

EFFECT OF SALTS OF DIFFERENT ORIGINS ON *STAPHYLOCOCCUS AUREUS* BIOFILM-FORMING ACTIVITY

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ABSTRACT

Background: In recent times, non-drug methods of the microbial status correction attract the attention of researchers all over the world. The efficiency of saline solutions for prevention of acute respiratory viral infections, treatment of surgical and skin diseases is widely known. The aim of our research was to study the influence of the Ancient Perm Sea, Dead and Red seas salts on biofilm production of *S. aureus* isolated from carriers' pharynx. **Methods:** The study included 41 people, the average age was 19,6 ± 0,2 years. Microbiological sampling for *S. aureus* isolation was performed using the system with Amies transport medium in the morning on an empty stomach. For selective cultivation of *S. aureus* yolk-salt agar was used. The affiliation of microorganisms to *S. aureus* species was determined by lecithovitellase and plasma coagulase activity, fermentation of glucose and mannitol. Biofilm production activity was determined by O'Toole G.A. method. **Results:** During the research it was found that *S. aureus* was present in the pharynx mucosa of 36.6% observed people. All *S. aureus* strains isolated from the carriers expressed biofilm-forming activity. Adding the Ancient Perm Sea and Dead Sea salts in the incubation medium do not allow *S. aureus* to form biofilms. In assessing the salts impact on the formed biofilm it was found that the solution of the Ancient Perm Sea and Dead Sea salts are able to destroy it – there was a reduction in the biomass of films formed by *S. aureus*. **Conclusion:** Salts of the Ancient Perm and Dead Sea have pronounced anti-biofilm activity against *S. aureus* strains. The use of these salts in solutions for gargling can be effective for sanitizing staphylococcal carriers.

KEYWORDS

Ancient Perm Sea Salt, Dead Sea, Red Sea, *Staphylococcus aureus*, Biofilm

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INTRODUCTION

Nowadays there is a proliferation of *Staphylococcus aureus* bacteria carrying. It is known that 20% of people in the world are permanent carriers of *S. aureus* and 60% are transient carriers [1]. The main reasons of *S. aureus* carriage are frequent contact with sources of infection and persistent damages of skin and mucous membrane integrity.

According to scientific studies, bacilli-carriers are the main source of infection [2]. This contributes to the decrease of the activity of antibacterial drugs and changes in the properties of pathogens, especially as a part of biofilms. It is known that *S. aureus* strains have a highly mutable genetic apparatus that leads to antibiotic resistance. It should be noted that regular antibiotic treatment can lead to such side effects as

dysbiosis, superinfection, bacteriolysis reactions and others [3]. There are many contraindications in antibiotic treatment [4]: pregnancy, childhood, diseases of the gastro-intestinal tract, disorders of the liver and kidneys, etc. That is why it is necessary to search new ways of correction of *S. aureus* carriage, which would not have side effects. Some of the most important aspects for choosing antibacterial therapy are a drug cost, ease of use and the possibility of its acquisition. The study of *S. aureus* persistent characteristics and selection of new ways of bacilli-carriers rehabilitation are very topical.

In recent times, non-drug methods of the microbial status correction attract the attention of researchers all over the world. Natural sources are the most available therapeutic agents, among which peloids, climate and physical factors have an important value. Therapeutic properties of natural salts, consisting mostly of *NaCl*, *MgCl₂*, *KCl*, *CaCl₂*, *MgSO₄*, etc., are widely studied [5]. The efficiency of saline solutions for prevention of acute respiratory viral infections, treatment of surgical and skin diseases is widely known. Antibacte-

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rial, adsorbent, immunostimulatory, anti-cancer and others effects of salts are revealed [7, 8]. However, little attention is paid to the effects of salts on microorganisms which are composed of biofilms. According to scientific studies, microorganisms in biofilms composition are more resistant to antimicrobial factors [9].

The aim of our research was to study the influence of the Ancient Perm Sea, Dead and Red seas salts on biofilm production of *S. aureus* isolated from carriers' pharynx.

METHODS

The study was carried out at Perm State Medical University at the immunology department in 2015. The selection included 41 people, the average age was $19,6 \pm 0,2$ years. The criteria for the patient's inclusion were the absence of acute infection and inflammatory diseases. People were chosen on voluntary agreement. The exclusion criteria were surgery on ENT organs, acute phase of infectious diseases, antibiotic treatment.

Microbiological sampling for *S. aureus* isolation was performed using the system with Amies transport medium in the morning on an empty stomach. For selective cultivation of *S. aureus* yolk-salt agar was used. The affiliation of microorganisms to *S. aureus* species was determined by lecithovitellase and plasma coagulase activity, fermentation of glucose and mannitol.

Biofilm production activity was determined by O'Toole G.A. method [10]. We used three varieties of natural salts. The first solution contained Ancient Perm Sea salt, which is composed of NaCl - not more than 84%, Mg^{2+} - at least 1,5%, K^+ - not less than 2.7%. The salt concentration in the experiments was 10 mg/ml. The concentration of the second solution consisting of Dead Sea salt was 300 mg/ml. The Dead Sea salt composition includes $MgCl_2$ - at least 30%, KCl - no more than 28%, NaCl - at least 4%, $CaCl_2$ - less than 1% [11].

The third solution containing Red Sea salt was in the concentration of 40 mg/ml. The Red Sea salt composition includes NaCl - not more than 98%, Ca^{2+} - not less than 0,05%, Mg^{2+} - at least 0,04%, SO_4^{2-} - not less than 0.1%. The last solution (control №1) comprises chemically pure salt NaCl at the concentration of 14

mg/ml. As a control of biofilm growth distilled water (control №2) was added to the basins.

Statistical processing of the results was performed using Student's t-test. The threshold of significance was the value of $p < 0.05$.

RESULTS

During the research it was found that *S. aureus* was present in the pharynx mucosa of 36.6% observed people. The carriers' group consisted of 53% of men and 47% of women (23 and 77%, respectively, among the people without detected *S. aureus*). The incidence of acute respiratory infections in the group of carriers was $2,4 \pm 0,6$, and in the non-carriers group - $2,3 \pm 0,4$ cases per year ($p > 0,05$). The morbidity was more common in men. Thus, the number of cases per year amounted to $2,6 \pm 0,9$ for men, and $1,9 \pm 0,3$ for women ($p < 0,05$).

All *S. aureus* strains isolated from the carriers expressed biofilm-forming activity. Adding the Ancient Perm Sea and Dead Sea salts in the incubation medium do not allow *S. aureus* to form biofilms. The solution of the Red Sea salt does not express anti-biofilm effect – there was biofilm formation of *S. aureus* (Fig. 1).

In assessing the salts impact on the formed biofilm it was found that the solution of the Ancient Perm Sea and Dead Sea salts are able to destroy it – there was a reduction in the biomass of films formed by *S. aureus*. However, the Red Sea salt solution does not affect the biofilms weight after incubation (Fig. 2).

DISCUSSION

The results of this study are probably due to the presence of magnesium and potassium ions in the salt compositions, which have a stronger antibacterial effect than sodium ions [12]. Red Sea salt solution does not possess a pronounced anti-biofilm effect, as it does not contain the necessary quantity of Mg^{2+} ions and K^+ in the structure.

It is believed that the use of Ancient Perm Sea and Dead Sea salts may be effective in complex rehabilitation of bacteria carriers, due to its easy use, ability to use solutions at home, availability and cost.

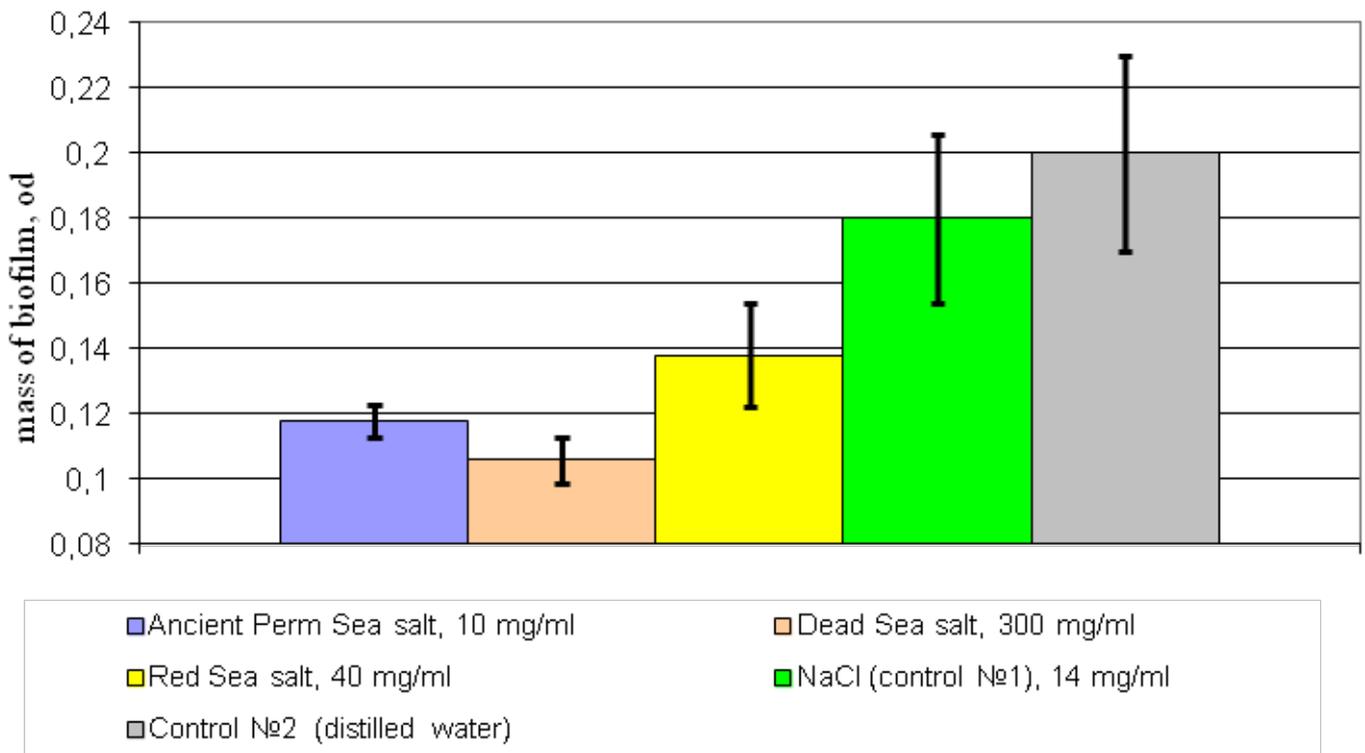


Fig. 1. Salts impact on *S. aureus* biofilm formation activity

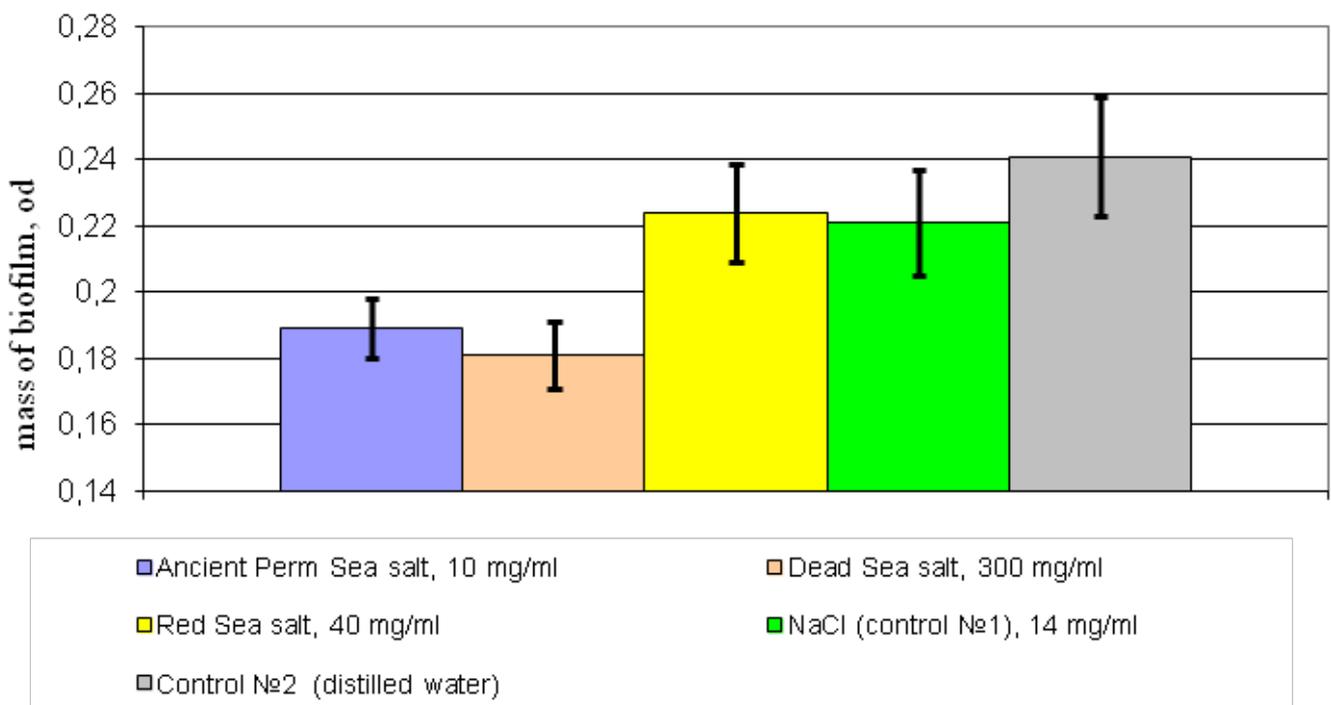


Fig. 2. Salts impact on *S. aureus* biofilm

CONCLUSION

Consequently, during the research it was found that a third of young people are carriers of *S. aureus* in throat mucosa. *S. aureus* isolated from a carrier has an expressed biofilm-forming activity. Salts of the Ancient Perm and Dead Sea have pronounced anti-biofilm activity against *S. aureus* strains. The use of these salts in solutions for gargling can be effective for sanitizing staphylococcal carriers.

CONFLICT OF INTEREST

The author confirms that this article content has no conflicts of interest.

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