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Abstract (poster session)

Directed evolution of Escherichia coli AcrB may contribute to a better understanding of the mechanisms of action of known bacterial RND-type efflux pump inhibitors

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Objectives: Combination therapy with efflux pump inhibitors (EPIs) is thought to be an attractive strategy combatting bacterial multidrug resistance (MDR) caused by efflux pumps. However, so far no EPIs have been developed for clinical use. 1-(1-naphthylmethyl)-piperazine (NMP) and phenylalanine-arginine-ß-naphthylamide (PAßN) are EPIs with well-characterized in vitro activity in gram-negative bacteria, and it is hypothesized that they directly affect AcrB, the pumping part of the AcrAB-TolC efflux complex from E.coli. Here, we study the feasibility of generating “EPI-resistant” AcrB-mutants revealing putative EPI binding. Methods: In-vitro random mutagenesis was performed by MutazymII® error prone PCR using acrB as template. The products harbouring 2 mutations on average were reintroduced into the corresponding site in acrB of an AcrAB-TolC over-expressing E.coli strain by a homologous recombination method. For pre-selection 5×10e4 - 2×10e5 recombinants were plated on agar containing a known AcrB substrate supplemented with EPI. Screening for altered EPI efficacy was done by MIC assays. Mutants with confirmed phenotype were sequenced and further examined by accumulation assays. Results: Random mutagenesis of the first periplasmic loop of AcrB yielded 0.04-0.1% of mutants growing in the presence of the selected drug/NMP combination. 60% of mutants preselected on linezolid/NMP revealed an at least 4-fold decrease in EPI activity with a minimum of 3 substrates. 38 from 45 sequenced mutants were found to harbour an alteration near the phenylalanine-rich putative substrate binding pocket most frequently from a non-polar to a polar amino acid. Particularly Gly288 was identified as a key site since mutation usually to Ser occurred in 19 independent derived mutants. Ala279 and Gly141 mutations were observed in 8 mutants each. A major effect on NMP efficacy was seen in two independent mutants with the double mutation Gly141Asp-Asn282Tyr. In contrast, stable phenotypes with regard to reduced EPI activity rarely appeared after novobiocin/PAßN selection (7% versus 90% after drug/NMP selection) and 3 out of 7 mutants did not contain any mutation in acrB. Conclusions: The results suggest strongly that directed evolution targeting an MDR transporter is a useful tool for contributing data potentially valuable for rational EPI design. Further investigations should include the whole efflux pump, more random mutagenesis passages and further drug/EPI combinations.