MULTIPLEX PCR TO DETECTION OF DIARRHEAGENIC *Escherichia coli*

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Considered the second world cause of infant mortality, diarrheagenic disease is considerate as a neglected disease, especially in prolonged microbiological diagnosis. This disease is caused by a range of pathogens like diarrheagenic *Escherichia coli* (DEC), including the pathotypes: enterotoxigenic *E. coli* (ETEC), enteroinvasive *E. coli* (EIEC), enteroaggregative *E. coli* (EAEC), Shiga toxin-producing *E. coli* (STEC) and enteropathogenic *E. coli* (EPEC), differing from each other by the virulence factors, resulting in different forms of aggression of human enterocytes. Taking these together our aim was to develop a reaction of Multiplex Polymerase Chain Reaction (mPCR) to detect DEC, making the most simple and effective diagnosis to this disease. For development, we used bioinformatics program (MPprimer 2.0), where we designed oligonucleotide primers specific to each *E. coli* pathotypes: *bfpA* and *escV* (EPEC), *elt* (ETEC), *aaiC* (EAEC), *ipaH* (EIEC) and *stx1* and *stx2* (STEC). Standardization of the method was performed in a thermocycler (Applied Biosystems®) using PCR reagents (Invitrogen®) and total DNA of reference strains for each pathotype. Amplification temperatures were performed between 58º-62ºC. The reaction was standardized into three distinct systems constituted by the following primers: Reaction 1: *aaiC*, *escV* and *bfpA;* Reaction 2: *stx1* and *ipaH*; Reaction 3: *stx2* and *elt.* PCR products were analyzed in 2.5% agarose gel eletrophoresis stained with Syber SAFE (Invitrogen®). The amplification sizes suitable for diagnosis: *aaiC* (183pb), *escV* (266pb), *bfpA* (478pb), *stx1* (130pb), *ipaH* (393pb), *stx2* (346pb) and *elt* (529pb). In conclusion, this mPCR system can be an useful method for the diagnosis of DEC, requiring validate this method with the strains isolated from stools and foods.

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KEY WORDS: Multiplex PCR, diarrhea, diarrheagenic *Escherichia coli*.