LMB-1, a novel subclass B3 metallo-beta-lactamase from a carbapenem-resistant Enterobacter cloacae isolate

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Background: Metallo-β-lactamase are β-lactamases that require one or two zinc ions for their catalytic activity. All metallo-β-lactamases are carbapenemases conferring resistance to almost all β-lactam antibiotics. As no efficient inhibitors of metallo-β-lactamase activity are available for clinical use, expression of metallo-β-lactamase strongly diminishes treatment options for bacterial infections.

Material/methods: Carbapenemase detection was performed by the modified Hodge test, combined disk test with boronic acid or EDTA and a bioassay based on cell-free extracts. Whole-genome sequencing was performed by MiSeq 2x300 bp paired-end sequencing. Draft-genome assembly was performed using the SPAdes-assembly-software. The Draft-genome was then screened for sequences showing homologies to known metallo-β-lactamases using tblastn. Homologous regions were screened for in-frame start-codons and likely promoter-regions. Suspicious sequences were amplified by PCR and cloned in the vector pBK-CMV. After transformation in Escherichia coli Top 10, comparative susceptibility studies were performed by disk diffusion, Etest and microdilution with the strain carrying the putative metallo-β-lactamase-gene and E. coli Top 10 carrying the pBK-CMV vector without an insert.

Results: E. cloacae strain 10170 was isolated in 2013 from a rectal swab of an inpatient in an Austrian hospital. The strain was resistant to carbapenems, synergy with EDTA could be demonstrated and the modified Hodge test was positive. No metallo-β-lactamase-gene could be detected by PCR and attempts to identify the carbapenemase gene by shotgun-cloning failed. After whole-genome sequencing a sequence with low-level homology to known subclass B3 metallo-β-lactamases was identified in the draft-genome. After cloning the putative metallo-β-lactamase coding gene, synergy with EDTA could be demonstrated and comparative susceptibility analysis showed
increased MICs for carbapenems. The novel metallo-β-lactamase was named Linz Metallo-β-lactamase (LMB-1).

**Conclusions:** In this report, we describe the novel metallo-β-lactamase LMB-1. This study highlights the importance of an ongoing surveillance for new resistance mechanisms which will be overlooked by PCRs targeting only the most frequent resistance genes.