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

Vera Manageiro¹,², Eugénia Ferreira¹,², João Rodrigues³, Daniel A. Sampaio⁴, Luís Vieira⁴, Patrícia Pereira⁵, Paulo Rodrigues⁵, Carlos Palos⁵, Manuela Caniça¹,²

1 National Reference Laboratory of Antibiotic Resistances and Healthcare Associated Infections (NRL-AMR-HAI), Department of Infectious Diseases, National Institute of Health Dr. Ricardo Jorge, Lisbon, Portugal.

2 Centre for the Studies of Animal Science, Institute of Agrarian and Agri-Food Sciences and Technologies, Oporto University, Oporto, Portugal.

3 Laboratory of Bacteriology, Department of Infectious Diseases, National Institute of Health Dr. Ricardo Jorge, Lisbon, Portugal.

4 Innovation and Technology Unit, Human Genetics Department, National Institute of Health Dr. Ricardo Jorge, Lisbon, Portugal.

5 BA Hospital, Loures, Portugal

**Objective:** *Providencia stuartii* is an opportunistic pathogen typically associated with urinary infections, and is intrinsically resistant 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*<sub>NDM-1</sub>, *bla*<sub>CMY-4</sub> and Δ*bla*<sub>DHA-1</sub>), aminoglycosides (*aac(2')-Ia*, *armA*), tetracycline (*tetB*), macrolides (*mphE* and *msrE*), chloramphenicol (*catB3*), and sulfonamides (*sul*). PlasmidFinder-v1.2 analyses revealed the presence of an IncA/C2, which has been associated with wide dissemination of *bla*<sub>NDM-1</sub>. In the 3' region, the *bla*<sub>NDM-1</sub> gene was adjacent to a bleomycin resistance-encoding gene (*ble*<sub>MBL</sub>), followed by a *trpF* and part of the *bla*<sub>DHA-1</sub>-*ampR* region. The IS*Aba*125 element upstream of *bla*<sub>NDM-1</sub> was interrupted by an IS26 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.