

Biofilm Formation by *Staphylococcus Aureus* Isolation from Atopic Dermatitis Patients on Defined Lewis Types Saliva

Fatima Rammadan Abdul

Department of Biology ;College of Science ;Al-Mustansiriyah University

Baghdad –Iraq

Email :qetasyshah@gmail.com

Abstract

Bacteria adherence and biofilm formation vary depending on strain of microorganism as well as the substrate .Here ,we evaluated the ability of different isolates of *Staphylococcus aureus* (*S. aureus*) ,isolated. from atopic dermatitis patients skin and stool to form biofilm on different Lewis types saliva ,including Le (a) , (b) ,(c) and Le a+b+ saliva. *S. aureus* isolates were cultured on tryptose soy broth .The bacteria were used to form biofilm on 96- well flat bottom Microtitration plate that was coated with the respective , optimally diluted,processed saliva of Lewis blood groups (a),(b),(c) and Le a+b+ .The intensity of biofilm formation was assessed by measuring the biofilm – associated optical density reading of bacterial bound dye. The results indicated a superior ability of biofilm formation on Lewis(a) saliva type .The highest binding gave a mean of 1.25 ± 0.39 compared to biofilm formation on non – coated wells that showed a mean of 0.23 ± 0.016 ($P=0.01$, $CL= 0.390 - 1.635$). On the other hand however, a variable degree of binding to Le(b) saliva was demonstrated for the isolates. The isolates didn't demonstrate binding on Le(a)-b-(Lec) and Le a+b+ -(Led) saliva.

We conclude that biofilm formation on Lewis types saliva of the atopic dermatitis bacterial isolates requires Le(a) in a ligand – receptor based system for biofilm establishment.

Keywords:-Biofilm- Lewis blood groups –atopic dermatitis– *Staphylococcus aureus*

الخلاصة

تغاير التصاق البكتريا وتكوين الغشاء الحيوي يعتمد على سلالة الكائن المجهرى وكذلك المادة الاساس .تم تقييم قدرة عزلات المكورات العنقودية الذهبية (*S. aureus*) المختلفة والمعزولة من جلد وخروج مرضى التهاب الجلد التأتبي لتكوين الغشاء الحيوي على انماط لويس للعابية المختلفة ,والتي تشمل لويس (a) , (b) , (c) و (a+b+) .زرعت عزلات المكورات العنقودية على وسط tryptose soy broth لدراسة قدرتها على تكوين الغشاء الحيوي ,حيث استعملت صفائح التسحيح الدقيق Microtitration plate ذات القعر المسطح flat bottom والتي تحتوي على ٩٦ خفرة .غطيت جميع الحقن باللعاب المعالج والمخفف بشكل مثالي لمجاميع الدم لويس (a),(b),(c) و a+b+ .قيمت شدة تكوين الغشاء الحيوي بواسطة قراءة قياس الكثافة الضوئية (O.D) المصاحبة لارتباط البكتريا للصبغة.

اظهرت النتائج القدرة العالية لتكوين الغشاء الحيوي على لعاب مجموعة الدم لويس (a). اعطى اعلى معدل ارتباط 0.23 ± 0.016 مقارنة مع تكوين الغشاء الحيوي للحفر الغير مغطاة والتي اظهرت معدل 1.25 ± 0.39 . من جانب اخر, اظهرت العزلات درجة ارتباط متغايرة لنمط (b) اللعابية ($P=0.01$, $CL= 0.390 - 1.635$). لم تظهر العزلات أي ارتباط على مجاميع لويس a-b- (c) و لويس a+b+ (d) اللعابية.

استنتجنا ان تكوين الغشاء الحيوي على انماط لويس اللعابية للعزلات البكتيرية لالتهاب الجلد التأتبي يحتاج الى لويس (a) في نظام تعتمد على المستقبل - النضيد لاستقرار الغشاء الحيوي. الكلمات المفتاحية: الغشاء الحيوي , مجاميع دم لويس, التهاب الجلد الرثوي, المكورات العنقودية الذهبية.

1-Introduction

Biofilm is a microbial structure that causes numerous issues in restorative field, in nourishment industry and even to nature. Along these lines, biofilm could cause numerous many public health problems. In reality, almost 80% of constant bacterial infections are related with the presence of biofilm [1].

Staphylococcus aureus (*S. aureus*) is regular pathogen related with different diseases and biofilm of the bacterium was observed to be associated with an assortment of microbial infection and to expand harmfulness [2] [3] [4]. *S. aureus* biofilm is made out of three noteworthy segments, the polysaccharide poly-N-acetylglucosamine (PNAG), otherwise called polysaccharide intercellular attachment (PIA), cell surface and emitted bacterial proteins, and extracellular nucleic acid, which shape extracellular polymeric substances [5]. Atopic dermatitis (AD) is an interminable fiery skin illness typically beginning in early youth with a revealed lifetime commonness of 15 – 30% in youngsters, while the comparing figure for adults is 2– 10%. [6]. *Staphylococcus aureus* is accounted for to rule in AD injuries and reports have uncovered the nearness of *Staphylococcal* biofilms. These infections add to irritation of the eczema [7]. Nonetheless, the part of *S. aureus* in the pathogenesis of AD remains ineffectively poorly understood [8].

Biofilm formation on synthetic saliva was demonstrated for mixed *Candida albicans* and *Streptococcus gordonii* [9], *S. aureus* predominate in the skin of atopic dermatitis patients [10].

Lewis blood group molecules expressed in a variety of surfaces [11] and their role in bacterial colonization and biofilm formation is well defined [12] [13] [14]. In this work it was tried to explore the role of Lewis blood groups determinants carried on salivary mucins on biofilm formation of *S. aureus* isolates from atopic dermatitis patients.

2-Materials and Methods

3-Bacterial isolates

The isolates used in this study included (9) isolates from skin and one isolate from stool of atopic dermatitis patients. They were maintained on Tryptose soy slants. Bacteria were grown on tryptose soy broth at 37°C for 24 hours and used in biofilm formation.

4-Saliva coating

Saliva of typed Lewis blood groups were used. Lewis typing was performed as described [15] (Ahmed *et al.*,2009). The saliva obtained were diluted 1:2 with saline and boiled at 100°C for 10 minutes, centrifuged at 4000 r.p.m and the supernatant was used as a source of saliva. Saliva were stored at -20°C until used.

To find optimal dilution of saliva coat, experiment was set using serially diluted saliva in 0.05M bicarbonate buffer pH 8.5. Optimal dilution of saliva was used to coat wells of 96 – well polystyrene microtitration plate. 100 μ l of saliva in bicarbonate buffer was used to coat wells. Uncoated wells included and served as controls.

5-Biofilm assay

Biofilm formation and assessment were adopted from [16]. Briefly bacteria were grown on tryptose soy broth supplemented with 0.25% glucose at 37°C for 24 hours. An overnight culture reached an average optical density (O.D at 578nm) of between 8-9 (O.D of 0.1 = 10⁸) (CFU/ml).

The culture was diluted to 1:20 in medium and 200 μ l of this suspension was used to inoculate 96-well polystyrene plate. The plate was incubated at 37°C for 24 hours, washed twice with 200 μ l sterile saline, air dried then stained with 0.1% safranin for 30 second. The O.D of the adhered bacteria was measured at 490 nm using a microplate ELISA reader. Each assay was performed in triplicates.

6-Biostatistical analysis

Significant differences between mean were evaluated using student t-test using a computer program of epidemiological statistics. $p \leq 0.05$ were regarded significant.

7-Results and Discussion

8-Saliva optimal coating

Saliva of Le(a) and Le(b) serially diluted and used to coat 96-wells of polystyrene plate. As seen in (Figure 1). There was a dose response in biofilm formation that depends on the coating concentration. The intensity of biofilm for a selected isolate of the bacterium was performed. Accordingly an optimal (1:8) coating dilution was used in subsequent experiments.

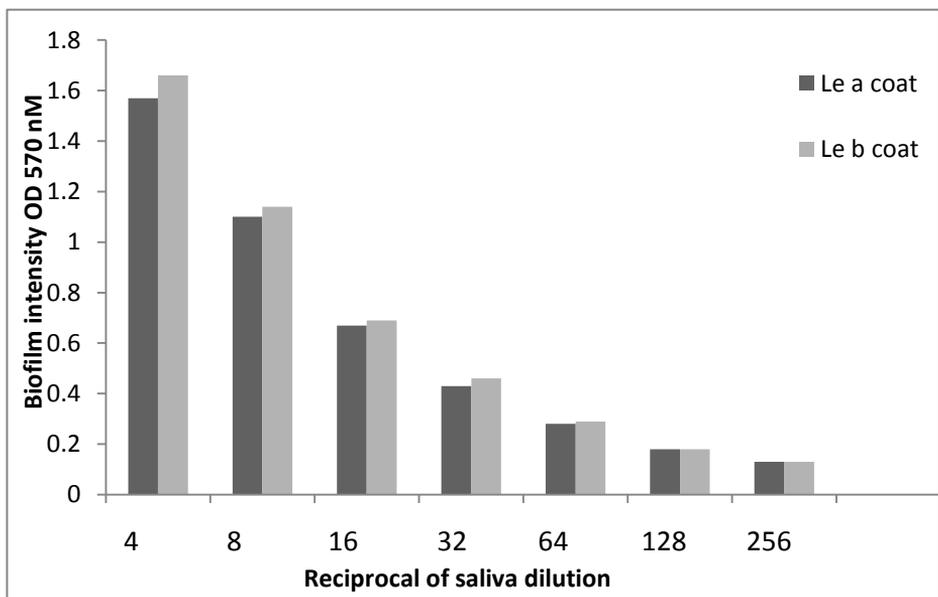
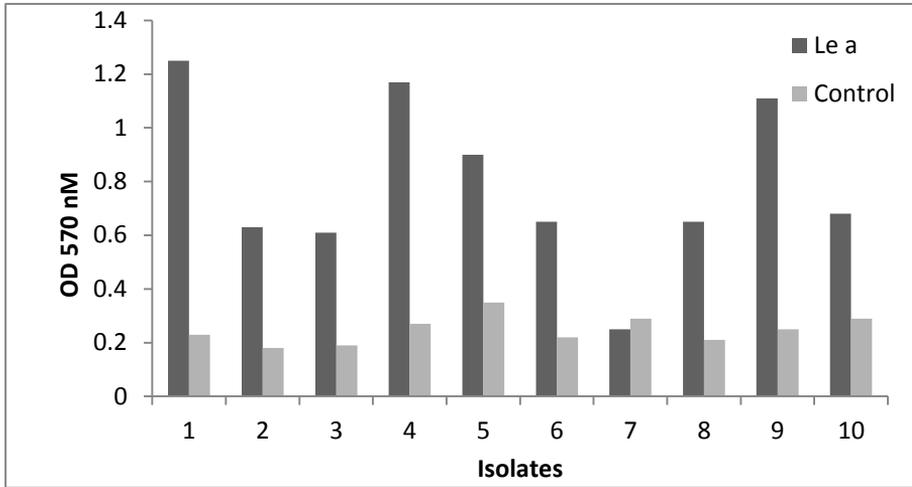


Figure 1 . Titration of saliva of Lewis (a) and (b) coating for optimal biofilm formation by *S. aureus*.

9-Biofilm formation by the isolates

A total of (10) isolates from atopic dermatitis patients were assayed for biofilm formation on a defined set of Le(a) ,(b), (c) saliva . The majority of isolates were potent biofilm formers on Le(a) saliva compared to binding on non saliva –coated wells(Figure 2). Nonetheless, however ,the degree of biofilm formation vary between isolates .Some were strong biofilm formation as seen isolates No.4 (Binding mean was 1.17 ± 0.14) in Le(a) coated wells compared to 0.27 ± 0.04 in non-coated wells. ($P=3.1 \times 10^{-4}$, $CL=0.74-1.203$) .This gave a percent increase of 334.07 in binding (Table 1).Other isolates showed a high degree of biofilm formation also.



isolates	% increase binding of biofilm intensities of saliva group			
	Lewis saliva			
	a	b	c (Lea-b-)	d (Le a+b+)
1	436.63	58.18	-42.24	-42.24
2	245.85	213.81	40.88	-3.31
3	206.59	272.58	-28.42	-37.56
4	334.07	112.96	-34.07	-41.85
5	154.80	101.12	-45.19	-18.36
6	197.70	79.81	-14.67	31.19
7	-8.27	28.57	60.15	-46.24
8	214.63	275.60	25.36	50.24
9	352.86	370.90	-31.55	-51.63
10	150.00	31.59	-21.52	-42.70

Figure 2 . Binding of *S. aureus* and biofilm formation of isolates from atopic dermatitis patients to saliva of Lewis (a) coating compared to non- coated wells

Table (1) Augmentation of *S. aureus* biofilm on different types of Lewis saliva

% increase in binding was calculated based on mean of triplicate reading of biofilm intensities of saliva coated test(t) and saliva non-coated (c), and derived using the formula $T-C / C \times 100$.

Negative value was statistically not analyzed. % increase in Le (a) gave highly significant results.

Biofilm formation on Le(b) saliva was studied also. As depicted in (Figure 3) ,the ability to form biofilm on this substrate was high also ,but a bit less compared to that seen with Le(a) saliva(Table 1).

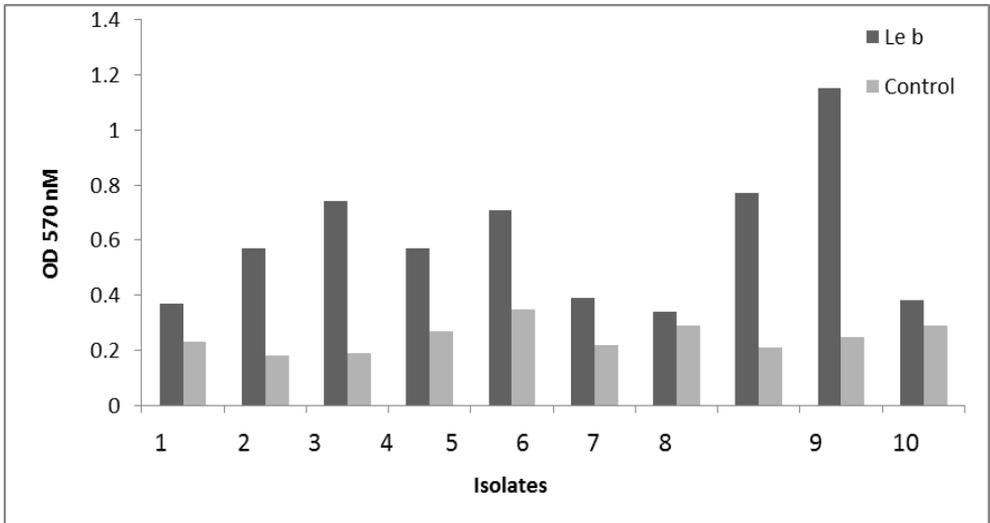


Figure 3. Binding of *S. aureus* and biofilm formation of isolates from atopic dermatitis patients to Lewis (b) saliva coating compared to non- coated wells

Interestingly ,biofilm formation on Le (a+b+) and Le (a-b-) was mostly negative on these substrates.

The finding presented in this report demonstrated clearly that *S. aureus* colonizing atopic dermatitis patients required a defined ligand for adherence .

As show here Le(a) is a strong presumptive ligand for the bacteria as this ligand which is represented in the salivary mucin [17] was involved in biofilm formation besides Le (b) moiety in mucin was also involved.

What strengthen then this finding is that neither Le (a+b+) nor Le (a-b-) acted as having ligands ,a notion emphasizes that a defined structural specificity is preferred among Lewis molecular types that are structurally well defined [18].

S.aureus processes adhesins [19], that are used to bind substrate in biofilm formation .In atopic dermatitis, the most suspected bacteria involved in the pathogenesis of this disease was shown to be *S. aureus* [20].

The novel findings presented here ,uncover a peculiar system for *S. aureus* binding that might initiate colonization in skin of affected individules.

References

- [1] J. E.-M. S. Jardak, M. Elloumi and S. Mnif, "Chemical composition, anti-biofilm activity and potential cytotoxic effect on cancer cells of rosmarinus officinalis l. essential oil from tunisia. lipids health dis," *Lipids Health Dis*, vol. 16, p. 190, 2017.
- [2] G. A. N. L. N. D. H. J. F. M. G. D. P. D. B. B. A. W. P. S. P. P. J. C. E. G. D. K. J. E. . Hall-Stoodley L, Hu F Z, "Direct detection of bacterial biofilm on the middle –ear mucosa of children with chronic otitis media," *JAMA.*, vol. 296, pp. 11–202.
- [3] A. Agarwal, S. K. P, and undefined Jain, "Medical significance and management of staphylococcal biofilm." *FEMS. Immunol .Med .Microbiol*, vol. 58, pp. 147–160, 2010.
- [4] M. O. Slany and undefined J., "Formation of staphylococcus aureus biofilm in the presence of sub-lethal concentrations of disinfectants: a transcriptomic analysis using rna-seq," *AEM*, pp. 01 643–17, 2017.
- [5] L. H. Yu, J. Hirakawa, H. Mizumachi, E. Toyoda, A. Yahara, and K. Sugai, "Complete genome sequence of super biofilm-elaborating staphylococcus aureus," *Genome Announc.*, vol. 5, no. 41, pp. 01 043–17, 2017.
- [6] A. P. Sonesson, K. Eriksson, S. Mörgelin, M. Kjellström, S. Davies, J. Potempa, and J. Schmidtchen, "Identification of bacterial biofilm and the staphylococcus aureus derived protease, staphopain, on the skin surface of patients with atopic dermatitis," *Sci Rep*, vol. 7, p. 8689, 2017.
- [7] S. Eriksson, M. Van der Plas, M. Mörgelin, and A. Sonesson, "Antibacterial and antibiofilm effects of sodium hypochlorite against staphylococcus aureus isolates derived from patients with atopic dermatitis," *J. Dermato*, vol. 177, no. 2, pp. 513–521.
- [8] Y. N. Yamazaki, Y., , and G. Núñez, "Role of the microbiota in skin immunity and atopic dermatitis," *Allergol Int.*, vol. 66, no. 4, pp. 539–544, 2017.
- [9] M.-J. D, S. A, R. A. K, and L.-R. J. L, "An in vitro model for oral mixed biofilm of candida albicans and streptococcus gordonii in synthetic saliva," *Front. Microbiol*, vol. 7, no. 1-13, pp. 48–553, 2016.
- [10] G. Baviera, L. M. C, L. Capra, C. F, G. Longo, N. Maielo, G. Ricci, and E. Gall, "Microbiota in healthy skin and atopic eczema," *Bio Med. Res. Interna*, p. 436921, 2014, article ID 436921.
- [11] H. S, O. R, and S. B, "Lewis histo-blood group system and associated secretory phenotypes," vol. 69, pp. 82–166, 1995.

- [12] Y. Ikehara, S. Nishihara, H. Yasutomi, T. Kitamura, K. Matsuo, S. N. K. Inada, Y. Kodera, Y. Yamamura, H. Narimatsu, N. Hamajima, and M. Tatematsu, "Polymorphisms of two fucosyltransferase genes (Lewis and secretor gene) involving type 1 Lewis antigen are associated with the presence of anti-*Helicobacter pylori* IgG antibody," *Cancer. Epidemiol. Biomarkers. Prev.*, vol. 10, pp. 971–977, 2001.
- [13] L. Lindesmith, C. Moe, S. Marionneau, R. N. X. Jiang, L. L. P. Stewart, J. LePendu, and R. Baric, "Human susceptibility and resistance to norwalk virus infection," *Nat. Med.*, vol. 9, no. 5, 2003, pp. 9:5.
- [14] H. E. A. M. J. M., H. G. C., and D. S. E., "Gastrointestinal mucins of fucose-deficient mice lack terminal fucosylation without affecting colonization by *Candida albicans*," *Glycobiology*, vol. 15, pp. 1002–1007, 2005.
- [15] A. T. L. A., A. M., Q. F., T. S., and S. A. M., "Children with the Le(a+b-) blood group have increased susceptibility to diarrhea caused by enterotoxigenic *Escherichia coli* expressing colonization factor I group fimbriae," *Inf. Immun.*, vol. 77, pp. 2059–2064, 2009.
- [16] S. Flahaut, E. Vinogradov, K. K. A., B. S., H. K., and L. J. C., "Structural and biological characterization of capsular polysaccharide produced by *Staphylococcus haemolyticus*," pp. 1649–1657, 2008.
- [17] T. K. A., S. B. L., P. N. H., and K. N. G., "Muc5b glycosylation in human saliva status," *Glycobiology*, vol. 15, pp. 791–804.
- [18] I. Brockhausen, "Mucin-type O-glycans in human colon and breast cancer: glyco-dynamics and functions," *EMBO. Rep.*, vol. 7, pp. 599–604, 2006.
- [19] M. Burian, M. Rautenberg, T. Kohler, M. Fritz, K. B., C. Unger, H. W. H., P. A., W. C., and G. C., "Temporal expression of adhesion factors and activity of global regulators during establishment of *Staphylococcus aureus* nasal colonization," *J. Infect. Dis.*, vol. 201, pp. 1414–1421.
- [20] O. P. Y., O. T., B. C., S. L., B. M., G. T., G. R. L., and L. D. Y., *N. Engl. J. Med.*, vol. 347, pp. 115–116, 2002.