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Review Article

Molecular Characterization of Virulence Factors in Enterotoxigenic *Escherichia coli*

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Abstract

Millions of diarrheal disease is made by *Enterotoxigenic E. coli* (ETEC) each year, specifically in developing countries. In the pathogenesis of ETEC infections, the first phase is sticking of the bacterium to the minute intestinal epithelium, as a result of colonization factors (CFs) mediation and subsequently generate enterotoxins. These CFs in accordance with their structure are diverged into discrete groups. CFA/I and CS6 are two of the most typical CFs. CFA/I is a fimbriae consists of a superior subunit, CfaB and inferior subunit, CfaE. CS6 is non-fimbrial which includes two main subunits, CssA and CssB. The enterotoxins caused by ETEC are related to two eminent classes of heat-labile toxins (LT) and heat stable toxins (ST). LT is formed of five B subunits and a single enzymatically active A. Its B subunits tied up to the enteral GM1 ganglioside receptors in the intestinal epithelium and A subunit whose ADP-ribosylating activity culminates in cellular adenylcyclase activation and an increase in cAMP, efflux of chloride ions and water and succeeding watery diarrhea. Guanylatecyclase (GC) is receptor for the ST toxin. Intracellular levels of cyclic guanosine monophosphate (cGMP) increase when ST binds to GC. Such increase in cGMP permits activation of cystic fibrosis transmembrane conductance regulator (CFTR) by phosphorylation-dependent cGMP protein kinase II producing an escalation in salt and secretion of water and prevention of sodium absorption through h the apical Na/H channel.More information about the CFs and enterotoxins of pathogen leads to more founding of ETEC virulence, and the founding is important to designing an appropriate vaccine.

Keywords: Enterotoxigenic *Escherichia coli*, Colonization Factors, Heat-Labile Toxins, Heat Stable Toxin

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Introduction

It has been approximated that about 650 million episodes of diarrhea and over 380,000 deaths annually in children less than five years old are made by diarrhea because of ETEC [1, 2], but It is prevalent in adults in endemic countries [3] and in travelers to such areas as well [4, 5]. The clinical signs of the illness encompass watery diarrhea often have been seen with abdominal cramps, malaise, and low grade fever.

The disease duration is possibly from 3-7 days and symptoms differs from mild diarrhea to dehydrating cholera like disease, which have been seen in about 5% of cases and foremost in adults [6].

Metabolism

ETEC as a chemoheterotroph facultative anaerobic bacteria is able to extract energy by aerobic breathing on the present of Oxygen but it can also convert to fermentation or anaerobic respiration under anaerobic circumstances.

For the bacteria, the nutritional requirements are plain and simple. To be versatile makes it possible for *E. coli* to adjust to both intestinal (anaerobic) and extra intestinal (aerobic or anaerobic) milieus. ETEC in the laboratory can grow on various media and it can be endure for a long period in the environment [7].

Virulence factors of ETEC

Two types of virulence factors are accompanied with ETEC; heat-labile and heat stable toxins (LT and ST, respectively) and the factors of colonization (CFs) which acts as a mediator of adhesion to the enterocytes of the intestine (Figure 1). ETEC joins to particular receptors of enterocytes in intestinal lumen by virtue of their CFs which are typically hair-like fimbriae. Surface antigens (CS) or colonization factor antigens (CFAs) as types of fimbrial antigens are more than 20 [8]. The colonized ETEC on the surface of the small bowel mucosa by virtue of the CFs elaborates enterotoxin which cause intestinal secretion [9, 10].

As mentioned before, ST and LT are two produced toxins of ETEC Despite the fact that one or both of these toxins can be secreted by different strains of ETEC, the diseases made by each toxin are the same [11].

ETEC, without the colonization factor adhesions, would probably be removed by the peristaltic motion of the small intestine ending in less diarrhea even if the enterotoxins are made [6, 8-10, 12].

Colonization factors

The colonization factors can act as mediator for the bacteria to adhere to the host small intestinal epithelium. Approximately twenty various colonization factors have been illustrated so far [6, 8]. Two different groups are formed by division of these colonization factors according to their structure (Table 1). Since CFA/I-like, type-IV-like, CS5 groups do not have any sufficient resemblance to other CFs, they do not belong to any group. CFA/I, CS1, CS2, CS4, CS14, CS17, CS19 and PCFO71 can be compiled by CFA/I-like group [6, 8, 12]. CS6 is a distinct colonization factor (Table 1).

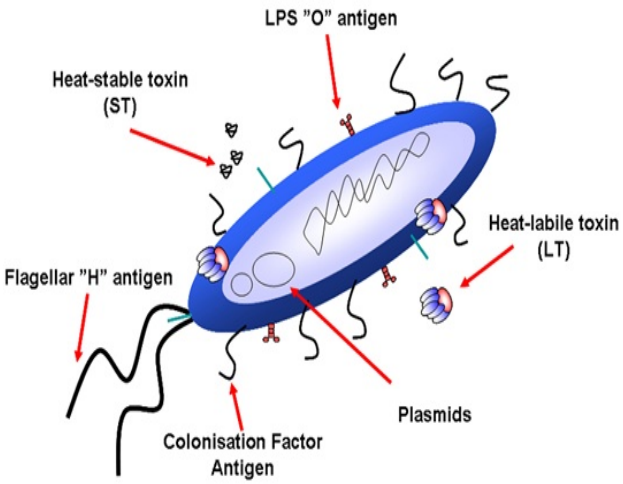

Figure 1. Scheme of the components of ETEC [12].

About one third of the ETEC strains evinces CFA/I [13]. Two third of these strains express just LT and the remained third expresses both LT and ST. By the expression of CS3 and/or CS1 or CS2, the CFA/II group is confirmed and is discovered on 23% of the strains. Both ST and LT are usually expressed by CFA/II strains. CFA/IV group owns 21 % of the strains which means that they express CS6 and/or CS4 or CS5. The mentioned strains usually express ST [10, 13].

Table 1: ETEC colonization factors in their groups. The last column CFs which do not belong to any group.

CFA/I-LIKE	CSS	TYPE-IV-LIKE	DISTINCT
CFA	CS5	CS8 (CFA/III)	CS3
CS1	CS7	CS15	CS6
CS2	CS13	CS21	CS10
CS4	CS18	--	CS12
CS14	CS20	--	--
CS17	--	--	--
CS19	--	--	--
PCFO71	--	--	--

CFA/I

The first described CF was colonization factor antigen I (CFA/I) which is the typical CFs in ETEC strains [6, 8, 13]. CFA/I is in a family of eight CFs (Table 1), they are segregated into three subgroups; CFA/I, CS4 and CS14, CS1, CS17, CS19 and PCFO71, and CS2. CFA/I as a fimbriae is made of a major subdivision, CfaB and a minor subdivision, the tip protein CfaE. The operon consists of cfaA, cfaB, cfaC and cfaE. A chaperone-like protein is encoded by cfaA and the cfaC gene is a protein which is involved in transporting the fimbriae across the outer membrane [14].

CS6

One of the most popular CFs is coli surface antigen 6 (CS6) [6] which is non-fimbrial and its structure has not yet been specified. CssA and CssB are two different subunits of CS6. The operon embodies cssA, cssB, cssC and cssD. CssC is a chaperone and transporting CssA and CssB across the outer membrane is done by CssD which is an usher [13].

Toxins

Host activity and bacterial regulation of the LT toxin

LT is a 86 kDa AB5 toxin which is popular with its homologous activities, immunogenicity and similar features as the cholera toxin (the proteins have in common 82% amino acid homology). LT is comprised of five B subdivisions that bind to the enteric GM1 ganglioside receptors in the intestinal epithelium, and a single enzymatically active A subdivision whose ADP-ribosylating activity results in activation of cellular adenylcyclase and an increase in cAMP, efflux of chloride ions and water and subsequent watery diarrhea [15] (Figure 2).

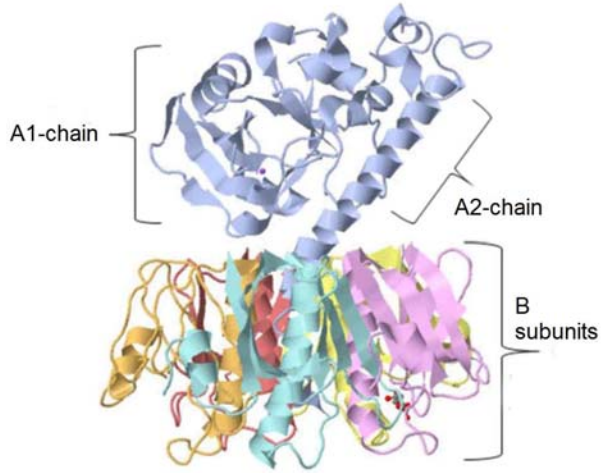

Figure 2. Crystal structure of ETEC LT [15].

The eltAB operon which is regulated by the global bacterial regulators (CRP) and histone-like nucleoid structuring protein encodes LT. (H-NS) [16, 17]. By the Sec dependent pathway, the translated peptides move through the inner membrane[18]. The subunits, once in the

periplasm, are rapidly congregated to the matured form of the AB5 holotoxin in a procedure that is DsbA dependent protein [19]. For LT secretion through the external membrane, the type II secretion system in the outer membrane of the gram negative ETEC is indispensible. (Figure 3A). The mechanism of LT secretion by ETEC into the extracellular area is still debatable. Recent studies determined that LT stays associated to the membrane [20, 21]. Latest studies have proposed that the toxin binds to lipopolysaccharide (LPS) on the extracellular surface of the bacteria through the B subdivisions after secretion through the outer membrane and through the spread of outer membrane vesicles charged with LT on their surface and periplasmic interior is the chief delivery of the toxin [22]. Although, this has been disputed, [23, 24] we and others have indeed proved LT secretion into the outward in some strains [25].

Host activity and bacterial regulation of the ST toxin

The ST which is 18-amino acid (STh primarily segregated from humans) or 19-amino acid (STp primarily segregated from pigs but causing illness in humans) eminently folded peptide and made disruption of chloride channels in the cell ending in secretory diarrhea.

Either alone or in association with LT, ST is expressed in almost 66% of ETEC strains, therefore it is seriously liable for disease load of ETEC all over the world [26]. By various genes, the ST toxin is encoded for STh and STp and STh at least has been determined to be under catabolite repression by the regulation of CRP [16] The ST genes are transcribed into “preproprecursors” [27] but the small mature ST is secreted along the TolC channel and folded into its mature shape by 4 cystein bridges [27] (Figure 3B).

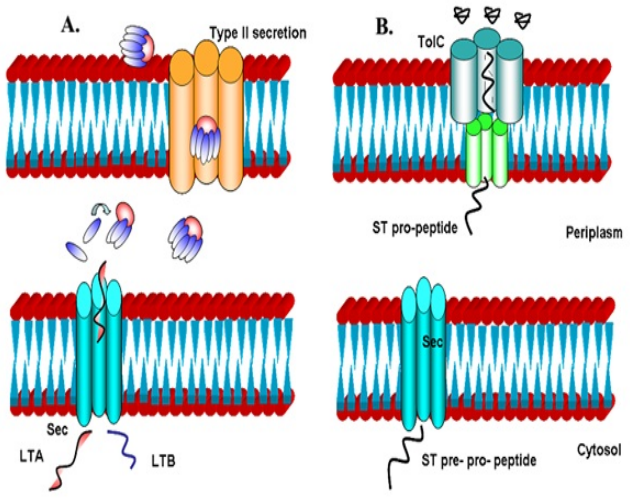

Figure 3. Mechanisms of assembly and secretion of A. LT and B. ST enterotoxins through the inner and outer membranes of ETEC [16].

Guanylatecyclase (GC) as transmembrane enzyme is situated in the apical membrane of the intestinal cells is the main receptor for the secreted ST toxin. Binding ST to GC increases intracellular levels of cyclic guanosine monophosphate (cGMP). The mentioned increase in cGMP permits activation of cystic fibrosis transmembrane conductance regulator (CFTR) by means of phosphorylation-dependent cGMP protein kinase II which makes salt and water secretion and inhibition of sodium absorption through the apical Na/H channe (Fig. 4) [28].

Host factors influencing ETEC infection

Obviously, numerous parameters like expression of specific virulence factors by the bacteria, both inborn and achieved host defenses and the genetic background of the host effects the clinical presentation of ETEC infection following ingestion of an enough inoculums. However, early studies just started to dealt with host factors related to acquisition of ETEC infections or different clinical presentations that have been seen from asymptomatic colonization to severe, life-threatening diarrhea.

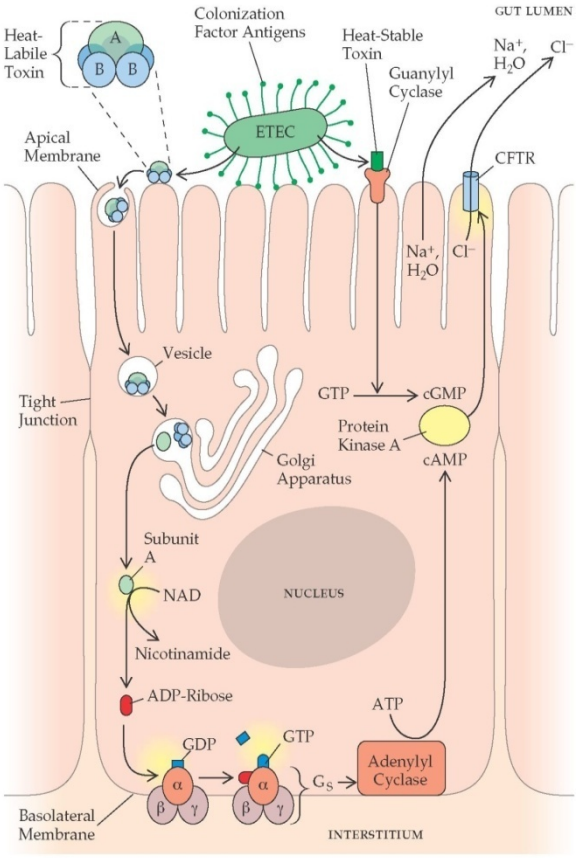

Figure 4. Mechanism of virulence of ETEC [21].

According to previous studies, individuals with type O blood were at high risk for severe *Vibrio cholera* infection and then several investigations have now focused on the correlation of ABO blood group determinants to ETEC infections. Data from Bangladeshi children in contrast to *V. cholerae* infections showed no relationship between blood group O and diarrhea [29].

Anyway, a succeeding study conducted in a birth cohort of more than 300 children in Dhaka specified that blood groups A or AB were connected to a ETEC diarrhea than those with group O blood [30]. Furthermore, children with Lewis blood group antigen-a positivity ($Lea^{+}Leb^{-}$) in

Bangladesh more commonly had symptomatic ETEC infections [31].

On the surface of red blood cells, Glycoconjugates may also be expressed on intestinal epithelia where they behave as receptors from one or more bacterial adhesins, and infactCfaB, the major subdivision of Cfa/I fimbriae binds to glycosphingolipids involves Lea [32]. Studies of traveler's diarrhea, in addition to the relationship with blood group antigens, have also determined a synonymous single nucleotide polymorphism in the human lactoferrin gene that was accompanied with an intensified risk of diarrheal illness [33].

Conclusion

There is strong evidence to support that ETEC vaccine can be developed, although there is no effectual one available so far. Since ETEC is a major cause of diarrheal disease worldwide, it is of great interest to develop a vaccine against it. One problem is that there are numerous ETEC strains with various characteristics and virulence parameters. However, to construct a vaccine based on the enterotoxins is one feasible approach, the most popular colonization factors and a common denominator for them [34]. There are different ways to do that. The carbohydrate receptor on the host cell can be obstructed so that adhesion by the pathogen is prevented or carbohydrate receptor analogs can be formed which stick to the adhesins of the pathogen and put it out of action. Several studies have tested Carbohydrates to prevent diverse bacterial infections in different animals such as infections in *E. coli* F18 fimbriae in pigs [35] and *E. coli* P pili in mice [23, 36]. It is recommended that such strategy can also be a good one for ETEC infection in humans. As a result, this study produced a better view on the virulence traits of ETEC and can might be useful for planning further studies about ETEC and vaccine development strategies.

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