Mutational analysis of AdeB transporter function

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In recent years, Gram-negative bacterial species such as *Acinetobacter baumannii* have caused life-threatening infections not treatable with antibiotics. In *A. baumannii*, the best characterized multidrug efflux system is the prevalent *Acinetobacter* drug efflux (Ade)ABC tripartite system. The *adeA*, *adeB* and *adeC* genes form an operon, encoding membrane fusion protein, multidrug transporter and outer membrane channel protein structure, respectively. PCR amplification showed that the detection rate of *adeB* was highest in clinical isolates (Lin, Ling et al. 2009, Choi, Choi et al. 2019). We constructed ten mutated AdeB variants containing single amino acid substitutions in the functionally important regions of AdeB. Purification and protein analyses showed that all mutated AdeB proteins were produced at similar levels. However, cells producing AdeB mutants varied in their susceptibilities to four antibacterials ethidium bromide (Et), gentamicin, zeocin and azithromycin, the known substrates of AdeABC efflux pump. The F178 and D664 residues were identified to be crucial. Cells producing AdeB with D644C showed a rare phenotype: although this mutation reduced the effectiveness of Et efflux, it also had a role in macrolides efflux. F178C enhanced efflux of gentamicin and zeocin as seen from MICs and growth inhibition curves. The concentration- and time-dependent changes of Et fluorescence were detected for all mutants and compared with wild type of AdeB and efflux-deficient hyperporinated cells. Transporter kinetic parameters (Km and B) were analyzed for each mutant. Our results provide a novel insight into the mechanism of AdeB and demonstrate that this transporter is an attractive target for pharmacological development.