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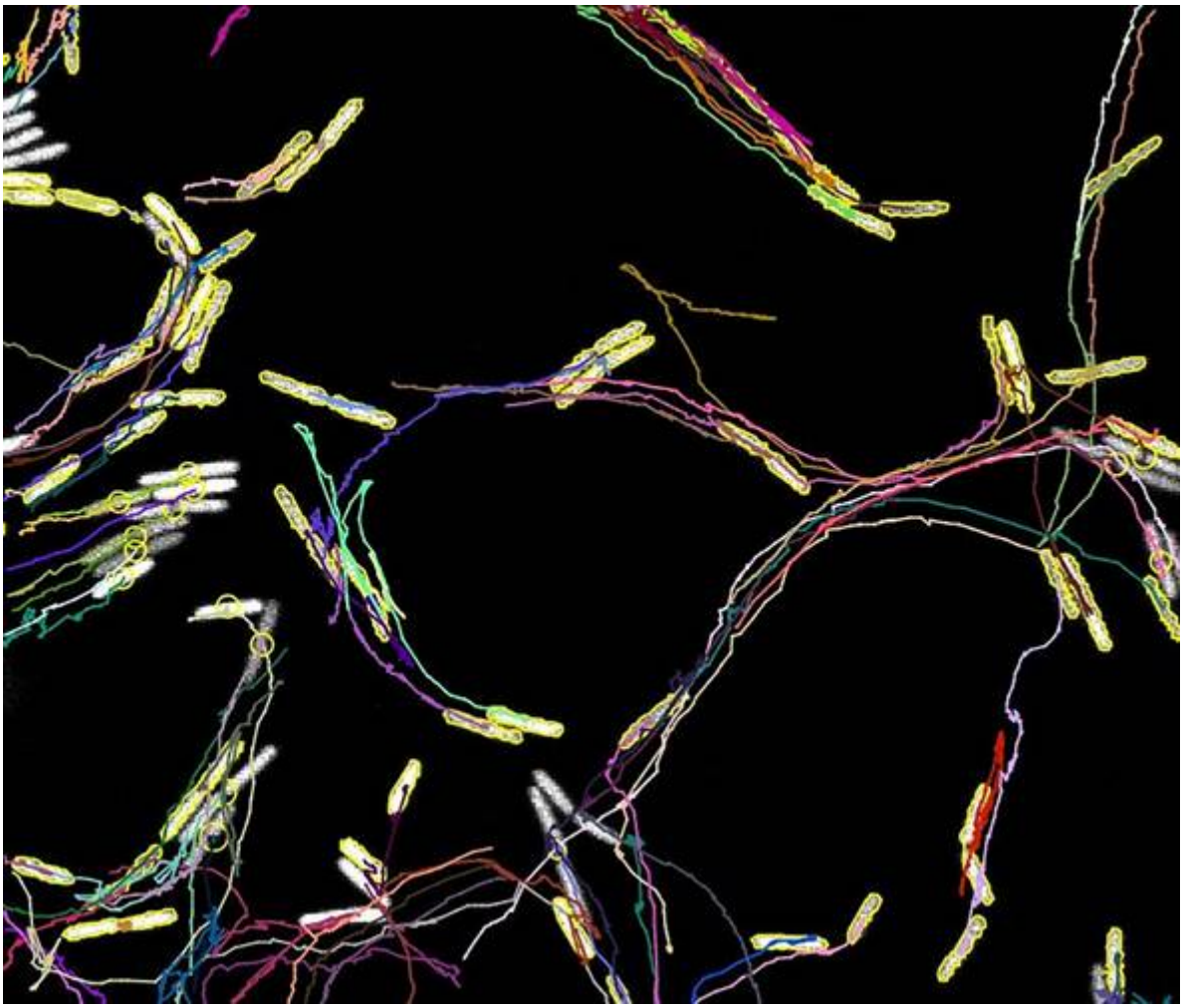

Foto: Se hace camino al andar. Celeste Dea. 1er puesto. Concurso fotográfico SAMIGE 20 años.

ZN(II) DEPRIVATION AS A DRIVING FORCE ACTING ON CLINICAL EVOLUTION OF NEW DELHI METALLO-?-LACTAMASES

Pennesi, Jazmín - Zuccato, Francesco - Delmonti, Juliana - Vila, Alejandro J.

Instituto de Biología Molecular y Celular de Rosario (CONICET-UNR) - Rosario - Santa Fe. Argentina.
Contacto: pennesi.jz@gmail.com

New Delhi metallo-?-lactamase (NDM) is the most widely and rapidly disseminated carbapenemase globally, with Enterobacteriaceae and non-fermenting organisms being the predominant producers of NDM strains. It is a periplasmic metallo-?-lactamase (MBL) anchored to the outer membrane with two Zn ions in its active site, which are essential for both catalysis and stability. These enzymes bind Zn(II) in the periplasm after its translocation through the Sec system. Zn(II) levels in the periplasm are not regulated and depends on the availability of this ion in the external environment. During an infection, the immune system withdraws Zn(II) from the host-pathogen interface, aimed to limit bacterial growth. In these conditions, MBLs accumulate in the periplasm as inactive and partially unfolded species, thus becoming susceptible to degradation by proteases.

Since its discovery in 2009, 68 allelic variants have been reported in clinical isolates worldwide, differing by a few point mutations located outside the active site. Our group reported that most of the first identified 16 variants exhibit improved tolerance to Zn(II) deprivation, and among the most frequent substitutions, M154L increases the protein's affinity for this metal ion.

In this study, our aim is to continue investigating the evolutionary pathways of proteins in this family and assess whether Zn(II) deprivation prevails as a selective pressure in the evolution of the new variants ranging from 16b to 42. To achieve this, we determined the minimum inhibitory concentration (MIC) values of specific ?-lactam antibiotics (?LA) under both Zn-replete and deprivation conditions. Additionally, protein levels were assessed for selected variants as a function of time after the addition of the chelating agent dipicolinic acid (DPA), in order to evaluate their stability through the respective half-lives.

The MBLs genes were cloned into pMBLe and expressed in *Escherichia coli* DH5-?. MICs were determined using the plate dilution method with the antibiotics cefepime, ertapenem, meropenem, imipenem, piperacillin, and cefotaxime. Metal restriction was induced by the addition of 500 µM DPA. Protein levels were determined by immunodetection.

None of the variants increased resistance to the 6 ?-lactams tested. However, most were more capable than NDM-1 of conferring resistance under Zn(II) limitation conditions. This suggests that NDM is evolving to withstand the lack of this ion imposed by the immune system during an infection, rather than incorporating mutations that enhance its catalytic efficiency. On the other hand,

all selected variants, except one, exhibited slightly or significantly longer half-lives compared to NDM-1.
Overall, our results suggest that NDM accumulates substitutions to maintain resistance under Zn(II) limitation conditions, which is achieved either by stabilizing the apoenzymes in vivo or by increasing Zn(II) affinity compared to NDM-1.

Palabras clave: metallo-?-lactamases – NDM – evolution - Zn(II) deprivation.