NDM-1-producing *Providencia stuartii* isolates in a Portuguese Hospital

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Objective: *Providencia stuartii* is an opportunistic pathogen typically associated with urinary infections, and is intrinsically resistance to a wide range of antibiotics. The main aim of this study was to characterize five carbapenemase (CA) NDM-1-producing *P. stuartii* isolates obtained during an outbreak detected in a Hospital.

Methods: MICs were obtained by the reference microdilution broth method, according to EUCAST guidelines. PCR amplification and DNA sequencing were applied to identify the presence of CA genes from class A, B and D. Direct transfer of the CA resistance phenotype was attempted by mating-out assays. Genetic relatedness was examined by PFGE. One isolate, INSRA21868, recovered from the urine of an 88-year-old male patient admitted to the intensive care unit, was selected for genetic characterization using whole-genome sequencing (WGS), performed using 150 bp paired-end reads on a MiSeq (Illumina). A set of bioinformatic web tools were used to estimate the presence of pathogenicity determinants, antibiotic resistance (AR) genes, and clinically relevant mobile genetic elements.

Results: All isolates, genetically indistinguishable by PFGE, presented multidrug-resistance with non-susceptibility to all carbapenems tested. Transconjugants had AR profiles similar to those of their parental clinical isolates. All NDM-1 determinants tested were found to be carried on conjugative plasmids. *In silico* AR analyses using ResFinder-v2.1 revealed genes conferring resistance to β-lactams [bla_{NDM-1}, bla_{CMY-4} and Δbla_{DHA-1}), aminoglycosides (*aac(2')-Ia*, *armA*), tetracycline (*tetB*), macrolides (*mphE* and *msrE*), chloramphenicol (*catB3*), and sulfonamides (*sul1*). PlasmidFinder-v1.2 analyses revealed the presence of an IncA/C2, which has been associated with wide dissemination of bla_{NDM-1}. In the 3' region, the bla_{NDM-1} gene was adjacent to a bleomycin resistance-encoding gene (ble_{MBL}), followed by a *trpF* and part of the bla_{DHA-1}-*ampR* region. The IS*Aba125* element upstream of bla_{NDM-1} was interrupted by an IS*26* element.

Conclusion: This study emphasizes the elements involved in dissemination of nosocomial infections and the potential of WGS in epidemiological investigations in the prevention of CA dissemination among hospitals as well as to other bacterial genera.