Sociedad Argentina de Microbiología General

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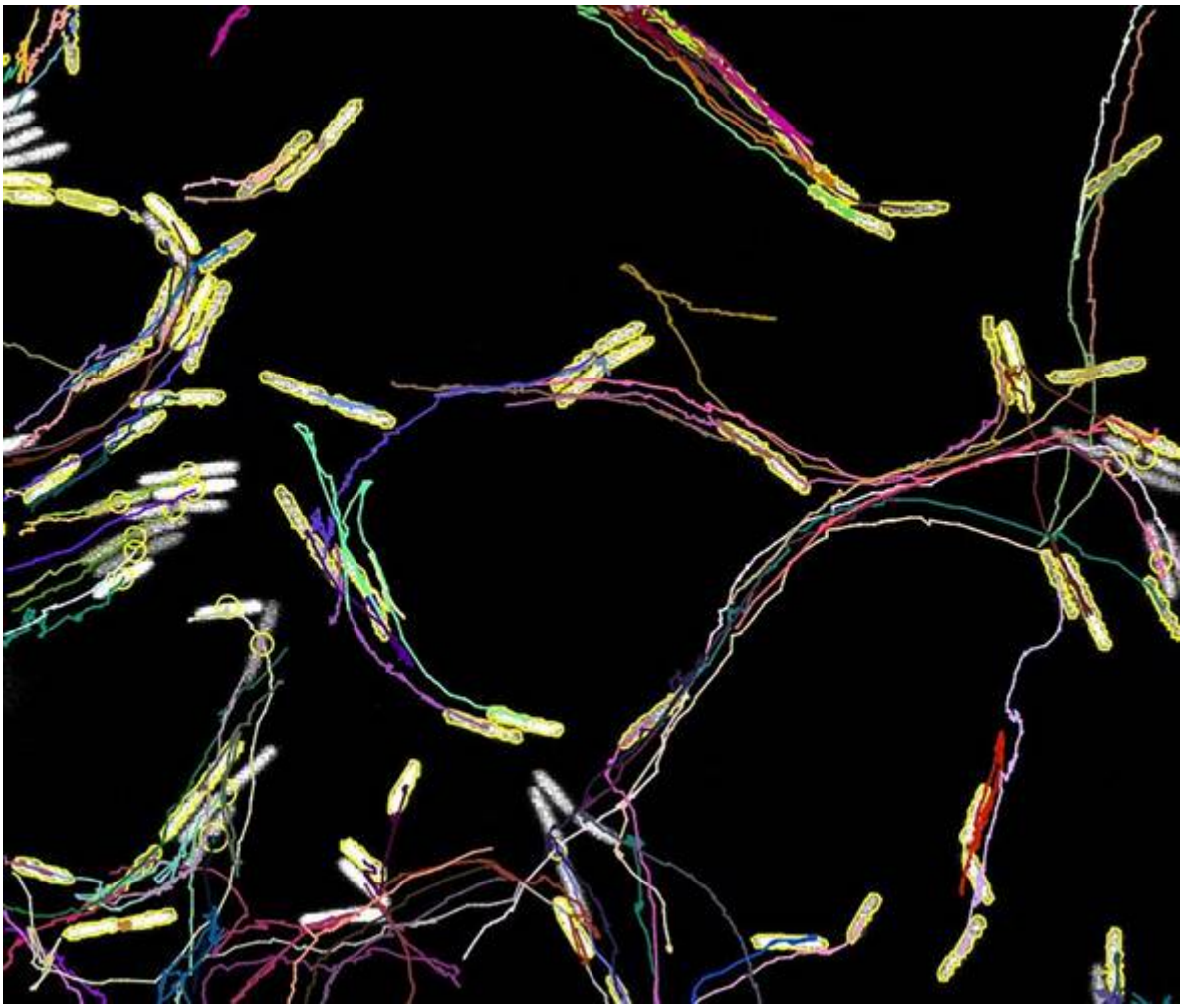

Foto: Se hace camino al andar. Celeste Dea. 1er puesto. Concurso fotográfico SAMIGE 20 años.

CEFIDEROCOL (FDC) RESISTANCE MECHANISMS BY METALLO-BETA-LACTAMASES (MBLs)

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FDC is a recently developed cephalosporin antibiotic featuring a chlorocatechol moiety that enhances its uptake into the periplasmic space through iron transporters, leading to increased levels of the drug within bacterial cells. It FDC has been identified as the only cephalosporin that resists hydrolysis by MBLs. However, resistance to FDC is emerging. This encompasses mutations in the iron transport, combined with the expression of MBLs such as NDM-1 and NDM-5. To better understand this resistance mechanism, we explored how FDC interacts with NDM and other MBLs. The MIC values of FDC against Escherichia coli DH10B cells expressing the NDM-1 to NDM-28 variants, which were cloned into a pHSG298 vector, were assessed using the microdilution method in iron-depleted cation-adjusted Mueller-Hinton broth. The NDM-1, NDM-5, IMP-1, and VIM-2 enzymes were produced in *E. coli* BL21(DE3) pLysS and subsequently purified through affinity chromatography. Steady-state catalytic parameters were obtained either by measuring initial reaction rates or by analyzing progress curves, all of which were conducted in HEPES buffer at pH 7.5 and 25°C. NMR spectra were recorded using a 700 MHz Bruker spectrometer. FDC exhibited MIC values of 2 µg/ml against *E. coli* cells expressing NDM variants, comparable to NDM-1. Kinetic analysis under steady-state conditions indicated that both NDM-1 and NDM-5 effectively hydrolyze FDC, with kcat/KM values of 0.19 and 0.12 µM-1s-1, respectively. In contrast, the catalytic efficiency of IMP-1 and VIM-2 was significantly lower, with kcat/KM values of 0.005 and 0.002 µM-1s-1, respectively. Mass spectrometry analysis revealed that FDC incubation with various MBLs leads to the formation of an adduct, which is more stable adduct in the case of IMP-1 and VIM-2, correlating with their lower turnover rates. Pre-steady state stopped-flow experiments demonstrated that IMP-1 and VIM-2 indeed form a stable adduct with FDC, leading to enzyme inhibition, whereas NDM-1 experiences a moderate inhibition due to the formation of a more labile enzyme-product adduct. HSQC NMR experiments further confirmed the presence of this adduct. NDM-1 and NDM-5 demonstrate significant hydrolytic

activity against FDC, unlike the limited activity observed with IMP-1 and VIM-2. These results indicate that clinical resistance is primarily associated with the overexpression of NDM variants rather than IMP-1 or VIM-2. This distinction suggests that the active site of NDM variants contains unique features that account for their activity against FDC.

Palabras clave: metallo-beta-lactamase – cefiderocol – antibiotic resistance