

ABSTRACT

The research presented in this doctoral dissertation focuses on using advanced technologies in microbiological laboratories to identify, analyze, and determine the drug susceptibility of microorganisms that play a significant role in spreading infections and the disseminating antibiotic-resistant genes. It is possible to ensure food safety, maintain public hygiene, and implement therapies and preventive strategies in human and animal health protection through precise and accurate microbiome analysis.

The main objective is to develop effective diagnostic tools that precisely identify pathogenic factors and determine their antibiotic-resistance level, mainly through laser desorption/ionization techniques and electromigration techniques. The dissertation aims not only at methodological development but also at verifying the effectiveness of various omics approaches in clinical sample analysis, enabling assessment of their utility in routine microbiological diagnostics. The research focuses on microorganisms of clinical significance, including those involved in diabetic foot infections and those associated with the development of prostate cancer, which affect a significant portion of the population.

Regarding electromigration techniques, the potential use of capillary electrophoresis for pre-concentration of bacterial cells as an innovative method for assessing their sensitivity to various classes of antibiotics is presented. In the section dedicated to mass spectrometry, particular attention is paid to MALDI technique, which is witnessing an increasing trend of implementation in hospital settings. In the section on mass spectrometry, special attention was given to the MALDI technique, which has been steadily increasing in hospital settings. The possibilities and limitations of proteomic and lipidomic approaches in the identification process and the metabolic approach based on enzymatic hydrolysis detection enabling the determination of pathogen susceptibility/resistance to antibiotics are discussed. The dissertation also addresses sample preparation issues, particularly the impact of implementing the culturomic approach on the microbiome identification process. To overcome some of the problems associated with the use of classical organic matrices in laser desorption/ionization techniques, an approach utilizing silver nanoparticles as an effective ionization tool is proposed. The synthesis of nanoparticles was conducted using the chemical vapor deposition method, allowing the production of a layer characterized by high uniformity and showing potential in analyzing low molecular weight compounds. The possibility of their application in distinguishing closely related strains is also presented. Comparative analyses of newly developed methods with traditional approaches, allowing determination of their advantages, limitations, and potential application areas, are an integral part of the work.

The research presented in this dissertation introduces an innovative and interdisciplinary approach to microbiological diagnostics based on advanced analytical methods, which can significantly contribute to improving healthcare quality and the effectiveness of bacterial infection treatment, particularly crucial in the face of contemporary medical challenges.

Ewelina Sibińska

The significance of developing spectrometric methods for microorganism's identification