Membrane composition and anionic phospholipid abundance affect activity of single component cationic antimicrobial selective efflux pumps in *Escherichia coli*

Carmine J. Slipski, George G. Zhanel, Denice C. Bay.

Medical Microbiology and Infectious Diseases, University of Manitoba.

Introduction: Multidrug-resistant (MDR) bacteria are a concerning threat to health globally and are worsening by constant overuse of cationic antiseptics in clinical, industrial, and household applications. Exposure to sub-inhibitory concentrations of antiseptics can lead to membrane adaptations, reducing efficacy of clinically relevant antibiotics. Antiseptic resistance in bacteria is predominantly conferred by efflux pump proteins, comprising different families with varying substrates. Here, we examine cationic substrate specific efflux pumps, and the role anionic phospholipids play in activity of these single-component-transporters. Phospholipid-protein interactions influencing activity of cation-selective efflux pumps is poorly understood, knowledge gained will help generate novel targets for efflux pump inhibitors and guide antimicrobial stewardship.

Methods: Efflux pumps EmrE(SMR family), MdtK(MATE family), MdfA(MFS family), and AceI(PACE family), were cloned into the plasmid vector pMS119EH and transformed into *E. coli* mutants containing different phospholipid biosynthesis gene deletions. Phospholipid content of *E. coli* was confirmed by Thin-Layer-Chromatography and Peptidisc® isolation of proteins along with annular lipids for Mass-spectrometry analysis. The activity of each pump was tested in these lipid mutants against varying concentrations of common cationic antimicrobials: quaternary ammonium compounds, bisbiguanides, and relevant antibiotics; utilizing high-throughput assays to determine if efflux activity is affected by varying membrane phospholipid compositions.

Results: low-abundance cardiolipin (CL) species were predominantly detected from Mass-spec. This may be due to close dependence on CL for efflux activity, or preferential isolation by the Peptidisc®. Diversity of remaining lipid types was highest in MdtK, which also demonstrated the largest changes in efflux activity in lipid mutant strains. MdfA demonstrated the lowest lipid diversity and corresponded to the least amount of susceptibility changes in lipid mutant strains.

Conclusions: these results provide evidence that specific phospholipid type abundance, mainly anionic CL species, have a significant role in the insertion, folding, and activity of cationic efflux proteins, and provide promising new drug targets.