

**Title:** Contribution of  $\beta$ -lactamases, porins and efflux pumps to carbapenem and/or fluoroquinolone resistance in clinical isolates

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## Introduction

Carbapenems, a class of  $\beta$ -lactam antibiotics with very broad activity, are often last resort antibiotics used to treat infections due to extended-spectrum  $\beta$ -lactamase (ESBL)- or plasmid-mediated AmpC (PMA $\beta$ )-producing Gram-negative bacteria. Considering the emerging rates of resistance to these antibiotics, the investigation of related resistance mechanisms constitutes an important assignment. Thus, this study aimed to characterize the carbapenem resistance mechanisms harbored by Gram-negative isolates and the co-resistance to structurally unrelated antibiotics (such as fluoroquinolones).

## Methods

A total of 426 isolates (2 *Acinetobacter baumannii*, 1 *Citrobacter freundii*, 7 *Enterobacter* spp., 153 *Escherichia coli*, 251 *Klebsiella* spp., 3 *Morganella morganii*, 6 *Proteus* spp., 3 *Pseudomonas* spp.) were screened for the susceptibility to ertapenem by disc diffusion, and interpreted by SFM guidelines. Non-susceptible isolates were retained and studied against other classes of antibiotics, using the same phenotypic method.

Mechanisms justifying the resistance to carbapenemes were searched: 1) carbapenemase production by using molecular methods and isoelectric focusing; 2) modification of outer membrane porins (OMPs) after migration in SDS-PAGE (OmpK35/OmpK36/OmpK37 for 15 *Klebsiella pneumoniae*, OmpC/OmpF for 1 *E. coli*, 1 *Enterobacter cloacae* and 1 *Enterobacter cancerogenus*, Omp35/Omp36 for 4 *Enterobacter aerogenes*) and complete characterization of OMP-encoding genes, performed by PCR amplification and sequencing. Deduced amino acid modifications were interpreted by comparing both clinical and wild-type sequences, using the EMBL database. In addition to carbapenemase-encoding genes, other *bla*, as well as plasmid-mediated quinolone-resistance (PMQR)-encoding genes were also searched by PCR and sequencing.

## Results:

Among the isolates studied, 22 (5%) revealed to be non-susceptible to ertapenem of which 4% were multidrug resistant. Their antibiotic susceptibility evaluation enabled the prediction of the respective resistance mechanisms and guided the remaining biochemical and molecular studies. We identified and characterized the expression of carbapenemases (3 KPC-3 and 1 GES-5), PMA $\beta$  (MIR-type) and ESBLs CTX-M-15 (n=11), co-expressed with OXA-1 and TEM-1, and one SHV-12 plus GES-5. Amino acid substitutions were identified in 21 out of 22 isolates, due to insertions, deletions and/or mutations in the nucleotide sequence of OMP-encoding genes in the main regions related to the porin functions, suggesting a role in carbapenem resistance. The acetyltransferase Aac(6')-Ib-cr variant (n=8) and the efflux pump OqxAB (n=5) were also identified, contributing to fluoroquinolone resistance.

## Conclusions:

Globally, this study provides meaningful insights towards the understanding of emergent carbapenem resistance, as well as PMQR mechanisms, particularly in a worrying multidrug resistant scenario.