

## COMBATting EMERGING BIOLOGICAL THREATS – PREPARING FOR THE FUTURE TODAY

# Tracking The Emergence And Fate Of Antibiotic Resistance Mutations In Heterogeneous *Yersinia Pseudotuberculosis* Cultures

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Bacteria are dynamic organisms that form divergent and competing subpopulations, even when propagated from a single colony pick. This behavior has profound consequences both for the loss of virulence in laboratory-passed strains and the emergence of antibiotic resistance in environmental and clinical isolates. These phenomena are of particular interest to the Biodefense Reference Material Repository at USAMRIID, which is tasked with faithfully maintaining collections of virulent bacterial and viral pathogens for use in medical countermeasures development. In order to visually and simultaneously track the emergence and fate of several multiple mutations in divergent bacterial populations, we recently designed the PopGen application for potential use on Los Alamos National Laboratory's EDGE bioinformatics platform. We tested PopGen by tracking genetic shifts in *Yersinia pseudotuberculosis* cultures that originated from a clonal population and were passed in the presence of multiple antibiotics at defined sub-inhibitory concentrations. Our data highlights the intense competition that takes place among trans- and cis-encoded mutations and shows that many mutations, which benefit the organism early-on in the mutation process, do not survive to be represented in subsequent populations. Our analysis suggests that the informative PopGen plots can be used to understand how selective pressures drive the emergence and fate of both transformative and silent hitchhiker mutations as they are either eclipsed by competing mutants or are permanently locked into the general population. We believe PopGen will be helpful in understanding how bacteria evolve in natural and artificial environments and in distinguishing inconsequential mutations from the beneficial mutations that share the genome with them, thereby, driving the subpopulation to prominence as the culture responds to acute environmental stressors.

Availability: <https://gitlab.com/chienchi/popgen> and <https://nm-covid19.edgebioinformatics.org/PopGen/>

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