Sociedad Argentina de Microbiología General

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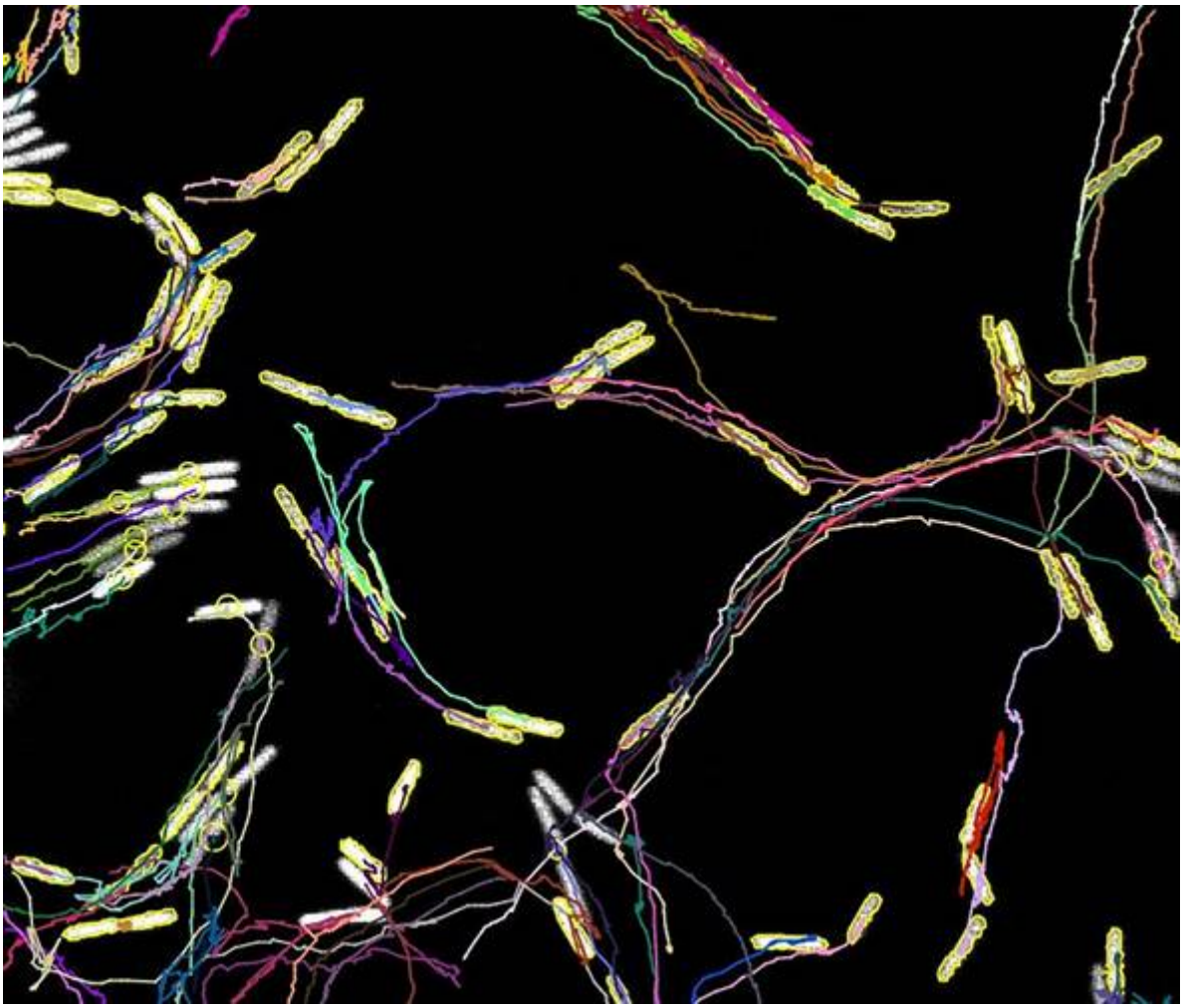

Foto: Se hace camino al andar. Celeste Dea. 1er puesto. Concurso fotográfico SAMIGE 20 años.

Exploring adaptive pathways: the role of the host environment and hypermutability in *Pseudomonas aeruginosa* ?-lactam resistance.

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We have investigated the influence of the microenvironment and the bacterium's inherent mutation rate on the evolution of antibiotic resistance in *Pseudomonas aeruginosa* (PA). We employed air-liquid interface (ALI) models mimicking the human airway epithelium (ex vivo models), alongside traditional *in vitro* cultures, to explore the impact of environmental complexity. Our findings highlight the interplay between these factors in shaping the evolutionary trajectories of PA resistance to ceftazidime (CAZ). Firstly, the results emphasize the combined influence of hypermutability and environmental complexity on fostering diversity and, consequently, higher levels of resistance. Strains with a higher mutation rate displayed greater phenotypic diversity than wild-type strains across all conditions. Interestingly, the *ex vivo* environment significantly increased diversity compared to *in vitro* cultures. Secondly, the study revealed a trend towards complex phenotypes in the ex vivo environment. While both environments led to increased CAZ resistance (MIC), *ex vivo* evolution appeared to favor strategies that also minimize damage to the host tissue, evidenced by lower cytotoxicity and reduced immune response activation. Our whole genome sequencing (WGS) results further support these findings. While both *in vitro* and *ex vivo* evolved strains primarily exhibited mutations in genes related to ?-lactam resistance, the two conditions led to mutations in distinct functional clusters. *In vitro* evolution was associated with mutations in genes involved in amino acid metabolism and biofilm formation. In contrast, ex vivo evolution selected for

mutations in genes related to cellular respiration (potentially adapting to a less aerobic environment), type IV pili, the type III secretion system, and quorum sensing (QS)—these latter two mutations might explain the "stealthier" phenotypes observed in ex vivo populations. Finally, phylogenetic and multivariate analyses revealed a greater functional convergence among parallel-evolved *in vitro* populations, while ex vivo populations displayed a broader spectrum of distinct evolutionary pathways. This suggests that environmental complexity allows for the exploration of a wider range of adaptive strategies. Our findings underscore the importance of considering the microenvironment's complexity and the bacterium’s mutation rate in the evolution of antibiotic resistance, with ex vivo evolution potentially driving more complex and varied resistance mechanisms compared to simpler in vitro settings.

Palabras clave: Pseudomonas aeruginosa - Antibiotic resistance - Hypermutability - Cystic Fibrosis - Ex vivo models