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Epidemiology and zoonotic potential of *Escherichia coli* CTX-M-15-producing isolates in Portugal

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Background: The recent spread of plasmid-located CTX-M-ESBL-encoding genes is a serious threat to the clinical efficacy of expanded-spectrum cephalosporins. This study proposes to identify the epidemiology of plasmid-mediated CTX-M-encoding genes between an *Escherichia coli* strain isolated from a dolphin and several *E. coli* strains of human origin and, explain the responsible mechanism and reservoirs of current spread of CTX-M-type enzymes. Molecular typing of these strains establishes the linkage of dissemination between human and animal isolates.

Methods: Sixty two ESBL-positive *E. coli* strains isolated from different clinical specimens in seven hospitals (2004 to 2009), from four different geographic regions, were screened for the presence of CTX-M encoding genes. An *E. coli* isolated from a respiratory exsudate in a dolphin in 2009, at the National Laboratory of Veterinary Research (LNIV) and characterized as CTX-M-producer, was also included in this study. Antimicrobial susceptibility was performed by broth-microdilution method. PCR and sequencing were used to screen and identify *bla* genes. Genetic relatedness among all isolates was examined by PFGE using XbaI enzyme. MLST was performed among the clinical epidemic human isolates clustering together and the isolate from the dolphin, according to the MLST database.

Results: Forty eight human clinical isolates (77%) were CTX-M producers. Susceptibility towards beta-lactams confirmed all isolates as ESBL producers and also suggesting CTX-M enzymes expression. We detected $bla_{\text{CTX-M-15}}$ (*n*=34), $bla_{\text{CTX-M-1}}$ (*n*=4), $bla_{\text{CTX-M-3}}$ (*n*=3), $bla_{\text{CTX-M-32}}$ (*n*=3), $bla_{\text{CTX-M-14}}$ (*n*=4), $bla_{\text{TEM-1}}$ (*n*=39), and *bla*SHV-12 (*n*=8) genes; the dolphin isolate presented the $bla_{\text{CTX-M-15}}$ gene. 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 the dolphin isolate, which clustered together with this clone A and, exhibited the same combination of MLST alleles across the seven sequenced loci, corresponding to the ST131. Twenty-one clinical isolates corresponding to the CTX-M-15-positive clone A were multidrug-resistant (95%), as well as the dolphin isolate.

Conclusions: This study illustrated that genetic relatedness between human and animal *E. coli* CTX-M-15-producing clone strengthens its zoonotic potential. It may also explain the current spread of CTX-M-type enzymes worldwide among species from different origins and highlights the importance to identify antimicrobial resistance reservoirs, contributing to a single health for all.