Austin Journal of Clinical Immunology

Austin Publishing Group

Open Access Full Text Article

Editorial

Detection of the Enteroaggregative Heat-Stable Enterotoxin 1 Nucleotide Sequences among Diarrhegenic Escherichia coli

Shabana II*

Department of Bacteriology, Immunology and mycology, Suez Canal University, Egypt

***Corresponding author:** Shabana II, Department of Bacteriology, Immunology and mycology, Suez Canal University, Egypt, Email: imanibrahim50@yahoo.com

Received: February 11 2014; **Accepted:** February 14 2014; **Published:** February 18 2014

Keywords: *E. coli*; **astA**

Escherichia coli strains are important causes of diarrhea in human and animals **[1].** *E. coli* isolates associated with enteric diseases have been classified into major categories, on the basis of their distinct virulence properties: ETEC, EPEC, EHEC, EIEC and EAEC **[2]**. Enteroaggregative *Escherichia coli* (EAggEC) heatstable enterotoxin 1 (*EAST1*) is a 38-amino- acid protein **[3].** It was originally found as an enterotoxin of EAggEC **[4].** Consequently, it is not clear if production of this toxin was relevant to the manifestation of diarrhea due to EAggEC. EAST1 gene, or its variants, were present not only in EAggEC but in other diarrheagenic *E. coli* including some EPEC and ETEC **[5,6].** Recently it detected additionally in DAEC and EHEC **[7].**

A total of 76 E. coli strains isolated from human and calves with diarrhea were tested for the presence of virulence genes including shiga toxins 1 and 2, intimin, aggR, hemolysin, heat labile toxin, heat stable toxin, F5 and F5 genes and the Enteroaggregative E. coli heat-stable enterotoxin 1 gene (EAST1) by PCR, The EAST1 gene was found in most human strains accompanied with LT and ST gene in Enterotoxigenic strains (O6:H16), aggR (Fimbrial antigen-specific gene) in Enteroaggregative strains (O126:H27) and with shiga toxins and intimin genes in Enterohemorraghic strains (O157:H7 and O26:H11). Only 4 bovine strains (3 Enterotoxigenic strains and 1 enteropathogenic strain) with adherence factor F5 (K99) were also positive for the EAST1 gene, therefore the study concluded that the presence of EAST1 gene not restricted to Enteroaggregative *E. coli* but also present in other pathotypes in combination with certain virulence marker **[7].**

The amplified astA PCR products then purified using a MSB1 Spin PCRapace kit (Invitek GmbH, Germany) according to the instructions of the manufacturer. Sequencing was per-formed using Big Dye Terminator v3. 11. Briefly, 1 ml purified PCR product was added to 1 ml Big Dye terminator, 1 ml of 5-fold-concentrated buffer and 1.3 ml single primer, and then completed to 19 ml using nuclease free water (Promega), and subjected to amplification in a thermal cycler (Applied Biosystems) under the following condi-tions, 96 8C (30 s), 96 8C (10 s), 50 8C (5 s), 60 8C (4 min). The labeled products were then analyzed by an ABI Prism1 3100 Genetic Analyzer (Applied Biosystems, USA). Sequences were edited and assembled using SeqMan Pro software included in the DNASTAR Laser gene package (Bioinformatics pioneer DNASTAR, Inc., Wisconsin, USA).

To determine the relationships among different STs Then the sequences were aligned with Bio Edit version 7.0.9. (http://www.mbio.nc-su.edu/Bio Edit/bioedit. html) **[8].**

The accession numbers of the nucleotide sequences for this study are HM099887-98 in the Gen Bank Database.

The nucleotide sequences of the EAST1 genes were almost identical among ETEC and EPEC and EAEC While for STEC only 1 base at the 31st codon (ACT replaced by AAT), resulting in a deduced amino acid change Threonine (T) to Asparagine (N) (Figure 1).To confirm the 31st-codon sequence (ACT), the nucleotide sequences upstream from the EAST1 gene were highly conserved among the strains. In contrast, the nucleotide sequences downstream from the

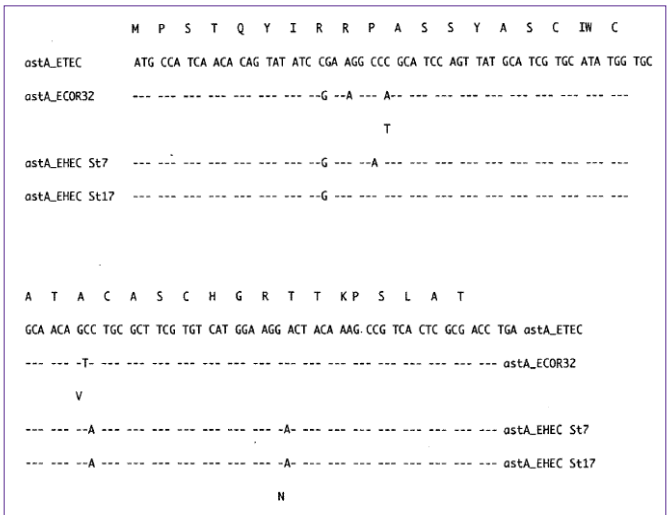

Figure 1: Only one base change at the 31st codon (ACT replaced by AAT), resulting in a deduced amino acid change Threonine (T) to Asparagine (N).

EAST1 gene were somewhat divergent between the ETEC and STEC strains, Enteroaggregative Heat-Stable Enterotoxin 1 supposed to be associated with various categories of diarrhea-associated *E. coli*, and its sequence is conserved among different diarrhea-associated *E. coli* pathotypes.

References

1. Holland RE. Some infectious causes of diarrhea in young farm animals. Clin Microbiol Rev. 1990; 3: 345-375.

Citation: Shabana II. Detection of the Enteroaggregative Heat-Stable Enterotoxin 1 Nucleotide Sequences among Diarrhegenic Escherichia coli. Austin J Clin Immunol. 2014;1(2): 1009.

2. Nataro JP and Kaper JB. Diarrheagenic Escherichia coli. Clin Microbiol Rev. 1998; 11: 142-201.

3. Savarino SJ, Fasano A, Robertson DC, Levine MM. Enteroaggregative Escherichia coli elaborate a heat-stable enterotoxin demonstrable in an in vitro rabbit intestinal model. J Clin Invest. 1991; 87: 1450-1455.

4. Savarino SJ, Fasano A, Robertson DC, Levine MM. Enteroaggregative Escherichia coli elaborate a heat-stable enterotoxin demonstrable in an in vitro rabbit intestinal model. J Clin Invest. 1991; 87: 1450-1455.

5. Yatsuyanagi J, Kinouchi Y, Saito S, Sato H, Morita M. Enteropathogenic Escherichia coli strains harboring enteroaggregative Escherichia coli (EAggEC) heatstable enterotoxin-1 gene isolated from a food-borne like outbreak. J Jpn Assn Infect Dis. 1996: 70: 73-79.

6. Yamamoto T, Wakisaka N, Sato F, Kato A. Comparison of the nucleotide sequence of enteroaggregative Escherichia coli heat-stable enterotoxin 1 genes among diarrhea-associated Escherichia coli. FEMS Microbiol Lett. 1997; 147: 89-95.

7. Shabana II, Algammal AM and Suzuki H. Molecular typing of the enteroaggregative E. coli heat-stable enterotoxin 1 gene (EAST1) in E. coli strains isolated from human and calves with diarrhea. 2013: 1: 1128-1138.

8. Hall TA. BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. Nucleic Acids Symp. Ser. 1999; 41: 95–98.

Austin J Clin Immunol - Volume 1 Issue 2 - 2014
ISSN : 2381-9138 | www.austinpublishinggroup.org

Citation: Shabana II. Detection of the Enteroaggregative Heat-Stable Enterotoxin 1 Nucleotide Sequences among Diarrhegenic Escherichia coli. Austin J Clin Immunol. 2014;1(2): 1009.