TITLE: A NOVEL AUTOTRANSPORTER ADHESIN IDENTIFIED IN AN ATYPICAL ENTEROPATHOGENIC *ESCHERICHIA COLI* OF SEROTYPE O2:H16 CONTRIBUTES TO THE AUTOAGGREGATION PHENOTYPE

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ABSTRACT:
The main virulence property of Enteropathogenic *Escherichia coli* (EPEC) is the formation of the attaching and effacing lesion, which is characterized by microvilli destruction and the formation of pedestal-like structures rich in polymerized F-actin. Based on the presence of the *bfp* operon, responsible for encoding the proteins involved in the biogenesis of a type IV fimbria known as bundle forming pili (BFP), EPEC isolates can be subdivided into two groups: typical EPEC (tEPEC) and atypical EPEC (aEPEC), in which only the former group harbors the *bfp* operon. Previously, we demonstrated the association of some aEPEC serotypes with diarrheal outbreaks in Brazil, among which we can highlight the serotype O2:H16. A comparative gene content analysis of 106 sequenced aEPEC, including 7 aEPEC of serotype O2:H16, revealed a set of 31 genes exclusively detected in this serotype. Among these genes, we identified one responsible for encoding an uncharacterized autotransporter protein (ATP). Therefore, the objective of the present study was to characterize the involvement of this novel ATP in bacterial autoaggregation and biofilm formation phenotypes. To this end, this ATP-encoding gene, from the aEPEC BA92, was amplified by PCR, cloned in the pBAD/Myc-His-A vector, thus generating the pIC recombinant plasmid and transformed in the MS427 (MG1655Δ*agn43*). The novel ATP-encoding gene encodes a 733 amino acid length protein, which belongs to the AIDA-I family. In the autoaggregation assay, performed in static condition using Brain Heart Infusion (BHI) broth supplemented with 0.2% arabinose, the OD_{600} of the MS427(pIC) decreased from 1.0±0.03 to 0.37±0.09 in a range of 2 hours. Distinctly, the OD_{600} from the MS427 and MS427(pBAD/Myc-His-A) remained constant throughout the assay. Biofilm formation assay, performed in polystyrene surface with 24, 48, and 72 hours of incubation and using Dulbecco's Modified Eagle Medium high glucose, did not show a role for this novel ATP for this phenotype. Together, these data support that the novel ATP identified in aEPEC strains of serotype O2:H16 mediates the bacterial autoaggregation phenotype, but not biofilm

formation. Further studies are necessary to understand better if this ATP can mediate epithelial cell adherence and determine the distribution of its encoding genes in distinct pathogenic *E. coli*.
Keywords: autotransporter proteins, atypical EPEC, virulence.

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