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Effect of adhesive properties and content on the level teichoic acids are capable of forming biofilms strains of *staphylococcus aureus*

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ABSTRACT

Most of inflammatory diseases which are caused by Staphylococcus aureus, proceed with the formation of biofilms. The microorganisms in biofilms composed of 50-500 times increase their resistance to disinfectants, antimicrobials, bacteriophages, phagocytes and antibodies. To date, the mechanisms of biofilm formation are not well understood, therefore the purpose of this study was to determine the relationship between the adhesive properties, the level of teichoic acids and the ability to form biofilms of clinical and reference strains of Staphylococcus aureus.

The study found that all the indicators that were studied were higher ($p < 0,05$) in clinical isolates of S. aureus compared to the referent. The index of adhesion of microorganisms in clinical isolates was – (4,98 ± 1,3), indicators of average optical density levels of teichoic acids (0,373 ± 0,031) un. OP, the ability to form biofilms and above was also determined at the level (1,0683 ± 0,006) un. OP. Moreover, a direct correlation ($r = 0,643$) between the indices of the adhesive properties and teichoic acid content level S. aureus cells.

Consequently, we can assume that the adhesive properties and increase the level of teichoic acids from clinical isolates of S. aureus is a sign of pathogenicity and virulence, which may affect the ability of microorganisms to biofilm formation.

Keywords: *Staphylococcus aureus, biofilm, adhesion, teichoic acid.*

INTRODUCTION

A special place among the causes of inflammatory diseases takes *Staphylococcus aureus*. Typically, these infections are accompanied by the formation of biofilms, which are attached to biotic or abiotic surfaces and have a high degree of resistance to environmental factors. [2, 9, 16]. It is known that from 67 % to 78 % of clinical isolates of *Staphylococcus aureus* biofilm may form. Their education play a crucial role in the pathogenesis of osteomyelitis, endocarditis, urinary disorders of the reproductive system, as well as the leading cause of nosocomial infections [11, 20].

The process of adhesion of bacteria to the surface is one of the first steps in the formation of biofilms. It is known that aggressive strains depend on their degree of pathogenicity and virulence. Adhesion of microorganisms – one of their virulence factors that determines the first step of colonization of substrates. [14, 15]. Consequently, the first stage of biofilm formation of bacteria adhesion occurs to the surface. Then begins the colonization, production of extracellular matrix and the formation of the biofilm. Microorganisms which are in the upper layer of the biofilm matrix multiply and produce planktonic cells which freely escape the biofilm and colonize other surfaces [12, 13]. Certain anionic polymers cell walls of bacteria are involved in cell-cell adhesion processes. These processes are based on the ionic interaction of the proteins of one cell with adhesions specific receptors other. These receptors can be teichoic acid [6, 18, 19].

According to the literature revealed that *S. aureus* teichoic acids involved in the binding of the bacteria to the epithelial cells of the mucous membranes [1, 17]. The molecular basis of primary bacterial adhesion to artificial surfaces is not fully understood. However, we know that the key role in the first stage of biofilm formation belongs teichoic acids [7, 8]. On the interaction between biomaterials and teichoic acids affect the ionic strength, and van der Waals forces, which can be both attracting and repelling [4, 5].

Thus, with a degree of esterification teichoic acid linked adhesive properties of the virulent bacteria and their ability to interact with a variety of positively charged molecules, polymers that can affect the ability of microorganisms to film formation. Research objective. Determining the relationship between the adhesion properties, the level teichoic acid content and the ability to form biofilms and clinical reference strains *Staphylococcus aureus*.

MATERIALS AND METHODS

The object of the study were 55 strains of *S. aureus* isolated from patients with various inflammatory processes in the first 48 h. after hospitalization and 4 reference strain (ATCC 25923) as a control group. Isolation and identification of *S. aureus* strains in pure culture was performed by conventional microbiological methods. To determine the adhesive properties of microorganisms was used method of Brilis V.I. et al. [10]

Extraction teichoic acids *S. aureus* cells was carried out by adding to the agar cultures washout with 10% trichloroacetic acid, then precipitated with cold ethanol, washed with cold acetone, ethanol, and ether in a desiccator. The content of teichoic acid levels was determined by optical density at the SF-46 at wavelength $\lambda = 254$ nm [3].

The ability of microorganisms to biofilm formation was determined in plastic plates for enzyme-linked immunosorbent assay. Formed biofilms were washed and stained with 1% alcohol solution of gentian violet [21]. The results were assessed by optical density at a wavelength of $\lambda = 545$ nm on analyzer LabLine-90. These values are expressed in units optical density (un. OD.). Viability of the microorganisms in the biofilms was determined by counting the colony forming units (CFU) in 1 ml of biomass. The obtained data were processed using the software package Excel.

RESULTS AND DISCUSSION

When studying adhesive properties of *S. aureus* strains was determined that all parameters which characterize the process was higher ($p < 0,05$) in clinical strains, than the reference (table 1).

Table-1: Mean adhesive properties of the clinical and reference strains of S. aureus

S.no	Groups studied strains ***S. aureus***	Erythrocytes participation ratio, % **(M ± m)**	The median adhesion **(M ± m)**	Index adhesiveness of microorganisms **(M ± m)**
1.	Clinical isolates *S. aureus*, n = 23	81,15 ± 1,24*	3,15 ± 0,33*	4,98 ± 1,3*
2.	Rreference strains *S. aureus*, n = 4	74,7 ± 1,12	2,05 ± 0,33	2,44 ± 0,54
Note: * – the difference is reliable with the control (reference strains) ($p < 0,05$), presented the results of studies of 3 repetitions.				

Thus, the participation rate of red blood cells clinical strains of *S. aureus* was (81,15 ± 1,24) %, for the reference strains, this figure is set at (74,7 ± 1,12), median adherence to established clinical isolates – (3,15 ± 0,33), for the reference strains – (2,05 ± 0,33). Adhesiveness index of microorganisms in clinical isolates was – (4,98 ± 1,3), this indicator was (2,44 ± 0,54) for the

reference strains. Thus, it was found that the clinical isolates of *S. aureus* were has high adhesion properties, and reference – low adhesion that may be due to the degree of virulence and evidence of an increase in aggressive colonization properties first.

In determining the content of the level of teichoic acids *S. aureus* cell parameters in clinical and reference strains were significantly different ($p < 0,05$). For example, the average absorbance values for clinical strains made (0,373 ± 0,031) un. OP, reference to – (0,147 ± 0,014) un. OP., that may indicate a higher virulence first. The increase of the level of teichoic acids can affect complement activation, which in turn causes a systemic reaction, which is a consequence of a decrease in phagocytic activity and suppression of host immune response.

In the study of the relationship between the indices of the adhesive properties and the level of teichoic acids *S. aureus* cells, a direct correlation (r = 0,643), which may affect the ability of microorganisms to form biofilms (figure 1).

In the experimental determination of the ability to form biofilms revealed that this feature of *S. aureus* was different. Thus, the average optical density that characterized this process were higher ($p < 0,05$) in clinical isolates and made (1,0683 ± 0,006) un. OP. reference strains for these indicators were lower – (0,0550 ± 0,007) un. OP.

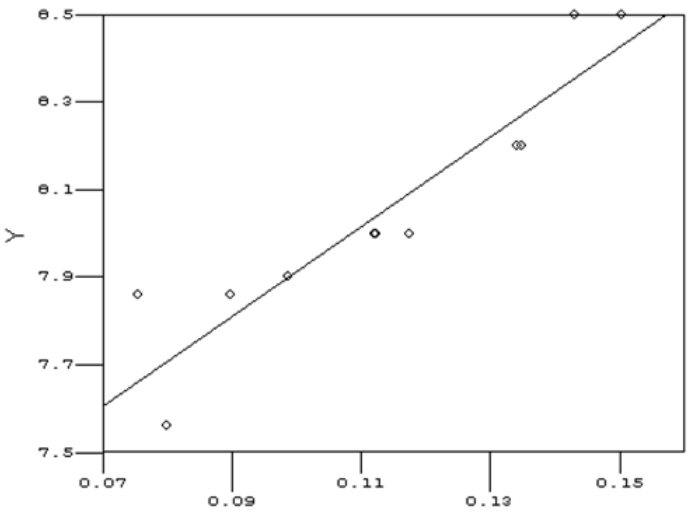

Figure-1: Analysis of the correlation dependence of the content of teichoic acids upon adhesive properties of *S. aureus* strains (a direct stable correlation dependence).

Note: n = 11. Regression coefficient : deviation b – 0.1112, shift a – "+"3.8 ; Correlation coefficient : r = 0.643, t – 10.62.

CONCLUSIONS

According to the results of the study it was found that all the indicators that were studied were higher ($p < 0,05$) in clinical isolates of *S. aureus* than the reference. Thus, the index of adhesion of microorganisms in clinical isolates was – (4,98 ± 1,3),

indicators of average optical density levels of teichoic acids (0,373 ± 0,031) un. OP, the ability to form biofilms and above was also determined at the level (1,0683 ± 0,006) un. OP. Moreover, a direct correlation (r = 0,643) between the indices of the adhesive properties and teichoic acid content level *S. aureus* cells. Consequently, we can assume that the adhesive properties and increase the level of teichoic acids from clinical isolates of *S. aureus* is a sign of pathogenicity and virulence, which may affect the ability of microorganisms to biofilm formation.

REFERENCES

1. Aly, R., et al., *J Infect Dis*, **1980**. 141(4), p. 463-465.
2. Archer, NK., *Virulence 2*, **2011**. P. 445-459.
3. Archibald, AR., *Adv Microb Physiol*, **1974**. 11, p. 53-59.
4. Archibald, AR., *Bath University Press*, **1988**. 47, p. 159-173.
5. Archibald, AR., *American Society for Microbiology*, Washington D.C., **1993.** p. 381-410.
6. Archibald, AR., et al., *Nature*, **1961**. 24, p. 570-572.
7. Bera, A., *J Bacteriol*, **2007**. 189, p. 280-283.
8. Biswas, R., et al., *PLoS ONE*, 7 (7), e41415.
9. Boles BR., *Trends Microbiol*, **2011**. 19, p. 449-455.
10. Brilis, VI., *Laborie Business*, **1986**. 4, p. 210-212.
11. Chatterjee, S., *Ann Med Health Sci Res*, **2014**. 4, p. 100–104.
12. Chen, C., *J Biol Chem*, **2013**. 288, p. 29440–29452.
13. Conlon, BP., et al., *Nature*, **2013**. 503, p. 365-370.
14. Corrigan, RM., *Microbiology*, **2007**. 153, p. 2435-2446.
15. Cue, D., *Infect. Microbiol*, **2012**. 2, p. 38.
16. *de la Fuente-Nunez, CF., Current Opinion in Microbiology, **2013**. **16**, p. 580-589.*
17. Ellis MW., et al., *Infection Control* **and** *Hospital Epidemiology***, 2014.** 35, p. 1251-1256.
18. Matthias, G., et al., *Infection* **and** *immunity*, **2001**. 69(5): p. 3423-3426.
19. Naumova, IB., et al., *FEMS Microbiol Rev*, **2001**. 25 (3): p. 269-284.
20. Naumova, IB., *FEMS Microbiol Rev*, **2001**. 25: p. 269-284.
21. Romanov, YM., *Journal of Microbiology***,** *Immunology* ***and Infection***, **2006**. 4: p. 38-42.