

Zoonotic potential of multidrug resistant *Escherichia coli* clonal groups in Portugal

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**D. Jones-Dias¹, L. Clemente², V. Manageiro¹, P. Themudo², T. Albuquerque², A.P. Francisco¹,
D. Louro¹, E. Ferreira¹, M. Caniça¹**

¹ National Institute of Health Dr. Ricardo Jorge, Lisbon, Portugal

² National Laboratory of Veterinary Research, Lisbon, Portugal

Multidrug-resistant (MDR) plasmids together with clonal dissemination of resistant isolates are possibly the most successful combination of factors contributing to the spread of antibiotic resistance genes. Our aim was to identify clones associated with multidrug-resistance among human and dolphin isolates, in order to evaluate zoonotic potential and risk.

Sixty two MDR human *Escherichia coli* isolates were randomly selected from NIH collection, being previously isolated from different clinical specimens in seven geographically apart Portuguese hospitals from 2004 to 2009. Two *E. coli* isolated from dolphin's respiratory exudates in 2009 and 2010, at the National Laboratory of Veterinary Research, were also included in this study for their zoonotic portencial analysis. Antimicrobial susceptibility was performed by broth-microdilution method (EUCAST). PCR and sequencing were used to screen and identify β -lactamase and Aac(6')-Ib-cr encoding genes, while PCR-based replicon typing was used to characterize plasmids from MDR isolates. Genetic relatedness of human and dolphin isolates was examined both by PFGE and MLST. Mobile genetic elements were also investigated through PCR mapping assays.

Regarding the human isolates, 48 (77%) were CTX-M producers. We detected *bla*_{CTX-M-1} (n=4), *bla*_{CTX-M-3} (n=3), *bla*_{CTX-M-14}, *bla*_{CTX-M-15} (n=34), *bla*_{CTX-M-32} (n=3), (n=4), *bla*_{TEM-1} (n=39), and *bla*_{SHV-12} (n=8) genes as well as *aac(6')-Ib-cr* (n=26). Concerning the isolates recovered from the dolphins, one of them produced TEM-1, OXA-30, CTX-M-15 and Aac(6')-Ib-cr and the other TEM-1, Aac(6')-Ib and Aac(6')-Ib-cr. Replicon-typing revealed a severe predominance of IncF plasmids in both animal and human isolates; IS26 and ISEcp1 were also detected in both groups, being associated with *bla*_{CTX-M-15} and Aac(6')-Ib-cr plus OXA-30, respectively, in one of the dolphin isolates. Genetic relatedness analysis by PFGE revealed one major cluster corresponding to a single epidemic clone A, which included 22 (35%) of all human isolates and both dolphin isolates. They exhibited the same combination of MLST alleles, corresponding to ST131.

This study illustrated the dominance of common antibiotic resistance genes, plasmids and clonal groups, specifically *bla*_{CTX-M-15}, *aac(6')-Ib-cr*, IncF plasmids and ST131 in both human and animal isolates, reflecting their linkage and enhancing their zoonotic potencial. Studies should be performed to further deepen their role as hotspots of resistance.