Chromobacterium spp. harbour Ambler class A beta-lactamases showing high identity with KPC - DTU Orbit (23/10/2019)

**Chromobacterium spp. harbour Ambler class A beta-lactamases showing high identity with KPC**

**Objectives:** The origin of KPC is unknown. The aim of this study was to detect progenitors of KPC in silico and to functionally verify their beta-lactam hydrolysis activity.

**Methods:** The sequence of KPC-2 was used to mine the NCBI protein sequence database. The best non-KPC hits were analysed by amino acid (aa) alignment and phylogenetic tree construction. Genes encoding KPC-2 homologues were expressed in Escherichia coli. The carbapenemase activities of the recombinant strains were characterized by the CarbaNP test and UV spectrophotometry and MICs of selected beta-lactams were determined.

**Results:** Genes encoding the closest KPC-2 homologues were identified on the chromosome of Chromobacterium piscinae strain ND17 (CRP-1, 76% aa identity), Chromobacterium sp. C-61 (CRS-1, 70% aa identity) and Chromobacterium haemolyticum DSM19808 (CRH-1, 69% aa identity). All three Chromobacterium beta-lactamases were phylogenetically more related to KPC than to other Ambler class A beta-lactamases. The 27 bp region preceding the start codon of bla(CRP-1) displayed high nucleotide identity to the corresponding region upstream from bla(KPC) (74%). Heterologous expression of bla(CRP-1) and to a lesser extent of bla(CRH-1) in E. coli significantly increased the MICs of meropenem and most cephalosporins. The CarbaNP test was positive for both recombinant strains, but spectrophotometric analysis confirmed higher carbapenemase activity for CRP-1-producing clones.

**Conclusions:** The recovery of three class A beta-lactamases with up to 76% aa identity to KPC from distinct Chromobacterium species is highly indicative of the role played by this genus in the evolution of KPC.