

Adhesive properties of film forming and non-film forming strains of *Staphylococcus epidermidis*, isolated from upper respiratory tract of human

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Objective: to compare the adhesive properties of film forming and non-film forming strains of *Staphylococcus epidermidis*, isolated from upper respiratory tract of human.

Materials and methods. An ability to adhesion of 13 film forming and 11 non-film forming strains of *Staphylococcus epidermidis*, isolated from upper respiratory tract of human, was studied. Ability to adhesion of film forming and non-film forming strains of *S. epidermidis* was studied with use of human buccal epithelial cells. There were determined: average rank of adhesion (ARA), index of participation of epithelial cells in the adhesion (K) and index of adhesion of microorganisms (IAM).

Results. Studied strains showed the ability to adhere to buccal epithelial cells. It was established that all 13 of film forming strains of *S. epidermidis* were high-adhesive with ARA amounted to 7.53 ± 2.30 . Among these strains highest level of ARA was 11, while participation of epithelial cells was 85%, and the IAM was 12.94. Lowest level of ARA among film forming strains equal to 3, K was 89%, and the IAM was 3.37, but it was only one strain. Among film forming strains 1 strain (7.7%) had IAM less than 4.0 and it was a strain with intermediate adherent ability. The rest of the strains were high-adhesive and had IAM more than 5.81. Among them IAM between 5 and 9 units had 8 (66.7%) and 4 (33.3%) strains had IAM more than 10 units. Among the 11 studied non-film forming strains non-adhesive were 2 (18.2%) strains, low-adhesive was 1 (9.1%), intermediate level of ability showed for 2 (18.2%) and 6 (54.5%) were high-adhesive.

The ARA of non-film forming strains of *S. epidermidis* was 1.84 times lower compared to the same index of film forming strains and amounted 4.09 ± 2.55 . Maximal ARA among surveyed non-film forming strains was 9, while the K was 89% and the IAM was 10.11. Lowest indexes were 0 that was fixed for one non-adherent strain.

Conclusions. It was established that all investigated film forming and non-film forming strains of *S. epidermidis* adhered to human buccal epithelium cells. It was found that all film forming strains were high-adhesive, among non-film forming strains high-adhesive were 54.5% of strains.

Among film forming strains IAM was 8.66 ± 2.66 that is 1.87 times higher compared to non-film forming strains, IAM of that was 4.62 ± 2.87 .

Key words:

Staphylococcus epidermidis, biofilm formation, adhesion, level of adhesion, upper respiratory tract.

The existence of bacteria in the biofilm as form of difficult complex community takes place both in the environment and in the human body. In this form microorganisms have an increasing level of resistance to environmental factors, including antibiotic and disinfectants, and can cause different lesions during infection [1–5].

One of the important factors during the formation of biofilm is the ability of microorganisms to

adhesion [6–8]. Adhesion to biological objects (surfaces of cells, tissues, walls of blood vessels etc.) is associated with a specific interaction of adhesion proteins [9] or fimbriae lectins with receptors or membrane domain of host cells surface [10, 11]. Herewith colonization resistance of macroorganism is close dependent on a combination of factors that prevent multiply and attachment of bacteria to mucous membranes. A significant role in this process

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Table. Adhesive properties of film forming and non-film forming strains of *S. epidermidis*, isolated from upper respiratory tract of human, to human buccal epithelium cells

Studied strains	Average rank of adhesion (ARA)	Index of participation of epithelial cells in the adhesion (K) [%]	Index of adhesion of microorganisms (IAM)
Film forming strains (n = 13)	7.53 ± 2.30*	87.15 ± 2.82	8.66 ± 2.66*
Non-film forming strains (n = 11)	4.09 ± 2.55	80.45 ± 26.92	4.62 ± 2.87

Asterisks [*] denotes significant at $p < 0.05$ between groups.

belongs to the normal microbiota of the mucous membrane and its relationships with competitive pathogenic microorganisms, and also the physiological state of the macroorganism's cells [12]. Therefore, the process of microbial adhesion is the initial step in the development of infection and biofilm-formation.

Objective: to compare the adhesive properties of film forming and non-film forming strains of *Staphylococcus epidermidis*, isolated from upper respiratory tract of human.

Materials and methods

An ability to adhesion of 13 film forming and 11 non-film forming strains of *S. epidermidis* was studied. Studied strains were isolated from the upper respiratory tract of human.

Ability to adhesion of film forming and non-film forming strains of *S. epidermidis* was studied with use of human buccal epithelial cells [13, 14]. The strains were grown overnight in meat-peptonic broth (MPB), and then centrifuged 5 min at 6000 rpm by laboratory centrifuge (ОПН-3 У 4.2). Pellet was resuspended in phosphate-buffered saline (PBS) (g/100 ml): NaCl – 0,85; Na₂HPO₄ – 1,42 (pH 7,2). Suspension of bacteria containing $1,0 \cdot 10^9$ CFU/ml. Sample of buccal epithelial cells transferred to a buffer and centrifuged at 6000 rpm 5 min. The supernatant was removed, and the resulting pellet was again resuspended in buffer and centrifuged in the same mode. The number of cells counted with the use of Horiaev–Toma chamber. Make a suspension of epithelial cells with $1,0 \cdot 10^8$ cells/ml. Suspension of bacterial and epithelial cells were mixed in equal quantities both and incubated 30 min at 37 °C. After incubation with use of centrifugation for 5 min at 6000 rpm cells washed twice with PBS to separate the epithelial cells and unattached bacterial cells. The microscopic preparation make from pellet stained by Gram method and the number of bacteria adhered on the surface of epithelial cells was counted.

There were determined:

- average rank of adhesion (ARA) – the average number of bacteria that adherent to one epithelial cell;

- index of participation of epithelial cells in the adhesion (K) – the percentage of cells, on the surface of which microorganisms were adhered;
- index of adhesion of microorganisms (IAM) – the average number of bacteria on one epithelial cell that is involved in adhesion. IAM calculated as: $IAM = (ARA \cdot 100) / K$.

Adhesiveness was considered as zero if ARA was 0–1,0; low if ARA was 1,01–2,0; intermediate if ARA was 2,01–4,0 and high if ARA was > 4,0. Microorganisms considered as non-adhesive if IAM was ≤ 1,75; low-adhesive – if IAM was 1,76–2,50; intermediate – if IAM was 2,51–4,0 and high-adhesive – if IAM was ≥ 4,00.

Statistical analysis of the results was made using the t-Student test with significance level of 0.05.

Results and discussion

One of the main factors of colonization of biotope by staphylococci is the ability of these bacteria to adhere on the cell surface and then resist to host defense mechanism. That's why the research of ability to adhesion is significant for studying of microorganisms.

We established an ability to adhesion of film forming and non-film forming strains of *S. epidermidis*, isolated from upper respiratory tract of human, to buccal epithelium cells of human.

In a result of study was found that all film forming strains of *S. epidermidis* capable to adhere on human buccal epithelial cells (table).

It was established that all 13 of film forming strains of *S. epidermidis* were high-adhesive with ARA amounted to 7.53 ± 2.30 . Among these strains highest level of ARA was 11, while participation of epithelial cells was 85%, and the IAM was 12.94. Lowest level of ARA among film forming strains equal to 3, K was 89%, and the IAM was 3.37, but it was only one strain.

IAM is a key parameter that determines the adhesiveness of microorganisms. Therefore, it is appropriate to use to further the general characteristics of adhesive properties studied strains of *S. epidermidis*.

Thus, among these 13 film forming strains 1 strain (7.7%) had IAM less than 4.0 and it was a strain with intermediate adherent ability. The rest of the

strains were high-adhesive and had IAM more than 5.81. Among them IAM between 5 and 9 units had 8 (66.7%) and 4 (33.3%) strains had IAM more than 10 units.

It was determined that adhesive potential of non-film forming strains of *S. epidermidis* was lower compared to film forming strains. During the studies it was found that in group of non-film forming strains were low-, intermediate- and high-adhesive strains. Among the 11 studied strains non-adhesive were 2 (18.2%) strains, low-adhesive was 1 (9.1%), intermediate level of ability showed for 2 (18.2%) and 6 (54.5%) were high-adhesive. This indicates on the prevalence among film forming and non-film forming strains cultures with intermediate level of adhesion activity. But indexes of adhesion of non-film forming strains are significantly lower compared to film forming isolates.

The ARA of non-film forming strains of *S. epidermidis* was 1.84 times lower compared to the same index of film forming strains and amounted 4.09 ± 2.55 . Maximal ARA among surveyed non-film forming strains was 9, while the K was 89% and the IAM was 10.11. Lowest indexes were 0 that was fixed for one non-adherent strain.

Thus, it can be assume that more pronounced adhesive properties of film forming strains, indicating their high colonization potential compared to non-film forming strains. This data indicate aside on higher pathogenic potential. It's known that the adhesion of pathogenic and opportunistic bacteria, including staphylococci, to mucosal epithelial cells is the initial stage of inflammation. During this stage the colonization resistance of the organism largely depends on a combination of factors that prevent the attachment and multiply of bacteria on mucous membranes. Essential role in realization of this mechanism play normobiota of mucosa and its competitive relationship with the pathogenic microorganisms, and also the physiological state of cells.

Analyzing the results it can be note the following: all film forming strains of *S. epidermidis* were characterized by high adhesive properties, as well

as the highest IAM was 12.94, the lowest IAM — 3.37. Among non-film forming strains of *S. epidermidis* were found non-adhesive, low-, intermediate- and high-adhesive strains with maximal IAM — 10.11 and minimal rank — 0. According to the another data [12] among strains of staphylococci, isolated from different biotopes of the human body, were both high- and intermediate-adhesive culture; from other sources [13] it's known that among staphylococcal strains, isolated from the skin of people suffering from chronic dermatitis, took place prevalence of high-adhesive strains with the maximal ARA rank 4.1 ± 0.08 , for intermediate-adhesive strains ARA equaled 2.73 ± 0.03 . Also there were low-adhesive strains and culture with zero levels: ARA were 46 ± 0.25 and 0.88 ± 0.12 respectively.

The problem of biofilmformation is a subject of many research, the results of which are shown in a number of reviews [8, 14, 15], but in the totality of the literature virtually no direct information about the relationship between the ability of microorganisms to form biofilm and to adhere to buccal epithelial cells. Our results indicate that strains that showed the ability to biofilmformation, characterized by approximately 1.84 times higher ARA to human buccal epithelium cells compared to non-film forming strains of *S. epidermidis*. In this regard, we can assume that the activity of adhesion to epithelial cells can provide the enhanced ability to form biofilm by high-adhesive strains that can be used in laboratory diagnostics as a rapid test for the evaluation of pathogenic potential of strain.

Conclusions

It was established that all investigated film forming and non-film forming strains of *S. epidermidis* adhered to human buccal epithelium cells. It was found that all film forming strains were high-adhesive, among non-film forming strains high-adhesive were 54.5% of strains.

Among film forming strains IAM was 8.66 ± 2.66 that is 1.87 times higher compared to non-film forming strains, IAM of that was 4.62 ± 2.87 .

No conflict of interest.

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Адгезивні властивості біоплівкотвірних і небіоплівкотвірних штамів *Staphylococcus epidermidis*, виділених із верхніх дихальних шляхів людини

Мета дослідження: порівняння адгезивних властивостей біоплівкотвірних та небіоплівкотвірних штамів *Staphylococcus epidermidis*, виділених із верхніх дихальних шляхів людини.

Матеріали та методи. Досліджено здатність до адгезії 13 біоплівкотвірних та 11 небіоплівкотвірних штамів *S. epidermidis*, виділених із верхніх дихальних шляхів людини. Здатність до адгезії біоплівкотвірних та небіоплівкотвірних штамів *S. epidermidis* вивчали на клітинах букального епітелію людини. Визначали середній показник адгезії (СПА), індекс участі епітеліальних клітин в адгезії (К) та індекс адгезивності мікроорганізмів (ІАМ).

Результати. Досліджені штами показали здатність адгезуватися до клітин букального епітелію. Встановлено, що всі 13 біоплівкотвірних штамів *S. epidermidis* були високоадгезивними (СПА — $(7,53 \pm 2,30)$ ум. од.). Серед цих штамів найвищий рівень СПА склав 11, тоді як участь епітеліальних клітин становила 85%, а ІАМ — 12,94. Найнижчий рівень СПА серед біоплівкотвірних штамів дорівнював 3, К становив 89%, а ІАМ — 3,37, але це був лише один штам. Серед біоплівкотвірних штамів 1 (7,7%) мав ІАМ менше 4,0, і це був штам з проміжною адгезивною здатністю. Решта штамів були високоадгезивними і мали ІАМ більше 5,81. Серед них ІАМ між 5 і 9 одиницями мали 8 (66,7%), а 4 (33,3%) штами мали ІАМ більше 10 одиниць. Серед 11 досліджених небіоплівкотвірних штамів неадгезивними були 2 (18,2%) штами, низькоадгезивними — 1 (9,1%), середній рівень адгезії встановлено для 2 (18,2%) штамів і високий — для 6 (54,5%) штамів. СПА небіоплівкотвірних штамів *S. epidermidis* був у 1,84 разу нижчим порівняно з тим же показником біоплівкотвірних штамів і становив $4,09 \pm 2,55$. Максимальний показник СПА серед досліджених штамів, що не утворюють плівки, становив 9, тоді як К — 89%, а ІАМ — 10,11. Найнижчі показники становили 0, що було зафіксовано для одного небіоплівкотвірного штаму.

Висновки. Встановлено, що всі досліджені біоплівкотвірні та небіоплівкотвірні штами *S. epidermidis* мали адгезивну здатність до клітин букального епітелію людини. Всі біоплівкотвірні штами були високоадгезивними, серед небіоплівкотвірних — високоадгезивними було 54,5% штамів. Серед біоплівкотвірних штамів ІАМ був $8,66 \pm 2,66$, що в 1,87 разу вище порівняно з небіоплівкотвірними штамми, ІАМ — $4,62 \pm 2,87$.

Ключові слова: *Staphylococcus epidermidis*, біоплівкотворення, адгезія, рівень адгезії, верхні дихальні шляхи.

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Адгезивные свойства биопленкообразующих и небиопленкообразующих штаммов *Staphylococcus epidermidis*, выделенных из верхних дыхательных путей человека

Цель работы: сравнение адгезивных свойств биопленкообразующих и небиопленкообразующих штаммов *Staphylococcus epidermidis*, выделенных из верхних дыхательных путей человека.

Материалы и методы. Изучена способность к адгезии 13 биопленкообразующих и 11 небиопленкообразующих штаммов *S. epidermidis*, выделенных из верхних дыхательных путей человека. Способность к адгезии биопленкообразующих и небиопленкообразующих штаммов *S. epidermidis* изучали на клетках букального эпителия человека. Определяли средний показатель адгезии (СПА), индекс участия эпителиальных клеток в адгезии (К) и индекс адгезивности микроорганизмов (ИАМ).

Результаты. Исследованные штаммы показали способность адгезироваться к клеткам букального эпителия. Установлено, что все 13 биопленкообразующих штаммов *S. epidermidis* были высокоадгезивными (СПА — $(7,53 \pm 2,30)$ усл. ед.). Среди этих штаммов максимальный СПА составил 11, тогда как участие эпителиальных клеток

составляло 85%, а ИАМ – 12,94. Самый низкий уровень СПА среди биопленкообразующих штаммов был равен 3, К составлял 89%, а ИАМ – 3,37, но это был только один штамм. Среди биопленкообразующих штаммов 1 (7,7%) имел ИАМ меньше 4,0: это был штамм со средними адгезивными свойствами. Остальные штаммы были высокоадгезивными и имели ИАМ больше 5,81. Среди них ИАМ между 5 и 9 единицами имели 8 (66,7%) штаммов, а 4 (33,3%) штамма имели ИАМ более 10 единиц. Среди 11 исследованных небиопленкообразующих штаммов неадгезивными были 2 (18,2%) штамма, низкоадгезивными – 1 (9,1%), средний уровень адгезии установлен для 2 (18,2%) штаммов и высокий – для 6 (54,5%) штаммов. СПА небиопленкообразующих штаммов *S. epidermidis* был в 1,84 раза ниже по сравнению с этим показателем биопленкообразующих штаммов и составил $4,09 \pm 2,55$. Максимальный показатель СПА среди исследованных штаммов, не образующих биопленку, составил 9, тогда как К – 89%, а ИАМ – 10,11. Самый низкий показатель составил 0, что было зафиксировано для одного небиопленкообразующего штамма.

Выводы. Установлено, что все исследованные биопленкообразующие и небиопленкообразующие штаммы *S. epidermidis* имели адгезивную способность к клеткам буккального эпителия человека. Все биопленкообразующие штаммы были высокоадгезивными, среди небиопленкообразующих – высокоадгезивными было 54,5% штаммов. Среди биопленкообразующих штаммов ИАМ был $8,66 \pm 2,66$, что в 1,87 раза выше по сравнению с небиопленкообразующими штаммами, ИАМ – $4,62 \pm 2,87$.

Ключевые слова: *Staphylococcus epidermidis*, биопленкообразование, адгезия, уровень адгезии, верхние дыхательные пути.