Shiga toxin producing *E. coli* (STEC) is an important emerging foodborne pathogen, frequently when associated with meat products of bovine origin. In humans, STEC can cause illness ranging from mild to bloody diarrhea, hemorrhagic colitis and hemolytic-uremic syndrome (HUS). Infection by STEC occurs by the ingestion of contaminated food or water, very often with low contaminating doses. Ruminant animals are the major reservoir of STEC what demonstrates the zoonotic potential of this pathogen. STEC from several serotypes has been detected in cattle and associated with human disease like the O113:H21 STEC strains detected in many countries around the world, and including related to HUS. Despite a high frequency in cattle of Rio de Janeiro State (RJ), human cases are not already described. The objective of this study was to compare and evaluate the virulence and genetic relatedness of 12 STEC strains belonging to serotypes O113:H21 (5), OR:H19 (4) and O22:H16 (3) isolated from food and cattle origin in different timelines (1989 – 1995 and 2014 – 2016). The strains were screened for *eae*, *toxB*, *saa*, *pilS*, *iha*, *ehx*, and *subA*, virulence genes. The genetic relatedness was assessed by random amplification of polymorphic DNA (RAPD) and pulsed field gel electrophoresis (PFGE). From PCR assays six virulence profiles were observed, with strains isolated from different periods showing similar virulence markers, pointing to a maintenance of such STEC strains for a long time. Additionally, the same virulence genes (*ehx, iha and saa*) remained the most frequent. The RAPD and PFGE assays revealed genetic similarity between O113:H21 and O22:H16 STEC strains isolated in distinct timelines while a genetic diversity between OR:19 strains was observed. The data suggest that STEC strains associated with human disease remain stable for a long time in the animal reservoir and represent a public health problem.

**Keywords**: Genetic diversity, human disease, STEC serotypes, virulence, zoonosis

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