TITLE: MOLECULAR CHARACTERIZATION OF CELLULAR DETACHMENT BY ATYPICAL ENTEROPATHOGENIC *Escherichia coli* (aEPEC)

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ABSTRACT: The term atypical enteropathogenic *Escherichia coli* (aEPEC) is used to define EPEC strains that do not carry the EAF plasmid. aEPEC constitutes an important pathotype of *E. coli* involved with diarrheal disease in Brazil and in other countries. Serine Protease Autotransporters of *Enterobacteriaceae* (SPATEs) have been identified in pathogenic *E. coli*, and implicated as accessory factors of virulence, including adhesion, autoaggregation, invasion, biofilm formation and cytotoxicity. SPATEs that display cytotoxic activity include EspC, EspP, Pet, Sat and SigA. In a previous study, we identified a group of five aEPEC isolates that exhibit cytotoxic phenotype, characterized by cellular detachment after 3 hours of contact with the bacterium. The strains were evaluated for hemolysin and none of them showed hemolytic activity, through the formation of hemolysis halos after 24 h of incubation on blood agar plates. In this study, we aimed to identify the genetic determinants involved in the cellular detachment phenotype of these strains. The PCR assays showed the presence of *espC* in one strain, and *espP* and *sat* in other strain. Plasmid content analysis showed the presence of plasmids with variable molecular weights in four of the five strains. Three strains were resistant to ampicillin and kanamycin, and one strain was resistant only to ampicillin; all of these strains harboring F-pili genes (*traA*). We performed conjugation experiments with *traA*-positive A99 strain, carrying only one large plasmid and the plasmidless non-adherent *E. coli* MA3456 strain. The resulting transconjugants were tested for adherence to HeLa cells, and a transconjugant (Tc) resistant to kanamycin and nalidixic acid harboring the large plasmid and the cellular detachment phenotype was identified. In attempt to identify genes involved in cytotoxic phenotype, we randomly mutagenized Tc strain with the kanamycin resistance (Km^r)-encoding transposome EZ::TN<R6Kyori/KAN-2> Tnp. Analysis of mutants for cellular detachment led to the identification of four mutants adherent to HeLa cells. Cloning experiments are underway to identify the DNA sequence flanked by the transposon insert. Furthermore, conjugation experiments with the other *traA*-positive strains are underway.

Keywords: Atypical Enteropatogenic *E. coli* (aEPEC), SPATEs, cellular detachment

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