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SATURDAY - AAR-644 / SATURDAY - AAR-644 -

Mutational Effects in Carbapenem Hydrolysis of YEM-1, a New Sub-Class B2 Metallo-beta-Lactamase from *Yersinia mollaretii*

June 22, 2019, 10:30 AM - 5:00 PM Exhibit and Poster Hall

Presentation Time 1:
11 am-12 pm

Presentation Time 2:
4 pm - 5 pm

Authors
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Disclosures
P. Mercuri: None. **R. Esposito:** None. **S. Bletard:** None. **S. Di Costanzo:** None. **M. Perilli:** None. **F. Kerff:** None. **M. Galleni:** None.

Abstract
Background The genome of *Yersinia mollaretii ATCC43969* was totally sequenced (NCBI NZ_AALD0200 0006.1). Its analysis indicated the presence of two β-lactamase genes, namely ***blaB*** and ***yem-1*** coding for an AmpC-like and a metallo β-lactamase respectively. Up to date, many studies on BlaB were performed (1,2). Our work aimed to characterize the kinetic profile of YEM-1 and the residues essential for its activity. ***Methods*** YEM-1 was produced in ***E. coli*** Rosetta and purified to homogeneity. A survey of its kinetic properties was performed against different β-lactam antibiotics. A model of the three-dimensional structure of YEM-1 was build using the known structure of ***Aeromonas hydrophila*** CphA as template. Its analysis highlighted the major residues substitution between CphA and YEM-1. These residues were mutated by site-directed mutagenesis. The kinetic properties of the different mutants were analyzed. ***Results*** We noted that, at 37°C, YEM-1 is produced as inclusion bodies. We obtained a soluble form of YEM-1 when the culture was grown at 18°C in presence of IPTG 0.1 mM. The MBL was purified in three chromatographic steps that include a HiTRAP SP, an IMAC and a molecular sieve. The kinetic analysis of the YEM-1 showed that only imipenem was hydrolyzed efficiently. We confirmed that it belongs to the subclass B2 MBL family. As expected, the structure of YEM-1 has a similar fold than CphA. We found that the main differences between CphA and YEM-1 were the residues in position 67, 156 and 236 (BBL numbering) respectively. By site-directed mutagenesis, we made the YEM-1 single mutants (Y67V, T156F, S236F), the double mutant T156F-S236F and the triple mutant (Y67T156FS236F) in order to design the active site of CphA in YEM-1. The substitution Y67V yielded a global increase of the catalytic efficiency to carbapenems, with the exception of the imipenem. The mutations of T156 and/or S236 in phenylalanine globally decrease the enzymatic activity of the enzyme. Finally the double mutant and triple mutants did not increase the catalytic efficiencies of YEM-1 against carbapenems. ***Conclusions*** Despite a high sequence's similarity between CphA and YEM-1 (57%), we showed that the Yersinia MBL was less effective than CphA against carbapemens. We also highlighted the substitution Y67V in YEM-1 that increased its efficiency against all the carbapemen tested and in particular ertapenem and meropenem.