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ePoster Viewing

Antimicrobials: mechanisms of action and resistance

Measuring efflux in MDR *Escherichia coli* using 1,2`DNA and BM27 as dyes in real-time fluorescence assays

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Objectives

Overexpression of efflux pumps in multidrug (MDR)-resistant gram-negative bacteria contributes to the development and level of resistance against many antimicrobial agents. In order to better understand resistance mechanisms and support drug development, accurate efflux measurement which needs to avoid confounding by different membrane permeability is crucial and would overcome the limitations of MIC testing and conventional drug accumulation assays that determine only a net total of influx and efflux. We previously reported a real-time assay using 1,2'-dinaphthylamine (1,2'-DNA) that was able to measure different efflux half times in *E. coli* AcrB mutants. We now extend the findings with this method and report the feasibility of using BM27 as a new alternative dye with this technique.

Methods

A series of well-defined laboratory *E. coli* strains included TolC and AcrB knockouts strains, a number of selection mutants expressing various MDR wild-type and mutant efflux pumps and MDR clinical isolates. After overnight growth in LB medium, the proton-motive force inhibiting agent carbonyl cyanide m-chlorophenylhydrazone (CCCP) was added to the cells to achieve de-energization and avoid pre-energization efflux. The cells were then loaded with fixed concentrations of either 1,2'-DNA or BM27 dyes and further incubated. CCCP and the dyes were removed from the medium before starting the measurement using a flat-bottom black 96-well plate in a microplate reader. After a scanning period of ~100 seconds, glucose was added to energize the cells. Fluorescence measurement continued for 500 seconds. Active efflux was considered when post-energization fluorescence levels dropped significantly.

Results

Both ?*tolC* and ?*acrB* strains accumulated the two dyes but showed no efflux when energized. *E.coli* K12-derived selection mutant 3AG100 with elevated expression of *acrB* showed high efflux capacity for both 1,2'-DNA and BM27. BM27 was shown to be a substrate of AcrF in experiments with an ?*acrB* strain with *acrF* overexpression and also of wild-type YhiV (strain DKO1/20) but not (in contrast to 1,2`-DNA) in strain DKO1/17 overexpressing mutant (Val610Phe) YhiV. The reference strains *E. coli* ATCC25922 and ATCC35218 both exhibited BM27 efflux, but only the latter responded to 1,2'-DNA. In a collection of 33 MDR *E. coli* clinical isolates with differing MDR efflux and porin gene expression levels, we found high BM27 efflux capacities in all except one isolate, but there was no consistent relationship between efflux capacity and multipanel MIC levels, susceptibility to efflux pump inhibitors (EPIs) or gene expression profiles.

Conclusion

Both dyes may be useful to screen for and measure efflux capacity in enteric bacteria. BM27 - when confirmed in subsequent studies - may have the advantage to be a substrate of several RND-type pumps. Whether and how chemical library screening for EPI activity can be integrated into the new real-time efflux assays remains to be determined.

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