O585
2-hour Oral Session
Resistance mechanisms in Gram-negatives

The TRIC target evaluation platform revealed an AdeR unrelated tigecycline resistance mechanism in XDR Acinetobacter baumannii clinical isolates

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Background: The opportunistic pathogen *Acinetobacter baumannii* is one of the major threats in hospital settings due to the emergence of multidrug-resistant (MDR), extensively drug-resistant (XDR), and even pan drug- resistant (PDR) strains. The underlying resistance mechanisms require a metabolic investment of the bacteria and are therefore regulated at a transcriptional level. In tigecycline-resistant *A. baumannii* the expression of the AdeABC efflux pump is controlled by the AdeRS two component system (TCS) and plays a major role in the efflux of tigecycline, a drug of the last line treatment. Mutations in the regulatory TCS were described to cause an *adeABC* overexpression leading to a MDR phenotype. These data suggest that AdeR may be a promising target for discovering Transcriptional Regulator Inhibitor Compounds (TRICs) capable of switching-off tigecycline resistance in *A. baumannii*. Purpose of this study was to assess the AdeR importance in XDR clinical *A. baumannii* isolates using BioVersys' proprietary TRIC target evaluation platform.

Material/methods: Ten recently isolated clinical *A. baumannii* strains with XDR phenotypes were studied for their tigecycline resistance mechanism. Scarless *adeR* knockout mutants were produced and the tigecycline resistance profile was compared to their parental strains. Sequencing of the AdeRS TCS and quantification of *adeB* transcript levels by qRT-PCR were used to assess the importance of AdeR in regulating the efflux pump expression. *In vitro* tigecycline resistance development in a wildtype and Δ*adeR* strain and subsequent whole genome sequencing were used to further characterise potential *adeABC*-independent alternative tigecycline resistance mechanisms in *A. baumannii*.

Results: Deletion of *adeR* in ten XDR *A. baumannii* strains reduced the MIC_{90} of tigecycline from 25µg/ml to 3.1µg/ml. With qRT-PCR we demonstrated that AdeR is essential for the expression of the *adeABC* efflux pump. The absence of AdeRS mutations in strains that remained non-susceptible to tigecycline after *adeR* deletion suggested the presence of an alternative resistance mechanism. Whole genome sequencing of strains evolved for tigecycline resistance *in vitro* highlighted the disruption of the *trm* methyltransferase as a potential alternative resistance mechanism. This result was confirmed by the presence of disrupted *trm* in the primarily tested tigecycline resistant clinical isolates.

Conclusions: By applying BioVersys' proprietary TRIC platform we demonstrated that inhibition/modulation of AdeR by a small molecule may not be sufficient to switch-off tigecycline resistance in *A. baumannii*. AdeRS-driven overexpression of AdeABC is the main tigecycline resistance pathway. However, we demonstrated that there is an alternative tigecycline resistance mechanism that confers moderate resistance (MIC=3.1µg/ml) in 50% of the tested clinical

A.baumannii isolates. The disruption of *trm* in the majority of tigecycline resistant strains suggested its implication in an alternative resistance mechanism.