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Evolution of high-level aminoglycoside resistance in *Escherichia coli* under high and low mutation supply rates

Claudia Ibacache-Quiroga
National Center for Biotechnology, Spain

Antibiotic resistance is a major concern in public health worldwide, generating 25,000 death per year only in Europe, thus there is much interest in characterizing the mutational pathways through which susceptible bacteria evolve resistance. Among most important antibiotics in human health are those that belong to the aminoglycoside family, whose are effective for the treatment of infections caused by gram negative pathogens like Escherichia coli. The usage of experimental evolution to explore the mutational pathways toward aminoglycoside resistance, using gentamicin as a model, under low and high mutation supply rates, allowed to identify that normo and hypermutable strains of *Escherichia coli* are able to develop resistance to drug dosages > 1,000 fold higher than the minimal inhibitory concentration for their ancestors. In this approach, this level of resistance has been associated with changes in susceptibility to other antibiotics. Whole-genome sequencing of gentamicin-resistant strains revealed that all resistant derivatives presented diverse mutations in five common genetic elements: fhuA, fusA and the atpIBEFHAGDC, cyoABCDE and potABCD operons. In contrast to recent studies, in this study the mutation supply rate mainly affected the speed (tempo) but not the pattern (mode) of evolution: Both backgrounds acquired the mutations in the same order, although the hypermutator strain did it faster. This observation is compatible with the adaptive landscape for high-level gentamicin resistance being relatively smooth, with few local maxima; which might be a common feature among antibiotics for which resistance involves multiple loci.

e: claudia.ibacache@uv.cl

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