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## Investigating the Role of the DUF445-Containing Putative Membrane Protein in Albicidin Resistance in *Acinetobacter baumannii* IMT51508

### Content

Albicidin, a promising antibacterial peptide, functions by inhibiting the activity of bacterial DNA gyrase. This study aims to elucidate the mechanisms underlying albicidin resistance in ESKAPE pathogens, which are leading contributors to nosocomial infections. Specifically, we focus on investigating the resistance mechanisms in *Acinetobacter baumannii* (IMT51508), a clinically significant multidrug-resistant pathogen.

The MIC of albicidin was determined, followed by laboratory evolution, where the albicidin concentration was increased by two-fold. The evolution of bacterial strains was confirmed by MIC assays of all independently evolved replicates. Subsequently, genomic DNA was extracted from the wild-type strain and eight evolved strains, and WGS was performed. Genome analysis revealed a consistent mutation in an uncharacterized protein, containing a DUF445 domain, YjiN, in 7 out of the 8 evolved strains. Bioinformatics analysis was employed to analyze YjiN protein, suggesting its role as a 2-3 transmembrane domain containing protein. To further elucidate the operon structure of gene cluster, RT-PCR was conducted. In addition, mutations in YjiN and MATE in *Acinetobacter baumannii* were performed, and their effects were measured through MIC assays. The involvement of the YjiN protein in *Acinetobacter baumannii* will provide valuable insights into the role of this putative membrane protein in the bacterial resilience to albicidin.

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