Unveiling clones and integrons dynamics associated with carbapenemase-producing *P. aeruginosa* clinical isolates in a Portuguese hospital

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Particular epidemic lineages (e.g. 235, 111, ST175 and 244) are associated with carbapenemase-producing *P. aeruginosa* (CPPA) isolates causing infections. Due to limited therapeutic options and possible carbapenemase spread, CPPA infections are of great concern. Nevertheless, the occurrence, genetic background and clonal dynamics over a long period of time of CPPA strains in Portuguese hospitals are unknown. The study aimed to assess the occurrence and the genetic background of CPPA isolates obtained from a Portuguese University Hospital.

Carbapenem-resistant *P. aeruginosa* isolates obtained from different biological samples from inpatients attending the Hospital Geral de Santo António, Porto during 2006 (n=27) and 2011-3 (n=135) were included. Carbapenemase production was searched by Blue-Carba. Carbapenemase and associated integrons were characterized by PCRs and sequencing. Antimicrobial susceptibility was performed by disc diffusion, E-test and agar dilution methods. Clonality was assessed by MLST. The *bla* genes location was assessed by I-*Ceu*I/S1 PFGE and hybridization with specific probes. Plasmid analysis included identification of incompatibility groups by PCR and electrotransformation of *P. aeruginosa* PAO1.

Eleven isolates were Blue-Carba positive and presented bla_{VIM-2}. These isolates were non-susceptible to imipenem, meropenem, ceftazidime, ciprofloxacin, amikacin, tobramycin and gentamicin, but presented variable susceptibility to cefepime, aztreonam and piperacillin+tazobactam. All were susceptible to colistin. Four different integron structures were identified: In*58*, In*100*, In58-like, and In796 (Figure 1). Five ST's were found: 179(n=5; 2011-3), 175(n=2,2006), 244(n=2;2012-3), 111(n=1;2013) and 1284 (n=1;2011). In58 was the most widespread integron, being associated with ST's 179, 175 and 111. bla_{VIM-2} gene was chromosomally located for nine isolates, whereas in two was plasmid located (~29 and 33 kb plasmids). The plasmids were not associated with searched incompatibility groups.

Despite the occurrence of widespread clinically relevant clones, ST179 has been an important contributor to the emergence of VIM-2 producing *P. aeruginosa*, suggesting a peculiar population of this opportunistic pathogen in the studied hospital. Also, the integron structures carrying bla_{VIM-2} were similar or highly related to previously described ones in *Pseudomonas* spp. from different Portuguese settings, suggesting an interconnection between them and/or a great stability of genetic structures encoding this carbapenemase.

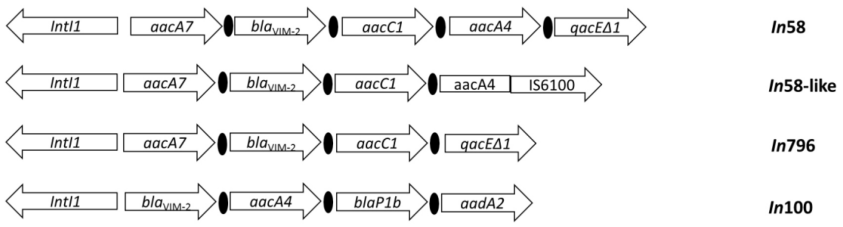

Figure 1. *P. aeruginosa* integrons described in this study. The arrows indicate the translation orientation of the coding genes. The 59 base elements are indicated by black circles.