

**MODERN ASPECTS OF DEVELOPMENT
OF ANTI-STAPHYLOCOCCAL DRUGS
OF A NEW GENERATION BASED ON *S. AUREUS* ADHESINS
AND PROBIOTIC STRAINS OF LACTOBACILLI**

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INTRODUCTION

One of the most relevant nosocomial infections are infections of Staphylococcal origin, which leads to complications of almost 30% of all surgical interventions, which is a serious problem in clinical medicine. The most common source of *Staphylococcus aureus* is practically healthy carriers among healthcare professionals and patients. It is known that the bacteriocarriers there is a restructuring of the mechanisms of protection of the macro body with the formation of immunological imbalance. Traditional methods remediation of bacteriocarriers with antibacterial drugs are ineffective – carrier is eventually restored and requires repeated courses of treatment, and the use of repeated courses of antibiotics leads to even more oppression of the immune system of the carrier and the formation of antibiotic resistant strains of microorganisms. The strategic direction of WHO considers the gradual replacement of antibiotics with prophylactic drugs of new generations.

The infectious process is initiated after the adhesion and colonization of mucosal mucous membranes by microorganisms. That is, if it is possible to influence the pathogenic bacteria in the initial stage of colonization of the macroorganism - suppress the adhesion of bacteria, it is possible to suppress the development of the infectious process. The above forms the basis of the anti-adhesive strategy for the development of new generation immunobiological drugs.

Regarding natural stimulation of local immunity and natural anti-infectious resistance of macroorganism mucus membranes, it is now widely used to treat the representatives of normal microbiota (bacteria of the genus *Lactobacillus*) with pronounced antagonistic and immunomodulatory properties.

The combination of these two strategies provides the opportunity for the development of new generation immunobiological drugs that, on the one hand, will suppress the persistent properties of pathogens, and on the other hand, will stimulate local immunity and anti-infectious resistance of the mucous membranes.

The concept of the presented work is to combine probiotics and / or their biologically active compounds with *S. aureus* adhesins, which will enable, on

the one hand, to block receptors for attachment of *S. aureus* to the epithelial cells, and on the other to stimulate the local link of the immune response.

1. Microbiological aspects of chronic staphylococcal upper respiratory tract infection

S. aureus plays a significant role in the occurrence of suppurative and inflammatory diseases of different localization and occupies leading positions in the etiological structure of nosocomial infections¹. Thus, hospital staphylococcal genesis infections complicate surgical intervention, increasing the length of stay of a patient in a medical institution by an average of 12–16 days and playing a significant role in fatal cases in hospitals of surgical and obstetric-gynecological profile (mortality from various nosological forms of lethality in various nosological forms of nosocomial infections ranges from 35% To 60%, and in case of generalization of the process reaches 90–100%)². It is important to note that the most dangerous source of staphylococcal infection are practically healthy carriers³. Intranasal carrier of staphylococcus aureus, especially among such representatives of decreed groups as medical workers, is very dangerous for a wide circle of the population⁴. At the same time, it is known that the detection of staphylococcal carriers among patients and medical personnel and the timely eradication of the pathogen can control the outbreaks of staphylococcal infections in inpatient facilities and thus reduce the risk of developing purulent-inflammatory complications, including fatal ones. It has been established that among medical personnel nasal carrier of staphylococcus aureus can reach 35%⁵. Mostly, at the expense of these

¹ Пономаренко С.В. Микробиологические аспекты стафилококковой инфекции на современном этапе (обзор литературы). *Анналы Мечниковского Института*. 2013. № 3. С. 13–17.

² Colburn N.E., Cadnum J., Flannery E., Chang S.. Perception vs Reality: Methicillin-Resistant Staphylococcus aureus Carriage Among Healthcare Workers at a Veterans Affairs Medical Center. *Infection Control & Hospital Epidemiology*. 2016. V. 37, №1. P.110-112. (208). DOI: 10.1086/646174.

³ Chen B., Dai X., He B., Pan K., Li H., Liu X., Bao Y., Lao W., Wu X., Yao Y., Huang S. Differences in Staphylococcus aureus nasal carriage and molecular characteristics among community residents and healthcare workers at Sun Yat-Sen University, Guangzhou, Southern China. *BMC Infectious Diseases*. 2015. V. 15, № 1. P. 303. DOI: 10.1186/s12879-015-1032-7.

⁴ Drilling A., Coombs G.W., Tan Hui-leen, Pearson J.C., Boase S., Psaltis Alkis, Speck P., Vreugde S., Wormald P.J. Cousins, siblings, or copies: the genomics of recurrent Staphylococcus aureus infections in chronic rhinosinusitis. *International Forum of Allergy & Rhinology*. 01 Oct. V. 4, № 12. 2014. P. 953–960. DOI: 10.1002/alar.21423.

⁵ Egyir B., Guardabassi L., Nielsen S. S., Larsen J. Prevalence of nasal carriage and diversity of Staphylococcus aureus among inpatients and hospital staff at Korle Bu Teaching Hospital, *Ghana Journal of Global Antimicrobial Resistance*. 2013. V. 1, № 4. P.189–193. DOI: 10.1016/j.jgar.2013.05.006.

individuals, there is the circulation and accumulation of hospital strains of staphylococci characterized by polyresistance to antibiotics.

The risk groups for the development of staphylococcal intestinal infection include pregnant women, newborns (especially preterm infants), persons of the elderly and senile, patients with immunodeficiency states, including iatrogenic ones (therapy with corticosteroids, cytostatics etc.), patients who undergo surgical intervention with the installation of plastic catheters, dentures, drainages, etc.

Another risk group that needs to detect and eradicate *Staphylococcus aureus* is a person with frequent exacerbations of chronic inflammatory diseases, especially the upper respiratory tract (URT) associated with *S. aureus*. According to the literature, among the most common chronic diseases of staphylococcal genesis in otolaryngology, a significant part belongs to chronic tonsillitis (CT) and chronic rhinitis (CR) and sinusitis (CS)⁶.

In connection with the above, we investigated the species, quantitative composition and frequency of extraction of individual representatives of the microflora of the mucous membranes of the nasopharynx of practically healthy persons, patients with ENT pathology and carriers of *S. aureus*. 36 patients were examined on CT and 33 persons on CR and CS in the stage of exacerbation. Among the examined patients 34 men and 35 women aged 18 to 25 years old. All patients were treated at the Department of Otolaryngology of the Communal Healthcare Establishment "Kharkiv City Student Hospital" (CHE KCSH). The control group consisted of 10 practically healthy persons (5 men and 5 women) aged 18 to 20 years. Also, 89 health workers of the specified institution were examined in order to identify healthy carriers of *S. aureus*.

The study material was mucus from the surface of the tonsils (135 samples) and nasal passages (132 samples) obtained from the abovementioned persons. Totally 267 samples were selected and investigated.

In the course of the research, 24 strains of *S. aureus* were isolated from patients with CT, 21 strains – from patients with CR and CS and 24 strains from carriers.

It has been found that in practically healthy persons from the mucous membranes of the throat most often isolated coagulase negative staphylococci, α -hemolytic streptococci (*S. mitis* and *S. dysagalactiae*), non-hemolytic streptococci (*S. mutans*), *Lactobacillus spp.*, Nonpathogenic corynebacteria and bacteria of genera *Peptostreptococcus*, *Veillonella*, *Fusobacterium*, *Bacteroides*. Coagulase-negative staphylococci, non-pathogenic Neisseria,

⁶ Мані Ханс. Оптимізація органозберігаючого лікування хронічного тонзиліту з використанням високоенергетичного лазера : автореф. дис. ... канд. мед наук : 14.01.19. Київ, 2014. 20 с.

non-pathogenic corynebacteria and aerococcus were most often sown from the nasal mucosa of almost healthy people. The concentration of microbiota ranged from 1.7 to 7.6 lg CFU / g of the material studied.

When exacerbation of chronic tonsillitis was most often isolated by strains of *S. pyogenes* – in (89,3 ± 2,8)% of patients, the second place was taken by strains *S. aureus* – the frequency of their withdrawal was observed in (69,4 ± 3,1)% of patients in the association With other conditionally pathogenic microorganisms. When exacerbations of chronic forms of rhinitis and sinusitis were most often isolated by strains *S. aureus* – in (63,6 ± 2,9)% of patients, both in monoculture and in association with other opportunistic pathogens. Lactobacilli in the period of exacerbation were removed only in (6.4 ± 2.1)% of patients.

We also conducted a survey of the medical personnel of the CHE KCSH for the presence of *S. aureus* bacterioses, with the degree of staphylococcal colonization of their mucous membranes. According to the obtained data, among 89 examined medical workers, 24 had *S. aureus* isolated from the nose, which accounted for 30.3% of the examined personnel. Moreover, in 9 people *S. aureus* was isolated from both the nose and throat. The results obtained do not contradict the literature⁷.

Comparative analysis of indicators revealed a significant difference between the degree of colonization of mucous membranes with *Staphylococcus aureus* in patients with ENT pathology (during exacerbation) and carriers. Thus, the rate of microbial population of *S. aureus* (lg CFU / g) of the mucous membranes of the throat and nose of patients was, respectively, 1.8 times (p <0,01) and 1.2 times (p <0,05) higher than the corresponding indicator defined for disease carriers. However, despite the above fact, the degree of population of the mucous membranes of medical staff was quite significant – 3.4–5.4 lg CFU / g.

For the development of an infectious process, in the first stage, microorganisms should attach to eukaryotic cells (adhesion). This process is considered as the initial stage of colonization, which provides the development of invasive lesions. Most scientists believe that the potential pathogens of infections are microorganisms that have high adhesion⁸.

⁷ Пономаренко С.В. Микробиологические аспекты стафилококковой инфекции на современном этапе (обзор литературы) *Анналы Мечниковского Института*. 2013. № 3. С. 13–17; Пономаренко С.В. Біологічні властивості *Staphylococcus aureus* ізольованих із різних осередків вегетування, та оптимізація лабораторної діагностики стафілококової інфекції: автореф. дис. ... канд. мед наук: 03.00.07. Харків, 2015. 26 с.; Egyir B., Guardabassi L., Nielsen S.S., Larsen J. Prevalence of nasal carriage and diversity of *Staphylococcus aureus* among inpatients and hospital staff at Korle Bu Teaching Hospital, *Ghana Journal of Global Antimicrobial Resistance*. 2013. V. 1, № 4. P. 189–193. DOI: 10.1016/j.jgar.2013.05.006.

⁸ Бухарин О.В. Усвяцов Б.Я. Бактерионосительство: Медицина. Екатеринбург : Вид. УрО РАН, 1996. 208 с.

It was found that among isolated *S. aureus* strains ($9.9 \pm 0.7\%$) were low-adhesion, ($36.3 \pm 1.4\%$) were medium- adhesion, and ($53.8 \pm 1.6\%$) had high Adhesive activity. Non-adhesive strains of *S. aureus* from the examined biotopes were not allocated.

The fact that the vast majority of *S. aureus* isolates were middle and high capacity for adhesion, in our opinion, can be explained from the perspective of the pathogenesis of infection, a respiratory tract is a constant and rapid renewal mucous layer due ciliary cells because microorganisms capable of sufficiently firmly attached to epithelial cells, are more likely to expansion.

Study of the adhesive potential of clinical strains of *S. aureus* has allowed to establish the difference in this activity between isolates that have been removed from the mucous membranes of the mouth and nose. Thus, *S. aureus* isolates isolated from the mucous membranes of the nose of patients and carriers had significantly higher rates compared to isolates removed from the mucous membranes of the pharynx. AAI (average adhesion index) and IAM (index of adhesion of microorganisms) strains from the mucous membranes of the nose exceeded the corresponding indices of isolates from mucous membranes of pharynx in 1,35–2,1 times ($p < 0,01$). It should be noted that despite the mentioned differences in AAI and IAM, CA (the coefficient of involvement of red blood cells in adhesion) for different groups of staphylococci was not significantly different (by criterion $\chi^2 p = 0.08-0.9$). Correlation analysis determined the absence of a linear relationship between the number of passages and the adhesion indicators of the studied cultures ($r = -0.68$ and -0.69 , respectively, for the indicators of SPA and IAM)⁹.

Bacteria adherent to the epithelial cells begin to produce specific enzymes that cause their further migration ability and ability to withstand protective factors macroorganism. Enzymes that promote invasion of microbes and their subsequent persistence in the host belongs to the pathogenicity factors (PF). To PF bacteria causing ability to resist the defense mechanisms of macroorganism, especially anticomplementary (ACA) and antilysozyme activity (ALA), which is associated with migrating to other organisms and their habitats further vegetation¹⁰. By the level of activity of these persistence factors, evaluate the virulent potential of pathogens.

In determining the parameters of *S. aureus*, it was found that ($9.7 \pm 0.3\%$) strains of staphylococci isolated from patients were

⁹ Антушева Т.І., Калініченко С.В. Вплив ультразвукових хвиль на адгезію патогенних коринебактерій Стратегія і тактика боротьби з інфекційними захворюваннями : матеріали міжнар. наук.-практ. конф., Харків, 17–18 жовт. 2012 р. Харків, 2012. С. 90.

¹⁰ Пономаренко С.В. Біологічні властивості *Staphylococcus aureus* ізольованих із різних осередків вегетування, та оптимізація лабораторної діагностики стафілококової інфекції : автореф. дис. ... канд. мед наук: 03.00.07. Харків, 2015. 26 с.

characterized by a very high level of the indicated activity ($52.8 \pm 0.4\%$) of strains – high ($36, 1 \pm 0.5\%$) – in the middle and ($1.4 \pm 0.1\%$) of strains – had a low level of anticomplementary activity. In the study of ACA strains of *S. aureus* isolated from the medical staff, a very high level of activity was observed in ($11,1 \pm 0,3\%$) isolates, ($60,0 \pm 0,1\%$) of strains had a high level of activity, and ($28, 9 \pm 0.4\%$) of isolates had an average activity level. Low levels of anti-complementarity in strains of *S. aureus*, extracted from the medical staff, were not detected.

The evaluation ALA of *S. aureus* showed that ALA was absent in ($29.8 \pm 1.7\%$) strains isolated from patients. In isolates approached from medical personnel, this attribute was absent only in ($2,2 \pm 0,1\%$).

To PF, which contribute to the invasion of bacteria in the tropical tissue of the macroorganism, belongs to lecithinase and plasmocoagulase. The presence of lecithinase facilitates the penetration of staphylococci through the tissue barrier, since it acts on lecithin and other phosphoglycerides, and the products of hydrolysis of lecithin toxicly affect the macroorganism. Plasmocoagulase causes blood clotting with the formation of a thrombin-like substance that interacts with the prothrombin and envelops the bacterium in a fibrin film, which has considerable resistance to the protective factors of the immune system of the macroorganism and serves as an additional capsule for staphylococci¹¹.

Lecithinase was detected in all isolates of *S. aureus* strains isolated from the mucous membranes of the pharynx and the nose as carriers and patients. However, the rate of its synthesis by bacterial cells was different. When comparing the time of appearance of this trait, it was found that after 24 hours it was detected in ($85.2 \pm 1.6\%$) *S.aureus* isolates removed from carriers and only in ($78.1 \pm 1.2\%$) of cultures removed from sick persons after 48 hours lecithinase activity was detected in ($14.8 \pm 0.8\%$) and ($21.9 \pm 1.1\%$) isolates, respectively. That is, the greater proportion of clinical isolates *S.aureus* produced this enzyme of pathogenicity in a day ($p < 0,05$).

It has been determined that all strains of *S. aureus* we studied had plasmocoagulase activity. However, the quantitative determination of the activity of extracellular plasmocoagulase showed differences in its activity in different strains. Thus, ($89,0 \pm 1,8\%$) isolates of *S. aureus* had plasmocoagulase activity at the level of – (120 ± 5) UM / ml, others ($11,0 \pm 0,9\%$) – at the level (60 ± 5 μmol / ml. Moreover, among strains removed from patients, all *S. aureus* possessed high activity of plasmocoagulase, and among strains isolated from carriers only ($88,5 \pm 1,4\%$) of isolates.

¹¹ Стафілокок. Класифікація і лабораторна діагностика : навч.-метод. посіб. / Волянський Ю.В. та інші. Харків : Стиль Издат, 2012. С. 75–76.

The degree of activity of the manifestation of these enzymes characterizes, to a large extent, the pathogenicity of clinical strains of staphylococci¹².

At the present stage of science development, many scholars support the idea that the main form of the existence of microorganisms in biocenoses is biofilms, in which the exchange of information occurs with the help of specialized signaling molecules, which makes the microbial community work as a single organism¹³. In this case, the microorganisms that are part of the biofilms are generally more resistant to antimicrobials, have the ability to counteract the factors of immune defense and are characterized by the ability to exchange information between cells and the collective coordination of gene expression¹⁴.

The study of the ability to biofilm formation in *S. aureus* cultures showed that all clinical isolates were able to form biofilms. At the same time (33,3 ± 1,6)% of strains showed weak ability to biofilm formation, (41,7 ± 1,8)% – characterized by an average level of biofilm formation, and (25,0 ± 1,5)% high.

The strains isolated from the mucous membranes of the carriers were predominantly weak in the formation of biofilms. Whereas (97.3 ± 1.7)% of the carriers isolated from the mucous membranes of the nose were characterized by moderate and high biofilm formation potential. *S. aureus* isolated from the mucous membranes of the pharynx and nose of the patients mostly had a moderate and high degree of formation of biofilms – respectively (89,9 ± 1,6)% and (96,6 ± 1,8)%.

Thus, it was determined that among the medical staff of the CHE KCSH the level of carriers of *S. aureus* was 30.3%. It has been experimentally established that the degree of secretion of *S. aureus* in the mucous membranes of carriers of *S. aureus* was at the level of 3.4–5.4 lg CFU / g. Isolated strains had high levels of anti-complementary, anti-lysozyme, lecithin and plasmocoagulase activity and had the ability to biofilm formation. The above data largely explain the processes of formation of bacteriological and chronic forms of infectious diseases.

2. Lactobacilli as a component of "nasal" probiotic drugs to restore anti-infectious resistance of the mucous membranes of the upper respiratory tract

Lactobacilli are fairly common in the environment, have high biological activity and are involved in the colonization resistance of the mucous

¹² Бухарин О.В. Усвяцов Б.Я. Бактерионосительство : Медицина. Екатеринбург : Вид, УрО РАН, 1996. 208 с.

¹³ Бухарин О.В. Персистенция патогенных бактерий. Медицина : Екатеринбург: Вид, УрО РАН, 1999. 367 с.

¹⁴ Там само.

membranes¹⁵. The population level of lactobacilli of the genital and digestive tract is well studied, but regarding the population of lactobacilli of the mucous membranes of the upper respiratory tract and their population level – in the literature there are only isolated reports¹⁶. In connection with the above, we have studied the frequency of extracting lactobacilli from the mucous membranes of the nasopharynx of practically healthy persons, patients with chronic forms of ENT pathology and carriers of *S. aureus*.

Comparison of our results on lactobacilli colonize the mucous membranes of the upper respiratory tract contingent surveyed showed that in patients with chronic tonsillitis number of lactobacilli was, on average, 2.5 times ($p = 0.001$) lower than the number in the mucous membranes of healthy people : (2.7 ± 0.4) lg CFU / g vs. (6.6 ± 0.9) lg CFU / g. Number of lactobacilli in patients with XP and cholesterol was at $(3,7 \pm 0,9)$ lg cfu / g, which is also lower compared to the practically healthy people, on average, 1.8 times ($p < 0.01$). The level of lactobacilli colonize the mucous membranes of the upper respiratory tract carriers *S. aureus* was also lower compared to the practically healthy people, on average, 1.5 times ($p < 0.05$) and was $(4,4 \pm 0,4)$ lg CFU / g. So determined that the level of lactobacilli population in the nasopharynx of healthy people is much higher than in patients and carriers. Such violations in ecological niche can promote more intensive development of pathogenic microorganisms, including staphylococci. From our point of view, patients with low population levels of lactobacilli nasal microbiota may require correction.

Currently sanitation *S. aureus* carriers occurs with the use of antimicrobials and not always effective¹⁷. The effects of antibiotic treatment lead to deterioration microecology all internal human environment and, above all, reducing representatives of normal microflora. As set habitat of the human body is a kind of extracorporeal body, the formation dysbiotic changes on any parts of the mucous membranes leads to disorders of the immune system and homeostasis of the whole macroorganism and increases the likelihood of complication. The key to this system is to provide

¹⁵ Маслянюк Р.П., Божик Л.Я., Романович М.С. Мікробіоз кишечника та імунітет. *Науковий вісник ЛНУВМБТ імені С.З. Гжицького*. 2011. Т. 13, № 4(50). С. 134–139; Парфенов А.И. Бондаренко В.М. Что нам дал вековой опыт познания симбиотной кишечной микрофлоры. *Архив патологии*. 2012. № 2. С. 21–25; Войда Ю.В., Солонина Н.Л. Микроэкология человека и роль пробиотических препаратов в терапии гнойно-воспалительных заболеваний в акушерстве и гинекологии. *Annals of Mechnikov Institute*. 2012. № 2. С. 27–37.

¹⁶ Рижкова Т.А. Мікробіологічна характеристика мікрофлори мигдаликів, *Corynebacterium diphtheriae* та особливості між бактеріальних взаємовідносин за аеробних і мікроаерофільних умов : автореф. дис. ... канд. мед наук : 03.00.07. Харків, 2009. 24 с.

¹⁷ Чанышева Р.Ф. Оптимизация технологий борьбы со стафилококковыми инфекциями : автореф дис. ... к. мед. н. : 14.02.02 : Нижний Новгород, 2014. 22 с.

antiinfectious resistance, which manifests itself in antagonism to the alien flora of microorganisms impede adhesion of pathogens to the epithelial cells of the mucous membranes and the regulation of the immune system.

In recent years, data on the effectiveness of the use of probiotic strains of lactobacilli in the prophylaxis and treatment of not only diseases of the gastrointestinal and genital tract (GI tract and GT), but also respiratory system diseases¹⁸. Overseas multicenter, double-blind, controlled, placebo-controlled trials have demonstrated that course administration of oral probiotics containing lactobacilli helps to increase the body's resistance to upper respiratory tract diseases, shorten the duration of respiratory infections, and reduce the need for antibiotic therapy¹⁹.

On the basis of lactobacilli, there are many pharmaceutical preparations for the restoration of biocenoses of the urogenital and digestive tract²⁰, but virtually no drugs are being used to restore the microflora of the upper respiratory tract. Known only work units, in which studies were conducted on the ability to normalize the biocenoses of palatine tonsils with a probiotic of "local" use – a solution of lactobacillus²¹.

¹⁸ Янковский Д.С., Дымент Г.С. Микрофлора и здоровье человека: ТОБ «Червона Рута-Тур», 2008. 552 с.; Metehan Ozen, Gonca Kocabas Sandal, Ener Cagri Dinleyici. Probiotics for the prevention of pediatric upper respiratory tract infections: a systematic review Expert Opinion on Biological Therapy 2015. V. 15, № 1. P. 9–20. DOI: 10.1517/14712598.2015.980233; Vrese M., Winkler P., Rautenberg P. Effect of Lactobacillus gasseri PA 16/8, Bifidobacterium longum SP 07/3, B. Bifidum MF 20/5 on common cold episodes: a double blind, randomized, controlled trial. Clin Nutr. Vol. 24. 2005. P.481–491. DOI: 10.1016/j.clnu.2005.02.006; Tapiovaara L., Pitkaranta A., Korpela R. Probiotics and the Upper Respiratory Tract – A Review Pediatric Infectious Diseases. 2016. V. 1 (3:19). P. 1–7. DOI: 10.21767/2573-0282.100019

¹⁹ Shan Liu, Pengwei Hu, Xiaoxin Du, Tao Zhou, Xiaofang Pei. Lactobacillus rhamnosus GG Supplementation for Preventing Respiratory Infections in Children: A Meta-analysis of Randomized, Placebo-controlled Trials. Pediatrics. 2013. V. 50. P. 377–381. DOI: 10.1007/s13312-013-0123-z; Shah M.M., Saio M., Yamashita H., Tanaka H., Takami T., Ezaki T., Inagaki N. Lactobacillus acidophilus strain L-92 induces CD4(+)CD25(+)Foxp3(+) regulatory T cells and suppresses allergic contact dermatitis. Biological and pharmaceutical bulletin. 2014. V. 35, № 4. P. 612–616. DOI: 10.1248/bpb.35.612.

²⁰ Lazarenko L., Babenko, Shynkarenko-Sichel L., Pidgorskyi V., Mokrozub V., Voronkova O., Spivak M. Antagonistic action of lactobacilli and bifidobacteria in relation to Staphylococcus aureus and their influence on the immune response in cases of intravaginal Staphylococcosis in mice. Probiotics and Antimicrobial Proteins. 2012. V. 4., № 2. P. 78–89; Mokrozub V.V. Antistaphylococcal action of lacto- and bifidobacteria and interleukin-2. *Мікробіологічний журнал*. 2013. Т. 75, № 6. С. 17–21; Калініченко С.В., Бабич Є.М., Рижкова Т.А., Маслій І.Г., Коротких О.О., Даніліна С.С., Солянік О.Г., Шикова О.А., Скляр Н.І., Ткач Л.М., Балак А.К. Сучасний стан розробки та застосування пробіотичних, пребіотичних та синбіотичних препаратів (огляд літератури). *Анали Мечниковського Інституту*. 2013. № 3. С. 5–12.

²¹ Заболотна Д.Д. Клініко-експериментальне обґрунтування застосування пробіотиків в комплексній терапії хронічних неспецифічних запальних та алергічних захворювань верхніх дихальних шляхів : автореф. дис. ... докт. мед. наук : 14.01.19 : Київ, 2010. 38 с.

Therefore, in our opinion, the perspective direction of modern microbiology is the development and creation of new highly effective probiotic drugs “local” for the regulation of microbiota of the mucous membranes of the upper respiratory tract.

For most lactobacilli, microaerophilic cultivation conditions are optimal. In view of this, for the restoration of the biocenosis of the digestive tract, industrial strains of probiotics are often encapsulated. In capsules, lactobacilli strains have the ability to continue to be stored and act only in certain areas of the digestive tract, where the capsule is destroyed. In gynecology for the restoration of normal flora vagina use suppositories or pills with lactobacilli, which slowly “melt”, gradually releasing lactobacilli. However, in contrast to the oropharynx, in the genital, and in the digestive paths, there is no permanent aeration of mucous membranes. Therefore, given the specific conditions for the use of probiotics for the restoration of the mucous membrane, it was advisable to study the biological properties of lactobacilli under aerobic cultivation conditions and compare them with similar properties in microaerophilic conditions.

We tested probiotic strains of *Lactobacillus* spp., Isolated from industrial preparations that are freely available in drugstores in Ukraine.

It was established that under aerobic conditions of cultivation in investigated lactobacilli strains, the inhibition of glucose consumption processes was 1,2–1,3 times ($p < 0,05$) compared with microaerophilic conditions. Similar results were obtained for indicators of the growth of lactobacilli: under aeration conditions, the accumulation of biomass strains was 1.2–1.8 times lower ($p < 0.05$). That is, under the aerobic conditions in lactobacilli, the level of metabolic and growth processes decreased. This may be due to less favorable vegetative conditions.

Study of adhesion of *Lactobacillus* under aerobic and microaerophilic culture conditions are not established significant changes in adhesion performance, indicating the ability of lactobacilli colonize the mucous membranes of the oropharynx in conditions of constant aeration.

The findings are positive for determining the applicability of lactobacilli in the treatment of chronic inflammatory processes of the upper respiratory tract staphylococcal origins, because despite the unfavorable conditions for lactobacilli (for gas composition of the atmosphere cultivation), they have the ability to adhesion and sufficient potential for further development.

3. Prerequisites for the development of antistaphylococcal agents based on probiotic strains of lactobacilli and *S. aureus* adhesives

Currently, the prophylaxis of staphylococcal infections is based on the use of either staphylococcal vaccine or staphylococcal ataxin, which relates to prophylactic immunobiological drugs of the first generation, which can

cause significant reactivity [Мани Ханс]. In addition, these immunobiological drugs have a short “protective” duration of 1 year and do not affect the processes of colonization resistance of macroorganism.

The strategic direction of WHO considers the gradual replacement of such immunobiological drugs with second-generation prophylactics aimed at addressing the fundamental problem of prophylactic and infectious medicine – the fight against colonization and persistence of pathogens on the mucous membranes of various ecological niches. Preparations of such a direction may include agents based on adhesins of bacteria²² and lactobacilli strains²³.

We obtained experimental specimens that contained either adhezine *S. aureus* or cells of probiotic strains of *Lactobacillus spp.* or a mixture of adhesives and lactobacilli in a ratio of 1:1.

For experimental samples were prepared microbial suspension adhesins reference strain *S. aureus* ATCC 25923 in accordance with standard optical density of 1.0; 5.0 and 10.0 units per McFarland scale using the device Densi-La-Meter (production PLIVA-Lachema, Czech Republic, wavelength 540 nm). For adhesins suspension of *S. aureus* ATCC 25923 were irradiated for 3 hours, ultrasound (US) with a frequency of 60 kHz and a power of 5 watts, ultrasonic frequency of 18 kHz and a power of 16 W and electromagnetic radiation range of 61.0 GHz band and 42, 2 GHz. Physical inductors were provided by the specialists of O. Ya. Usikov Institute for Radio Physics and Electronics National Academy of Sciences of Ukraine under the agreement on scientific and practical cooperation.

The adhesion can be evaluated by the number of teichoic acid (TA) in the samples. Experimentally determined that the TA level was higher in samples obtained using ultrasound at a frequency of 60 kHz and a power of 5 W and electromagnetic wave frequency range of 61.0 GHz.

To prove the presence of adhesins in experimental samples, conducted experiments in vitro, designed to study the effect obtained drugs on human

²² Єлісеєва І.В., Бабич Є.М., Ждамарова Л.А., Білозерський В.І., Колпак С.А., Балак О.К. Дослідження дії дифтерійного бактерійного антигенного препарату, одержаного за допомогою електромагнітного випромінювання надзвичайно високої частоти, на формування гуморального антитоксичного імунітету та колонізаційну резистентність в піддослідних тварин. *Вісник проблем біології і медицини*. 2015. Вип. 3, Т. 2(123). С. 277–282; Єлісеєва І.В., Бабич Є.М., Білозерський В.І., Ждамарова Л.А., Колпак С.А. Вплив експериментальних бактеріальних препаратів збудника дифтерії, одержаних за допомогою фізичних чинників, на адгезію тест-штамів *S. Diphtheria*. *Вісник проблем біології і медицини*. 2016. Вип. 4, Т. 1(133). С. 264–268; Антушева Т.І. Біологічні властивості штамів *Corynebacterium diphtheriae* та оцінка повноти детоксикації екзотоксину при застосуванні фізичних чинників : автореф. дис. ... канд. мед. наук : 03.00.07. Київ, 2014. 24 с.

²³ Калініченко С.В., Коротких О.О., Антушева Т.І. Протиінфекційна резистентність слизових оболонок верхніх дихальних шляхів. *Вісник проблем біології і медицини*. 2016. Вип. 4(1). С. 269–273.

erythrocytes. Treatment of human erythrocytes formalized supernatants control samples did not lead to significant changes in adhesion properties of *S. aureus* clinical strains – the interval was within $2,5 \leq \text{IAM} \leq 4,0$ (group medium adhesive microorganisms), indicating a lack of control samples adhesins. Treatment of red blood cells supernatants experimental models resulted in significant changes in adhesion properties of *S. aureus* clinical strains – the interval was within $0,74 \leq \text{IAM} \leq 2,1$ (group low adhesion microorganisms). The results give reason to believe that the supernatants derived experimental samples containing surface antigens, adhesins that block the relevant receptors of erythrocytes, as a result, does not allow test cultures attach to the surface structures of erythrocytes.

The next stage was the study of the possibility of using adhezins *S. aureus*, whole cells of the probiotic strain *L. rhamnosus GG* and their mixtures for the sanitation of staphylococcal carriers on the model of nasal staphylococcal carriage developed in animals²⁴.

Rabbits from chronic rhinitis of staphylococcal genesis were divided into the following groups: Group I (n = 5) rabbits, which for 6 days three times a day, 4% aqueous solution of erythromycin was caught in the nose; Group II (n = 5) rabbits, which for 6 days were swallowed up to 0.1 ml of *S. aureus* adhezins in the nose three times a day; Group III (n = 5) rabbits, which received a 0.1 ml suspension of lactobacillus *L. rhamnosus GG* in physiological saline at a density of 3 units on the McFarland scale for 6 days; IV group (n = 5) of rabbits, which for 6 days three times a day, a 0.1 ml suspension of *L. rhamnosus GG*, in a physiological solution, a density of 3 units on the McFarland scale and adhezins *S. aureus* (1:1); V group (n = 3) of rabbits – as carrier control (for 6 days, three times a day, the nasal cavity was injected into 0.1 ml of physiological saline). Subsequently, after 1, 7, 14 and 30 days, an animal survey was carried out to determine the population level of *S. aureus* and the values of the local immunity index – lysozyme and secretory immunoglobulin A (sIgA).

It has been established that the rabbits sanitation with antibiotics did not lead to complete eradication of staphylococci. The examination of animals of this subgroup in 7, 14 and 30 days found in all rabbits the presence of *S. aureus* in the amount of 0.9–3.2 lg CFU / g. This may be due to the persistence of the pathogen in deeper layers of the mucous membrane and its subsequent development in connection with the absence of the agent of negative influence. The incidence of lactobacilli inclusion mucous nose in

²⁴ Спосіб одержання лабораторної моделі хронічного назального носійства стафілококового генезу у кролів: пат. UA 113116 U G09B 23/28 Україна. u 2016 07616: заявл. 11.07.2016; опубл. 10.01.2017, Бюл. № 1, 2017.

rabbits of a given group decreased to 0.6-1.2 lg CFU / g, the lysozyme level was $6.9 \pm 1.8 \mu\text{g} / \text{ml}$, and the level of sIgA was $1.52 \pm 0.23 \text{ mg} / \text{ml}$.

Rabbits sanitation by cells of lactobacillus also resulted in the gradual eradication of *S. aureus*. The examination of animals in this group after 7, 14 and 30 days established the presence of *S. aureus* in the amount of 0.9–1.6 lg CFU / g in animals for 7 days and in the amount of 0.3–0.6 lg CFU / g on 14 Day. On day 30, *S. aureus* was not detected.

At 30 days in animals of this group, the degree of population of lactobacilli of the nasal mucosa increased by 1.8-2.0 times ($p < 0.05$), the level of lysozyme – 2.8–3.3 times ($p < 0.01$), the level of sIgA – 1.6–2.0 times ($p < 0.05$) compared with the control group. In our opinion, this may be due to the active population of lactobacilli ecological niche and the production of biologically active substances that inhibited the growth and development of staphylococci and stimulated the development of its own normoflora.

Rabbits sanitation by *S. aureus* adhesives also led to the gradual eradication of *S. aureus*. The animals in this group tested the presence of *S. aureus* in the amount of 0.6-1.2 lg CFU / g in animals at 7 days and in the amount of 0.3 lg CFU / g for 14 days. At 30 days *S. aureus* was not detected. The extent of lactobacillus placement of the nasal mucosa for 30 days in the rabbits of this group was at the level of the control group, the level of lysozyme increased in 1.2–1.4 times ($p < 0.01$), the level of sIgA – in 1.2–1.6 times ($P < 0.05$) compared with the control group.

Rabbits sanitation with a mixture of adhesins with lactobacilli cells resulted in the complete elimination of *S. aureus*. The examination of animals in this group after 7 days showed the absence of *S. aureus*. The same results were obtained after 14 and 30 days after the rehab. In animals of this group for 30 days the degree of lactobacilli inclusion in the nasal mucosa increased in 2.2-2.5 times ($p < 0.05$), lysozyme level – in 3.2–3.9 times ($p < 0.01$), The level of sIgA – 1.8–2.2 times ($p < 0.05$) compared with the control group.

The indicated points to the expediency of purposeful search and development of new immunobiological drugs that would have participated in the formation of anti-infectious resistance of the upper respiratory tract mucous membranes, suppressed the colonization properties of pathogens and were safe for humans.

CONCLUSIONS

Taking into account the WHO announced in 2011–2020 a strategy aimed at replacing the first generation of antibiotics and immunobiological drugs with new, more effective means, the concept of the creation of novel agents based on adhesins of bacteria and representatives of human normophytes is very promising.

In the structure of nosocomial infections, *S. aureus* occupies leading positions, which is due, first of all, to carriers among medical personnel. Currently, immunobiological drugs to prevent carriers and methods for the sanitation of carriers of *S. aureus* are outdated. In connection with what we conducted experimental research on the preconditions for the development of anti-staphylococcal immunobiological drugs of the new generation.

As a result of the research, it was determined that the population level of *Lactobacillus spp.* On the upper respiratory tract mucous membranes of patients with chronic tonsillitis (CT), chronic rhinitis (CR), chronic sinusitis (CS) and carriers of *S. aureus* is much lower than that of practically healthy persons, whereas the population level of *S. aureus* is sufficient High – 3.4–9.7 lg CFU / g. The study of the biological properties of *S. aureus* strains isolated from patients and carriers has shown their high persistent and virulent properties and the ability to level the effect of the protective forces of the macroorganism.

Taking into account long-term experience of using probiotic strains of lactobacilli to restore microbiota of mucous membranes of the alimentary canal and genital tract, which contributes to the restoration of anti-infective resistance of the mucous membranes, and the absence of such preparations in Ukraine for correction of microbiocenosis of the upper airway mucous membranes, we have been conducting studies on the possibility of developing new generation antistheel drugs. – on the basis of adhesins *S. aureus* and cells of the probiotic strain *L. rhamnosus GG*. According to experimental data, the samples obtained suppressed the colonization properties of *S. aureus* isolates and, in experiments on laboratory animals, contributed to the restoration of normal flora and anti-infectious resistance of the mucous membranes of the upper respiratory tract.

Thus, it has been shown that this research direction is promising and needs further development.

SUMMARY

Nosocomial infections of staphylococcal genesis play a leading role in the occurrence of complications with prolonged stay of patients in the infectious institution. For the prevention / treatment of infectious diseases caused by *Staphylococcus aureus*, antibiotics or immunobiological preparations of the 20th century are used.

Today, the WHO strategy for antimicrobial therapy aims at the development of a new generation of immunobiological drugs. These drugs can be classified anti staphylococcal products based on bacterial adhesins and representatives of the humans flora. We were studies experimental samples containing adhesins of *S. aureus*, or cells probiotic strain *L. rhamnosus GG*, or a mixture thereof. In experiments *GG* on laboratory

animals determined by the effectiveness of the samples: nasal readjustment of rabbits that have been artificially reproduced staphylococcal chronic rhinitis, the samples for 6-7 days resulted in irradiation of *S. aureus*, restoration of normal flora and anti-infectious resistance of the mucous membranes of the nose animals.

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