Title: Characterization of genes involved in the aggregative adhesion phenotype of atypical enteroaggregative *Escherichia coli* (EAEC)

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Introduction: Enteroaggregative *Escherichia coli* (EAEC) is defined by a characteristics "stacked-brick" aggregative adherence (AA) pattern to cultured cells. In typical EAEC strains, the AA phenotype requires aggregative adherence fimbriae (AAFs) encoded on a large plasmid called pAA. However, previous studies suggest known AFF alleles are not found in all EAEC strains. In a previous study, we found a high prevalence of EAEC pAA-negative strains in quilombola children with and without diarrhea. In this study, we evaluated the presence of EAEC-specific genes among these strains.

Methods. Thirty-two strains were tested by PCR for the presence of adhesin genes related to the AA phenotype. Insertion mutagenesis was performed by using the EZ::TN<R6K*ori*/KAN-2>Tnp transposome (Epicentre), and transposon-inserted bacterial colonies were screened for their adhesion phenotype to HeLa cells.

Results. All strains were PCR negative using primers specific to *aggR* (regulator), *aap* (dispersin), *aggA* (AAF/I), *aafA* (AAF/II), *agg3C* (AAF/III), *hdaA* (AAF/IV), *ap58* (outer membrane protein), and *pilS* (type IV pili) genes. In order to identify a gene responsible for the AA phenotype, we performed transposon mutagenesis on three selected strains (Q010, Q015, and Q212) and screened for adhesion-defective mutants. Among 3,000 transposon-inserted mutants screened, 16 mutants (3 Q010 mutants, 3 Q212 mutants, and 10 Q015 mutants) that did not adhere to HeLa cells were isolated. Transposon insertion sequences of mutants revealed similarity with biosynthetic arginine decarboxylase (one Q015 mutant, and one Q212 mutant), serine endoprotease (one Q010 mutant), surface antigen family protein (one Q015 mutant), stbA family protein (one Q212 mutant), fis family transcriptional regulator (one Q212 mutant), shikimate kinase (one Q010 mutant), threonine synthase (one Q010 mutant), hypothetical protein (one Q010 mutant), and mannosyltransferase/hypothetical protein (one Q010 mutant). Transposons in seven Q015 mutants were found to have inserted into different sites of the same 4,380-bp open reading frame (ORF) encoding a 1,460-amino-acid polypeptide. Blast analysis revealed that the ORF was highly similar to filamentous hemagglutinin family N-terminal domain protein of *E. coli.*

Conclusion. Our data point toward an involvement of the filamentous hemagglutinin in the AA phenotype of QO15 strain. Further studies are under way to address this question.

Keywords: Enteroaggregative *E. coli* (EAEC)**,** atypical EAEC; adherence of atypical EAEC.

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