ABSTRACT

Healthcare-associated infections represent a significant cause of morbidity and mortality in hospital settings. The risk of nosocomial infection differs significantly in different group of patients, depending on the character of their primary illness, the co-morbidities, the type of care provided, the length of hospitalization, or the medical procedures used. Artificial surfaces such as central venous catheters, shunts, urinary catheters, valve and joint replacements or controlled lung ventilation play a major role. The majority of nosocomial infections is caused by several representative of *Enterobacteriaceæ* family, *Pseudomonas* spp., *Acinetobacter* spp., or some Gram-positives, especially *Staphylococcus* and *Enterococcus* spp. This is largely due to their ability to retain and transfer different types of resistance to antibiotics. The identification and subtyping of these pathogenic microorganisms is an essential tool of modern public health infectious disease surveillance not only for appropriate and efficient treatment of infections, but also in case of an outbreak. Understanding clonal continuity among investigated strains is essential to determine the source and routes of infections, confirm or rule out outbreaks, trace cross-transmission of healthcare-associated pathogens, or recognize virulent strains. For this purpose, a series of simple phenotypic methods such as determination of minimal inhibition concentration, biochemical testing or serotyping can be used, utilizing differences in morphology, biochemical and enzymatic activity, or antigenic composition of given microorganism. Furthermore, more complex genotypic methods can be used to provide more sensitive strain differentiation and higher levels of standardization, reproducibility, typeability, and discriminatory power compared with phenotypic typing methods. These techniques include not only the number of PCR-based methods, often associated with conventional, pulsed or capillary electrophoresis, but also sequenation, ranging from Sanger sequencing of individual genes to exacting whole-genome sequencing.

The present dissertation thesis summarizes the results of five selected publications published in impact factor journals, with two first-author publications. The first work led to the development and validation of a novel automatic technique for bacterial and yeast deposition on MALDI target using "wet-deposition" into a droplet of 70% formic acid. This automatic deposition was compared to conventional manual spotting using a wooden toothpick, semi-extraction routinely used for yeasts, and manual "wet-deposition". Spotting by MALDI Colonyst robot significantly increased identification score of bacteria compared with the routine diagnostic process. Following four publications are focused on the epidemiology of several of the most common carbapenemases in the Czech Republic (KPC, OXA-48, NDM, and IMP), either within hospital outbreaks or their occurring across the country. In particular, we focused on detecting the possible clonal spread and tracking the path and the source of their transmission with the use of a number of typing methods, both phenotypic and genotypic, including whole-genome sequencing.

Keywords: bacterial typing – phenotypic methods – genotypic methods – NGS – MALDI-TOF MS – outbreak – HAI