Sociedad Argentina de Microbiología General

XIX CONGRESO DE LA SOCIEDAD ARGENTINA DE MICROBIOLOGÍA GENERAL

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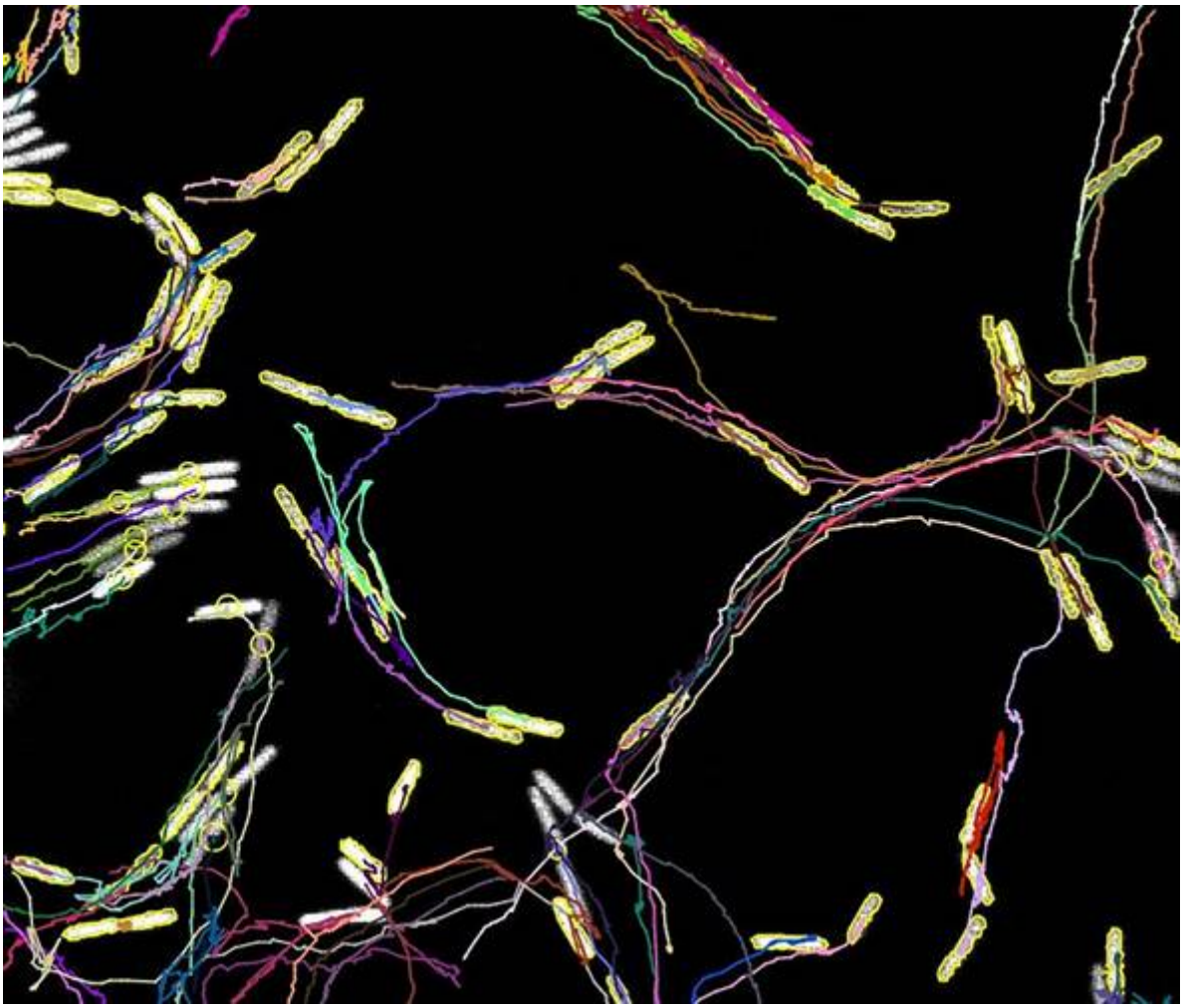

Foto: Se hace camino al andar. Celeste Dea. 1er puesto. Concurso fotográfico SAMIGE 20 años.

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Kinetic and structural analysis of OXA-567 ?-lactamase, a novel variant derived from OXA-163

Arturo Gonzales Rodriguez[1,2] - Barbara Ghiglione[1,3] - Juan Faya Castillo[2] - Juan Manuel de Mendieta[4] - Gabriel Gutkind[1,3] - Fernando Pasterán[4] - Sonia Gómez[3,4] - Pablo Power[1,3]

1) Universidad de Buenos Aires, Facultad de Farmacia y Bioquímica, Instituto de Investigaciones en Bacteriología y Virología Molecular (IBaViM), Buenos Aires, Argentina.
2) Universidad de Piura, Facultad de Medicina Humana, Perú.
3) Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Buenos Aires, Argentina.
4) Laboratorio Nacional de Referencia en Resistencia Antimicrobiana, INEI-ANLIS-Malbrán, Buenos Aires, Argentina.
Contacto: ao.gonzalesr@gmail.com

The class D ?-lactamase OXA-163, derived from OXA-48, presents a greater hydrolytic capacity against oxyimino-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 ?5-?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 ?5-?6 loop. The catalytic efficiency (kcat/Km) for nitrocefin, cephalothin, imipenem and ertapenem were 0.4 µM-1 s-1, 2.3 µM-1 s-1, 3.4 µM-1 s-1 and 0.5 µM-1 s-1, respectively. The crystallographic structure showed a shortened ?5-?6 loop, compared to OXA-48. Molecular dynamics evidenced that K214 was found outside the active site, oriented towards D159 (? 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 Km app.

Palabras clave: ?-lactamase - carbapenems