A novel robust genome engineering platform to unravel pathogenic mechanisms in drug resistant *Acinetobacter baumannii*.

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Infections with the Gram-negative coccobacillus *Acinetobacter baumannii* are a major threat in hospital settings. The progressing emergence of multidrug resistant clinical strains significantly reduces the treatment options for clinicians to fight *A. baumannii* infections. The current lack of robust methods to genetically manipulate drug resistant *A. baumannii* isolates impedes research on resistance and virulence mechanisms in clinically relevant strains. In this study we developed a highly efficient and versatile genome editing platform enabling the markerless modification of the genome of *A. baumannii* clinical and laboratory strains, regardless of their resistance profile.

We applied this method by deleting AdeR, a transcription factor that regulates the expression of the AdeABC efflux pump in tigecycline resistant *A. baumannii*, to evaluate its function as a putative drug target. Loss of *adeR* reduced the MIC_{90} of tigecycline from 25 µg/ml in the parental strains to 3.1 µg/ml in the Δ*adeR* mutants indicating its importance in the drug resistant phenotype. However, 60% of the clinical isolates remained non-susceptible to tigecycline after *adeR* deletion. Evolution of artificial tigecycline resistance in two strains followed by whole genome sequencing revealed loss of function mutations in *trm,* suggesting its role in an alternative AdeABC-independent tigecycline resistance mechanism. This finding was strengthened by the confirmation of *trm* disruption in the majority of the tigecycline resistant clinical isolates.

This study highlights the development and application of a powerful genome editing platform for *A. baumannii* enabling future research on drug resistance and virulence pathways in clinical relevant strains.