**Investigation Efflux Pump Activity In *Acinetobacter baumannii* Clinical Strains Through A Single-cell Approach**

Dieu Do-Mai-Xuan1, Thai Nguyen-Minh1, Thao Le-Thi-Thanh1, **Anh Nguyen-Tu1**

Department of Microbiology and Parasitology, Faculty of Pharmacy, University of Medicine and Pharmacy at Ho Chi Minh City, Ho Chi Minh City, Vietnam;

**Background and aims.** *Acinetobacter baumannii* is a commensal and opportunistic pathogen that has emerged as a problematic hospital pathogen due to its multiple-drug resistance. Biofilm-forming capacity and overexpression of efflux pumps have been described as essential mechanisms causing antibiotic resistance in most clinical isolates. Our study aimed to investigate these abilities at the phenotypic and single-cell levels under non-induced and induced antibiotic conditions among 65 clinical isolates.

**Methods.** The susceptibility to tetracycline, cefoperazone-sulbactam, ciprofloxacin, cefipime, meropenem, and amikacin of the *A. baumannii* strains was first tested by the disk diffusion test. We then employed the flow cytometry technique (FC) to monitor the bacterial growth rate, determine the MIC values of 6 antibiotics, and use microdilution as a reference. Accumulation and secretion of Ethidium bromide (EtBr) by the efflux pump were closely studied at the subpopulation level under limiting energy supply and in the presence of carbonyl cyanide m-chlorophenyl hydrazone (CCCP) as an efflux pump inhibitor.

**Results.** The most effective antibiotics against *A. baumannii* were tetracycline and cefoperazone-sulbactam, with 45 % and 50 % resistance rates. Meanwhile, the highest resistance rate was observed in ciprofloxacin at 85 %. The cefipime, meropenem, and amikacin rates were 75 %, 80 %, and 80 %, respectively. Thirty-five strains removed more than 60% of the preloaded EtBr, twenty-six removed 30-60%, and four removed less than 30%. We observed heterogeneity in efflux pump activity in 17 strains. Ciprofloxacin slightly enhanced (from 5.66 to 11.66%) the efflux pump activity but strongly reduced (9 times) the coefficient of variation of accumulated EtBr between cells in the population.

**Conclusion/Discussion.** These results suggest that a single-cell approach is a promising method for rapidly assessing drug pump efflux activity, screening efflux pump inhibitors, and studying the causes of heteroresistance.

**Acknowledgements.**

**References:**

1. Viveiros M, Martins M, Couto I, et al. New methods for the identification of efflux mediated MDR bacteria, genetic assessment of regulators and efflux pump constituents, characterization of efflux systems and screening for inhibitors of efflux pumps. Current drug targets. Sep 2008;9(9):760-78. doi:10.2174/138945008785747734
2. Paixão L, Rodrigues L, Couto I, et al. Fluorometric determination of ethidium bromide efflux kinetics in Escherichia coli. J Biol Eng. Oct 16 2009;3:18. doi:10.1186/1754-1611-3-18