

# Contribution of Lewis Blood Groups Molecules in Biofilm Formation of *Pseudomonas aeruginosa* Isolated From Atopic Dermatitis Patients.

**Khedhir H. Ali, Fatima R. Abdul\***

Department of Biology, College of Science, Mustansiriyah University, IRAQ.

\*Correspondent author email: [qetasyshah@gmail.com](mailto:qetasyshah@gmail.com)

## Article Info

Submitted  
01/11/2017

Accepted  
18/12/2017

## Abstract

Biofilm formation is a mechanism for bacterial community defense against insults including antibiotics. In this report we evaluated the potency of *Pseudomonas aeruginosa* (*P. aeruginosa*) isolates from atopic dermatitis patients skin as well as stool to colonize different Lewis types saliva, manifested by biofilm formation. The bacteria were cultured on tryptose soy broth, 96-well polystyrene plate were used. Coating with heat inactivated Le (a), (b), (c) a-b- and Le(d) a+b+ saliva was performed.

Biofilm intensity was measured using crystal violet stained films compared to non-saliva coated situation. The results showed a superior capability of most isolates to form biofilm on Le (a) followed by Le (b) saliva. The highest binding mean was for isolate (4). Le (a) saliva binding (mean  $\pm$  SD was  $0.66 \pm 0.25$  for test compared to  $0.21 \pm 0.04$  for control non coated wells),  $p=0.04, cl=0.041-0.864$ . Other isolates demonstrated variable degree of biofilm formation on this substrate. In contrast to Le (c) a-b- saliva, Le (d) a+b+ saliva demonstrated weak biofilm formation.

**Keywords:** Lewis blood groups, *Pseudomonas aeruginosa*, atopic dermatitis, Biofilm.

## الخلاصة

تكوين الغشاء الحيوي هو ميكانيكية دفاعية للمجتمع البكتيري ضد الاعتداءات وبضمنها المضادات الحيوية. في هذا البحث تم تقييم (تقدير) قدرة عزلات بكتيريا *Pseudomonas aeruginosa* (*P. aeruginosa*) المعزولة من جلد وخروج مرضى التهاب الجلد التأتبي على تكوين الغشاء الحيوي بوجود انواع مجاميع الدم اللويسية اللعابية. زرعت البكتريا على الوسط السائل Tryptose soy broth, واستعملت صفائح التسحيح الدقيق Microtitration plate ذات القعر المسطح flat bottom والتي تحتوي على 96 خفزة. غطيت جميع الحفر بلعاب مجاميع الدم اللويسية (a), (b), (c) و (c) غير الفعالة بالحرارة. تم قياس قدرة الغشاء الحيوي باستعمال الاغشية المصبغة بصبغة crystal violet مقارنة بالانواع غير المغطاة باللعاب.

اظهرت النتائج القدرة العالية لمعظم العزلات لتكوين الغشاء الحيوي على لعاب مجاميع الدم لويس (a) وبتبعها مجاميع الدم لويس (b). معدل الارتباط الاعلى كان للعزلة رقم (4). اعطى اعلى معدل ارتباط  $0.66 \pm 0.25$  لمجاميع الاختبار مقارنة مع تكوين الغشاء الحيوي للحفر الغير مغطاة والتي اظهرت معدل  $0.21 \pm 0.04$  ( $p=0.04, cl=0.041-0.864$ ). من جانب اخر, اظهرت العزلات درجة ارتباط متغايرة لنمط (b) اللعابية. لم تظهر العزلات أي ارتباط على مجاميع الدم لويس (c) و a-b- و لويس d (+a+b) اللعابية.

## Introduction

*Pseudomonas aeruginosa* is a Gram-negative opportunistic pathogen capable of various infection including bacteremia sepsis, pneumonia and other affecting immune compromised patients [4].

The organism has the ability to form biofilm leading to chronic infection [5]. *P. aeruginosa*

used its lectins to form biofilm [6]. The bacterium's lectins specificity is galactose for lectin (A) [7] and fucose for lectin (B) [8].

These ligands are represented in mucins and carried on a defined glycan structures represented by Lewis (a) and Lewis (b) [9]. Atopic dermatitis patients skin are usually colonized by *Staphylococcus aureus*, which is

implicated in the process of disease pathogenesis [10].

In the process of *S. aureus* isolation from atopic dermatitis patients skin, *P. aeruginosa* frequently isolated, their role in disease process is not known.

In this report we seemed to see if *P. aeruginosa* isolated from atopic dermatitis patients skin uses Lewis blood groups determinants to colonize and form biofilm associated with this disease.

## Materials and Methods

### Bacterial isolates

Eight isolates from atopic dermatitis were characterized to be, *P. aeruginosa*. These four isolates from stool and four isolates from skin, The bacteria were maintained on trypticase soy slants. For biofilm formation the isolates were cultivated using tryptose soy broth, at 37°C for 24 hours.

### Saliva coating

Saliva of Lewis blood groups a, b, c, and Le(d) (a+b+) were obtained from atopic dermatitis patients. Lewis typing was performed on red blood cells [1]. Saliva obtained from patients were diluted 1:2 with saline, boiled at 100°C for 10 minutes, centrifuged at 4000 r.p.m for 15 minutes and the supernatant they were stored at -20°C until used.

To coat polystyrene surface a dilution of 1:8 of supernatant in 0.05M bicarbonate buffer pH 8.6 was used. 96-well microtiterplates wells were coated with 100µl of supernatant, left at 4°C overnight. These plates were used to perform biofilm production from *P. aeruginosa*. Uncoated wells served as controls.

### Biofilm assay

Biofilm formation procedure was adopted from Subhi, 2015 [2]. Briefly bacteria were grown on tryptose soy broth at 37°C for 24 hours. An overnight culture was used to inoculate wells. The culture was diluted to 1:20 in tryptose soy broth medium containing 1% glucose. Wells was inoculated with 200µl of culture. Incubated at 37°C for 24 hours, washed for three times, dried, then stained with 100µl of 1% crystal violet, for 5 minutes.

Unbound dye was removed by (3x) washing with distilled water. The water was removed and plate was dried for 2 hours. Finally, all wells were filled by 200µl ethanol (95%) to release the dye from the cells and Optical density (OD) of stained adherent biofilm was obtained by using micro ELISA auto reader at wavelength 570 nm. Triplicate assays were performed for each treatment.

### Biostatistical analysis

Student t-test was used to compare means of test (biofilm on saliva coated wells) and controls (biofilm on non-coated wells), using a statistical program for epidemiological [3].

## Results and Discussion

Eight isolate of *P. aeruginosa* (4 from skin and 4 from stool) of patients of atopic dermatitis were screened for biofilm formation on defined Lewis groups saliva. The majority of the isolates showed binding and biofilm formation on Le(a) coats. The most prominent biofilm former was isolates No.4, 6 and 8, giving means of  $0.66 \pm 0.25$ ,  $0.5 \pm 0.12$  and  $0.39 \pm 0.06$  binding intensities respectively compared to  $0.21 \pm 0.04$ ,  $0.29 \pm 0.01$  and  $0.24 \pm 0.04$  for control respectively.

The highest percent increase of binding was for isolate No.4 which showed 222.06% increase over control (Figure 1 and Table 1).

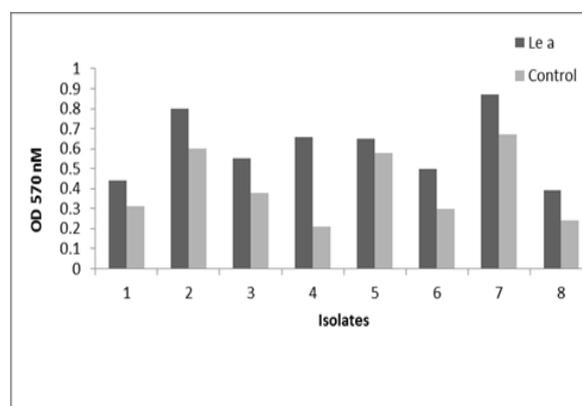


Figure 1: *P. aeruginosa* biofilm formation to Lewis (a) saliva supernatant coated wells compared to non-coated wells.

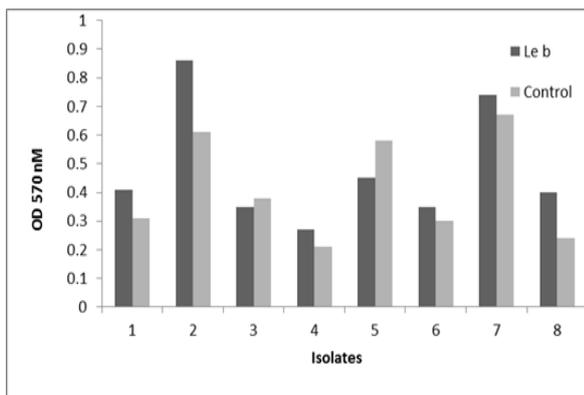


Figure 2: Binding of *P. aeruginosa* and biofilm formation of isolates from atopic dermatitis patients to Lewis (b) saliva coating compared to binding to non-coated wells.

In contrast Le(b) saliva coat showed a biofilm formation less than that seen in Le(a) saliva coat. Two of the isolates showed negative biofilm establishment on this substrate. The other isolates however, showed moderate degree of biofilm formation (Figure 2 and Table 1).

On Led (a+b+) saliva coating isolate (4) demonstrate appreciable binding intensity (mean  $0.38 \pm 0.15$  in tests versus  $0.21 \pm 0.04$  in control). On the other hand however, Lec (a-b-) coated saliva did not have any biofilm formation, except for isolate (4) (Table 1).

The novel findings presented in this report demonstrated clearly that *P. aeruginosa* utilized a substrate associated ligand, mostly of Le(a) variety. Which is purely represented in a defined structure of Le(a) saliva. Le(b) saliva contained Le(a) structure but the second fucose might somehow impair binding of bacteria that resulted in decrease of biofilm. This conclusion is strengthened when Le (a-b-) saliva formation didn't enhance or give any biofilm. What is peculiar in this investigation is that atopic dermatitis manifested increase isolation frequency of *P. aeruginosa*, a situation that might implicate this microorganism in the pathogenesis of this multifactorial-based disease [11].

*P. aeruginosa* have Lec(A) and Lec(B) (PA1L and PA11L) Lectins, mediating bacterial pathogenesis through biofilm formation [12-15].

Table 1: Augmentation of *P. aeruginosa* biofilm on different types of Lewis saliva..

isolates	% increase binding of biofilm intensities of saliva group			
	Lewis saliva group			
	a	b	c (Lea-b-)	d (Lea+b+)
1	*43-65	*33.88	-26.06	-28.34
2	*31.8	*41.64	-72.95	-48.69
3	44.39	-8.35	-45.43	-28.98
4	*222.06	*30.4	*82.85	*39.70
5	10.78	-23.28	-38.18	-70.2
6	*68.35	16.49	15.15	-33.33
7	*29.61	9.82	-70.38	-73.21
8	*63.33	*67.08	-4.58	-7.08

\*Binding percent increase was obtained based on the formula of  $T-C/C \times 100$ , were T represents mean of triplicate reading of biofilm density (O.D) and C is the mean of triplicate biofilm density on uncoated wells. Negative results in the table weren't statistically analyzed. \*refers to statistically significant result at  $p \leq 0.05$ . Le(a) group a, Le(b) group b, Le(a+b+) group c and Le(a-b-) group d.

The Ligand presumed to be Le(a) which is involved in biofilm formation as seen in this report could mediate colonization of the bacteria to skin and colon of atopic dermatitis[16].

Mucin is important carbon and nitrogen nutrient source and triggers changes in expression; decreases surface motility and promote biofilm formation [17, 18]. Also [19] found that *P. aeruginosa* strains form biofilm varied according to medium and strain type. Our results were compatible with [18] who found that *P. aeruginosa* biofilm formed on mucin-coated surfaces. Also published data suggested that a specific adhesin-mucin interaction immobilizes the bacterium on the surface [19].

## Conclusion

We concluded that, among atopic dermatitis patients skins, *P. aeruginosa* Lec (A) or Lec (B) lectins might be involved in colonization in such patients.

There seem to be a relationship between bacterial adherence and biofilm formation and saliva coat concentration. On the other hand, the formation of biofilm is influenced by Lewis blood groups.

## Acknowledgment

This study has been achieved in facilities of the Mustansiriyah University laboratories and deepest gratitude to all doctors and staff of bacteriology laboratories at Al-Sader general hospital.

## References

- [1] Ahmed, T., Lundgren, A., Arifuzzaman, M., Qadri, F., Teneberg, S. and Svennerholm, A. M. Children with the Le(a+b-) blood groups have increased susceptibility to diarrhea caused by enterotoxigenic, *Escherichia coli* expressing colonization factor1 group fimbriae. *Inf. Immun.*77:2059-2064. 2009.
- [2] Subhi, H.T., Inhibition of biofilm formation of *Pseudomonas aeruginosa* isolated from colorectal cancer and ulcerative colitis using iron oxide nanoparticle composites. Ph.D. thesis, University of Al-Mustansiriyah. College of Science. 2015.
- [3] SAS, Statistical Analysis System, Users Guide. Statistical. Version 9.1<sup>th</sup> ed. SAS. Inst. Cary. N.C. USA. 2012
- [4] Kipnis, E., Sawa, T. and Wiener-Krmish, K. Targeting mechanisms of *Pseudomonas aeruginosa* pathogenesis. *Med. Mal. Infect.* 36:78-91. 2006.
- [5] Bjarnsho, H. T. The role of bacterial biofilm in chronic infections. *APMIS.*1211-58. 2012.
- [6] Grishin, A.V., Krivozubou, M. S., Karyagina, A. S. and Gintsburg, A. L. *Pseudomonas aeruginosa* lectins as targets for novel antibacterials. *Acta Nature.*7:29-41. 2015.
- [7] Cioci, G., Mitchell, E.P., Gaatier, C., Wimmerova, M., Sudakevitz, D., Perez, S., Gilboa-Garber, N. and Imberty A. Structural basis of Calcium and galactose recognition by the lectin PA-1L of *Pseudomonas aeruginosa*. *FEBS let.*4:297-301. 2003.
- [8] Mitchell, E, Houles, C., Sudakevitz, D., Wimmerova, M., Gautier, C., Pérez, S., Wu, A.M, Gilboa-Garber, N. and Imberty A. Structural basis for oligosaccharide-mediated adhesion of *Pseudomonas aeruginosa* in the lungs of cystic fibrosis patients, *Nature Struct. Biol.*, 9: 918–921. 2002.
- [9] Thomsson, K.A., Schulz, B.L., Packer, N.H. and Karlsson, N. G. MUC5B glycosylation in human saliva reflects blood group and secretor status, *Glycobiology*, 15:791-804. 2005.
- [10] Agarwal, A., Singh, K. P. and Jain A. Medical significance and management of *Staphylococcal* biofilm. *FEMS. Immunol. Med. Microbiol.*58:147-160. 2010.
- [11] Baker, B.S. The role of microorganisms in atopic dermatitis. *Clin. Exp. Immunol.* 14 4:1-9. 2006.
- [12] Derrien, M, VenPassel, M. W. J., Van de Boven Kamp, J. H. B. , Schipper, R. G., de Vos, W. M. and Dekker, J. Mucin – bacterial interactions in the human oral cavity and digestive tract. *Gut. Microbes.*1:254-268. 2010.
- [13] Sommer R., Hauck, D., Varrot, A, Wagne, r S, Audfray, A, Prestel, A., Molker, H.M, Imberty, A. and Titz, A. Cinnamide derivatives of d-Mannose as inhibitors of the bacterial virulence factor lec B from *Pseudomonas aeruginosa*. *Chemistry. Ooen.*4:756-67. 2015.
- [14] Johansson, E. M. , Crusz, S.A., Kolomiet, E., Buts, L. , Kadam, R. U., Cacciarini M, Bartels, K. M. , Diggle, S. P., Camara M, Williams, P., Loris, R., Nativi, C., Rosenau, F., Jaeger, K. E., Dorbre, T. and Reymond, J. L. Inhibition and dispersion

- of *Pseudomonas aeruginosa* biofilms by glycopeptide dendrimers targeting the fucose-specific lectin Lec B. *Chem. Biol.* 15:1249-57. 2008.
- [15] Tielker, D., Hacker, S., Loris, R., Strathmann, M., Wingender, J., Wilhelm, S., Rosenau, F. and Jaeger, K.E. *Pseudomonas aeruginosa* lectin B IS located in the outer membrane and is involved in biofilm formation. *Microbiology* .151:1313-23.2012.
- [16] Biesbrock, A., Reddy, M.S. and Levine, M. J. Interaction of a salivary mucin – secretory Immunoglobulin A complex with mucosal. *Pathogens. Infect Immunil.* 59: 3492-3497, 1991.
- [17] Sriramulu, D. D., Lunsdorf, H., Lam, J. S. and Romling, U. Microcolony formation: a novel biofilm model of *Pseudomonas aeruginosa* for the cystic fibrosis lung, *J Med Microbiol*, 54: 667–676. 2005.
- [18] Landry, R. M., An, D., Hupp, J. T., Singh, P. K., and Parsek, M. R., Mucin–*Pseudomonas aeruginosa* interactions promote biofilm formation and antibiotic resistance, *Molecular Microbiology*, 59 (1): 142–151. 2006.
- [19] Aboukacem, A., Barguigua, I., Maroui, M., T., and Belhaj, A., Influence of both media carbon source and strains variety on the Biofilms formation ability by *Pseudomonas aeruginosa*, *International Journal of Innovation and Scientific Research*, 12(1): 190-198. 2014.