Summary

The growing antimicrobial resistance (AMR) is one of the greatest challenges that infection medicine faces today. Opportunistic Gram-negative pathogens, such as *Pseudomonas aeruginosa*, pose a particular risk because these perfectly adapt to hospital environments, easily acquiring AMR determinants, including metallo-β-lactamase (MBL) genes. MBL production is one of the most dangerous AMR mechanisms due to the ability of the enzymes to hydrolyse all of the commonly used penicillins, cephalosporins and carbapenems, and their resistance to all β-lactamase inhibitors of currently clinical use. MBLs are the most clinically and epidemiologically relevant source of *P. aeruginosa* resistance to carbapenems, until recently the only last-resort drugs in the treatment of nosocomial infections caused by multidrug-resistant Gram-negative pathogens. The first studies on the spread of MBL-producing *P. aeruginosa* (MPPA) in Poland, including 53 isolates from 1998-2004 from 16 hospitals, showed regional epidemiology of around 10 individual MPPA genotypes in seven main administrative areas. After 2004 there has been observed a dramatic increase in the MPPA incidence in Poland: from 2005 to 2015 1,314 cases in 258 hospitals throughout the country were confirmed.

The aim of this study was to explain the basis of this extensive growth of the MPPA cases in 2005-15, with respect to the phenomena observed already in the early phase of the dissemination (2000-2004). By the molecular and genomic analysis of the MPPA strain collection (n=454) from the whole country, it was planned to reveal the clonal structure of the population, and indicate the crucial epidemic genotypes largely responsible for the MPPA spread overall. Characterization of the MBL genes and their genetic platforms was aimed at assessing the role of the horizontal gene transfer in the MPPA epidemiology, with a specific focus on large, conjugative MBL-encoding plasmids, originally observed in the early 2000s.

The analysis showed high diversity and remarkable specificity of the Polish MPPA population in terms of the clonal structure and MBL genetic determinants (class 1 integrons with MBL genes). Around 75% of the isolates were classified into four sequence types (STs): the pandemic clones ST235 and ST111, and ST273 and ST654 scarcely reported so far. The ST235 and ST111 subpopulations were internally diverse themselves, and arose from the international circulation of several genotypes and from their intense differentiation in the country. Among the ST235, ST111, ST273 and ST654 isolates overall, 10 major genotypes (ST-pulsotype-MBL integron combinations) were distinguished, each characterised by different spatiotemporal distribution, accounting for approximately 55% of all MPPA cases.

The comparative analysis of representative isolates from the early period (2000-2004) showed that two of these emerged in individual hospitals in the early 2000s. The vast majority of the Polish MPPA strains produced VIM-type MBLs, with VIM-2 being the predominant variant. All of the MBL genes were located within class 1 integrons, the number and structural diversity of which were very high. Fourty-six such elements were identified altogether, including 29 new ones, greatly contributing to the overall specificity of the Polish MPPA, given that 40 integrons occurred mainly or only in the country. Along with regional and/or interregional clonal outbreaks of the individual genotypes, the extensive horizontal spread of IncP-2-type conjugative megaplasmids with a specific VIM-2-encoding integron In461 was observed. More than 15% of all MPPA isolates carried these plasmids (pPUVs), thus documenting possibly the largest ‘plasmid epidemics’ ever reported in *P. aeruginosa*. The genomic analysis allowed positioning the pPUV lineage as a distinct branch in the phylogenetic tree of the IncP-2-like plasmids, and gaining insights into their structure and function of particular regions. Finally a single *P. aeruginosa* strain with a large set of 25 acquired AMR genes was identified during the study, including those of DIM-1 and NDM-1 MBLs observed rarely in the species. As the only one in the collection, the isolate belonged to the ST234 clone, and according to the phylogenetic analysis results, it was closest related to *P. aeruginosa* isolates from West Africa.

The results clearly indicated the largely clonal nature of the MPPA expansion in Poland. The multidirectional evolution of *P. aeruginosa* populations and MBL determinants in Polish hospitals, commenced in the early 2000s and replenished by the international exchange of strains, produced 10 major genotypes with possibly enhanced epidemic potential. Another significant factor was the spread of a specific lineage of IncP-2-type conjugative megaplasmids. The entire study, carried out on a large representative collection of MPPA isolates, has provided a unique picture of epidemiology of these alarming pathogens on a country scale over more than decade.