## SP26 Prix poster Jeunes Microbiologistes



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### Resistance to antibiotics by efflux in multidrug resistant *Enterobacteriaceae*: detection, prevalence and development of a technique allowing use for diagnostic purposes

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**Introduction and objectives:**
The emergence of multidrug-resistant clinical strains is a public health problem in the management and treatment of associated infections. Several resistance mechanisms are present in a single strain, and antibiotic efflux is often found at the same time as enzyme resistance and / or target mutations. RND (Resistance Nodulation cell Division) efflux pumps, with AcrAB as the leader, are the pumps mainly involved in Enterobacteriaceae. Routinely in the laboratory, only enzyme resistance and target mutations can be identified. The development of a diagnostic system to detect the presence of efflux could help in improving the management of infected patients.
**Materials and methods:**
Clinical strains of *Escherichia coli, Klebsiella pneumoniae, K. aerogenes, K. oxytoca, Enterobacter cloacae* and *Citrobacter koseri* were used in our study.
Fluoroquinolones and chloramphenicol were used to perform antibiotic sensitivity tests to determine the minimum inhibitory concentrations (MIC) in the presence or absence of a pump inhibitor, phenylalanine arginine β-naphthylamide (PAßN) at 20µg / mL . Broth microdilution in Mueller Hinton II, in the presence of iodonitrotetrazolium chloride (INT) after an incubation of 18 hours at 37° C, make it possible to obtain the different MICs.
Enrofloxacin at a concentration of 3 µg / mL was used to perform intracellular accumulation tests in intact *Enterobacteriaceae* with or without carbonyl cyanide m-chlorophenylhydrazone (CCCP) at 20 µM (collapsing pump energy). The results were monitored by spectrofluorimetry after incubation at 25° C., overnight, in 0.1M glycine-HCl lysis buffer.
**Results discussion and conclusion:**
The determination of MICs with or without PAßN makes it possible to discriminate the strains according to this phenotype. Optimization of the spectrofluorimetric determination of intracellular accumulation level of enrofloxacin has enabled us to refine a reproducible protocol for routine use. The availability of a semi-quantitative efflux threshold makes it possible to consider the application of this test for diagnostic purposes in the laboratory.

*Mots clés :* Enterobacteriaceae - Efflux pumps - Multidrug resistance - Clinical strains - Fluoroquinolones.