

**Title:** Occurrence of *stx*<sub>2f</sub> and virulence profiles in disease-associated and animal O145 *E. coli* strains from Brazil.

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**Abstract:**

*Escherichia coli* belonging to serogroup O145 is associated mainly with Shiga toxin producing *Escherichia coli* (STEC) pathotype. However, in Brazil strains of such serogroup from cases of human infection are classified as atypical enteropathogenic *E. coli*, as they harbor only the *eae* gene. As there is a close association between 2f subtype of *stx*<sub>2</sub> gene with O145 STEC strains, Brazilian isolates could harbor the gene of this toxin subtype once it is not sought in the routine characterization of diarrheagenic *E. coli* strains. Alternatively, O145 aEPEC Brazilian strains could represent STEC isolates excised of *stx* genes. This study evaluated the occurrence of *stx*<sub>2f</sub> and other virulence markers in a collection of 23 O145 strains classified as aEPEC, isolated from humans (n = 22) and animal (n = 1). Standard PCR was employed to search for *stx*<sub>2</sub> subtypes, including *stx*<sub>2f</sub>, *bfp*, *ehx*, *espP*, *katP*, *iha*, *toxB*, *astA*, *cdtV*, *terE* and *etpD*, as well as the *fliC* genes H25, H28, H34 and H45. The Z2098, Z2099, *espK*, *espM1*, *espN*, *espV* and *ureD* genes, which are highly specific STEC genotypic markers were investigated by real-time PCR. The allelic types of the *eae* gene were investigated by Sanger sequencing. Of the 23 strains analyzed, two (9%) presented *stx*<sub>2f</sub>, confirming the hypothesis that some O145 strains in our settings are erroneously identified as aEPEC. The only strain from animal source was positive for *stx*<sub>2a</sub>. The *fliC*<sub>H28</sub> gene occurred in 13 (56%) of the strains, while the *fliC*<sub>H34</sub> and *fliC*<sub>H45</sub> occurred in eight (35%) and two (9%) isolates respectively. All O145 *fliC*<sub>H28</sub> strains were positive for *eae* gamma while strains with *fliC*<sub>H34</sub> had *eae* iota, and the two isolates carrying *fliC*<sub>H45</sub> harbored *eae* mi. The other virulence genes searched, except for *bfp*, occurred exclusively in O145:*fliC*<sub>H28</sub> strains. In our settings, O145 *E. coli* are associated with flagellar antigens H28, H34 and H45. Some O145:H34 strains identified as aEPEC represent STEC strains carrying *stx*<sub>2f</sub>. Different allelic forms of the *eae* gene are present in the O145 serogroup, with an allelic specificity occurring as a function of the flagellar antigen. Several virulence markers, including those highly specific to STEC, were observed at high frequencies, but only in O145:H28 strains, suggesting the occurrence of phage excision events preferentially in this serotype.

**Keywords:** *Escherichia coli*, O145 serogroup, Shiga toxin.

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