ANTIBIOTIC AND PHAGE THERAPY TO CONTROL BIOFILM FORMATION OF *STAPHYLOCOCCUS AUREUS* STRAINS

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Introductions. The ability of staphylococci to form biofilms makes the treatment of many diseases more complicated and reduces the effectiveness of hemodialysis and hemotransfusion. These microorganisms can cover wounds and medical instruments (implants and catheters) and promote the proliferation of hospital-acquired infections. The main problem in the treatment of *Staphylococcus aureus* biofilm formation is its high antibiotic resistance. Existing methods of biofilm control are generally ineffective that leads to the accumulation of resistant microorganisms.

Traditional antibiotic therapy is focused on killing viable cells, but removal of matrix components and prevention of initial attachment stages are also potentially important mechanisms in the treatment against biofilms [1].

It is found that biofilm cells are 10-1000 times more resistant to antibiotics and antimicrobial peptides [1]. The low penetration of antibacterial drugs into biofilms associated with their highly complex structure, as well as internal antibacterial resistance, are main factors in the resistance of biofilms to antibacterial therapy.

The use of alternative therapies is very promising, one of which is the use of bacteriophages, a specific type of virus that can infect bacterial cells. Bacteriophages are characterized by their high specificity and are unable to multiply in mammalian cells due to receptor restrictions too, so they can be used in medical practice [2].

Staphylococcal bacteriophages are characterized by a high level of effectiveness, but their practical use in the medical sphere of Ukraine is limited.

The aim of our work was to analyze a biofilm formation control scheme using bacteriophages, to evaluate the effectiveness and safety of the proposed method.

Materials and methods. *S. aureus* strains were cultured at t = 35±2 °C on Colombian agar with 5 % sheep blood for 24 h. Inoculum with a bacterial concentration of $1,5\times10^8$ CFU/mL was prepared with sterile 0,85 % saline. Seeding was performed in horizontal lines on Mueller-Hinton agar, after which 20 μL of phage (PYO, STAPHYLOCOCCAL, INTESTI, and PHAGESTI) was added to each line. After 24 h of incubation at t = 35±2 °C, the lysis zone was assessed using the dot test [3].

Results and discussion. *Staphylococcus aureus* is a Gram-positive aerobic facultative pathogenic bacterium that can often lead to skin infections. However, while most staphylococcal infections are not serious, *S. aureus* can also cause serious infections such as blood poisoning, pneumonia, endocarditis, and osteomyelitis [4].

S. aureus has a specific virulence factor, coagulase, which promotes biofilm formation. The coagulase binds to host prothrombin and forms active staphylotrombin complexes that transform soluble monomeric fibrinogen into insoluble, self-polymerizing fibrin and activate the coagulation cascade. *S. aureus* uses the fibrin and fibrinogen formed by coagulase to form the biofilm framework. Microbial biofilms demonstrate increased resistance to disinfectants and antibiotics, and often only a

combination of chemical and physical methods can reduce the CFU and remove the biofilm from surfaces. Biofilms formed by resistant or intermediate categories of *S. aureus* reduce the penetration of oxacillin, vancomycin, and cefotaxime [5].

The high-level antibiotic resistance of biofilms makes it possible to use alternative therapies, such as phage treatment. The antibacterial effect of bacteriophages is caused by the penetration of the phage genome into the bacterial cell, followed by its reproduction and lysis of the infected cell. This leads to the death of pathogenic bacteria and determines the important role of bacteriophages in the control of infections caused by the formation of biofilms [6].

Studies of bacteriophage preparations *in vivo* and *in vitro* on invertebrates (in particular, *Galleria mellonella* and *Drosophila melanogaster*) have shown that the sensitivity of Staphylococcus strains ranges from 17,9 % to 50,6 %, depending on the specific preparation (Table 1).

Table 1. Sensitivity of Staphylococcus isolates to bacteriophages [3]

Sensitivity to bacteriophage	*S. aureus,* n = 66 (%, absolute values)	CoNS, n = 17 (%, absolute values)
PYO	54,5 % (36/66)	23,5 % (4/17)
INTESTI	56,1 % (37/66)	29,4 % (5/17)
PHAGESTI	21,2 % (14/66)	11,8 % (2/17)
STAPHYLOCOCCAL	17,9 % (10/56)	7,7 % (1/13)

Notes: CoNS – coagulase-negative staphylococci.

The inclusion of bacteriophage cocktails in a pure two-day culture of *S. aureus* led to a significant inhibition of biofilm formation. So, the optical density of biofilms decreased from 0,196±0,031 in pure culture to 0,111±0,018 when INTESTI phage cocktail was added at a dilution of 1/2 (Fig. 1).

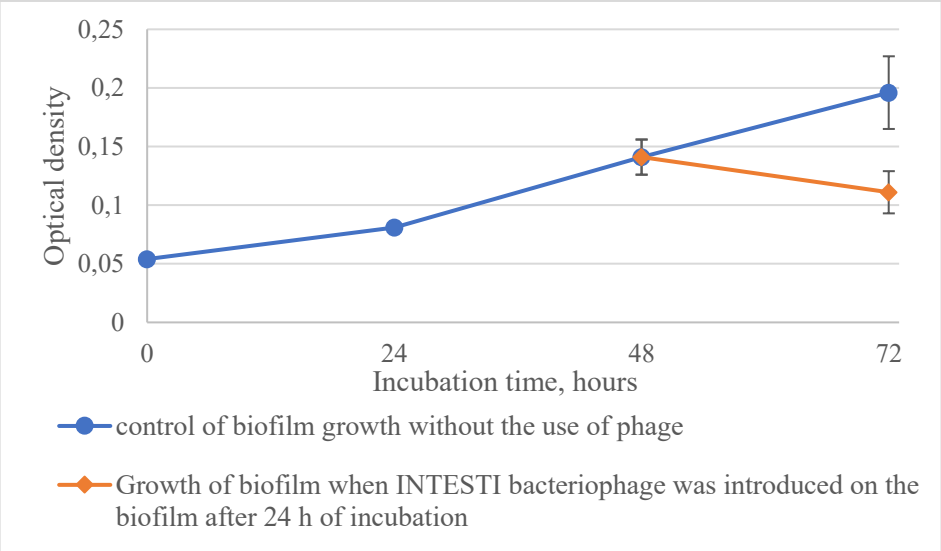

Fig. 1. Changes in optical density under the influence of INTESTI bacteriophage when applied to a 2-day biofilm after 24 h of incubation [6]

The results of the study demonstrate the sensitivity of antibiotic-resistant *S. aureus* strains to INTESTI and PYO (56,1 % and 54,5 % of isolates, respectively), and a 3,4 % decrease in the optical density of biofilms indicates the high efficiency of phage therapy. The obtained data are explained by the destruction of exopolysaccharide components of the biofilm matrix under the influence of phage endolysins, which facilitates the infiltration of the viral genome into bacterial cells.

A combination of antibiotic and phage therapy is a perspective method in the control of biofilm formation. *In vitro* studies have shown that incubation of *S. aureus* culture for 24 h with phage SAP 26 leads to the death of 26 % of biofilm cells, with azithromycin – 25 % of cells, while the combined use of these drugs led to the death of 60 % of bacterial cells [7]. This synergistic phenomenon is due to both a decrease in the minimum inhibitory concentration and the destruction of the biofilm matrix, which also reduces the antibiotic resistance of *S. aureus* strains. Despite the high efficiency of this method of therapy, further clinical studies are necessary to demonstrate the safety of this form of therapy.

Conclusions. The analysis results revealed that phage therapy is an effective method in the control of *Staphylococcus aureus* biofilm formation. The described phage cocktails PYO and INTESTI are clinically proven, characterized by high antimicrobial activity (inhibition of bacterial growth by ≈ 40 %) and therapeutic effect, which allows the use of these drugs in medical practice and for the prevention of biofilm formation on medical equipment. The most promising method is the combined use of antibiotics and phages, which increases the effectiveness of therapy from ≈ 25% to 60 %, but the absence of clinical studies makes it less possible to implement such treatment on a large scale.

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