From colistin persistence to resistance in carbapenem resistant *Acinetobacter baumannii*: evidence for a novel toxin–antitoxin mRNA–asRNA regulatory mechanism

Eleonora Chines[1,2], Ludovica Boscarelli[1], Viviana Cafiso[1]

[1]*Department of Biomedical and Biotechnological Sciences, University of Catania, 95123 Catania, Italy*

[2]*PhD National Program in One Health Approaches to Infectious Diseases and Life Science Research, Department of Public Health, Experimental, and Forensic Medicine, University of Pavia, 27100 Pavia, Italy*

Background: Bacterial persistence is a transient, non-heritable state of metabolic dormancy that allows susceptible sub-populations to survive lethal antimicrobial concentrations and represents a critical evolutionary reservoir facilitating the transition towards stable genetic resistance. In high-risk pathogens, such as Carbapenem-Resistant *Acinetobacter baumannii* (CRAB), understanding the molecular mechanisms underlying this transient-persistent state is essential to elucidate how bacteria survive antibiotic exposure. Here, we investigated colistin persistence subpopulations and their Toxin/Antitoxin (T/A) omics in ST2 clinical colistin-susceptible (COL-S) CRAB that subsequently developed full and stable *in-vivo* COL-R.

Methods: High-dose colistin time-kill assays were performed to detect persisters in 10 clinical CRABs. Genomics and basal transcriptomics of chromosomal/plasmid toxin–antitoxin systems (T/As) were conducted in two representative ST2 COL-S CRAB to investigate the genomics and basal T/A transcriptional profiles.

Results: All strains showed a persistent subpopulation (~1% survival at 8h) under 5X COL-MIC exposure. Genomic analysis identified ten type-II and one type-IV T/A systems. Basal transcriptomic profiling revealed active expression patterns mainly involving GNAT superfamily T/A modules, with consistently low toxin mRNA levels associated with toxin- or antitoxin-directed antisense RNAs (asRNAs) in chromosomal loci. This regulatory architecture supports novel dual-combined models in which asRNAs act as primary modulators of T/A transcript balance, potentially influencing persistence-associated dormancy transitions mainly via a translational-termination mechanism. Conversely, the plasmid-encoded BrnT/A module showed a highly balanced expression profile.

Conclusion: Our findings highlighted the type-II GNAT T/A superfamily as molecular switchers of T/A transcript-balance by a novel asRNA–mediated regulatory mechanism in high-risk developing colistin persistence and resistance CRABs.