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### **Kinetic and structural analysis of OXA-567 $\beta$ -lactamase, a novel variant derived from OXA-163**

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The class D  $\beta$ -lactamase OXA-163, derived from OXA-48, presents a greater hydrolytic capacity against oxymino-cephalosporins and a reduce activity against carbapenems compared to OXA-48, and it's increasingly found in Enterobacterales. Since its first identification in Argentina in 2011, different variants have emerged from OXA-163 by accumulation of substitutions and/or insertions-deletions in the  $\beta$ 5- $\beta$ 6 loop located in the close vicinity of the conserved "KTG" motif. In 2016, a new variant derived from OXA-163, OXA-567, was detected in a clinical isolate of *Klebsiella pneumoniae* in Argentina. The objective of this study was to evaluate the kinetic behavior and structure of OXA-567, and predict the molecular dynamics of the covalent complex with imipenem. The blaOXA-567 gene was cloned into pET28a and transformed into *E. coli* BL21(DE3) for expression and purification. Steady-state kinetic parameters were determined by spectrophotometry, in the presence of 50 mM sodium bicarbonate. The X-ray structure of OXA-567 was determined by X-ray diffraction, the refinement of the model was carried out using REFMAC5, TLS and Coot. The X-ray structure was used to model the acylated complex against imipenem and perform a molecular dynamics simulation for 150 ns, using the YASARA v24.4.10 program. For the interaction study, the PLIP v2.3.0 program was used, subsequently visualized in PyMOL. OXA-567, unlike OXA-48, is characterized by having three substitutions (S212D, R214K and P216G) and a deletion in E215, located in the  $\beta$ 5- $\beta$ 6 loop. The catalytic efficiency (kcat/Km) for nitrocefin, cephalothin, imipenem and ertapenem were 0.4  $\mu$ M<sup>-1</sup> s<sup>-1</sup>, 2.3  $\mu$ M<sup>-1</sup> s<sup>-1</sup>, 3.4  $\mu$ M<sup>-1</sup> s<sup>-1</sup> and 0.5  $\mu$ M<sup>-1</sup> s<sup>-1</sup>, respectively. The crystallographic structure showed a shortened  $\beta$ 5- $\beta$ 6 loop, compared to OXA-48. Molecular dynamics evidenced that K214 was found outside the active site, oriented towards D159 ( $\beta$  loop), and at 12.9 Å to the 6-hydroxyethyl moiety of imipenem; for OXA-48, this distance with R214 was 5.7 Å. Furthermore, the hydrophobic pocket formed by V120, L158 and A69 presented a reduced area compared to OXA-163, although

similar to OXA-48 (PDB:7KH9). On the other hand, a stable configuration of OXA-567 with imipenem was observed with an RMSD below 1 Å, compared to OXA-163. OXA-567 showed diminished kinetic behavior against carbapenems, in relation to what was reported for OXA-163 family in other studies. The mutation near the active site showed important modifications in the interaction with imipenem, presenting greater dynamic synchronicity compared to OXA-163, in agreement with the imipenem  $K_m$  app.

Palabras clave:  $\beta$ -lactamase - carbapenems