Title: COMPARATIVE ANALYSIS OF THE TYPE THREE SECRETION SYSTEM TRANSLOCON VARIANTS AND THEIR INFLUENCE IN THE EARLY ADHERENCE OF ATYPICAL ENTEROPATHOGENIC ESCHERICHIA COLI TO HELA CELLS

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Abstract:
The number of atypical Enteropathogenic *Escherichia coli* (aEPEC) infections has increased in developing countries; thereupon detailed knowledge on their virulence mechanisms is required. Previously, we showed that the Type III Secretion System (T3SS) translocon (EspA, EspB and EspD proteins) of aEPEC 1551-2 mediates early bacterial adherence to HeLa cells. The aim of this study was to analyze these proteins in aEPEC strains concerning their sizes, nucleotide sequence and influence in bacterial adherence to cultured cells. Protein sizes were calculated in ten strains by SDS-PAGE and Immunoblotting using the ABEletro software v.1.0. The sizes of EspB and EspD of eight strains differed from those of the 1551-2 and typical EPEC E2348/69 strains. To investigate the influence of the T3SS translocon in bacterial adherence, single Intimin (*eae*) mutant strains were obtained so far from three strains (BA4095, 3991-1 and 2012-1), which were analyzed in HeLa cells regarding any modification in their adherence efficiency (Student-t test) and ability to produce attaching and effacing lesions (A/E) as examined by Fluorescent Microscopy. Only the 2012-1 mutant remained adherent, producing a diffuse adhesion pattern, although unable to form A/E lesions. The adherence of this mutant was quantitatively similar to that of the wt strain ($p > 0.05$). Consequently, *escN* (the ATPase of the T3SS) and *eae*/*escN* double mutants were generated which were non-adherent, suggesting that the T3SS plays a role in the early adherence of the wt strain *in vitro*. In an attempt to sequence the *esp* genes in the three strains, primers based on the sequences of the following strains were used: E2348/69 (*espA*, *espB* and *espD*). Enterohemorrhagic *E. coli* EDL933 (*espB* and *espD*) and the aEPEC O26:H- (*espB*).The E2348/69 primers amplified *espA* in all, *espB* in one (BA4095) and *espD* in two (BA4095 and 2012-1) strains. The EDL933 primers amplified *espD* in two strains (2012-1 and 3991-1) and *espB* in none. The O26 primers amplified *espB* in the 2012-1 strain. These results reflect the genetic variability of *espB* and *espD,* which will be confirmed by the sequencing analyses that are in progress. Although the EspB and EspD sizes differ among strains, these proteins might carry an essential motif favoring a more efficient adherence. Alternatively, further studies comparing the amount of EspB and EspD production among strains could allow better understanding of the T3SS translocon mediated adherence in aEPEC.

Keywords: aEPEC, EspA, EspB, EspD, Type III Secretion System (T3SS), translocon, adhesion and polymorphism.

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