Title: CHARACTERIZATION OF ATYPICAL ENTEROPATHOGENIC *ESCHERICHIA COLI* ISOLATED DURING OUTBREAKS INVESTIGATION OR SPORADIC CASES OF DIARRHEA IN BRAZIL

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Abstract:

Enteropathogenic *Escherichia coli* (EPEC), one of the six pathotypes of diarrheagenic *E. coli*, represent an important agent of diarrheal diseases. Based on the ability to form compact microcolonies (localized adherence) on the surface of infected cells, the EPEC pathotype can be divided in typical and atypical, being the localized adherence (LA) present only in the former group. The main EPEC pathogenicity mechanism comprises the formation of a histopathological lesion termed attaching and effacing (AE), which consists of intimate bacterial attachment to enterocytes, microvilli effacement and the establishment of a structure similar to a pedestal, which is rich in F-actin and other cytoskeletal elements. The purpose of this study was to characterize 88 isolates of atypical EPEC (aEPEC), obtained during outbreaks investigation and sporadic cases of diarrhea occurred during the years of 2012 and 2013, in Brazil. The aEPEC isolates were serotyped (O1-O181 and H1-H56), and the ability to adhere to and promote cytoskeletal reorganization was investigated in infected epithelial cells (HeLa). Additionally, the pathway used to induce AE lesion and intimin subtypes (*eae*) were determined. Among the aEPEC studied, only 13 isolates (14.7%) could be serotyped employing antisera against the classical EPEC serogroups. Bacteria-cell interaction assays demonstrated that 62 isolates (70.5%) were adherent to HeLa cells, being 45 of them able to induce F-actin polymerization, which comprises a main characteristic of the AE lesion. The majority of the aEPEC FAS positive (43 isolates) demonstrated the potential to employ a tyrosine phosphorylated (Y_{474}) dependent pathway in order to induce AE lesions. Curiously, in 14 of these isolates, besides the phosphorylated-Y_{474} residue, the *tccP* and/or the *tccP2* genes, responsible for encoding the Tir-cytoskeleton coupling protein (TccP), were detected, indicating the ability of such isolates to employ an alternative mechanism (Tir-TccP) for AE lesion formation. Although the *eae* gene could not be typed in 26 (29.5%) of the aEPEC isolates, the subtypes theta, epsilon, lambda and beta were identified in 19.3%, 15.9%, 9.1% and 9.1% of the aEPEC isolates studied, respectively. In conclusion, our results reveal a high diversity among the aEPEC studied, probably reflecting the extensive virulence repertoire that could be employed by this group of bacteria in order to damage the host cell and induce diarrheal disease.

Keywords: intimin, attaching and effacing lesion, atypical EPEC, diarrheagenic *Escherichia coli.*

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