

## Occurrence of beta-lactamases, namely GES-5 carbapenemase, among gram-negative isolates from wastewater samples in Northern Portugal

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Manageiro, V., Ferreira, E., Figueira, V, Caniça, M., Manaia, C.M.

Antimicrobial resistant pathogens are profoundly relevant to human health and many were the studies that focused on their spread. However, natural and human associated environmental reservoirs of resistance are yet poorly understood. The main goal of this study was to evaluate the main antibiotic resistance mechanisms in Gram-negative bacteria isolates from different wastewater environments.

Water samples were collected from different environments within an urban water cycle in the region of Northern Portugal, which included treated and raw wastewater, water to the consumers and water surface. Screening of antimicrobial susceptibility of 48 Gram-negative isolates (20 *Escherichia coli*, 8 *Citrobacter* spp, 7 *Klebsiella* spp, 6 *Kluyvera* spp, 2 *Enterobacter* spp, 1 *Hafnia alvei*, 1 *Pantoea agglomerans*, 1 *Pseudomonas luteola*, 1 *Roultella ornithinolytica*, 1 *Serratia* spp) was performed by disk diffusion method. Interpretative reading of susceptibilities allowed to direct the search for antibiotic resistant genes. PCR and sequencing were used to screen and identify bla and plasmid-mediated quinolone resistance (PMQRs) genes. All isolates were also screened for the presence of class 1 integrons.

Overall, 29.2% of the isolates were multidrug resistant, suggesting a great diversity of resistance mechanisms. Noteworthy, 2 isolates showed non-susceptibility to carbapenems, which constitutes one of the last resorts on the antimicrobial therapy. Their phenotypic and molecular characterization revealed the expression of a chromosomal metallo-beta-lactamase in *P. luteola* and the presence of a GES-5 encoding gene in a *Klebsiella pneumoniae* isolate. Furthermore, we detected a vast variety of beta-lactamase encoding genes, specifically 12 blaTEM-1 with distinct promoters, 4 blaSHV (2 blaSHV-1 and 2 blaSHV-11), besides different chromosomal AmpC beta-lactamases, namely CMY-65. Class 1 integrons were detected among 6 of TEM-1-producing isolates. Together, these beta-lactamases explain the level of beta-lactam resistance. None PMQR genes were detected.

In conclusion, this study provides the first description of a class A carbapenemase in an environmental setting in Portugal, in addition to several other beta-lactam resistance mechanisms. The study highlights the need of surveillance of these resistance mechanisms in environmental backgrounds, since it represents a liable reservoir of potential pathogenic resistant bacteria.