Unraveling the role of the Central metabolism in mediating tolerance in Uropathogenic *Escherichia coli*.

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In recent decades, antimicrobial resistance (AMR) has been increasingly reported in patients suffering from urinary tract infections (UTI) caused by Uropathogenic *Escherichia coli* (UPEC). AMR research has predominantly focused on resistance, defined as bacteria's ability to withstand a drug's adverse effects. However, other resilient strategies such as tolerance are responsible for treatment failure. Tolerance describes bacterial populations that can survive for an extended period under antibiotic exposure, and mounting evidence reveals that this adaptation precedes and supports the emergence of resistance. Interestingly, tolerance can also arise in sub-populations, called persisters, of genetically identical bacteria. Phenotypes such as longer lag time and slower growth rate have been associated with persistent cells. Previous research showed that the regulation of fermentation and respiration is closely linked to growth dynamics. Therefore, the activity of the central metabolism is expected to play a significant role in mediating tolerance.

The first objective is to elucidate the role of the central metabolism in mediating tolerance in the BW25113 lab strain of *E. coli*. Our approach consists of manipulating fermentative and respiratory activity through the control of the ArcA transcription factor. ArcA plays a dual regulatory role, activating fermentative pathways while repressing respiratory ones. By genetically engineered strains expressing an inducible ArcA gene or lacking the endogenous ArcA, we aim to create distinct metabolic states and associate them with tolerance to elucidate the role of the central metabolism.

The second part of the project aims to find metabolic predictors of tolerance that enable clinical strains to develop resilient forms of metabolism. We will examine whether clinical strains exhibit similar adaptations in central metabolism as observed in BW25113 strains. We will analyse approximately 120 clinical UPEC isolates in *in-vivo* mimicking conditions, performing high-throughput non-targeted metabolome profiling. Each strain will present a set of changes depicted as "metabolic fingerprint," revealing metabolic adaptations that might explain clinical tolerance (Fig).

In summary, our project aims to uncover metabolic adaptations that contribute to the emergence of tolerance in UPEC and has the potential to advance novel treatment strategies – notably by uncovering metabolic pathways that could be targeted in synergy with existent treatment.

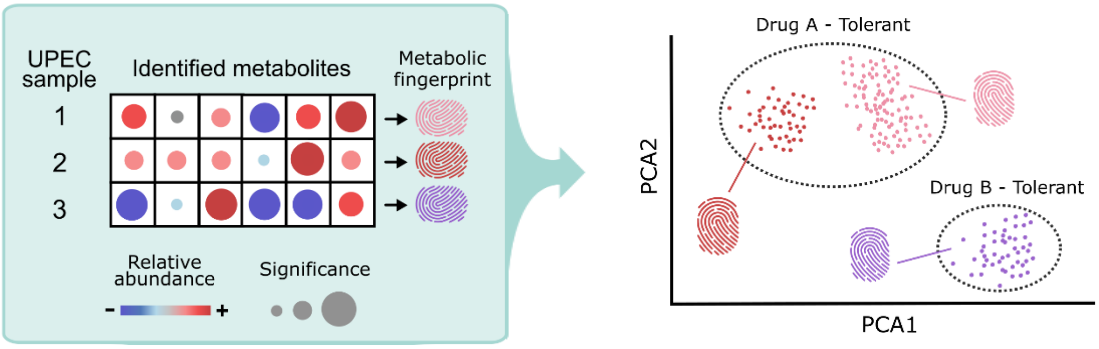

Figure: Non targeted metabolome profiling of UPEC clinical strains by flow injection Q-TOF MS1 will reveal relative changes in metabolites. These set of metabolic changes, depicted as “fingerprint”, are expected to cluster UPEC strain and associate with drug tolerance profile.