Background. The antibiotic resistant bacterial infections cause almost a million deaths each year globally. In spite of these horrifying statistics, and the fast spread of the problem, people continue to believe in the golden age of antibiotics. Immediately after being introduced in 1940's, antibiotics did increase the quality and length of our lives. Unfortunately, nowadays antibiotic resistant bacteria pose a real threat to mankind. For example, the New Delhi Metallobetalactamase (NDM) resistance was discovered in 2008 in India. The first NDM infection in Poland was reported in 2014. Just four years later we expect more than 3 thousand infections, and some specialists warn of an outbreak of an epidemic.

Dwelling in the comfort of the golden age of antibiotics, we settled with the picture that the response of bacteria to treatment is simple – in the absence, or at sufficiently low concentrations of antibiotics bacteria proliferate, while above a certain threshold concentration of the drug bacteria either stagnate or die. To this day the susceptibility of bacteria to antibiotics is characterized with a single value of the so-called minimum inhibitory concentration (MIC). This is the believed threshold between life and death of bacteria. Unfortunately, the reality is much more complex. Even in a single colony, bacteria are not all the same. Cells display genetic variations, and even in a genetically homogeneous population, there might be significant heterogeneity in how the cells 'behave' – i.e. in their phenotype. Some cells tolerate higher concentrations of the antibiotic than others. If dosed at a concentration that falls in the range of tolerance in the population, the antibiotic will not only fail, but worsen the situation by promoting growth of the more resistant cells. The second issue is the following: for some antibiotics, bacteria may show increased tolerance if they live in dense populations. The spots where bacteria form dense colonies will become the centers of resistance and proliferation. Finally, the MIC measurement itself is subject to error, because in a typical setting of a diagnostic laboratory, the starting density of bacteria is difficult to be prepared with high precision – and for some antibiotics, an imprecision in the density of bacteria results in an error in the measured value of MIC. This error may, in some cases, especially those of the more difficult infections, have important negative consequences – because it may exclude the use of antibiotics that could be effective, or prompt the use of ones that will fail.

Objective of our project. In our project we propose to develop a new set of analytical tools that will allow to characterize the complexity of how bacteria respond to antibiotics. We will use droplet microfluidics – a set of techniques that enables generation of thousands or millions of tiny, identical droplets. These droplets may be formed of a suspension of bacteria, resulting in encapsulation of single (individual) bacterial cells, or multiple cells in the droplets. By adding antibiotics at a range of concentrations, we will be able to prepare sets (libraries) of droplets, each library will contain droplets with a well-defined concentration of the antibiotic and number of bacterial cells.

Research to be carried out. In order to develop a platform for such measurements, we must develop microfluidic systems to handle multiple libraries of thousands of nanoliter droplets, develop a technique for detecting the growth of bacteria in such tiny droplets without the need for any dye additives or genetic modifications of bacteria and we must ensure that the antibiotics that we encapsulate in the droplets stay in the droplets.

Reasons for choosing the topic. Antibiotic resistant infections constitute one of the grimmest threats to human health. While we hope for new antibiotics to be developed, even if they are approved for use, the problem can only be sustainably solved by better understanding of the response of bacteria to antibiotics. It will help to avoid emergence of resistance and to guide effective treatment. In science, understanding can only be built with reliable and accurate measuring tools. We strongly believe that the tools that we propose to build and test are extremely needed and this fuels our motivation to pursue the topic.