**MAREK ŻYWICKI**

**PROJECT TITLE:**

**OPUS no 2017/25/B/NZ6/00642**

## Research project objectives

Drug-resistant bacteria are big challenge for modern medicine. One of the most common drug-resistant microbes is methicillin-resistant *Staphylococcus aureus* (MRSA). Bacteria exhibit great ability to adapt through spontaneous mutations and horizontal gene transfer, thus are able to acquire resistance even for novel antibiotics like vancomycin, linezolid and daptomycin. For this reason, study of regulatory mechanisms in bacteria is crucial and can contribute to development of novel antibiotics. Promising targets in antimicrobial treatment are regulatory RNAs, such as riboswitches. Riboswitches are common regulators in bacterial genome, which modulates gene expression on transcriptional or translational level by structural rearrangements in 5'UTR of mRNA induced by binding of small metabolites. They are composed of two major functional domains – expression platform and aptamer, which binds ligand. Since single family of riboswitches binding same ligand can control multiple mRNAs acting on different metabolic pathways, applying proper analog of natural ligand can target multiple genes. Such treatment would require more complex rearrangements in bacterial genome to gain resistance.

The main goal of this project is identification of RNA regulatory networks, which could be potential targets for antimicrobial compounds. Study will focus on methicillin-resistant *Staphylococcus aureus* (MRSA). In order to identify novel RNA regulators, computational methods for synergistic analysis of high throughput transcriptomic data derived from multiple high throughput experimental approaches will be developed.

The multidimensional analysis of experimental data will be applied to identify: i) riboswitches acting by transcription modulation, ii) riboswitches acting by translation modulation, iii) non-coding regulatory RNAs and their targets, iv) other regulatory RNA mechanisms.

## Research project methodology

Experiments will be performed on methicillin-resistant *Staphylococcus aureus* in four conditions: on culture grown on a standard LB medium, in addition of sub-lethal concentrations of methicillin or vancomycin and as co-culture with HaCaT keratinocytes as infection model. In order to identify RNA regulatory mechanisms, wi will analyze rearrangements of RNA secondary structure, changes in expression and translational activity of mRNAs. Identified regulatory changes will be used to connect the RNA regulators and their targets into the regulatory networks. We will attempt to predict the functional consequences of inhibition of such regulatory networks. Activity of regulators with strongest therapeutic potential will be validated experimentally with the use of *in vivo* assays based on reporter gene constructs. This will allow for extensive reconstruction and characterization of RNA regulatory networks with crucial importance in infection and drug resistance.

## Expected impact of the research project on the development of science

The realization of the project will enable for the first time high throughput identification of multiple RNA regulators, including translational riboswitches and translation atenuation by upstream open reading frames (uORFs) and others. Obtained results will enable identification and characterization of novel RNA-dependent regulatory mechanisms related to infection and drug resistance of bacteria. The final result of the project will be list of reliable targets for antimicrobial therapeutics. All those data will be released to public via publications and deposition in scientific databases, thus

facilitating further development of antimicrobial compounds against multidrug resistant *Staphylococcus aureus*.