

TITLE: CHARACTERIZATION OF VIRULENCE FACTORS PRESENT IN AN AGGREGATIVE-ADHERENT *ESCHERICHIA COLI* ISOLATED FROM URINARY TRACT INFECTION

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ABSTRACT

Uropathogenic *Escherichia coli* (UPEC) is the main agent of human urinary tract infections (UTI), while enteroaggregative *E. coli* (EAEC) comprises an important diarrheagenic pathotype characterized by the ability to produce the aggregative adhesion (AA) in epithelial cells. In a previous study, our group identified an UPEC strain (UPEC 46) producing the AA pattern and carrying some EAEC genetic markers (*aatA*, *aap*, *astA*, *pet* and *irp 2*). In the current study, we further characterized UPEC 46 in terms of genotypic and phenotypic properties. For this purpose, whole genome sequence was obtained by the Illumina HiSeq 1500 platform and *in silico* prediction of known virulence factors was performed using the *ecoli* VF collection database. Adhesion assays were performed using HEK-293 and 5367 cells (3 or 6 h of bacteria-cells incubation) in the presence or absence of 2% D-mannose. The production of type I fimbriae was assessed by means of *Saccharomyces cerevisiae* agglutination. Expression of Curli fimbriae was verified on Congo red agar plates; Pet toxin production was determined by immunoblotting and the hemolytic capacity was evaluated on sheep blood agar plates. Also, UPEC 46 was tested in a serum bactericidal assay using normal human serum (NHS). A total of 129 *E. coli* virulence associated genes were identified in the genome of UPEC 46: 83 associated with adhesion (e.g. Curli, Type I fimbriae and *E. coli* common pilus) and invasion (e.g. *ibeB* and *ibeC*), 30 with iron uptake, and 8 with serum resistance (*iss* and genes associated with group 4 capsule). Also, 6 autotransporter protein-encoding genes (*pet*; *aatA*, or APEC autotransporter adhesin A; *ehaC*; *ehaD*; *upaC*; *upal*) and 3 toxin-encoding genes (*astA*, *hlyE* and colicin E1) were found. In the adhesion assay, the AA phenotype was observed in bladder and kidney cell lines, in the presence or absence of D-mannose. Type I fimbriae and Curli expression were detected, as well as Pet toxin production. Hemolysin production was negative after 24 h incubation. UPEC 46 was able to resist in 50% NHS with a survival rate around of 80%. In summary, our data provide a more detailed understanding of the genotypic and phenotypic background of UPEC 46, defining it as an EAEC strain that was able to cause UTI.

Keywords: UPEC; EAEC; whole genome sequence; virulence factors

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