishment of multidrug-resistant infections, which are extremely difficult to cure.[5,7-9] *Pseudomonas aeruginosa's* biofilm matrix is a structure consisting of three distinct exopolysaccharides that includes: alginate, Psl, and Pel. The most studied structure, alginate, significantly contributes in the protection of the biofilm as well as structural stability, is a polymer composed of α-d-mannuronic acid and β-l-guluronic acid.[10] Previous studies reported that mucoid variants, frequently isolated from the lungs of chronically colonized cystic fibrosis patients, are

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mostly related with alginate overproduction.[11,12] On the other hand, non-mucoid bacteria rely on Pel and Psl polysaccharides.[13] The polysaccharide Psl is a structure of a repeating pentasaccharide comprising D-mannose, D-glucose, and L-rhamnose, is essential for the development and protection of biofilms. Glucose-rich Pel is the 3rd polysaccharide discovered in the biofilm of *P. aeruginosa*.[14] In 2016, a group of researchers found that *P. aeruginosa* biofilm development involves a large number of surface proteins.[10] This work aimed to examine the frequency of genes encoding biofilm exopolysaccharides (*pelF*, *algD*, and *pslD*) in *P. aeruginosa* chronic burn wound isolates to better understand the genetic basis for the production of extracellular matrices in distinct *P. aeruginosa* biofilms.

Materials and Methods

Samples collection and cultural characteristics

Throughout the period of January 2022 to May 2022, 70 swabs were taken from burn wounds and diagnosed in the labs of Al-Hillah Teaching Hospital. After incubating on blood agar and nutrient agar plates, all swabs were transferred to MacConkey agar (Hi-Media, India) and cetrimide agar media to grow as single colonies (Oxoid, UK). The VITEK® 2 technology was depended to confirm the identification of *P. aeruginosa* colonies, after which they had been identified on the basis of their morphological and biochemical characteristics.

Biofilm assay

According to O'Toole,[15] the biofilm development of *P. aeruginosa* isolates was studied. Biofilm experiments were performed by diluting overnight cultures of *P. aeruginosa* isolates from 5 mL of trypticase soy broth (Gibco) into 1:100 with fresh medium (all cultures were fixed at optical density (OD) = 0.06). For the purpose of quantitative analysis, four identical tubes were used for each overnight culture across all isolates. After incubation, the cells were dumped out by shaking the tubes upside down. Shake the water out of the tubes after placing them gently in a container of tank. Carry out these steps once more. This step helps reduce background staining by removing detached cells and medium components that can be stained. Three milliliters (mL) of a crystal violet solution (0.1%) were poured in each tube to stain the biofilm. The tubes should be incubated for 10–15 min at room temperature. Turn the biofilm tubes upside down and let them air dry for few hours or overnight after rinsing them three to four times with water by submerging in a tank of water as described above. OD was read by spectrophotometer at 550 nm. The calculations of biofilm results were interpreted as follows.[16]

If OD < ODc, the biofilm was not formed (negative)

If ODc < OD < 2× ODc, the biofilm was weak.

If 2× ODc <OD < 4× ODc, the biofilm was moderate.

If 4× ODc < OD. The biofilm was strong.

Detection of genes encoding biofilm exopolysaccharides

Using a modified protocol of the classic phenol/chloroform procedure, DNA from *P. aeruginosa* isolates was extracted and purified.[17] Genes encoding exopolysaccharides (*pelF*, *algD*, and *pslD*) were the targets of conventional polymerase chain reaction (PCR) for molecular identification. The predicted size of the amplified PCR product, the annealing temperature, and the primers used[10] for all genes are detailed in Table 1. The PCR procedure followed the manufacturer's instructions for the Taq Green master mix by Promega. These thermocycling conditions were: 5 min of initial denaturation at 95ºC, 1 min of denaturation at 95ºC, annealing temperature was variable among different genes as listed in Table 1 at 30 min, 45 s of extension at 72ºC, and 5 min at 72ºC for final extension. After 45 min of electrophoresis at 120 V on 1% of agarose for each PCR products, the amplicons were detected using UV eliminator.

Ethical approval

The study was conducted in accordance with the ethical principles that have their origin in the Declaration of Helsinki. It was carried out with patients verbal and analytical approval before sample was taken. The study protocol and the subject information and consent form were reviewed and approved by a local ethics committee according to document number B220105 in January 17, 2022 to get this approval.

Results

Identification of *Pseudomonas aeruginosa* isolates

Taking into account the morphological features of *P. aeruginosa* isolates grown on MacConkey and

Table 1: Primers sequence, annealing temperature, and the expected size of genes encoding biofilm exopolysaccharides (pelF, algD, and pslD)

Gene	Primer sequence (5'–3')	Annealing temp. (˚C)	Size of amplicon (bp)
algD	F-CTACATCGAGACCGTCTGCC	58	593
	R-GCATCAACGAACCGAGCATC		
pelF	F-GAGGTCAGCTACATCCGTCG	58	789
	R-TCATGCAATCTCCGTGGCTT		
pslD	F-TGTACACCGTGCTCAACGAC	56	369
	R-CTTCCGGCCCGATCTTCATC		

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Table 2: Biofilm evaluation among *P. aeruginosa* isolates

Biofilm evaluation		No.	%	
Non-adherent		2	7.4	
Adherent	Strength of adherent		% among adherent	% among total
	Weakly adherent	2	8	7.4
	Moderately adherent	6	24	22
	Strongly adherent	17	68	63
	Total	27	92.6	

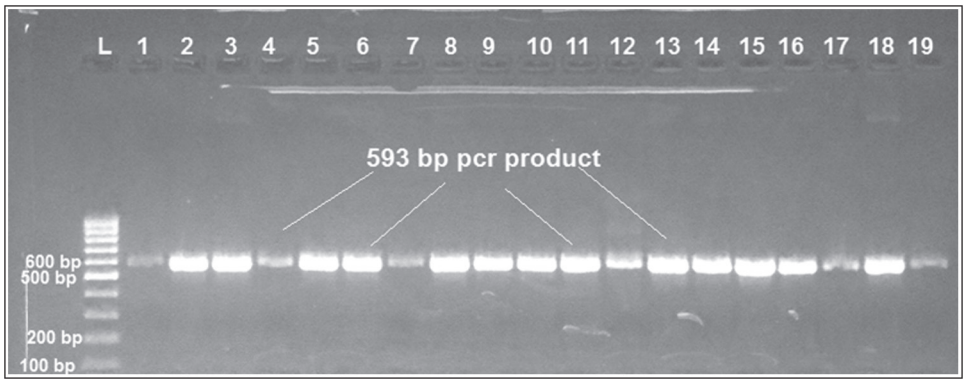

Figure 1: Agarose gel of algD polymerase chain reaction product (593 bp) among *P. aeruginosa* isolates. L lane 100 bp DNA ladder, lanes 1–19 the isolates at 85 volt for 60 min

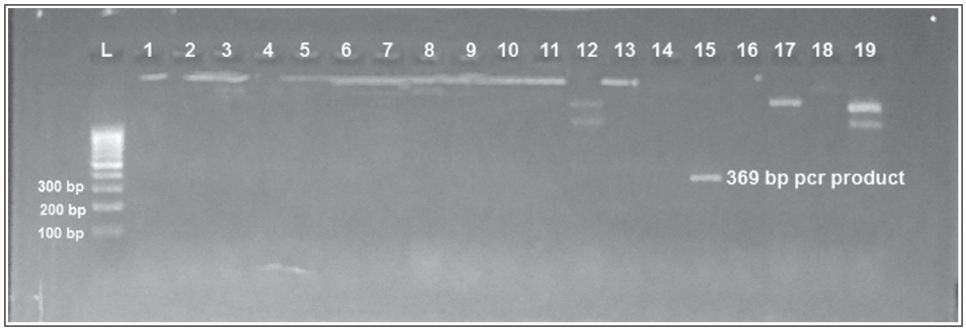

Figure 2: Agarose gel of pslD polymerase chain reaction product (369 bp) among *P. aeruginosa* isolates. L lane 100 bp DNA ladder, lanes 1–19 the isolates at 85 volt for 60 min

Cetrimide agar media, 27 isolates were initially identified. The Gram-negative, rod-shaped colonies on Cetrimide agar developed pyocyanin pigmentation, giving them a blue-green appearance. They were non-lactose fermented on MacConkey agar. Using the analytical profile index of the VITEK® 2 system and a biochemical test, their identification was verified (the identification percentage is id% = 93.8%).[18]

Evaluation of biofilm forming isolates

The findings revealed that 92.6% of *P. aeruginosa* isolates were biofilm former after growing on brain heart broth at 37ºC for 24 h. However, 7.4% of isolates were non-biofilm former [Table 2]. Biofilms produced by the tube method had a noticeable film lining the tube's wall and bottom, whereas non-biofilm producing strains were unable to form the lining. According to Stepanović *et al.*[16] formula: 68% of isolates were considered as strong former, whereas 24 and 8% of isolates were evaluated as moderate and weak biofilm production respectively among biofilm formers.

Identification of biofilm genes

The identification of exopolysaccharides genes including *algD*, *PslD*, and *PelF* has been investigated among 27 burn wounds isolates of *P. aeruginosa*. Gel electrophoresis result of PCR products [Figures 1 and 2] presented bands with sizes 593 and 369 for *algD* and *psID* respectively. However, there was no obvious bands for *pelF* gene among all isolates after electrophoresis. The results showed that the frequency for each virulence factor exopolysaccharides biofilm genes was as follow: *algD* was highly dominant among isolates with frequency reach up to (96%), whereas *pslD* was (3.7%), based on the presence of these genes in agarose gel [Table 3].

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Table 3: Frequency for each virulence factor exopolysaccharides biofilm genes

Gene	Frequency (%)
algD	96
pslD	3.7
pelF	0

Discussion

The species *P. aeruginosa* is among the most common pathogens causing severe infections in burn patients, and the rise of MDR strains of *P. aeruginosa* in healthcare institutions, particularly in burn units, is a main issue in the context of infection control.[1,19,20] After 24 h of growth at 37°C in brain heart broth, the majority of the isolates (91%) produced biofilms; of those, 61% were rated as strong formers, while the remaining 22% and 13% were recorded as moderate and weak biofilm producers, respectively. This characteristic may allow them to survive in the environment as being either commensal or pathogenic microorganisms, and it may also play a significant role in their virulence when they invasive wounds. Furthermore, due to the protective effect of the complex structure enclosing bacterial cells in an extracellular polymeric matrix, limited cellular metabolic activity in biofilm structure, and the function of efflux pumps that effectively eliminate antimicrobials from the bacterial cells, it can be considered one of the causes of treatment delayed of burn patients.[21,22] Therefore, whereas testing the anti-biofilm activity of anti-bacterial agents *in vitro*, it is important to take into account the variations in biofilm development among isolates. In consistent with previous research, we found that biofilm formation in *P. aeruginosa* isolates ranged from 83.75% to 98.4%.[7,10,23,24] Our research found that *P. aeruginosa* from burn wounds fell into one of three categories (high, medium, or low producers of biofilm) based on their varying biofilm-capabilities. This could be due to the presence of different biofilm production mechanisms within the same bacterial species or to the variations in their metabolic activity.[25]

The proteins of biofilm matrix in *P. aeruginosa* are mainly encoded by *algD*, *pel*, and *psl* genes.[14] The prevalence of these genes and their linkage with biofilm development were investigated in this study. The results showed that the frequency for each virulence factor exopolysaccharides biofilm genes was as follows: *algD* was highly dominant among different biofilm former categories with frequency reaching up to 95. This might be supported the evidence of relationship between the occurrence of this gene among *P. aeruginosa* isolates and their capability to produce biofilm. The prevalence of *alg*D in our study was compatible with the results of Banar *et al.*[10] who collected 57 isolates of *P. aeruginosa* from burn wound infections. However, other researches[26,27] found frequencies as high as 98% and 87.5%, respectively, whereas ours was high when compared with the findings of Zaranza *et al.*,[28] who reported a prevalence rate of 39%. Possible explanations for the observed variation in this gene's frequency are the differences in the clones commonly found in each region and the dispersion of those clones.[29] Only one of the strong former group isolates was tested positive for the presence of *pslD* based on the presence of these genes in agarose gel, for a frequency of 4.5%. According to the previous study by Branda *et al.*[30] there is no information about the incidence of *pslD* and *pelF* genes in other regions. Further research shows that the pel gene cluster is conserved among *P. aeruginosa* isolates, but the psl genes are not found, which is consistent with our results.

Conclusion

The prevalence of *algD*, the large operon necessary for alginate production, was high among *P. aeruginosa* biofilm producer in this study and it can be an essential agent in the pathogenicity of *P. aeruginosa* burn wound infections comparing with other biofilm genes (*pelF* and *pslD*) of exopolysaccharide structure.

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Conflicts of interest

There are no conflicts of interest.

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