Multilocus Sequence Typing for characterization of potential risk ESBLs-producing *Escherichia coli* isolated from pigs, including strains of new singletons ST2528, ST2524 and ST2525

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Infections caused by *Escherichia coli* harboring extended-spectrum beta-lactamases (ESBL) have a tremendous impact on public health, because of treatment complications. ESBL-producing *E. coli* are increasingly reported in healthy food-producing animals that can spread to humans either by direct contact or, more importantly, through the food chain. Here we describe a molecular survey aimed at determining the population structure and dynamics of ESBL-producing *E. coli* strains recovered from healthy pigs slaughtered for human consumption in Portugal.

For this purpose, a total of 71 faecal samples from pigs were collected (2008 to 2009) in different geographical regions of Portugal. Susceptibility to 16 antibiotics was tested by disk-diffusion method in all recovered isolates and ESBL detection was carried out by double-disk test. PCR and sequencing methods characterized bla_{ESBL} genes responsible for the ESBL-phenotype. In addition, we used multilocus sequence typing (MLST) to identify the genetic lineages of all ESBL-producing *E. coli* strains, which were characterized by sequencing the internal fragments of 7 housekeeping genes (*adk*, *fumC*, *gyrB*, *icd*, *mdh*, *purA*, *recA*); the MLST database was used to determine allelic profiles and for sequence type (ST) and clonal complex (CC) assignment.

Among the 35 ESBL-producing strains, MLST analysis revealed 9 different STs under 6 CCs and 9 singletons STs. The CC10 and CC155 were the most common CCs, with 4 and 11 isolates, respectively. Two other isolates were assigned to the CC101. Moreover, 5 strains were included in 3 new STs; 3 of them were identified in a new allele for the *fumC* gene that originated the new ST2528; in addition, 2 isolates were registered as ST2524 and ST2525 through new combination of alleles. Through the MLST database we found that ST656 (CC10) and ST8 (CC165) have a higher homology to ST2524 and ST2525, respectively. However, by the definition of CCs, ST2524 and ST2525 most likely belong to CC10 and CC165, respectively.

Our data shows the presence of ESBL producing *E. coli* isolates in pigs slaughtered for human consumption and raises important questions in the potential risk factors to public health due to the transmission of bacteria carrying resistance through the food chain, and spreading resistance to other bacteria of human clinical significance. A great heterogeneity of MLST types was observed, among which CC10, CC155 and CC101 have already been associated with human clinical isolates.