In vitro selection of *Aliarcobacter butzleri* with erythromycin produce genetic changes and affects fitness and virulence

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Contextualization

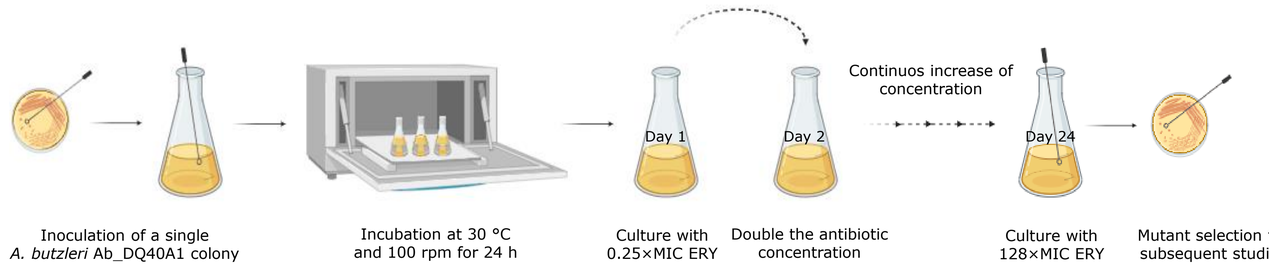
Macrolides are one of the recommended antibiotic classes in the treatment of the emerging enteropathogen *Aliarcobacter butzleri*[1], nonetheless this bacterium' isolates may present highly variable erythromycin resistance rates[2] and the mechanisms behind this resistance phenotype remain mostly unexplored.

Aim

In this work, aiming to gain insights into the **resistance mechanisms to macrolides** and its progression in *A. butzleri*, resistant strains resulting from adaptive laboratory evolution (ALE) under increasing concentrations of erythromycin were studied to unravel the associated phenotypic and genotypic changes, as well as the effects on the fitness of this species.

Experimental Design

ALE of *Aliarcobacter butzleri* under erythromycin stress



1. Cross-resistance and colateral sensitivity to antibiotics
2. Fitness evaluation
3. WGS and bioinformatic analysis
4. Ethidium bromide accumulation
5. RT-qPCR
6. Motility and biofilm formation assessment

Results

Evolution of resistance to erythromycin, and selection of cross-resistance and collateral sensitivity to antibiotics

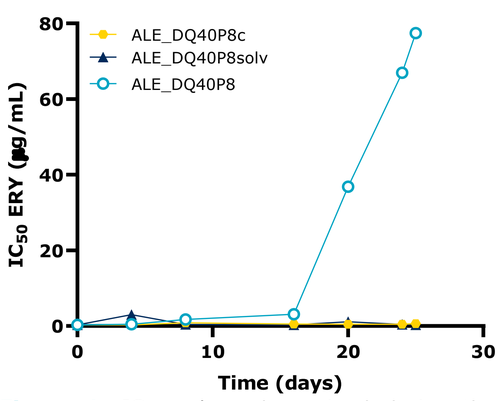


Figure 1. IC_{50} values determined during the ALE of the experimentally adapted strain ALE_DQ40P8 and a control with TSB only, ALE_DQ40P8c, and a solvent control with ethanol, ALE_DQ40P8solv.

Table 1. Susceptibility profile of the parental strain, *Aliarcobacter butzleri* Ab_DQ40A1, and evolved strains to antibiotics.

Strain	MIC (µg/mL)					
	ERY	GEN	TET	AMP	CIP	CTX
Ab_DQ40A1	1	0.5	1	4	≤ 0.03	8
ALE_DQ40P8c	**2**	**1**	**2**	4	**0.06**	8
ALE_DQ40P8solv	**2**	0.5	**2**	4	≤ 0.03	8
ALE_DQ40P8	**256**	0.5	**2**	4	**0.25**	**2**

ERY: Erythromycin; GEN: Gentamicin; TET: Tetracycline; AMP: Ampicillin; CIP: Ciprofloxacin; CTX: Cefotaxime.

Increase in resistance to ERY, decreased susceptibility to TET and CIP along with a collateral sensitivity to CTX

Role of efflux pumps in resistance to erythromycin

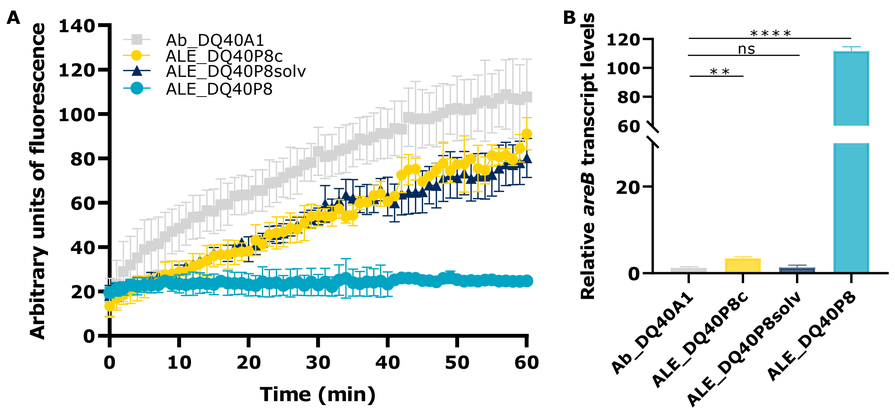


Figure 2. Evaluation of the contribution of efflux activity in macrolide resistance by (A) accumulation of ethidium bromide and (B) assessment of the relative *areB* transcript levels. p>0.05; *p<0.05; **p<0.01; ***p<0.001; ****p<0.0001.

The emergence of erythromycin resistance led to the accumulation of EtBr, potentially associated with increased expression of the AreABC efflux system

Effect of resistance to erythromycin in bacterial fitness and virulence potential

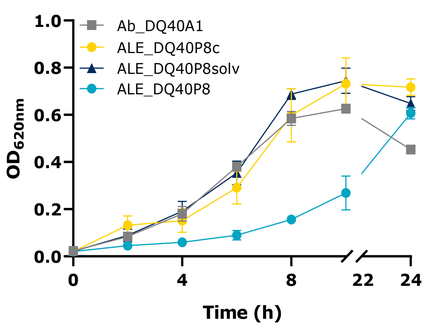


Figure 4. Growth profile in the absence of erythromycin.

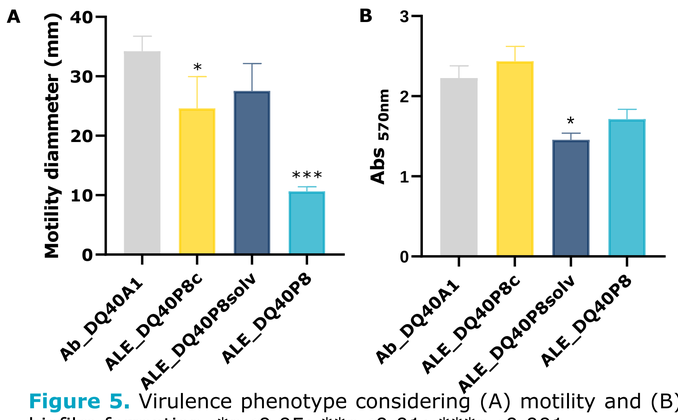
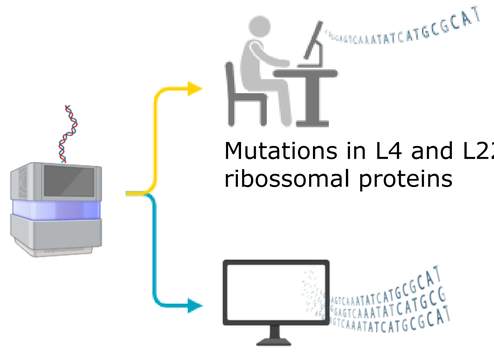


Figure 5. Virulence phenotype considering (A) motility and (B) biofilm formation. *p<0.05; **p<0.01; ***p<0.001.

Fitness and virulence are affected by the development of eryhtromycin resistance

Genetic basis of erythromycin resistance



WGS analysis revelead that combination of mutations may lead to erythromycin high-level resistance

Highlights

- **Efflux activity contribute synergistically with mutations in L4 and L22 ribosomal proteins to *A. butzleri* macrolide resistance, leading to high-level resistance;**
- **The evolution of erythromycin resistance impairs bacterial physiology and virulence;**
- **Fitness cost may justify the low prevalence of high-level resistant circulating strains.**

References

[1]Ferreira S, Queiroz JA, Oleastro M, Domingues FC. (2016) Crit Rev Microbiol. 42:364–383.
[2]Ferreira S, Luís Â, Oleastro M, Pereira L, Domingues FC. (2019) J Glob Antimicrob Resist. 16:130–9.

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