TITTLE: ADHERENCE PATTERNS AND ATTACHING AND EFFACING LESION PATHWAYS DETECTED IN ATYPICAL ENTEROPATHOGENIC *Escherichia coli*

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ABSTRACT:

Enteropathogenic *Escherichia coli* (EPEC), one of the six pathotypes of diarrheagenic *E. coli*, can be divided in typical and atypical, based on the presence of the pEAF in typical and the absence in atypical. The main EPEC virulence mechanism is the formation of a histopathological lesion, termed attaching and effacing (AE), which consists of the intimate bacterial attachment to enterocyte cells, microvilli effacement and forming of pedestal-like structures, which are rich in F-actin and other cytoskeletal elements. This study aimed to characterize 82 aEPEC isolates, obtained from outbreaks and sporadic cases of diarrhea, occurred during the years of 2012 and 2013, in Brazil. The adherence and FAS (Fluorescence Actin Staining) assays were performed in HeLa cells, and the determination of the AE lesion pathways was done through the detection of the phosphorylated tyrosine residue 474 (Y474), in the Tir protein, after translocation to the host cells, and PCR for *tir* genotyping and *tccP* (Tir-cytoskeleton coupling protein) genes detection. Regarding the adherence pattern, 12 isolates (14.6%) showed the localized adherence (LA)-like, 3 (3.7%) showed aggregative adherence (AA) and 4 (4.9%) produced a hybrid LA/AA-like pattern, despite the occurrence of 36 isolates with an undefined pattern (43.9%), 26 (31.7%) isolates unable to interact with epithelial cells *in vitro* and one (1.2%) producing cell detachment. Taken in consideration the 55 adherent isolates, 40 of them were able to trigger F-actin accumulation underneath the adherent bacteria (FAS-positive). Among the 82 aEPEC studied, the majority harbored *tir*Y-P (84.1%), suggesting that these isolates can use the Tir-NcK pathway, while 15.9% harbored *tir*S, i.e., they lacked the Y474 phosphorylated residue. The TccP-encoding genes (*tccP* and/or *tccP2*) were detected in 34 isolates (41.5%), 28 of which harbored the *tirY*-P genotype. Analyzing the 40 FAS-positive aEPEC studied, we observed that these isolates could use the Tir-NcK (70.0%), Tir-TccP (2.5%) or both pathways (25.0%) to promote F-actin accumulation during the establishment of the AE lesion. Curiously, one FAS-positive isolate harbored the *tir*S, but lacked *tccP* genes, probably indicating the existence of unknown strategies in the pathogenicity of some aEPEC. Our results reveal the diversity found among the aEPEC isolates studied, reflecting the potential of these isolates to use multiple mechanisms to damage the host cells and cause diarrheal disease.

Keywords: atypical EPEC, AE lesion, diarrheal disease.

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