

## Short Communication: Influence of the content level of teichoic acids in the cells of *Staphylococcus aureus* on their adhesive properties

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Manuscript received: 4 June 2016. Revision accepted: 25 April 2017.

**Abstract.** Minukhin VV, Kochneva EV. 2017. Short Communication: Influence of the content level of teichoic acids in the cells of *Staphylococcus aureus* on their adhesive properties. *Bioteknologi* 14: 9-11. Teichoic acids (TA) are known to be factors of pathogenicity and virulence. They take part in binding the bacteria *Staphylococcus aureus* to epithelial cells of mucous membranes. The purpose of the research consisted in determining the content of TA in cells of clinical and reference strains of *S.aureus* and studying the relationship between these acids and adhesive properties of the cell wall. TA from cells of *S.aureus* were extracted by the addition of 10 % trichloroacetic acid to washings of agar cultures; followed by precipitation with cold ethanol, washing with acetone, ethanol, and ether in a desiccator. The content of TA was determined at a SP-46 by optic density (OD) with wavelength  $\lambda = 254$  nm. When TA were isolated from cells of clinical and reference strains of *S.aureus*, it was revealed that their content levels differed. Values of the clinical strains were higher than those of the reference ones: respectively,  $(0.373 \pm 0.016)$  versus  $(0.147 \pm 0.014)$  OD units. This phenomenon could be explained by a high degree of aggressiveness of the clinical strains and could influence the activation of the complement that in its turn caused a systemic response resulting in a decrease of phagocytic activity. The course of the research revealed a direct correlation dependence of the content of TA upon adhesion values ( $r = 0.643$ ). This dependence could affect the ability of microorganisms to form biofilms.

**Keywords:** Adhesion, *Staphylococcus aureus*, teichoic acids

### INTRODUCTION

Teichoic acids (TA) compose a significant part of the cell wall in gram-positive bacteria and their proportion can reach to 60 % of its weight (Archibald 1993; Baddiley et al. 1961). These acids have covalent links with peptidoglycans and are an integral part of the cell wall. Thus, they are in close contact with all the processes that take place with its participation. These include growth and division of cells, binding and redundancy of cations required for the functioning of membrane enzymes, processes of intercellular recognition, reception of phages, pathogenicity. TA and other anionic compounds of the cell wall make a significant contribution to the formation of polyelectrolyte gel structure and determine its mechanical properties (Archibald et al. 1961; Archibald 1988).

The study of the cell walls of pathogenic microorganisms has led to the understanding of such important phenomena as adhesion, virulence, and formation of biofilms on implanted materials (Cramton et al. 1999; Xia 2010). Side by side with other components of cell walls, TA are responsible for sensitivity of bacteria to some antibiotics and their immunomodulatory properties (Sieradzki 1999; Stephanie et al. 2012). Some anionic polymers of cell walls of bacteria participate in processes of intercellular adhesion, which are based on ionic interactions of adhesin proteins of one cell with specific receptors of another one. TA can be such receptors (Archibald 1974; Sanderson 1962).

According to published literature, TA of *Staphylococcus aureus* take part in binding bacteria to epithelial cells of mucous membranes (Aly et al. 1980; Naumova et al. 2001; Scheffers 2005). They launch a complementary cascade along an alternative route, activate the clotting and kallikrein-kinin systems. *Staphylococcus aureus* is a common cause of infections associated with colonization of implanted biomaterials. The molecular basis of the initial adhesion of bacteria to artificial surfaces has not been fully clarified. But it is known that the key part of the first stage of biofilm formation is played by TA. The processes of interaction between TA and biomaterials are influenced by ionic forces and those of van der Waals, which can be both attractive and repulsive (Bera 2007; Matthias et al. 2001).

The negative charge of TA play a crucial part in the coaggregation of cations, binding autolysins as well as in pathogenic and adhesive properties, caused by the phosphate groups. Meanwhile, the role of certain structural elements of these polymers has not been studied enough, though it has been revealed that they affect the functional activity and some biological properties of TA (Biswas et al. 2012; Matthias et al. 2001).

Thus, the adhesive and virulent properties of bacteria, their ability to interact with different positively charged molecules, polymers, etc., depend upon the degree of esterification of TA. Hence one of the stages of this research consisted in revealing the relationship between adhesive properties and content of TA in the cell wall of *S.aureus*.

The objective of this research was to determine the content of TA in cells of clinical and reference strains of *S. aureus* and study the relationship between these acids and adhesive properties of the cell wall.

## MATERIALS AND METHODS

The research used 55 clinical strains isolated from patients with different proinflammatory infections and four reference strains (ATCC 25923) as the control group. The strains were then divided into four groups. The first group consisted of strains isolated from a purulent discharge of wounds in acute inflammatory processes (abscess, gangrene, acute purulent periostitis, phlegmon, acute appendicitis, acute hidradenitis, acute mastitis). The second group of strains were isolated from a purulent discharge of wounds in protracted inflammatory processes (furunculosis, carbuncle, infected wounds, panaritium, atheroma, hematoma, erysipelas, baroclinities). The third group of strains were isolated from mucus of the fauces and nose during an examination for carriage. The remaining reference strains ATCC 25923 were the control group.

Pure cultures were isolated in compliance with standard methods (MacFaddin 2000; David et al. 2004; Murray et al. 2011).

TA from cells of *S. aureus* were extracted by addition of 10 % trichloroacetic acid to washings of agar cultures followed by precipitation with cold ethanol, washing with acetone, ethanol, and ether in a desiccator. The content of TA was determined at an SP-46 by optic density (OD) with wavelength  $\lambda = 254$  nm.

## RESULTS AND DISCUSSION

The results of this study showed that the values of TA content in *S. aureus* in clinical and reference strains differed reliably ( $p < 0.05$ ) (Table 1).

The values of average optical density were ( $0.542 \pm 0.016$ ) OD units in the 1<sup>st</sup> group and ( $0.147 \pm 0.014$ ) in the 4<sup>th</sup> one, thereby making a statistical difference. The values in the 2<sup>nd</sup> and 3<sup>rd</sup> groups were less versus the 1<sup>st</sup> one, but higher than in the reference strains. The values for these groups were, respectively, ( $0.381 \pm 0.019$ ) and ( $0.196 \pm 0.013$ ) OD units.

Thus, it was revealed that the content of TA in cells of *S. aureus* was higher in clinical isolates versus the reference strains, which might be caused by the degree of their aggressiveness and influenced the course of an infectious disease.

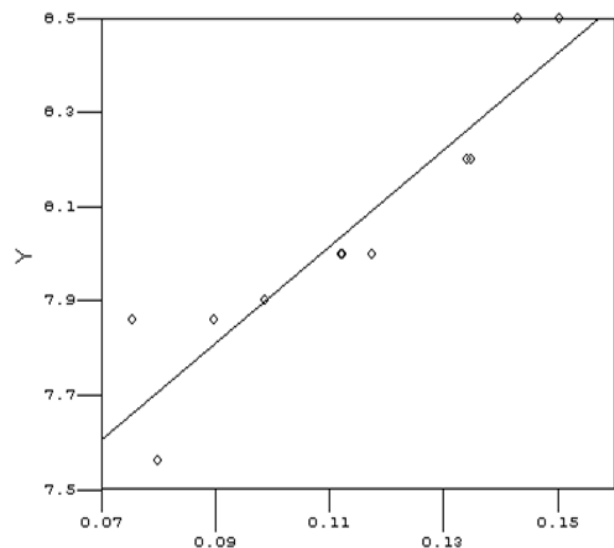
Taking into consideration that the content of TA is closely associated with adhesive properties of *S. aureus*, this research revealed a correlation between these indices (Figure 1).

The study of the relation between adhesive property values and TA content in cells of *S. aureus* revealed a direct correlation dependence ( $r = 0.643$ ), which reduced the ability of microorganisms to form microfilms.

**Table 1.** Mean values of TA content in cells of *S. aureus*

Groups of <i>S. aureus</i> strains studied	TA content (OD units)
Acute inflammatory processes (abscess, gangrene, acute purulent periostitis, etc.), n = 25	$0.542 \pm 0.016^*$
Protracted inflammatory processes (furunculosis, carbuncle, etc.), n = 25	$0.381 \pm 0.019^*$
Examination of carriers, n = 5	$0.196 \pm 0.013^*$
Reference strains ATCC 25923, n = 4	$0.147 \pm 0.014^*$

Note: \* – the difference is reliable,  $p < 0.05$ ; results of 3 repeated studies are presented



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

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

In conclusion, the results of this study demonstrated that the content of TA in cells of *S. aureus* was higher ( $p < 0.05$ ) in clinical isolates versus the reference ones: average OD was, respectively, ( $0.373 \pm 0.016$ ) and ( $0.147 \pm 0.014$ ) OD units. This phenomenon could be explained by a higher degree of aggressiveness of the clinical strains and could influence the activation of the complement that in its turn caused a systemic response resulting in a decrease of the phagocytic activity. A direct correlation dependence of the content of TA upon adhesion values ( $r = 0.643$ ) was found out. This dependence could affect the ability of microorganisms to form biofilms. Study of the ability of *S. aureus* strains to form biofilms and revealing of the correlation of the above formation with the content of TA are promising directions for further research.

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