# On the clustering and co-transfer of multidrug resistance genes

Gabriel Carvalho*[1,2], Jean-Philippe Rasigade*[1,2], and Natacha Lenuzza[1]

[1]Centre International de Recherche en Infectiologie (CIRI), Equipe PHE3ID, INSERM U111, CNRS UMR 5308, ENS Lyon, UCBL – Ecole Normale Supérieure de Lyon, Université Claude Bernard Lyon 1, Institut National de la Santé et de la Recherche Médicale, Centre National de la Recherche Scientifique – France

[2]Hospices Civils de Lyon – CIRI, Centre Internatinal de Recherche en Infectiologie – France

**Résumé**

Bacteria evolve multidrug resistance by accumulating antimicrobial resistance genes (ARG) targeting different drugs. ARGs are frequently aggregated in clusters on transposons, plasmids, and chromosomes, facilitating their simultaneous transfer across bacterial lineages. In turn, the transfer of ARG clusters accelerates the spread of multidrug resistance. The driving forces behind the clustering of ARGs are poorly understood. We propose a unifying model of the emergence and persistence of ARG clusters. Using simulations of bacterial evolution under this unifying model, we clarify the environmental conditions that favor clustering.
We combine previous theories of gene clustering, namely the selfish operon model (Lawrence & Roth 1996) and the essential gene model (Fang 2008), and we adapt them to the setting of antimicrobial resistance. The resulting model combines loss-preventing clustering, in which gene clustering reduces the risk of losing a beneficial multi-gene function, and transfer-promoting clustering, in which clustering facilitates the horizontal transfer of a complete multi-gene function. We implement a mathematical model of bacterial evolution and population dynamics under antimicrobial pressure patterns that can vary through time and space.

In *in silico* experiments of bacterial evolution, loss-preventing clustering required DNA deletion rates so high that they drove bacterial populations to extinction under antimicrobial pressure; indicating that it is unlikely to be the primary driver of ARG clustering. In contrast, transfer-promoting clustering efficiently drove cluster emergence, only requiring the sustained coexistence of multidrug-resistant and wild-type populations. This coexistence was maintained by temporal or spatial fluctuations of antimicrobial pressure. Finally, clustered ARGs provided a competitive advantage under periodic pressure fluctuations.
Temporal and spatial fluctuations of antimicrobial pressure may favor the clustering of ARGs through transfer-promoting clustering, which can be interpreted as a generalization of the selfish operon model. Identifying the conditions of such fluctuations, typically at the interface between antimicrobial-free and -contaminated environments, may help design evolution-informed interventions to mitigate the clustering of resistance genes and the diffusion of multi-drug resistance.

**Mots-Clés:** multidrug resistance, resistance gene cluster, mathematical modeling

*Intervenant