

Summary

Growing trends of antimicrobial resistance (AMR) are one of the greatest challenges in modern medicine. Currently, among numerous types of AMR bacteria, the carbapenemase-producing *Enterobacterales* (CPE) represent a major public health threat worldwide. The *Klebsiella pneumoniae* species complex (KpSC) is one of the most clinically significant CPE, being an object of extensive research on factors and mechanisms driving its expansion. Another important group is *Klebsiella oxytoca* species complex (KoSC), sharing a large common AMR and virulence gene pool with KpSC. KoSC members, overshadowed by KpSC, also contribute in the global dissemination of carbapenemases, but their actual role in the spread of AMR determinants remains unexplored. For more than a decade, Poland has been strongly affected by resistance to carbapenems, with the predominant position of KpSC, and a specific role of KoSC in the epidemiological situation.

The primary aim of this study was to elucidate the factors and mechanisms driving a part of the expansion of carbapenemase-producing (CP) *Klebsiella* spp. in Poland in the period 2006–2019, and during the first year of the full-scale war in Ukraine (February 2022–February 2023), with a particular emphasis on clonal dissemination, horizontal gene transfer of mobile genetic elements (MGEs), and potential importation of novel genotypes. The thesis comprises four original genomic-bioinformatic studies, utilizing representative groups of clinical isolates collected across Poland by the National Reference Centre for Susceptibility Testing. The methodology was predominantly based on the short-read whole genome sequencing (WGS) for large-scale analyses, supplemented by long-read sequencing to finely characterise the specific loci or MGEs contributing to the spread of carbapenemases.

The analyses highlighted the clonal diversity of the Polish CP KpSC population over the years, dominated largely by several well-known global “high-risk” clones. At the beginning of the CPE epidemic in Poland, *K. pneumoniae* ST147 was recognised as one of minor clones. Its growing role has been observed from 2015 to 2019, when an NDM-1 carbapenemase-producing ST147-Tn125F genotype of Tunisian origin has expanded from an index institution in Warsaw, causing a regional outbreak in the Warsaw area and Mazowsze. Moreover, several related but non-outbreak ST147-Tn125F organisms, detected at that time in different towns in the country, represented the same genotype from the endemic Mediterranean region, being likely results of independent imports by Polish tourists. In the similar period, ST147 has been involved in the spread of VIM-type carbapenemases, when its several genotypes with different VIM-encoding integrons, namely ST147-In916, ST147-In2245 and ST147-In238, caused

regional outbreaks in Małopolskie, Śląskie and Lubelskie, respectively. Later, during the first year of the war in Ukraine, NDM-1/-5- and/or OXA-48-producing ST147 isolates were one of the major *K. pneumoniae* groups linked with patients arriving from that country. The initial stage of dissemination of ST147 occurred when the entire Polish CPE population was immensely dominated by an endemic NDM-1-producing *K. pneumoniae* ST11-Tn125A genotype. This dissertation revealed also the broader epidemiological context of the ST11-Tn125A organisms in Europe which have been also recovered from patients arriving from Ukraine after the escalation of the war.

Several important epidemiological observations have been done for *K. pneumoniae* ST395, which has been a significant OXA-48-type spreader in Poland since 2013. The recent increase in its incidence might have been also influenced by the migration from Ukraine after February 2022, when NDM-1/ and/or OXA-48-producing ST395 isolates were the most numerous CPE linked with patients arriving from that country. These demonstrated close genetic relatedness. to such organisms identified in Russia and to the isolates obtained from Ukrainian war refugees in other European countries. Another *K. pneumoniae* genotypes originating likely from Eastern Europe and characterised in this thesis was ST307 with NDM-1 or KPC-2/-3. These were the second most common *K. pneumoniae* group gathered from patients arriving from Ukraine. Except for the clonal aspect of CP *K. pneumoniae* isolates linked with Ukrainian patients, another interesting feature emphasized in this dissertation was the relatively broad occurrence of hvKP (hypervirulence *K. pneumoniae*) genetic determinants present in ST23, ST147, ST307 and ST395 clones, resulting likely from acquisition and circulation of hybrid IncFIB+HI1B pNDM-MAR-type plasmids, combining AMR and virulence genes.

Other KpSC “high-risk” clones presented in this dissertation were *K. pneumoniae* ST437 and ST392, both contributing to the expansion of VIM-type enzymes in Poland. The ST437-In238 genotype has been recognized as a major Polish *K. pneumoniae* VIM producer from 2009 to 2019, comprising more than 25% of all KpSC VIM cases and being responsible for a large regional outbreak in Lubelskie, which in just one year expanded to 16 institutions of the region. ST392-In1667 has been detected in Kujawsko-Pomorskie and caused an outbreak in seven hospitals of the region. Interestingly, the phylogenetic analyses in the international context of ST437 and ST392 demonstrated, that both above mentioned genotypes likely emerged and disseminated on-site.

KoSC played a particularly important role in the early phase of VIM dissemination in Poland. A decade-long (2009-2019) multiregional expansion of a *K. oxytoca* ST145-In237-like

genotype was one of the most remarkable phenomena in entire VIM epidemiology in the country, being unprecedented also on the international scale. Another unique feature of the Polish *K. oxytoca* ST145-In237-like genotype, not reported previously, were frequent chromosomal deletions covering the natural β -lactamase *bla*_{OXY} gene, usually considered as core for all KoSC members and historically used for species determination. Although the molecular mechanisms of this phenomenon have not been unambiguously explained, a phage-induced deletion hypothesized in this thesis seems to be a probable cause for some of the examined strains at least.

The dissertation emphasized also the role of the horizontal transfer of various MGEs in the dissemination of different carbapenemases. Among the most important were three individual *bla*_{NDM}-carrying Tn125 transposon derivatives. Tn125A, characteristic for the endemic *K. pneumoniae* ST11 lineage in Poland, has been detected also in ST11 organisms obtained from patients migrating from Ukraine. Relevant associations with Eastern Europe have been observed also for the transposon variant Tn125K, prevailing in *K. pneumoniae* isolates of different STs gathered from patients arriving from Ukraine. Finally, Tn125F has been used as a molecular marker for the previously mentioned ST147-Tn125F genotype spreading in Warsaw and Mazowsze. The horizontal transfer of VIMs was facilitated primarily by In238 and In916 integrons-carrying plasmids, both of wide inter-species distribution. The study contributed also in characterisation of several types of carbapenemase-encoding plasmids. These were mainly IncFII_K+IncFIB_K, IncA, IncL and IncFIB+HI1B types, all demonstrating remarkable similarities to sets of international molecules of these types, and those identified previously in Polish CPE.

The overall findings of this thesis demonstrated that the expansion of CP-*Klebsiella* spp. in Poland has been a highly dynamic phenomenon, driven by the interplay of clonal spread of ‘high-risk’ genotypes, inter-species horizontal transfer of carbapenemase-encoding MGEs, and the importation of novel epidemic lineages by travelling and migration. All four original articles of the dissertation characterised the complex epidemiology of the CP *Klebsiella* spp. in Poland over the last 20 years and contributed significantly to the overall knowledge on the CPE situation in the country.

Title: Clonal structure of the population of carbapenemase-producing *Klebsiella* spp. in Poland: epidemic genotypes, their phylogeny, antimicrobial resistance mechanisms and virulence factors”