Abstract

The discovery of antibiotics and their subsequent clinical use has had a tremendous and beneficial impact on human health. β-lactam antibiotics are considered the most widely used therapeutic class of antibacterials prescribed in human and veterinary clinical practices due to their excellent safety profile and broad antimicrobial spectrum. β-lactams have undergone continuous development since their introduction in order to improve properties such as potency, spectrum of activity, pharmacokinetic and safety profiles and to counter the emergence of resistance. Resistance can occur by multiple mechanisms, including, notably, the production of β-lactamases and modification of β-lactam receptors - penicillin-binding proteins.

The resistance of bacterial pathogens to common antimicrobial therapies and the emergence of multidrug-resistant bacteria are increasing at an alarming rate in Czechia. Understanding the mechanisms of resistance in clinical isolates is critical to the design of novel therapeutics and the improvement of detection techniques. Insight into the genetic basis of resistance can also reveal drug design strategies for curtailing the spread of resistance and combatting multidrug-resistant organisms.

This dissertation thesis concerns different thematic investigations, beginning with the evolution of β-lactam antibiotics to a background of the biochemistry of β-lactam antibiotic resistance, and continuing with publications focused on a) the development and validation of assays for rapid detection of carbapenemase activity, b) the validation of a commercial automatic program for the detection of MRSA strains, and c) the molecular-epidemiological characterization of carbapenemase positive Gram-negative isolates detected in hospitals in the Czech Republic.

The first study compares the efficiency of imipenem and meropenem hydrolysis assay for the detection of carbapenemase-producing *Enterobacterales* and *Pseudomonas aeruginosa* by MALDI-TOF mass spectrometer. Validation supported the high sensitivity and specificity of both assays to both strains. However, the study showed that the addition of special compounds is necessary for higher sensitivity of detection of carbapenemases.

The second study describes the first case of IMI-2-producing *Enterobacter asburiae* identified in the Czech Republic in 2016. The isolate was obtained from a patient without previous hospitalization and travel history. The strain lacked an obvious source of origin, suggesting a silent spread via unknown pathways.

The third study characterizes NDM carbapenemases isolated from *Enterobacterales* during an outbreak in 2016. Until then, the occurrence of NDM carbapenemases was rare. The results of the plasmid analysis showed that bla_{NDM} genes were located on IncX$_3$ plasmids. These are the main factors contributing to the dissemination of NDM-like enzymes in the Czech Republic. Moreover, two distinct NDM-producing strains have been found in two different patients, suggesting the occurrence of horizontal gene transfer. These findings indicate that NDM-like producers pose an important public threat, mainly due to the rapid horizontal transfer of IncX3 bla_{NDM}-carrying plasmids.

The next study reports a case of VIM-1 producing *Enterobacter cloacae* isolate and description of the novel VIM-1-encoding plasmid. Until then the frequency of *Enterobacterales* with VIM carbapenemases has been rare with just a few sporadic cases detected in Czechia, although VIM carbapenemase has occurred frequently in Europe. Plasmid analysis suggests that this ColE1-type plasmid could be developed by obtaining a Tn*1721*-like transposon carrying the integron In*110* encoding VIM-1. This finding highlights the important role of mobile genetic elements in the spread of resistance determinants such as bla_{VIM-1}.

The last study focuses on validation of a commercial automatic program on MALDI-TOF MS-based identification of MRSA. The assay showed 90% sensitivity and 100% specificity; however, repeatability and reproducibility were determined to be poor. Based on our data, the method is not suitable for routine use.