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Abstract (publication only)

Carbapenemase-producing Enterobacteriaceae isolates collected in Portuguese hospitals

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Objectives: In Portugal, little is known on carbapenemase (CARB)-producing Enterobacteriaceae. The aim of this study was to identify the resistance mechanisms of Enterobacteriaceae isolates, identified at hospital laboratories as carbapenem (CA) non-susceptible. **Methods:** This study included 61 Enterobacteriaceae isolates (26 *Klebsiella* spp, 15 *Escherichia coli*, 9 *Enterobacter* spp, 6 *Morganella morganii*, 4 *Proteus mirabilis*, 1 *Serratia marcescens*), collected between 04/2006 and 09/2011 and sent to the NIH-Lisbon for CA susceptibility confirmation. Antimicrobial susceptibility of clinical isolates was performed by disk diffusion method (CA-SFM). Clinical isolates showing synergism between CA and boronic acid (BOR) (and/or clavulanic acid, CLAV) or with EDTA were considered presumptively CARB-producers from class A or Class B, respectively. PCR and sequencing were applied to detect and identify CARB-encoding genes; the respective genetic environment was revealed by sequencing using PCR mapping. Direct transfer of the CA resistance phenotype was attempted by mating-out assays. Antibiotics susceptibility (MIC) of transconjugants and respective isolates were tested by microdilution. **Results:** The majority of isolates were collected from the urine (57.4%) of elderly (≥ 65 years old) male patients (54.1%), admitted at the emergency room/ambulatory (24.6%) and at internal medicine (18.0%) wards. Among all isolates, 50.8% were nonsusceptible to at least one CA, being 67.2% multidrug-resistant; 16 isolates showed synergy between CA and BOR (and/or CLAV). Among those, 5 were KPC-3-producers (4 *Klebsiella pneumoniae* and 1 *Enterobacter cloacae*), collected in 2010 (2) and 2011 (3). The blaKPC-3 genes were confirmed to be carried by plasmids. Genetic environment of blaKPC-3 gene revealed the presence of a Tn4401 transposon in all but one isolate (*E. cloacae*), suggesting that this last gene was included in other Tn4401-like isoform. We also detected a VIM-2-producing *Klebsiella oxytoca*, collected in 2009, among the 7 isolates that showed synergy between imipinem and EDTA. No blaGES, blaNDM or blaIMP were detected. **Conclusion:** This study provides new data regarding the molecular epidemiology of CARB-producing Enterobacteriaceae in Portugal. Overall, our results emphasize the need of a concerted action to manage CA use. This is supported by EARS-Net, which reported an increase in CA nonsusceptibility of *K. pneumoniae* isolates from 0.72% in 2008 to 1.58% in 2010.