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Molecular characterization of MBL-producing Pseudomonas aeruginosa isolates in Czech hospitals

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Background: In Czech hospitals, carbapenem-resistant *Pseudomonas aeruginosa* isolates are currently a serious problem in the management of health-care associated infections. Metallo-β-lactamases (MβLs) are the most commonly acquired carbapenemases in *P. aeruginosa*. Therefore, the aim of this study was to compare the molecular characteristics of MβL-producing *P. aeruginosa* detected in Czech hospitals.

Material/methods: In 2015, a total of 222 *P. aeruginosa* isolates being non-susceptible to imipenem or meropenem were referred to the National Reference Laboratory for Antibiotics from Czech hospitals. All isolates were tested for carbapenemase production by matrix-assisted laser desorption/ionization time-of flight mass spectrometry (MALDI-TOF MS) meropenem hydrolysis assay. Carbapenemases were detected phenotypically and by PCR. MβL-producing isolates were typed by MLST. The MβL-encoding integrons were amplified and sequenced. For 28 isolates representing different hospitals and STs, the genetic location of the detected MβLs genes was defined by PFGE analysis of total DNA digested with S1 nuclease, followed by hybridization with MβL probes. Bacterial

genomes were sequenced using the Illumina MiSeq platform. Annotation and comparative analysis were performed using software available on the Internet.

Results: A total of 132 *P. aeruginosa* isolates showing carbapenemase-activity on MALDI-TOF MS meropenem hydrolysis assay were collected from twenty-two Czech hospitals. The population structure of the MβL-producing isolates studied by MLST was classified into 6 sequence types (STs). The international clone ST357 was the most prevalent, accounting for 116 isolates. Thirteen of the isolates were distributed in the pandemic STs 111 (n=9) and 235 (n=4). The remaining isolates belonged to distinct STs. One-hundred seventeen isolates produced IMP-type enzymes (IMP-7 [n=116] and IMP-1 [n=1]), while fifteen isolates produced the VIM-2 MβL. The bla_{IMP}-like genes were located in four types of class 1 integron, two of which were novel. The most prevalent IMP-encoding integron type was In-p110, identified in 93 isolates. Among the VIM-2 producers, the integrons In56 (n=7) and In-p385 (n=7) were found in fourteen of them. The ST253 VIM-2 producing isolate carried a new integron, including bla_{VIM-2}, *aadB* and *gcuD* gene cassettes. In selected isolates, S1 profiling and Illumina sequencing showed that MβL-encoding integrons were integrated into their chromosomes.

Conclusions: These findings underscore the clonal spread of ST357 *P. aeruginosa* isolates producing IMP-7 MβL, in Czech hospitals. Thus, in order to control the spread of this challenging pathogen, there is an immediate need: (i) to perform surveillance cultures for carbapenemase-producing bacteria upon admission of patients; (ii) to improve the used hygiene practices; and (iii) to avoid frequent transfer of patients between different hospitals. Furthermore, a few sporadic MβL-producing *P. aeruginosa* isolates that belonged to different STs or carried integrons of divergent or novel structures were identified, underlining their ongoing evolution.