TITLE: THE POLYMORPHISMS IN THE SECRETED PROTEIN ESPD COULD AFFECT THE EFFICIENCY OF ATYPICAL ENTEROPHATOGENIC *Escherichia coli* (aEPEC) TO ADHERE TO EPITHELIAL CELLS

AUTHORS: SANTOS, F. F.[1], YAMAMOTO, D.[1], BRYANT, J.[2] HERNANDES, R. T. [3], ELIAS, W. P. [4], PIAZZA, R. M. F.[4], HENDERSON I. R. [2], GOMES, T. A. T.[1]

INSTITUTIONS: [1]UNIFESP – UNIVERSIDADE FEDERAL DE SÃO PAULO, DEPARTAMENTO DE MICROBIOLOGIA, R. BOTUCATU, 862, 3° ANDAR, VILA CLEMENTINO, SÃO PAULO, SP; [2]INSTITUTE OF MICROBIOLOGY AND INFECTION, COLLEGE OF MEDICAL AND DENTAL SCIENCES, UNIVERSITY OF BIRMINGHAM, EDGBASTON, BIRMINGHAM, B15 2TT, UK, [3]UNESP – UNIVERSIDADE ESTADUAL PAULISTA, DEPARTAMENTO DE MICROBIOLOGIA E IMUNOLOGIA, INSTITUTO DE BIOCIÊNCIAS, BOTUCATU, SP; [4]IB, INSTITUTO BUTANTAN, LABORATÓRIO DE BACTERIOLOGIA, AVENIDA VITAL BRASIL,1500, BUTANTÃ, SÃO PAULO, SP.

ABSTRACT:

In the atypical enteropathogenic *Escherichia coli* (aEPEC) pathotype, the protein intimin is the main adhesin involved in intimate adherence to epithelial cells. Nevertheless, other secreted proteins could contribute to aEPEC adherence at the beginning of the colonization process, such as EspD, that binds in the host cell membrane. This study aimed to investigate whether EspD, one of the proteins of the bacterial Type Three Secretion System (T3SS)-translocon, plays a role in the initial adherence process of aEPEC to epithelial cells. In addition, we investigated whether the EspD polymorphisms could result in differences in the bacterial adherence efficiency. Intimin insertion mutants were constructed in eight aEPEC strains, using the suicide vector pJP5603, and tested in HeLa cells regarding any modification in their adherence efficiency. Two strains were then selected, one of which was non-adherent (BA4095) while the other remained adherent (2012-1) ($p>0.05$). The latter strain was thus used to generate an *eae/escN* (encoding intimin and the T3SS ATPase, respectively) double mutant and an *espD* single mutant, employing the Lambda Red recombineering system. Next, *espD* sequences of the 2012-1 and BA4095 strains were obtained and translated into amino acid sequences, which were compared with each other and with the corresponding sequences of typical EPEC prototype E2348/69 and *Escherichia albertii* 1551-2 strains, using ExPasy and Clustal Omega. It was previously shown that a derivative mutant of tEPEC E2348/69, which lacks the BFP fimbria and intimin, became non-adherent, while the 1551-2 intimin mutant strain remained efficiently adherent. Both the *eae/escN* and *espD* mutants of aEPEC 2012-1 were non-adherent, thus suggesting that EspD contributes to the adherence of this strain**.** The preliminary analyses of the two EspD transmembrane domains (around 20 aa each), which are important to anchor this protein in the epithelial cell membrane, showed that there are only three identical amino acids between the BA4095 and E2348/69 strains, which were different from those present in the same sequence positions of the 2012-1 and 1551-2 strains. Interestingly, these amino acids differ on their polarity. Our findings suggest that specific types of EspD could play an additional important role as an adhesin at the beginning of the colonization process. Furthermore, specific polymorphisms could render certain aEPEC strains more efficient intestinal colonizers.

KEYWORDS: Adhesion, aEPEC, EspD, Polymorphism and Type 3 Secretion System (T3SS).

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