

**Title: Phenotypic and molecular characterization of Enteroaggregative *Escherichia coli* (EAEC) isolated from outbreaks investigations and sporadic cases of diarrhea in Brazil**

**Authors:** dos Santos, B.C.<sup>1</sup>, Dias, R.C.B.<sup>1</sup>, Oliveira, S.L.<sup>1</sup>, Vieira, M.A.<sup>1</sup>, dos Santos, L.F.<sup>2</sup>, Pinheiro, S.R.<sup>2</sup>, Gomes T.A.T.<sup>3</sup>, Elias W.P.<sup>4</sup>, Hernandez, R.T.<sup>1</sup>.

**Institutions:** <sup>1</sup>Departamento de Microbiologia e Imunologia, Instituto de Biociências, Universidade Estadual Paulista “Júlio de Mesquita Filho” (UNESP), Botucatu, SP, Brasil

<sup>2</sup>Centro de Bacteriologia, Instituto Adolfo Lutz (IAL), São Paulo, SP, Brasil

<sup>3</sup>Departamento de Microbiologia, Imunologia e Parasitologia da Universidade Federal de São Paulo (UNIFESP/EPM), São Paulo, SP, Brasil

<sup>4</sup>Laboratório de Bacteriologia, Instituto Butantan, São Paulo, SP, Brasil

**Abstract:** Enteroaggregative *Escherichia coli* (EAEC), one of the six pathotypes of diarrheagenic *E. coli*, is defined based on the production of the aggregative adherence (AA) pattern in cultured epithelial cells *in vitro*. EAEC are recognized as agents of acute and persistent diarrhea in children and adults, and also as an important agent of outbreaks of diarrhea worldwide. Furthermore, EAEC infections have been associated with growth and intellectual shortfalls, especially in malnourished children from developing countries. EAEC strains colonize the intestinal mucosa via the aggregative adherence fimbriae (AAFs), which include at least five major antigenic variants that are transcriptionally regulated by an activator, called AggR. Several potential virulence factors have been described in EAEC; however the pathogenesis of the diarrhea caused by this DEC pathotype has not been fully understood. Many of the major virulence factors associated with EAEC are encoded by genes located on virulence plasmids, and detection of these genes has been routinely used in the diagnosis of this pathotype. The main aim of this study was to characterize 69 EAEC isolates, regarding their ability to adhere to HeLa cells, and the presence of important virulence factors-encoding genes. The EAEC pathotype was defined, in the present study, as *E. coli* isolates that harbor the *aatA* gene, located in the EAEC plasmid (pAA), and previously employed as a probe for EAEC diagnosis. The majority among the 69 EAEC isolates studied (91.3%) was *aggR* positive, thus classified as typical EAEC. Regarding the adherence pattern, 46 (66.7%) isolates were able to produce the characteristic AA pattern in 3 or 6 hours assays. Curiously, 6 isolates produced the chain like-adherence (CLA), and in 5 of them the AA pattern was detected simultaneously. Among the AAF-encoding genes investigated, the *agg5A* and *agg4A* were the most prevalent, and detected in 23.2% and 14.5% of the EAEC isolates. The *aafA* and *agg3A* were detected in the same frequency (7.2%) and the *aggA* identified in only 5.8% of the isolates. Toxin encoding genes were also detected: *pic* was present in 56.5% and *pet* and *sigA* were equally detected (4.35%). Our study indicates the prevalence of typical EAEC in sporadic and epidemic cases of diarrhea in our population and points out the contribution of AAF/IV and V as important virulence factors.

**Keywords:** Enteroaggregative *Escherichia coli*, aggregative adherence, diarrhea, outbreaks

**Financial support:** Fapesp Nº 2014/17286-7