Review Article

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Pathogenesis and Vaccines against Enterotoxigenic Escherichia Coli

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ABSTRACT

BACKGROUND AND OBJECTIVE: Enterotoxigenic Escherichia coli (ETEC) is the most common causes of diarrhea and its mortality is estimated at 157,000 deaths per year that equivalent 9 percent of all deaths attributed to diarrhea. Vaccine development against ETEC has been identified as an important primary prevention strategy against bacteria. Present study aims to provide importance of and pathogenesis of ETEC bacteria and research in the field of vaccine against it.

METHODS: In this study literature search was conducted in NCBI databases with ETEC, virulence factors, colonization factors, enterotoxins and vaccine as keywords. Related articles were collected and investigated.

FINDINGS: Survey of articles indicated that mucosal immunization could provide the secretary IgA antibody (sIgA) response and are of particular importance for protection against ETEC infection. Diversity and geographical distribution of bacterial strains is one of the main problems to deal effectively against disease caused by ETEC. One of the important points is expressing of different CFs on bacterial surface. Development of a vaccine against bacteria and toxins strains is dependent on careful investigation of the prevalence of colonization factors and toxin strains.

CONCLUSION: Scrutiny could reduce information gaps and expected to help appropriately for development strategies against bacteria. Design multivalent ETEC immunogens containing the most prevalent colonization factors and toxins may provide protection against a wide range of ETEC strains.

KEY WORDS: *Diarrhea, Pathogenic Escherichia coli, Enterotoxigenic E.coli, Virulence factors,Mucosal immunity, Vaccine.*

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Introduction

Diarrhea caused by bacteria and viruses is one of the most important health problems in human societies, especially in developing countries, and causes the deaths of hundreds of thousands of people, including children (1-3). Escherichia coli producing diarrhea in many parts of the world is widespread and is divided into six groups of enterogenic, enterohaemorrhagic, entero-enteric, enteropathogenic, diffuse and enterotoxicogenic (ETEC) based on antigenic differences and pathogenic mechanism (4-6). The prevalence of ETEC-induced diarrhea, especially in deprived areas and those who travel to such areas, is high (7, 8).

In 1956, De et al, showed that Escherichia coli isolated from people with cholera diarrhea could cause liquids and electrolytes to accumulate in closed loops of rabbit intestines (9). ETEC bacteria account for 15% to 20% of diarrhea in children under 5 years old in the poorest countries and the most common cause of 60% of travel diarrhea in Africa, Asia and Latin America (10, 11). In many of these countries, the prevalence in infants below the age of 12 months has been reported to be much higher (10-12). About 10 million of ETEC-induced diarrhea is reported annually in the world. In the reports of the world's health organizations, the estimated human casualties caused by ETEC diarrhea are estimated to be around 157,000 per year, which is roughly equivalent to 9% of deaths from diarrhea (11, 13, 14). In 2013, 42,000 children under the age of 5 were reported to die due to diarrhea caused by ETEC. Over the same year, more than 89,000 people over the age of 5 were reported to die in Africa and South Asia (1, 11). According to these reports, the prevalence of bacteria in communities over the age of 5 years has reached 44 million, which is much higher than that of the 6 million people with typhoid and 3 million with vibrio cholera (1, 10, 11).

The main problem with the ETEC disease is the diversification and geographical distribution of bacterial strains. The development of a vaccine against bacteria is dependent on the precise examination of the prevalence of colonization and toxin factors in the strains. However, the epidemiological studies that have been done so far make researchers more aware of the ETEC burden index, however, there are still some inaccuracies in information about mortality, bacterial outbreak, and diagnosis (1, 3, 15, 16). The present study addresses the importance of ETEC and its pathogenicity, the information and the results of research on vaccine against it.

Methods

The electronic search of articles in the NCBI database was performed using the key words of enterotoxicogenic E. coli, acute factors, colonization factors, enterotoxin, immunity and vaccine. The titles and abstracts of the articles presented in the database were reviewed and the abstracts of the articles were examined in terms of thematic relation. Studies based on the pathogenicity, disease outbreaks and immunity and Vaccines against ETEC were selected and studied. Also, the authors of this paper have also used the results of the research.

Results

The word "Escherichia coli" was found in the title, abstract, and key words of 3757 articles in the Pubmed database. By limiting the search using Boolean operators (And, Or, Not) to articles related to pathogenesis, disease outbreaks and immunity and vaccination against bacteria, the number of articles reached 75, which entered the study.

Acute Causes: Swallowing disease begins with 106 to 1010 bacteria, and then the bacteria binds to the epithelium of the intestine through its surface colonizing factors (8, 17, 18). The main factor for ETEC actinicity is the susceptible or heat-resistant enterotoxin (19-21). ETEC strains can produce only ST, LT toxin or both (Figure 1).

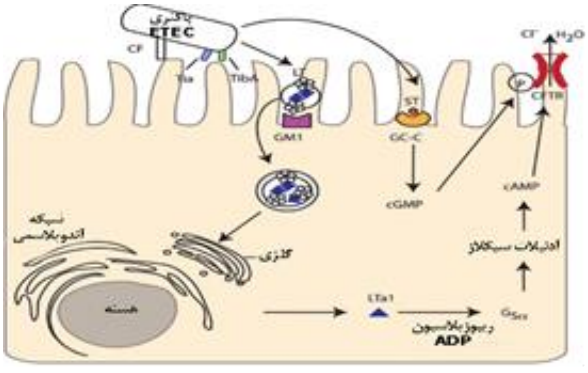


Figure 1. The mechanism of the disease by enterotoxicogenic E. coli (27).

In addition to enterotoxins, other acute factors such as adhesion factors, colonization and aggression factors are also present (22-24). Type II (LT-1) and II (LT-II) heat-sensitive enterotoxins are different in terms of genetic, biochemical and immunological characteristics. The LT-1 toxin is very similar to chlorotoxin, with a weight of 86 kilo daltons hetero

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hexamery is a molecule composed of Pentamer subunit B and a subunit A (Fig. 2) (26, 25).



Figure 2. Heat-sensitive toxin structure of Escherichia coli and subunits A and B (30)

Subunit B binding to GM1 ganglioside on the host cell surface causes endocytosis of toxin. Subunit A, by preventing GTPase activity of Gs proteins increase cAMP and stimulate chlora channels in the membrane. Diarrhea develops by secretion of electrolytes and water into the intestine (17, 18, 27). LT-II toxin with chlora toxin does not have an immunological cross-reactivity. The similarity of its amino acid sequence with chlora toxin and LT-1 is less than 14% (28, 25). The importance of type 2 toxin in human pathogenesis is not known (28, 25). Heat-resistant enterotoxin ETEC is a small amount of cysteine-rich peptides (Fig. 3) which, by increasing intracellular cGMP, disrupts the function of ionized channels, resulting in epithelial cells lose water and the patient gets diarrhea (29, 30).

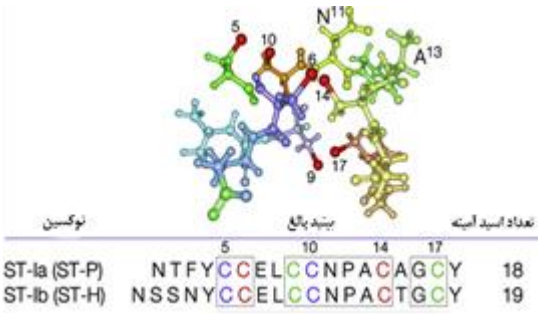


Figure 3. Heat-resistant toxin structure and sequence of bacteria from Escherichia coli (22).

Heat-resistant enterotoxin type I and II are produced by ETEC. The STI (STa) toxin, which is attached to Guanylian cyclase, is itself divided into ST-Ia (ST-P) and ST-Ib (ST-H) types (18, 30). One of the most important pathogens in enterotoxicogenic E. coli bacteria is colonization factors (CFs) and are considered as targets for vaccine preparation (31, 32). About 25 different CF types are known today, most of which are coded by plasmids (1, 5). The colonization factors are the protein content and are classified into three different groups. The first group or quasi-CFA / I group is CFA / I, CS1, CS2, CS4, CS14, and CS17. The second group or quasi- CS5 group is CS4, which includes the CS20, CS18, CS7, CS5 connection factors. Third non-matched group includes CS10-12, CS6, CS3 (34, 33, and 5). Most of the ETCI colonization factors are collected through the chaperone-guide and are presented on the surface of the bacteria (Fig. 4).

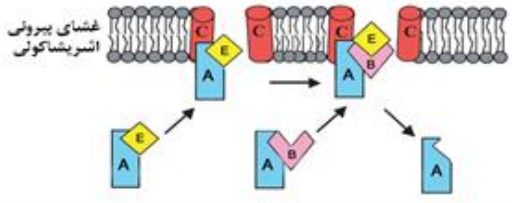


Figure 4. A schematic model for assembling the ETEC colonizing agents. Chaperon: A, subunit: B, external membrane protein: C, subsidiary sub unit: E (37).

One of the notable points is the presentation of some colonization factors on the bacterial cell. For example, CS5 + CS6, CS4 + CS6, CS1 + CS3 and CS2 + CS3 dual patterns can be presented on some of the strains. 30 to 50 percent of the ETEC strains isolated from patients do not have specific colonization factors (15, 33, 35).

Safety and Vaccine: The development of a vaccine against bacteria is dependent on a thorough examination of the prevalence of colonization and toxin factors in the strains. Due to its neighbors with countries where the health index is very low, Iran is at the risk of the spread of such diarrhea pathogens. Accordingly, the importance of ETEC in diarrhea in Iran has also been studied (36).

Studies in Iran show a 5 to 8 percent increase in ETEC in diarrhea samples (6). The prevalence of ST and LT toxins has varied in different studies, so that, in separate cases, more strains of LT, strains producing ST toxin are mentioned. In some reports, the more frequent strains of generative LT-ST are mentioned (6, 37-40). However, in regions of East Asia and Latin America, strains of ST toxin have a higher percentage (15, 41). The return and recurrence of ETEC-induced diarrhea decreases with age, which confirms the natural acquired immunity against bacteria after infection (1, 3, 12, 14). Effective immunization against this intestinal pathogen stimulates the secretion of mucosal antibodies. Therefore, evaluation of mucosal immune responses in immunogenicity testing of various vaccines against this bacterium is important. A study in volunteers showed that there is a relationship between the level of mucosal responses to CFs and the protection against the bacteria that are responsible for. Also, the serum antibody level against LT is dependent on the degree of protection (7,

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35, 42). Various antigens, including colonization factors and LT and ST toxins, are considered in the preparation of vaccine (3, 14, 43). O and H antigens can also play a role in protecting against ETEC, but they are not suitable candidates due to the many structures and variations (1, 34). Studies have shown that the use of colonization and toxoid agents can lead to acceptable immunity in humans. Based on the efficacy of the CF and LT antigen, the ETEC vaccine should contain CF antigens found in most strains. Multivalent vaccine containing CFA / I, CS1-6 and LT toxoid can protect against about 80% of the strains. Due to the fact that some strains have the ability to produce ST toxin, ST toxoid should also be considered. However, having ST that is not toxic and immunogenic is difficult (1, 3, 16, 44). So far, various methods for presentation of CF and toxin antigens to the human immune system have been investigated in order to provide protective immune responses against vaccine candidates.

Pure enterotoxins and CFs: Pure CFs are not as effective as oral immunogens because they are sensitive to proteolytic degradation. To prevent the destruction of these materials in the stomach, immunization is carried out by loading CFs into microspheres or nanoparticles. Studies of PLGA microspheres containing the commonly colonization factor CFA / I, CS3, and CS6 that have been administered orally have shown that microspheres are absorbable and processed in peyer's patches of topical and systemic immune responses (47-45, 34). In 1994, the antigens of colonization factors CS3 and CS1 (9 to 1) was encapsulated in the biodegradable polymer of PLGA and its immunization was studied in volunteers. The IgG and IgA immune response was evaluable up to 57 days after receiving a dose of vaccine (48). In another study, it was shown that with the increase in the stability of the chitosan nanocapsules containing the F4 fibrin antigen from ETEC, oral immunology in the animal also improved (49). A study conducted in Iran also showed that the administration of the chimeric protein containing the three CFA / I, CS2, CS3 and B subunit colonization factors resulted from LT toxin loading in PLGA nanoparticles caused immune responses against binding and toxin bacteria in experimental animal. Since LTB and CTB are non-toxic and non-immunogenic, as well as in the gastrointestinal tract, they are capable of binding to intestinal cells, they are a good candidate for immunity against LT. Antibody against recombinant LTB protein (rLTB) can neutralize LT-toxin activity (50-52). In the design of multiple immunogens, LTB is also used. In a research in Iran, LTB recombinant protein was used in the design of multivariate recombinant immunogenicity against ETEC. Antibodies produced against this subunit of toxin prevented the occurrence of normal toxin symptoms (16, 53, 54). In several other studies, such a function was observed for the LTB recombinant protein (2, 53, 55). The method of administration of ETEC antigens by transgenic plants has also been used (56).

Inactivated bacteria: An alternative method for the preparation of CF in mucosal vaccines, is the use of killed ETEC bacteria, which have all types of CF in immunogenic form and bacterial surface. CF molecules are more stable on surface of bacteria than purified CF molecules and maintain antigenicity and the structure of their fimbriae (13, 35). Some inactivated organisms are associated with the appropriate amounts of LT toxoid. Efforts to produce ST toxoid have not been successful, and the biggest reason for this failure is the small size and high content of the amino acid cysteine in the ST molecule (13, 35).

Live oral vaccines of ETEC: Various strategies have been used for this purpose, and it has been used from reduced rebate of Shigella, Vibrio cholera and Salmonella, which express various CF factors alone or in combination with LT toxoid. Alternatively, the use of reduced rebate ETEC bacteria as a carrier and supplier of protective antigens. The ETEC abbreviated vaccines are made by removing the ompF, ompC and aroC genes. PTLoo2 and PTLoo3 derived from the strain O6: H16, have CS1 + CS3 and have a mutation in aroc. The candidate for the CS4-CS6 and LTB vaccine with CFA / I and CS1-CS3 are also in this category (57-59). Hybrid vaccines based on Live Shigella / ETEC can produce CF or LT molecules. In a study, several reduced rebate Shigella such as Shigella Flexner II, Flexner 3a, Flexner 6 and Shigella Sonia were engineered to express the stability of the ETEC fimbriae and LTB subunits. The use of this vaccine in the clinical model resulted in the secretion of systemic IgG and mucosal IgA against Shigella and ETEC antigens (60, 61). Salmonella typhi and Vibrio cholera reduced rebate vaccines for immunogenicity and health testing in clinical trials have been investigated. These bacteria express CTB / LTB subunits and various CF molecules. For example, in the peru-15 vaccine, the strain expressing subunit B from chlora toxin can be a dual vaccine for chlora / ETEC. In other studies, CF factors have been produced in strains of Salmonella and Vibrio cholera vaccines and have been presented on the bacterial surface (62, 63).

Chimeric immunogens: Nowadays, design of vaccine candidates focuses on chimeric proteins. The chimeric

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proteins containing protein subunits, linkers, and adjuvant sequences not only can enhance the immunogenicity of recombinant proteins, but also induce and cause widespread immune responses to cellular and humoral proteins. If a chimeric protein has an antigen of several different pathogens or different strains of a pathogen, it is possible to create the same immunity against different agents with only one protein (23, 64). In the following study, the main components of CFA / I and CS2 factors were fusion based on the surface of Escherichia coli bacteria. Another chimeric protein is also designed and manufactured, which includes the K88 fimbriae and ETEC toxins. The fusion protein of LTB-ST was considered as nanocapsules for immunization and toxin protection (65-67). The results of the research on the chimeric protein containing three colonization factors of CFA / I, CS2, CS3 and B subunit of LT toxin from enterotoxicogenic E. coli showed that the immune system was stimulated against all four protein forming components of the chimer (16). Since CS6 colonization factor is also one of the most common colonization factors in ETEC strains, the chimeric protein composed of the main and secondary subunits of the CS6 and LTB coupling factor and its immunity was investigated (55). It has also been shown that the use of CfaE, CfaB and LTB chimeric proteins can reduce the amount of bacterial binding activity and the effects of heat-sensitive toxin (2). The design of the recombinant protein CstH and LTB fusion, the chimer protein having CS6 and CFA / I colonization factors, and resistant bacteria sensitive toxins show similar results in the efficacy of multiple immunogens against ETEC (44, 53, 54). Table 1 summarizes the status and development of vaccine against ETEC.

Table 1. Vaccine development approach against ETEC

Candidate for the vaccine	Developer	Progress status
Fourth complete inactivated cell with LTB-CTB toxoid	Swedish Biotechnology Laboratory	Phase 2 Clinical
Activated reduced rebate cell based on Omp, omp F, aroC genes with dmLT adjuvant	PATH	Phase 2 Clinical
Typhoid cell vaccine presenting of Toxoid LT-ST	Prokarium company	Preclinical
Second generation Shigella reduced rebate presenting of colonization and toxoid factors of LT	CVD Vaccine Development Center	Preclinical
Subunit Vaccine against binding-factors	NMRC	Phase 2 Clinical
Subunit vaccine against binding-factors and toxoid	University of Kansas	Preclinical
Fusion LT-ST and Conjugated LT and ST	International Intestinal Vaccine Consortium	Preclinical

Discussion

Considering the available evidence, including the fact that ETEC-induced diarrhea decreases with increasing age of children, as well as the observation of antibodies against acute bacterial factors in patients' blood, it seems that the immune response is normal in these individuals. On this basis, it can be concluded that the production of a safe and effective vaccine against ETEC is possible .However, the epidemiological studies that have been done so far have made scientists more aware of the ETEC burden index, however, there are still some inaccuracies in information about mortality rates, bacterial outbreaks, and diagnosis and coping with it. Precise studies can reduce information deficiencies and help in the development of coping strategies. Considering that studies on immunization against ETEC have not succeeded so far, the use of known virulence factors along with the bioinformatics of isolated strains genomes can be challenging in the development of vaccines protects against ETEC with high rates. The results of the research show that there is a challenge in the development of the vaccine against ETEC that there are different strains with different colonization factors, which greatly reduces the efficiency of individual immunosuppression. Accordingly, the use of chimer protein that has multiple candida can help. Another important topic for success in producing vaccine against intestinal pathogens is to consider the route of prescribing a vaccine to the body. The use of nanotechnology as well as herbs for oral administration provides an appropriate presenting of immunogens to the mucosal immune system. Supports from various organizations, such as the World Health Organization, can provide valuable assistance in obtaining appropriate protective mechanisms against ETEC.

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References

1.Bourgeois AL, Wierzba TF, Walker RI. Status of vaccine research and development for enterotoxigenic Escherichia coli. Vaccine. 2016;34(26):2880-6.

2.Gheibi Hayat SM, Mousavi Gargari SL, Nazarian S. Construction and immunogenic properties of a chimeric protein comprising CfaE, CfaB and LTB against Enterotoxigenic *Escherichia coli*. Biologicals. 2016;44(6):503-10.

3.Zhang W, Sack DA. Current progress in developing subunit vaccines against enterotoxigenic *Escherichia coli*-associated diarrhea. Clin Vaccine Immunol. 2015;22(9):983-91.

4.Kaper JB, Nataro JP, Mobley HL. Pathogenic *escherichia coli*. Nat Rev Microbiol. 2004;2(2):123-40.

5.Madhavan TV, Sakellaris H. Chapter Five-Colonization Factors of Enterotoxigenic *Escherichia coli*. Adv Appl Microbiol. 2015;90:155-97.

6.Nazarian S, Mousavi Gargari SL, Rasooli I, Alerasol M, Bagheri S, Darvish Alipoor S. Prevalent phenotypic and genotypic profile of enterotoxigenic *Escherichia coli* among Iranian children. Jpn J Infect Dis 2014;67(2):78-85.

7.Petri WA, Miller M, Binder HJ, Levine MM, Dillingham R, Guerrant RL. Enteric infections, diarrhea, and their impact on function and development. J clin Invest. 2008;118(4):1277-90.

8.Velarde JJ, Levine MM, Nataro JP. Hunter's Tropical Medicine and Emerging Infectious Disease. 9th ed. Chapter 42 - *Escherichia coli* Diarrhea. London: W.B. Saunders; 2013. p. 442-7.

9.De S, Bhattacharya K, Sarkar J. A study of the pathogenicity of strains of Bacterium coli from acute and chronic enteritis. J Pathol. 1956;71(1):201-9.

10.Lamberti LM, Bourgeois AL, Walker CLF, Black RE, Sack D. Estimating diarrheal illness and deaths attributable to Shigellae and enterotoxigenic *Escherichia coli* among older children, adolescents, and adults in South Asia and Africa. PLoS Negl Trop Dis. 2014;8(2): 2705.

11.Lanata CF, Fischer-Walker CL, Olascoaga AC, Torres CX, Aryee MJ, Black RE. Global causes of diarrheal disease mortality in children< 5 years of age: a systematic review. Plos One. 2013;8(9): 72788.

12.Lozano R, Naghavi M, Foreman K, Lim S, Shibuya K, Aboyans V, et al. Global and regional mortality from 235 causes of death for 20 age groups in 1990 and 2010: a systematic analysis for the global burden of disease study 2010. The Lancet. 2013;380(9859):2095-128.

13.Svennerholm A-M, Lundgren A. Recent progress toward an enterotoxigenic *Escherichia coli* vaccine. Expert Rev Vaccines. 2012;11(4):495-507.

14.Zhang W, Sack DA. Progress and hurdles in the development of vaccines against enterotoxigenic *Escherichia coli* in humans. Expert Rev Vaccines 2012;11(6):677-94.

15.Gupta S, Keck J, Ram P, Crump J, Miller M, Mintz E. Part III. Analysis of data gaps pertaining to enterotoxigenic *Escherichia coli* infections in low and medium human development index countries, 1984-2005. Epidemiol Infect 2008;136(06):721-38.

16.Nazarian S, Gargari SLM, Rasooli I, Hasannia S, Pirooznia N. A PLGA-encapsulated chimeric protein protects against adherence and toxicity of enterotoxigenic *Escherichia coli*. Microbiol Res. 2014;169(2):205-12.

17.Fleckenstein JM. Chapter 6 - Enterotoxigenic *Escherichia coli*. In: D M, editor. *Escherichia coli*. Boston: Academic Press; 2013. p. 183-213.

18.Fleckenstein JM, Hardwidge PR, Munson GP, Rasko DA, Sommerfelt H, Steinsland H. Molecular mechanisms of enterotoxigenic escherichia coli infection. Microbes Infect. 2010;12(2):89-98.

19.Gonzales-Siles L, Karlsson R, Kenny D, Karlsson A, Sjöling Å. Proteomic analysis of enterotoxigenic *Escherichia coli* (ETEC) in neutral and alkaline conditions. BMC Microbiol 2017;17(1):11.

20.Hajizade A, Ebrahimi F, Amani J, Arpanaei A, Salmanian AH. Design and in silico analysis of pentavalent chimeric antigen against three enteropathogenic bacteria: enterotoxigenic *E. coli*, enterohemorragic *E. coli* and Shigella. Biosci Biotechnol Res Com. 2016;9(2):229-43.

[DOR: 20.1001.1.15614107.1396.19.6.2.3]
[DOI: 10.22088/jbums.19.6.2]

21.Saldaña-Ahuactzi Z, Cruz-Córdova A, Rodea GE, Porta H, Navarro-Ocaña A, Eslava-Campos C, et al. Genome Sequence of Enterotoxigenic *Escherichia coli* Strain FMU073332. Genome Announc 2017;5(8):01600-16.

22.Clements A, Young JC, Constantinou N, Frankel G. Infection strategies of enteric pathogenic *Escherichia coli*. Gut microbes. 2012;3(2):71-87.

23.Nazarian S, Gargari SLM, Rasooli I, Amani J, Bagheri S, Alerasool M. An in silico chimeric multi subunit vaccine targeting virulence factors of enterotoxigenic *Escherichia coli* (ETEC) with its bacterial inbuilt adjuvant. J Microb Method. 2012;90(1):36-45.

24.Kharat VB, Ahmed M, Jiang Z-D, Riddle MS, DuPont HL. Colonization Factors in Enterotoxigenic *Escherichia coli* Strains in Travelers to Mexico, Guatemala, and India Compared with Children in Houston, Texas. Am J Trop Med Hygiene. 2017;96(1):83-7.

25.Joffré E, Sjöling Å. The LT1 and LT2 variants of the enterotoxigenic *Escherichia coli* (ETEC) heat-labile toxin (LT) are associated with major ETEC lineages. Gut Microbes. 2016;7(1):75-81.

26.Mudrak B, Kuehn MJ. Heat-labile enterotoxin: beyond G M1 binding. Toxins. 2010;2(6):1445-70.

27.Lasaro MA, Mathias-Santos C, Rodrigues JF, Ferreira L. Functional and immunological characterization of a natural polymorphic variant of a heat-labile toxin (LT-I) produced by enterotoxigenic *Escherichia coli* (ETEC). FEMS Immunol Med Microbiol. 2009;55(1):93-9.

28.Connell TD. Cholera toxin, LT-I, LT-IIa and LT-IIb: the critical role of ganglioside binding in immunomodulation by type I and type II heat-labile enterotoxins. Expert Rev Vaccines. 2007;6(5):821-34.

29.Nandre RM, Duan Q, Wang Y, Zhang W. Passive antibodies derived from intramuscularly immunized toxoid fusion 3xSTa N12S-dmLT protect against STa+ enterotoxigenic *Escherichia coli* (ETEC) diarrhea in a pig model. Vaccine. 2017;35(4):552-6.

30.Sato T, Shimonishi Y. Structural features of *Escherichia coli* heat-stable enterotoxin that activates membrane-associated guanylyl cyclase. J Pep Res. 2004;63(3):200-6.

31.Mansouri M, Mousavy SJ, Ehsaei Z, Nazarian S, Zali MR, Moazzeni SM. The codon-optimization of cfaE gene and evaluating its high expression capacity and conserved immunogenicity in *Escherichia coli*. Biologicals 2013;41(3):169-75.

32.Sincock SA, Hall ER, Woods CM, O'Dowd A, Poole ST, McVeigh AL, et al. Immunogenicity of a prototype enterotoxigenic *Escherichia coli* adhesin vaccine in mice and nonhuman primates. Vaccine. 2016;34(2):284-91.

33.Gaastra W, Svennerholm A-M. Colonization factors of human enterotoxigenic *Escherichia coli* (ETEC). Trend Microbiol. 1996;4(11):444-52.

34.Walker RI, Steele D, Aguado T, Committee AHETE. Analysis of strategies to successfully vaccinate infants in developing countries against enterotoxigenic *E. coli* (ETEC) disease. Vaccine. 2007;25(14):2545-66.

35.Svennerholm A-M, Tobias J. Vaccines against enterotoxigenic *Escherichia coli*. Expert Rev Vaccines. 2008;7(6):795-804.

36.Jafari F, Shokrzadeh L, Hamidian M, Salmanzadeh-Ahrabi S, Zali MR. Acute diarrhea due to enteropathogenic bacteria in patients at hospitals in Tehran. Jpn J Infect Dis. 2008;61(4):269-73.

37.Hoseinzadeh T, Ghanbarpour R, Rokhbakhsh-Zamin F. Phylogenetic background of enterotoxigenic and enteroinvasive *Escherichia coli* from patients with diarrhea in Sirjan, Iran. Iran J Microbiol 2016;8(3):187.

38.Katouli M, Jaafari A, Ketabi G. The role of diarrhoeagenic *Escherichia coli* in acute diarrhoeal diseases in Bandar-Abbas, Iran. J Med Microbiol. 1988;27(1):71-4.

39.Salmani H, Azarnezhad A, Fayazi MR, Hosseini A. Pathotypic and Phylogenetic Study of Diarrheagenic *Escherichia coli* and Uropathogenic *E. coli* Using Multiplex Polymerase Chain Reaction. Jundishapur journal of microbiology 2016;9(2): e28331.

[DOR: 20.1001.1.15614107.1396.19.6.2.3]
[DOI: 10.22088/jbums.19.6.2]

40.Shahrokhi N, Bouzari S, Jafari A. Comparison of virulence markers and antibiotic resistance in enterotoxigenic *Escherichia coli* isolated ten years apart in Tehran. J Inf Dev Count. 2010;5(04):248-54.
41.Isidean S, Riddle M, Savarino S, Porter C. A systematic review of ETEC epidemiology focusing on colonization factor and toxin expression. Vaccine. 2011;29(37):6167-78.
42.Neutra MR, Kozlowski PA. Mucosal vaccines: the promise and the challenge. Nat Rev Immunol. 2006;6(2):148-58.
43.Ahmed T, Bhuiyan TR, Zaman K, Sinclair D, Qadri F. Vaccines for preventing enterotoxigenic Escherichia coli (ETEC) diarrhoea. Cochrane Database Syst Rev. 2013;5(7):9029.
44.Zeinalzadeh N, Salmanian AH, Ahangari G, Sadeghi M, Amani J, Bathaie SZ, et al. Design and characterization of a chimeric multiepitope construct containing CfaB, heat-stable toxoid, CssA, CssB, and heat-labile toxin subunit B of enterotoxigenic *Escherichia coli*: a bioinformatic approach. Biotechnol Appl Biochem. 2014;61(5):517-27.
45.Byrd W, Cassels FJ. Intranasal immunization of BALB/c mice with enterotoxigenic *Escherichia coli* colonization factor CS6 encapsulated in biodegradable poly (DL-lactide-co-glycolide) microspheres. Vaccine. 2006;24(9):1359-66.
46.Katz DE, DeLorimier AJ, Wolf MK, Hall ER, Cassels FJ, van Hamont JE, et al. Oral immunization of adult volunteers with microencapsulated enterotoxigenic *Escherichia coli* (ETEC) CS6 antigen. Vaccine. 2003;21(5):341-6.
47.Lemoine D, Francois C, Kedzierewicz F, Preat V, Hoffman M, Maincent P. Stability study of nanoparticles of poly (ε -caprolactone), poly (d, l-lactide) and poly (d, l-lactide-co-glycolide). Biomaterials. 1996;17(22):2191-7.
48.Tacket CO, Reid RH, Boedeker EC, Losonsky G, Nataro JP, Bhagat H, et al. Enteral immunization and challenge of volunteers given enterotoxigenic *E. coli* CFA/II encapsulated in biodegradable microspheres. Vaccine 1994;12(14):1270-4.
49.Khan MS, Vishakante GD. Development and evaluation of porous chitosan nanoparticles for treatment of enterotoxigenic *Escherichia coli* infection. J Biomed Nanotechnol. 2013;9(1):107-14.
50.Khalesi R, Salimian J, Nazarian S, Ehsaei Z, Amini N, Moazzeni SM. Production and purification of heat-labile toxin of enterotoxigenic *Escherichia coli* and its detection by GM1 gangelioside receptor-ELISA based method. Arak Med Univ J. 2012;15(1):35-42.
51.Khalesi R, Sh N, Amani J, Ehsaei Z, Mansouri M, Moazzeni S, et al. Cloning and expression of enterotoxigenic *Escherichia coli* heat labile toxin B subunit (LTB) as a vaccine candidate. Trauma Month. 2009;2009(2):95-100.
52.Khalesi R, Sh N, Ehsaei Z, Mansouri M, Amani J, Salimian J, et al. Optimization of gene expression and purification of enterotoxigenic *Escherichia coli* recombinant LTB protein and antibody production against it. Trauma Month. 2010;15(3):141-7.
53.Alerasol M, Gargari SLM, Nazarian S, Bagheri S. Immunogenicity of a fusion protein comprising coli surface antigen 3 and labile B Subunit of enterotoxigenic *Escherichia coli*. Iran Biomed J. 2014;18(4):212.
54.Kazemi R, Akhavian A, Amani J, Salimian J, Motamedi M-J, Mousavi A, et al. Immunogenic properties of trivalent recombinant protein composed of B-subunits of LT, STX-2, and CT toxins. Microbes Infect. 2016;18(6):421-9.
55.Bagheri S, Gargari SLM, Rasooli I, Nazarian S, Alerasol M. A CssA, CssB and LTB chimeric protein induces protection against Enterotoxigenic *Escherichia coli*. Braz J Infect Dis. 2014;18(3):308-14.
56.Tacket C. Plant-based oral vaccines: results of human trials. Plant-produced Microbial Vaccines: Springer; 2009. p. 103-17.
57.McKenzie R, Bourgeois AL, Engstrom F, Hall E, Chang HS, Gomes JG, et al. Comparative safety and immunogenicity of two attenuated enterotoxigenic *Escherichia coli* vaccine strains in healthy adults. Infect Immun. 2006;74(2):994-1000.
58.McKenzie R, Darsley M, Thomas N, Randall R, Carpenter C, Forbes E, et al. A double-blind, placebo-controlled trial to evaluate the efficacy of PTL-003, an attenuated enterotoxigenic *E. coli* (ETEC) vaccine strain, in protecting against challenge with virulent ETEC. Vaccine. 2008;26(36):4731-9.

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[DOI: 10.22088/jbums.19.6.2]

59.Turner AK, Beavis JC, Stephens JC, Greenwood J, Gewert C, Thomas N, et al. Construction and phase I clinical evaluation of the safety and immunogenicity of a candidate enterotoxigenic *Escherichia coli* vaccine strain expressing colonization factor antigen CFA/I. Infect Immun. 2006;74(2):1062-71.

60.Barry EM, Wang J, Wu T, Davis T, Levine MM. Immunogenicity of multivalent Shigella-ETEC candidate vaccine strains in a guinea pig model. Vaccine. 2006;24(18):3727-34.

61.Osorio M, Bray MD, Walker RI. Vaccine potential for inactivated shigellae. Vaccine. 2007;25(9):1581-92.

62.Roland KL, Cloninger C, Kochi SK, Thomas LJ, Tinge SA, Rouskey C, et al. Construction and preclinical evaluation of recombinant Peru-15 expressing high levels of the cholera toxin B subunit as a vaccine against enterotoxigenic *Escherichia coli*. Vaccine. 2007;25(51):8574-84.

63.Ziethlow V, Favre D, Viret JF, Frey J, Stoffel MH. Assessment by electron-microscopy of recombinant *Vibrio cholerae* and Salmonella vaccine strains expressing enterotoxigenic *Escherichia coli*-specific surface antigens. Clin Microbiol Infect. 2008;14(3):282-6.

64.Berzofsky JA, Ahlers JD, Belyakov IM. Strategies for designing and optimizing new generation vaccines. Nat Rev Immunol. 2001;1(3):209-19.

65.Deng G, Zeng J, Jian M, Liu W, Zhang Z, Liu X, et al. Nanoparticulated heat-stable (STa) and heat-labile B subunit (LTB) recombinant toxin improves vaccine protection against enterotoxigenic *Escherichia coli* challenge in mouse. J Biosci Bioeng. 2013;115(2):147-53.

66.Tobias J, Svennerholm A-M, Holmgren J, Lebens M. Construction and expression of immunogenic hybrid enterotoxigenic *Escherichia coli* CFA/I and CS2 colonization fimbriae for use in vaccines. Appl Microbiol Biotechnol. 2010;87(4):1355-65.

67.Zhang C, Zhang W. *Escherichia coli* K88ac fimbriae expressing heat-labile and heat-stable (STa) toxin epitopes elicit antibodies that neutralize cholera toxin and STa toxin and inhibit adherence of K88ac fimbrial *E. coli*. Clin Vaccine Immunol. 2010;17(12):1859-67.

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