1. PHD PROJECT DESCRIPTION (4000 characters max., including the aims and work plan)

Project title: Analysis of antimicrobial drugs and clinical bacterial isolates using hyphenated analytical techniques based on mass spectrometry

1.1. Project goals

- assessment the possibility of using two types of diagnostic methods such as proteomic (matrix-assisted laser desorption ionization time-of-flight mass spectrometry, MALDI-TOF MS) and genotypic (16S rRNA gene sequencing) as w for the determination and identification of bacteria isolated from wound specimens and urine samples
- optimization of mass spectral properties in MALDI-TOF MS profiling of bacteria strains characteristic for clinical samples, identification of drug-resistant bacteria in clinical material
- development method for rapid detection of antibiotic resistance based on MALDI-TOF MS
- development of efficient methods of microbiological analysis and spectrometric method of bacteria cell isolated from patients under antibiotic therapy
- development of chromatographic method of selected antibiotic drugs and their metabolites from biological material

1.2. Outline Wound infections have long been a problem in the medical field, especially in surgery. Progress in the control/monitoring of post-operative wound infections has not entirely eliminated this problem due to the development of antibiotic resistance. Antimicrobial resistance can lead to increased complications and costs associated with surgery and treatment. An infected wound complicates the postoperative process and results in prolonged hospitalisation and delayed recovery. The skin bacterial flora is colonized by both commensal and pathogenic bacteria. Pathogens colonizing operative wounds may be part of the patient's normal flora (endogenous source) or may be derived from the hospital environment or other infected patients (exogenous source). Among the transition microorganisms are *S.aureus*, methicillin-resistant strains (MRSA) and hospital-acquired *E. coli* bacteria strain. Microbiological testing of clinical samples is an important tool/approach for effective surveillance of antimicrobial resistance. The quick and reliable identification of bacteria is essential to recognize and treat patients with infectious diseases. To date, biochemical tests, molecular methods and even antibiotic susceptibility have been used to identify genera and species. The main limitations of these methods include the necessary time and difficulty in distinguishing between very similar or difficult to cultivate microorganisms. Many of these problems have been solved with the matrix-assisted desorption laser desorption time in flight mass spectrometry (MALDI-TOF MS). The research has shown that rapid diagnosis of infectious diseases by mass spectrometry MALDI-TOF MS has enhanced the use of antimicrobial agents has improved, allowing targeted anti-microbial therapy to be administered quickly. Knowledge of the causal species of bacteria and their resistance profile allows targeted antimicrobial therapy, reduces ineffective of drug treatment as well as partly avoids unnecessary antimicrobial therapy against inactive bacterial pathogens. Thus, the data obtained contribute to the prevention and control of antimicrobial resistance.

Identification of pathogenic clinical bacterial isolates is mainly dependent on phenotypic and genotypic characteristics of the microorganisms. These conventional methods are costive, time-consuming, and need special skills and training. An alternative, mass spectral (proteomics) analysis method for identification of clinical bacterial isolates will be recognized

as a rapid, reliable, and economical method for identification. Hence, quickly advancing progress in the field of molecular biology methods contributes to the improvement of the process of microorganisms identification.

1.3. Work plan

- determination of the impact of cultivation conditions on the MS spectra of selected clinical bacterial strains
- optimization of an effective method of DNA isolation of different bacteria genus in 16S rRNA gene sequencing method
- determination of resistance mechanism of isolated bacteria to selected antimicrobial agents with the use of MALDI TOF MS approach
- study the molecular profile of bacteria strains forming biofilms cultivated/grown on various surfaces (plastic, glass) at different stages of biofilm progression, study of the possibility to prevent *in vitro* biofilm formation

1.4. Literature

[1] B. Li, T.J. Webster, J. Orthop. Res. 36 (2018) 22–32.
[2] M. Szultka-Mlynska, B. Buszewski, Anal. Bioanal. Chem. 408 (2016) 8273–8287.
[3] T.-Y. Hou, C. Chiang-Ni, S.-H. Teng, J. Food Drug Anal. (2019) 1–11.
[4] M. Beccaria, D. Cabooter, Analyst 145 (2020) 1129–1157.
[5] M. Szultka-Mlynska, P. Pomastowski, B. Buszewski, J. Chromatogr. B 1086 (2018) 153–165
[6] M. Szultka-Mlynska, B. Buszewski, Talanta 160 (2016) 694–703.
[7] L. Krásný, R. Hynek, I. Hochel, Int. J. Mass Spectrom. 353 (2013) 67–79.
[8] S. Leekha, C.L. Terrell, R.S. Edson, Mayo Clin. Proc. 86 (2011) 156–167.
[9] M. Szultka, R. Krzeminski, M. Jackowski, B. Buszewski, Chromatographia 77 (2014) 1027–1035.
[10]M. Szultka, R. Krzeminski, M. Jackowski, B. Buszewski, J. Chromatogr. B. 940 (2013) 66–76.

1.5. Required initial knowledge and skills of the PhD candidate:

knowledge of Polish and English, analytical thinking, eager to learn and work hard, knowledge of analytical chemistry and microbiology, methods of determination and identification of antibiotic drugs and bacterial strains from biological samples, knowledge about advanced instrumental techniques.

1.6. Expected development of the PhD candidate's knowledge and skills:

PhD candidate knows and can applied of two diagnostic approach (MALDI TOF-MS and 16S rRNA gene sequencing) for determination and identification of clinical isolates in the microbiology laboratory setting. Moreover, PhD candidate is able to discriminate bacterial isolates at the genus and species level as well as to detect and differentiate particular antibiotic resistance mechanisms based on MALDI TOF MS technique. PhD candidate is able to indicate bacterial strains characteristic of specific clinical matrices (urine, wound specimens). On the other hand, PhD candidate is able to develop a new, previously unknown procedures and analytical methods for the determination and identification of drugs from various therapeutic groups and their metabolites with the use of (U)HPLC in combination with a different mass spectrometers.