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 cloacae), 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 $\text{bla}_{\text{SHV-12}}$, $\text{bla}_{\text{SHV-14}}$, $\text{bla}_{\text{SHV-26}}$, $\text{bla}_{\text{SHV-36}}$, $\text{bla}_{\text{CTX-M-15}}$, and the $\text{bla}_{\text{SHV-164}}$. PMQR-encoding genes were also detected, namely $\text{qnrA}$, $\text{qnrB}$, $\text{aac(6')-Ib-cr}$ and the recently identified $\text{oqxAB}$.

All $\text{bla}_{\text{KPC-3}}$ genes were located on a Tn3-based transposon, $\text{Tn4401}$, while $\text{bla}_{\text{GES-5}}$ and $\text{bla}_{\text{VIM-2}}$ genes were associated with class 3 and 1 integrons, respectively. In our study, the majority of the $\text{bla}_{\text{CARB}}$-harbouring plasmids were nonconjugative, having been typed as IncF$_{\text{repB}}$ 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 $\text{bla}_{\text{CARB}}$ 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.