



Fund “Nauka” Project № 25024 Resume – Competition-based Session 2025:

“Investigation of the activity of peptides and proteins of natural origin and their potential for application in combination therapy with conventional microbial inhibitors”

Project leader: assoc. prof. Neli Mitkova Ermenlieva, PhD

Antimicrobial resistance represents one of the most serious challenges facing modern healthcare, as multidrug-resistant pathogens such as *Staphylococcus aureus* (MRSA), *Enterococcus faecium* (VRE), *Escherichia coli*, and *Klebsiella pneumoniae* significantly limit the effectiveness of antibiotic therapy. The present project aims to investigate the antimicrobial potential of natural bioactive molecules—bacteriocins (nisin, bacteriocin A), lactoferrin, and lysozyme—used alone and in combination with antibiotics against clinically relevant multidrug-resistant microorganisms and mixed bacterial–fungal biofilms.

The main objectives include: acquisition of reference multidrug-resistant strains; determination of minimum inhibitory and bactericidal concentrations; investigation of synergistic interactions using checkerboard and time-kill assays; and evaluation of activity against biofilms. The project also envisages the development of polymer-based pharmaceutical dosage forms containing bacteriocins, lactoferrin, and/or lysozyme, either alone or in combination with antibiotics, followed by assessment of their physicochemical stability and compatibility.

The studies will be conducted using state-of-the-art microbiological, pharmaceutical, and analytical methods, ensuring the reliability and reproducibility of the results. The project is expected to identify synergistic bioactive peptide–antibiotic combinations with enhanced efficacy and a reduced risk of resistance development, as well as to propose technologically applicable pharmaceutical formulation prototypes. The results will have both fundamental and applied significance, providing a basis for the development of new therapeutic strategies against multidrug-resistant microorganisms.

The present study is expected to provide new experimental data on the antimicrobial activity of bacteriocins (nisin, bacteriocin A), lactoferrin, and lysozyme against clinically significant multidrug-resistant strains. It is anticipated that combinations of natural bioactive proteins and antibiotics exhibiting pronounced synergistic effects will be identified, leading to a reduction in the minimum effective

doses and offering new therapeutic solutions for infections caused by multidrug-resistant pathogens.

As a result of studies using biofilm models, evidence is expected to be obtained demonstrating the effectiveness of bacteriocins, lactoferrin, and lysozyme, as well as their combinations, against infectious agents, thereby expanding current knowledge of their potential to overcome the barriers associated with biofilm organization.

From a technological perspective, prototypes of polymer-based pharmaceutical dosage forms containing bacteriocins, lysozyme, and combinations with antibiotics are expected to be developed. Data on their physicochemical and microbiological stability will be collected. These results will serve as a foundation for future preclinical and clinical development.

In summary, the project will contribute to the identification of the most promising combinations of bioactive peptides and proteins and their combinations with antibiotics suitable for the development of antimicrobial therapies. The obtained results will be of significance both for fundamental science, by advancing knowledge of bacteriocins, and for applied practice, by creating a basis for new therapeutic strategies for the treatment of infections caused by multidrug-resistant microorganisms.