

TITLE: PHENOTYPIC AND MOLECULAR CHARACTERIZATION OF PATHOGENIC *Escherichia coli* OF SEROTYPE O3:H2 HARBORING VIRULENCE TRAITS OF ATYPICAL ENTEROPATHOGENIC AND/OR ENTEROAGGREGATIVE *E. coli*

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ABSTRACT:

Enteropathogenic (EPEC) and enteroaggregative (EAEC) *Escherichia coli* are two of the major pathotypes of diarrheagenic *E. coli* (DEC) causing disease worldwide. Atypical EPEC (aEPEC) produces the attaching and effacing lesion in infected host cells, leading to the accumulation of F-actin and the formation of pedestal-like structures underneath adherent bacteria. EAEC adheres on epithelial cells forming an arrangement that resembles stacked bricks, known as aggregative adherence (AA) pattern, due to the production of several aggregative adherence fimbriae (AAF/I – AAF/V). Recently, we reported a diarrheal outbreak investigation, in which we obtained 1 aEPEC (*iae*⁺/*bfpB*⁻), 4 EAEC (*aggR*⁺), and 3 hybrid aEPEC/EAEC (*iae*⁺/*bfpB*⁻/*aggR*⁺), with all 8 isolates from serotype O3:H2. Here we attempted to characterize these isolates in terms of *E. coli* phylogroup classification, presence of virulence factor-encoding genes, associated with the pathogenesis of EPEC and EAEC, adherence pattern on HeLa cells, FAS (fluorescent actin staining) test and biofilm formation. All 8 isolates studied were assigned in the phylogroup A. We observed that the EAEC and the hybrid aEPEC/EAEC harbored several genes from the aggregative adherence plasmid (pAA), such as *aatA*, *aap*, *orf3*, *aar*, and *aggC* (encoding the AAF/I usher). On the other hand, the aEPEC and the hybrid aEPEC/EAEC isolates harbored two chromosomal genes, *nleC* and *nleF*, encoding for type 3 secreted effectors. Together, these data may suggest that the aEPEC background must have served as a receptor for the pAA, thus originating the hybrid aEPEC/EAEC isolates. The EAEC and the hybrid aEPEC/EAEC produced the AA pattern, while the aEPEC adhered only sporadically to the epithelial cells. As observed in the FAS assay, the aEPEC was unable to promote F-actin accumulation underneath adherent bacteria, while 2 of 3 hybrid aEPEC/EAEC were FAS-positive. Distinct from the aEPEC isolate, the 4 EAEC and the 3 hybrid aEPEC/EAEC produced biofilm on abiotic surface, thus pointing out the influence of the pAA for the establishment of this phenotype. Our study reveals the high plasticity of pathogenic *E. coli* of serotype O3:H2 in acquiring and maintaining virulence-encoding genes. However, the search for new hybrids is continually needed to better understand which events are involved in the emergence of these hybrids and how different genetic combinations can influence their pathogenicity.

Keywords: hybrid aEPEC/EAEC, diarrhea, virulence

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