TITLE: ANALYSIS OF THE VIRULENCE PROFILE AND PHENOTYPIC FEATURES OF TYPICAL AND ATYPICAL ENTEROAGGREGATIVE *Escherichia coli* (EAEC) ISOLATED FROM DIARRHEAL PATIENTS IN BRAZIL

AUTHORS: Dias, R.C.B.¹; Tanabe, R.H.S.¹; dos Santos, B.C.¹; Vieira, M.A.¹; Elias, W.P.²; Dos Santos, L.F.³; Hernandes, R.T.¹

INSTITUTION: ¹Instituto de Biociências, Departamento de Microbiologia e Imunologia, Universidade Estadual Paulista "Júlio de Mesquita Filho" (UNESP), Botucatu/SP, Brasil, ²Laboratório de Bacteriologia, Instituto Butantan, São Paulo/SP, Brasil, ³Centro de Bacteriologia, Instituto Adolfo Lutz, São Paulo/SP, Brasil

Enteroaggregative Escherichia coli (EAEC) is an important agent that causes acute and persistent diarrhea in children and adults worldwide. EAEC is defined as E. coli isolates that produce the aggregative adherence pattern (AA) in epithelial cells (HeLa and/or HEp-2) cultured *in vitro*. The EAEC pathotype can be divided in typical (tEAEC) and atypical (aEAEC) based on the presence of the aggR gene, which encodes a transcriptional activator, in the former group. The aim of this study was to characterize a collection of EAEC isolates obtained from diarrheal patients over a 7-year period of surveillance (2010-2016). A total of 220 EAEC isolates (193 tEAEC and 27 aEAEC) were evaluated regarding the presence of 20 virulence factor-encoding genes and adherence pattern produced in HeLa cells. In addition, the EAEC isolates were assigned to the distinct *E. coli* phylogroups, using a quadruplex PCR method. The majority of the EAEC isolates were assigned to phylogroups A (44.1%) and B1 (21.4%). Regarding the adherence pattern, we observed that 93.2% produced AA. Moreover, we identified 9 isolates (4.1%) that produced the chain-like adherence (CLA), with 8 of them producing concomitantly the AA pattern, besides EAEC isolates producing an undefined adherence (1.4%) and isolates non-adherent (1.4%). The genes encoding for the major pilin subunit of all five previously described aggregative adherence fimbriae (AAF) were detected among the EAEC isolates studied (50.5%), with agg5A (18.6%) and agg4A (15.0%) being the most prevalent. Among the 20 virulence factor-encoding genes investigated, aap (95.5%), orf3 (89.1%), aar (84.5) and pic (57.7%) were the most prevalent, while eleven of them (agg4A, pic, aap, aaiA, aaiC, aaiG, orf3, aar, air, capU and shf) were statistically more prevalent in typical than atypical EAEC (p<0.05). The afpA2 gene, one of the fifteen genes from the afp (aggregateforming pili) operon, that was recently described in a hybrid EHEC/EAEC, and implicated with the AA phenotype in this strain, was detected in 10 (37.0%) EAEC isolates from our study. Of importance, this gene was exclusively detected in atypical EAEC, and statistically associated with this subgroup of the EAEC pathotype (p < 0.001). In conclusion, we presented an extensive characterization of the EAEC isolates circulating in the Brazilian settings, and identified the afpA2 gene as a putative diagnosis marker for increasing the efficiency of atypical EAEC identification.

Keywords: Enteroaggregative *Escherichia coli*, virulence factors, aggregative adherence fimbriae, adherence pattern, diarrhea.

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