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Paper Poster Session
Deciphering carbapenem resistance

Biochemical characterization of KHM-2, a novel subclass B1 Metallo-β-Lactamase

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Background: The worldwide increase of multidrug-resistance in gramnegative bacteria has become an important clinical challenge. Carbapenem resistance can be caused by a variety of mechanisms, however the worldwide spread of carbapenemases is especially important. A worrying trend is the dissemination of Ambler class B metallo-β-lactamases (MBL). In 2013, a carbapenem-resistant clinical *P. aeruginosa* isolate was referred to the National Reference Laboratory for Multidrug-resistant Gram-negative Bacteria. This isolate harboured the novel bla_{KHM-2} MBL gene. Here we present the purification and biochemical characterization of KHM-2.

Material/methods: The KHM-2 encoding gene was cloned into the pBK-CMV vector and expressed in *E. coli* TOP10. The cells were lyzed by sonication and the lysate was cleared by centrifugation, followed by a desalting step. The enzyme was purified by a two-step Fast Protein Liquid Chromatography (FPLC). The first step was an ion exchange chromatography, followed by gel filtration. The purified enzyme was analyzed biochemically by *in vitro* hydrolysis assays by photometrically monitoring the absorbance changes with various β-lactam substrates. The kinetic parameters K_m and k_{cat} were determined by nonlinear regression using the Michaelis-Menten equitation. To serve as a reference, KHM-1 was purified and characterized using the same procedure.

Results: Biochemical analysis of KHM-2 showed that the enzyme was able to hydrolyze almost all tested β-lactam substrates. Penicillin G and ampicillin were hydrolyzed with high turnover numbers but with relatively low affinity towards the enzyme. KHM-2 showed a very weak piperacillin hydrolysis. Most cephalosporins were hydrolyzed with high efficiencies. Regarding carbapenems, imipenem showed the highest hydrolysis rates, while meropenem and ertapenem were rather poor substrates. In comparison to KHM-1, KHM-2 showed higher rates for ceftazidime and imipenem, but lower rates for cefotaxime, meropenem and ertapenem. Both KHM-2 and KHM-1 were not able to hydrolyze aztreonam.

Conclusions: The biochemical characterization of KHM-2 and the comparison to KHM-1 further underline the diversification of subclass B1 metallo-β-lactamases and the resulting differences in catalytic behaviour between enzymes of the same group. The kinetic data for KHM-2 suggest that this enzyme is a potent carbapenemase that most likely can confer high carbapenem resistance levels in Gram-negative species of clinical importance.