Molecular survey of 2109 carbapenem resistance *Enterobacteriaceae* isolates from Portuguese Hospitals: co-production of carbapenemase KPC-3 and the efflux pump OqxAB

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Objectives: Although there are important studies regarding the different carbapenemase (CARB)-producing Gram-negative bacteria, little is known concerning their molecular epidemiology in Portugal. The main aim of this study was to characterize, by phenotype and molecular typing methods, CARB-producing *Enterobacteriaceae* isolates recovered from Portuguese health care institutions, and evaluate its impact on treatment strategy.

Methods: This study included 2105 clinical isolates, collected between April/2006 and February/2013, in different Portuguese healthcare institutions. Screening of antimicrobial susceptibility was performed by disc diffusion method. Clinical isolates with resistance or with decreased susceptibility to ertapenem were considered presumptively CARB-producers; in these isolates, PCR and sequencing were applied to detect and identify CARB-encoding genes, as well as other *bla* and plasmid-mediated quinolone resistance (PMQRs) genes. MICs of CARB-producing isolates were tested by microdilution (EUCAST breakpoints). The plasmids obtained from clinical isolates were characterized by PCR-based replicon typing (PBRT). Clonal relatedness of *K. pneumoniae* isolates was investigated by multilocus sequence typing (MLST), using the protocol developed by the Institute Pasteur (www.pasteur.fr/mlst/Kpneumoniae.html).

Results: Among the 2105 isolates tested, 165 (7.8%) were putative CARB-producers and were selected for further analysis. Thirty-five (21.2%) of the 165 positive isolates were confirmed to be CARB-producers, of which the majority were collected from the urine (54.3%) of elderly (≥65 years old) male patients (54.3%), and admitted at the emergency room/ambulatory (22.9%) or internal

medicine (17.1%) wards. All were multidrug-resistant, with nonsusceptibility to at least one carbapenem, and with consistent susceptibility only to colistin. In those isolates was detected the following beta-lactamases: 30 KPC-3 (22 *K. pneumoniae*, 3 *Escherichia coli*, 2 *Enterobacter aerogenes* and 3 *Enterobacter clocae*), 4 GES-5 (K. pneumoniae) and one VIM-2 (*Klebsiella oxytoca*). CARB-encoding genes were present alone or in combination with other *bla* genes, such as bla_{SHV-12}, bla_{SHV-14}, bla_{SHV-26}, bla_{SHV-36}, $bla_{CTX-M-15}$, and the $bla_{SHV-164}$. PMQR-encoding genes were also detected, namely *qnrA*, *qnrB*, *aac(6')-Ib-cr* and the recently identified *oqxAB*. All bla_{KPC-3} genes were located on a Tn3-based transposon, *Tn*4401, while bla_{GES-5} and bla_{VIM-2} genes were associated with class 3 and 1 integrons, respectively. In our study, the majority of the bla_{CARB}-harbouring plasmids were nonconjugative, having been typed as $IncF_{repB}$ by PBRT. Clonal relatedness of the 26 *K. pneumoniae* isolates, obtained by MLST, showed that they were from distinct STs, namely ST14, ST15, ST34, ST59, ST147, ST416, ST698, and from the two novels ST: ST960 and, among all, the predominant ST1138 (corresponding to KPC-3 plus SHV-36 producers).

Conclusion: In conclusion, this study provides new data regarding the molecular epidemiology of CARB-producing *Enterobacteriaceae*, which appears to be widespread in Portugal. Dissemination of bla_{CARB} seems to be due to carriage of similar CARB-harbouring plasmids within genetically diverse clinical strains. Overall, our results emphasize the need of a concerted action to manage carbapenem use.