Evolution of *Aliarcobacter butzleri* under low ciprofloxacin concentrations associated with the selection of multidrug-resistant mutants

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Contextualization

0 investigation in this topic under gastrointestinal pathogens

By 2050 **ANTIBIOTIC RESISTANCE** could kill more than cancer, with **10 MILLION DEATHS** predicted

4th Species more frequently detected in diarrhoeal samples among *Campylobacter*-like organisms

SUBCLINICAL ANTIBIOTIC CONCENTRATIONS has recently been demonstrated to select for multidrug-resistant mutants

Aliarcobacter butzleri Emergent foodborne, waterborne and zoonotic enteropathogen

CIPROFLOXACIN
> indicated for *A. butzleri* infections
> **highest priority** among critically important antimicrobials
> member of **Watch Category**
World Health Organization

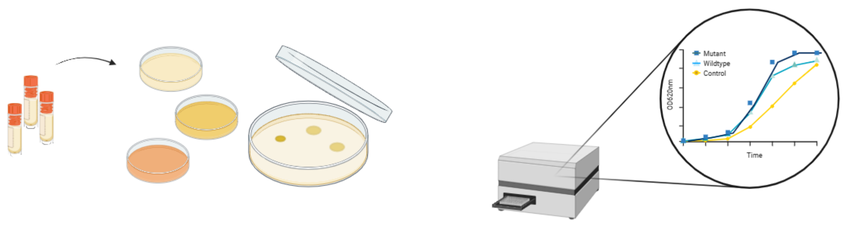
MULTIDRUG RESISTANCE rates ranging from **20** to **94%** in isolates from **humans**, **animals**, **food products** and **environment**

1st to unveil the role of low antibiotic concentrations on the resistance potential of *A. butzleri*, with a focus on an **ONE HEALTH** perspective

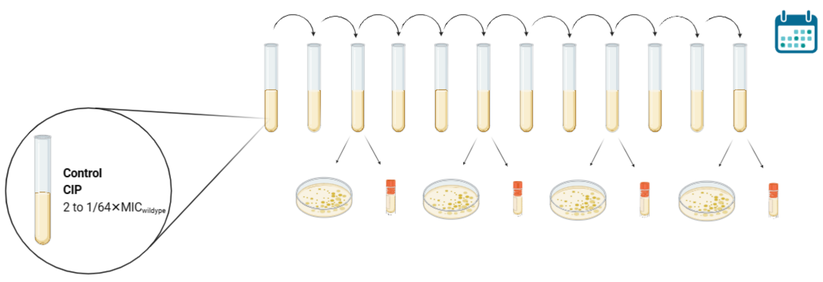
work aligned with **3** UN sustainable development goals

Experimental Design

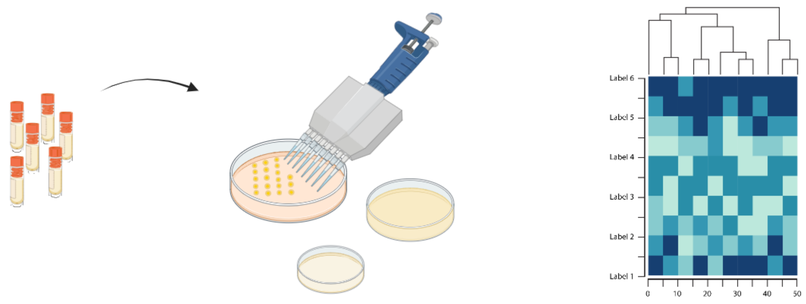
1 Phenotypic characterization of parental strains' resistance



2 Short-term adaptative laboratory evolution (ALE) assays



3 Determination of the cross-resistance profile of the evolved populations



Results

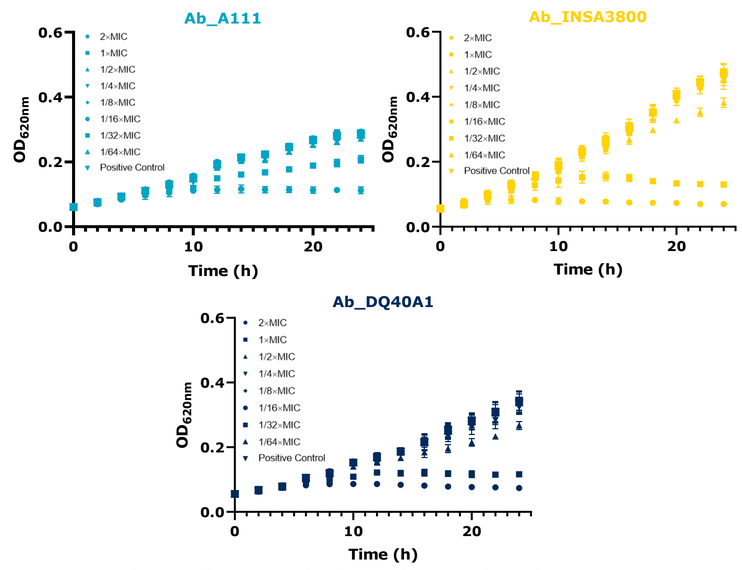


Figure 1. Relative fitness of *Aliarcobacter butzleri* strains grown for 24h in TSB in absence or presence of 2 to 1/64×MIC of ciprofloxacin, with the MIC corresponding to 0.03 µg/mL for the three strains under study.

Low ciprofloxacin concentrations, below those found in the environment, **impairs fitness** of *A. butzleri*.

A. butzleri strains showed an **increase in the number of mutants** selected upon exposure to subclinical ciprofloxacin concentrations, especially in populations evolved at the highest subclinical concentrations under study.

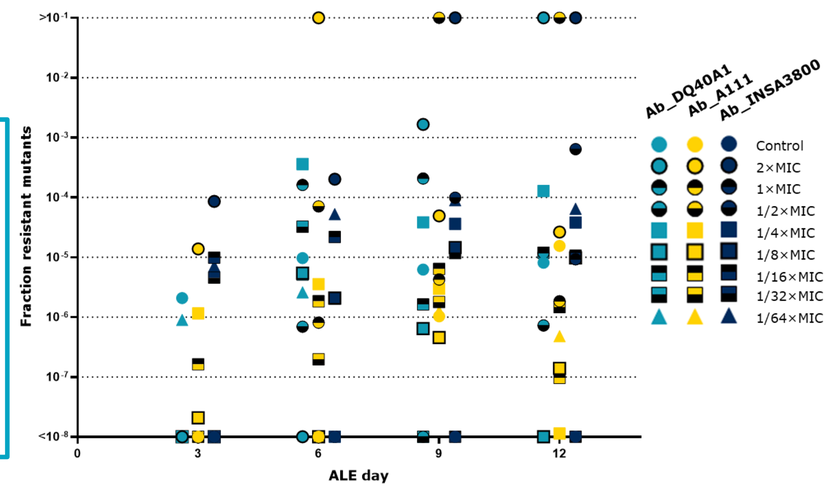


Figure 2. Selection of resistant mutants of *Aliarcobacter butzleri* strains evolved for 12 days under sub-inhibitory concentrations of ciprofloxacin. Data correspond to a representative ALE of each strain.

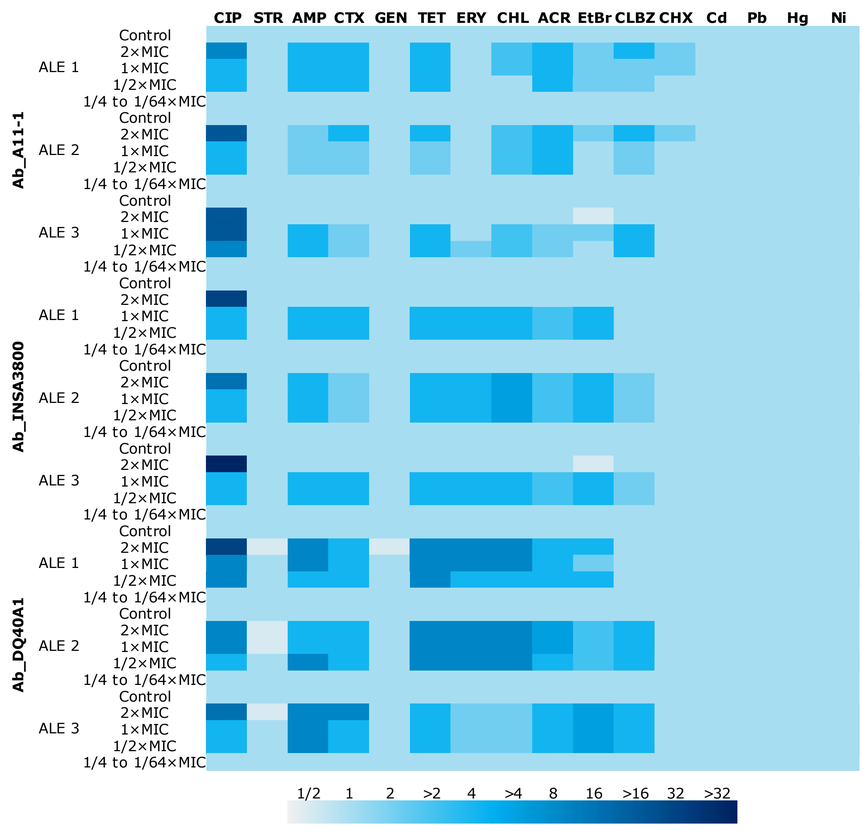


Figure 3. Heat map representing the fold changes between the MICs of *Aliarcobacter butzleri* strains evolved and corresponding parental strain to several antimicrobial agents and heavy metals. CIP: ciprofloxacin; STR: streptomycin; AMP: ampicillin; CTX: cefotaxime; GEN: gentamicin; TET: tetracyclin; ERY: erythromycin; CHL: chloramphenicol; ACR: acriflavine; EtBr: ethidium bromide; CLBZ: benzalkonium chloride; CHX: chlorhexidine; Cd: cadmium; Pb: lead; Hg: mercury; Ni: nickel.

Different ALE assays lead to **distinct resistance phenotypic profiles**, with clinically relevant **multidrug-resistance** emerging upon exposure to low concentrations of ciprofloxacin.
The changes in the susceptibility to ethidium bromide suggest the role of **efflux pumps activity** in the resistance phenotypes.

Conclusion

From a **One Health** perspective, these findings support the **role that low antibiotic concentrations may play in the selection of multidrug resistance** pathogens highly disseminated in the environment, such as *Aliarcobacter butzleri*.

Acknowledgements

Inês Martins is recipient of a doctoral incentive fellowship under the scope of the protocol established between UBI and Santander Totta Bank. This work was developed within the scope of the CICS-UBI projects UIDB/00709/2020 and UIDP/00709/2020, financed by national funds through the Portuguese Foundation for Science and Technology/MCTES.