

Title: Occurrence and characterization of Shiga toxin-producing *Escherichia coli* from human infections in Brazil

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Abstract: STEC is characterized by the production of potent cytotoxins, termed Shiga toxins (Stx) that inhibit protein synthesis in eukaryotic cells. STEC infection can lead to life-threatening complications such as hemorrhagic colitis (HC) characterized by severe cramps abdominal pain and bloody diarrhea, and hemolytic uremic syndrome (HUS), which is characterized by hemolytic anemia, thrombocytopenia and acute renal failure. There are two groups of Stx, Stx₁ and Stx₂, with Stx₂ being more prevalent in HC and HUS. Some STEC isolates, termed EHEC (enterohemorrhagic *E. coli*), can induce the AE lesion, which is characterized by intimate bacterial attachment, localized microvilli destruction, and accumulation of F-actin, resulting in pedestal-like structures on the infected epithelial cells. The main objective of this study was to characterize 29 STEC isolates obtained from cases of diarrhea (bloody or not) and HUS during 2007 to 2014. After the isolation procedures, the STEC isolates were serotyped, and the ability to adhere to and promote cytoskeletal reorganization was investigated in infected epithelial cells (HeLa). Additionally, several virulence factors-encoding genes, such as adhesins and toxins, were also investigated. Four isolates were O157:H7, and four of the top six most important non-O157 serotypes, O26:H11, O111:H8/NM, O103:HNM and O145:HNM were also detected. The *stx*₁ gene was found in 18 (62.0%) isolates and the *stx*₂ found in 11 (31.0%). Two STEC isolates (7.0%) harbor the *stx*₁ and *stx*₂ genes simultaneously, and all the isolates were able to produce Stx. The locus of enterocyte effacement, investigated on the basis of the occurrence of *eae* gene, was found in 18 isolates (62.0%), and all these *eae*-positive STEC were able to produce the localized adherence-like (LAL) pattern, and promote F-actin accumulation underneath adherent bacteria when in contact with HeLa cells. Regarding the additional virulence factors investigated, genes located in the O1-122 appeared as the most prevalent; being the *nleB* and *nleE* detected in 62.0% of the STEC isolates. The genes encoding the autotransporter EspP protein (*espP*) and the EHEC hemolysin (*ehxA*) were present in 51.7% and 24.1% of the isolates, respectively. In conclusion, our data demonstrated the occurrence of STEC isolates as a cause of human infections in Brazil, and also demonstrated the virulence potential of such isolates in adhere and damage host cells.

Keywords: Shiga toxins, attaching and effacing lesion, STEC, diarrheagenic *Escherichia coli*, hemolytic uremic syndrome

Financial support: Fapesp N° 2014/21064-0.