Abstract

In the era of increasing antibiotic resistance, diagnostics that can be a simple, quick and inexpensive identification of bacterial infection or determining antibiotic response are absolutely necessary to optimize individual patient therapy and reduce the risk of antibiotic resistance. Therefore, the presented doctoral dissertation involves assessment of therapeutic value and usefulness of antibiotics and their metabolites as resistance markers based on the analysis of protein and metabolic profiles.

The first stage of the research performed within the scope of the dissertation was the development of simple, relatively fast, as well as low-cost methods for the determination of antibiotics and their metabolites from different groups. To this purpose, the focus of attention was initially on the choice of separation conditions for selected antibacterial agents and their metabolites using high-performance liquid chromatography with diode array detector (HPLC-DAD). Further, a new analytical method was developed and validated for the simultaneous analysis of antibiotics and their metabolites in human urine. Capillary electrophoresis (CE) combined with mass spectrometry (MS/MS) was used to determine and identify all analytes. However, the effect of different analytical conditions (composition, concentration and pH value of separation buffer, injection time and pressure, capillary temperature and the effect of organic modifier) on the migration and separation of antibiotics and metabolites was investigated using CE-DAD. Subsequent studies were focused on estimating the efficacy of antibiotics with different action mechanism (amoxicillin, gentamicin, metronidazole) against a model of bacteria (*B. tequilensis*) forming a biofilm using capillary electrophoresis and related techniques. Capillary electrophoresis demonstrated the ability to characterize and show differences in electrophoretic mobility between untreated and antibiotic-treated biofilms. The stability of the dispersion study, the molecular profile analysis, the viability of bacterial cells and the scanning morphology imaging were also investigated. Microscopic and spectrometric studies indicated degradation of the extracellular polysaccharide substances (EPSs) matrix, inhibition of cell wall synthesis, and blocking of ribosomal protein synthesis by amoxicillin and gentamicin. It was observed that untreated and treated bacterial cells had high stability for the biofilm formation system. In addition, on the basis of the type of the antibiotic treatment, the mechanism of used antibiotics in cell clumping and degradation were proposed. The next stage of the study involved the application of the MALDI-TOF MS technique for identification of the saliva microbiome and observation of changes in molecular profiles in dependence of the administered antibiotic. Significant changes in the composition of the saliva microbiome were noted depending on the used culture media, antibiotic therapy and coexisting microbiota.

The methods presented in this paper can be applied in patient screening analysis to eliminate drug combinations that are not recommended for co-administration. In addition, the such approaches can help in developing the methods enabling a faster diagnosis of disease changes at the cellular level before clinical changes occur.

Katarzyna Pauta-Jurida