**Project goal**

The development of a new analytical procedure in order to assess the therapeutic value and usefulness of antibiotics and their metabolites as resistance markers based on the proteomic and metabolic profiles.

**The fundamental research**

The first stage of the research is to develop simple, relatively fast, cheap and environmentally friendly methods for the isolation of analytes from the clinical samples collected from patients with ulcerations in the diabetic foot syndrome and postoperative wounds. It is also important to choose the separation conditions and optimal parameters of the mass spectrometer that enable simultaneous determination of selected antibiotics and their metabolites in physiological fluids using the LC-MS / MS technique. Following research will be focused on developing a method for determining antibacterial agents in body fluids based on the MEKC-DAD technique by optimizing parameters affecting analyte separation as well as optimizing the extraction procedure of active substances depending on the type of biological matrix. Due to the fact that saliva has not been used so far as a matrix to detect the tested active substances, one of the tasks will be to determine the relationship between the concentrations of selected antibiotics in saliva and their levels in urine and plasma (correlation).

The next stage will be the optimization of the method of sample preparation for the influence of different biological matrices (blood, saliva, urine) on the type of identified bacterial strains using the MALDI TOF-MS/MS technique.

Due to the important role of bacterial biofilms in bacterial infections, the project will address the study of the influence of selected antibiotics and their metabolites on the acquisition of resistance by selected bacterial strains and the assessment of their ability to form a biofilm *in vitro*. The determination of intact bacterial cells as well as lysed cells will be performed using capillary electrophoresis coupled with mass spectrometry (molecular profile testing).

The following studies will be focused on metabolic changes *in vitro* of selected antibiotics against metabolic enzymes present in the microsomal fraction of human liver cells and electrochemical simulation of metabolism (phase I and II reactions) of selected antibiotics.

**The reasons for attempting the research topic**

The antibiotic treatment strategy is considered to be as milestone in the treatment of bacterial infections. Nevertheless, the misuse of antibacterial drugs, both in humans and animals, may be associated with recurrence of a single infection, direct toxicity followed by organ failure, and above all with the appearance of infection based on antibiotic resistance. In the time of the increasing antibiotic resistance a proper diagnostics tool, which can be a simple, quick and cheap identification of a bacterial infection or a response to antibiotics is a necessity. It is also very important to make people aware that the optimization of an antibiotic therapy does not only focus on the therapeutic strength, but it also involves minimizing the risk of resistance arising during the therapy, both for infectious pathogens and a natural microbiota. It is also important to develop diagnostic techniques that are more specific and less time consuming than current standard techniques.

**Substantial results expected**

The knowledge obtained during the implementation of the proposed research can be used to develop effective strategies for controlling drug resistance. Indeed, the research topic described above can be a necessity to develop analytical tools for early medical diagnostics and to monitor effective antibiotic therapy.