

## Abstract

Currently, the development of bacterial resistance is one of the major healthcare problems. Especially, the significant continuous increase of carbapenem-resistant Gram-negative bacteria, which most often affected seriously ill hospitalized patients, is a cause for great concern. Worldwide dissemination of carbapenemase-encoding genes is largely associated with mobile genetic elements and the clonal spread of high-risk clones.

The dissertation thesis is focused on a molecular-epidemiological mapping of carbapenemase-positive Gram-negative isolates detected in hospital settings throughout the Czech Republic.

Since 2015, a significant increase of carbapenemase-producing *Enterobacteriaceae* (CPE) was detected in our country, mainly attributed to the dissemination of OXA-48 and NDM enzymes. Studies focusing on the first big outbreaks as well as sporadic cases of OXA-48 and NDM carbapenemases were performed. Results showed that *bla*<sub>OXA-48</sub>-carrying plasmids, which are derivatives of the archetypal IncL plasmid pOXA-48 originally described in Turkey, play a major role in the dissemination of OXA-48 enzymes in Czech hospitals. This finding is in agreement with the data from previous studies reported worldwide. The study of NDM-positive isolates revealed that IncX<sub>3</sub> plasmids are the main factor contributing to the spread of NDM-like enzymes in the Czech Republic. The increasing incidence of NDM-positive isolates in Czechia is in concordance with the extremely successful spread of *bla*<sub>NDM</sub> genes detected worldwide. *In vivo* horizontal gene transfer of genes encoding for carbapenemases was observed in 4 and 3 patients infected or colonized by OXA-48 or NDM producers, respectively. Moreover, several novel derivatives of mobile genetic elements were identified during both studies.

The GES-5 and IMI-2 carbapenemases are sporadically reported from clinical settings. However, the association with mobile genetic elements should be a warning sign highlighting possible rapid dissemination. The first cases of IMI-2-producing *Enterobacter asburiae* and GES-5-producing *Enterobacter cloacae* identified in the Czech Republic in 2016 were described. Both isolates were obtained from patients without previous traveling abroad, lacking the obvious source of origin which indicates possible silent dissemination via unrecognized ways.

Carbapenemase-producing *Pseudomonas aeruginosa* isolates have been frequently reported from Czech hospitals. First nationwide surveillance of carbapenemase-positive *P. aeruginosa* isolates detected during 2015 throughout the hospitals in the Czech Republic was performed, including deep molecular genetic typing. The vast majority of the isolates harbored *bla*<sub>IMP-7</sub> genes, and sequence type ST357 was the most prevalent. The phylogenetic analysis indicates that IMP-7-producing ST357 *P. aeruginosa* isolates, recovered from different hospitals throughout the Czech Republic, were closely related. The study highlighted the importance of dissemination of high-risk clones in the Czech Republic.

Very few antibiotic options are left for patients infected with multidrug-resistant Gram-negative bacilli when resistance to carbapenems is concurrently expressed. Not rarely, bacterial isolates resistant to

almost all available antibiotics are detected, which complicate therapy and significantly limit treatment options. There is an urgent need for comprehensive intervention. Strict adherence to epidemiological precautions and an active approach of individual states, as well as international organizations, is essential. Epidemiological surveillance and molecular genetic typing at a national level can significantly contribute on the deep understanding of the rapid spread of carbapenemases, point out to possible ways of dissemination, and thoroughly map the evolution of carbapenemase-producing isolates frequently contributing to their ongoing successful dissemination.

The results of this dissertation thesis are summarized in 5 manuscripts which have been published in journals with impact factor, one of these studies is the first-author publication.