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Novel Antigens for enterotoxigenic *Escherichia coli* (ETEC) Vaccines

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Abstract

Enterotoxigenic Escherichia coli (ETEC) are the most common bacterial pathogens-causing diarrhea in developing countries where they cause hundreds of thousands of deaths, mostly in children. These organisms are leading cause of diarrheal illness in travelers to endemic countries. ETEC pathogenesis, and consequently vaccine approaches, have largely focused on plasmid-encoded enterotoxins or fimbrial colonization factors. To date these approaches have not yielded a broadly protective vaccine. However, recent studies suggest that ETEC pathogenesis is more complex than previously appreciated and involves additional plasmid and chromosomally-encoded virulence molecules that can be targeted in vaccines. Here, we review recent novel antigen discovery efforts, potential contribution of these proteins to the molecular pathogenesis of ETEC and protective immunity, and the potential implications for development of next generation vaccines for important pathogens.

These proteins may help to improve the effectiveness of future vaccines by making simpler and possibly broadly protective because of their conserved nature.

Keywords

ETEC; E. coli; enterotoxigenic; vaccines; enterotoxins; fimbriae; bacterial; bacterial adhesins; flagella

Introduction

Six recognized diarrheagenic categories or pathotypes of *Escherichia coli* have been characterized and include, enteropathogenic *E. coli* (EPEC), enterohaemorrhagic *E. coli*

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(EHEC), enteroinvasive *E. coli* (EIEC), enteroaggregative *E. coli* (EAEC), diffusely adherent *E. coli* (DAEC) and enterotoxigenic *E. coli* (ETEC). Of these, *Enterotoxigenic Escherichia coli* (ETEC) are among the most common causes of diarrhea in the developing world [1]. Here they are particularly important as a cause of diarrheal disease among young children, accounting for millions of infections and hundreds of thousands of deaths each year [2]. These pathogens also cause significant morbidity in adults, and are perennially an important etiology of traveler's diarrhea (TD) [3].

ETEC are defined by the production of heat-labile enterotoxin (LT) and/or heat-stable enterotoxin (ST) These toxins, encoded on transmissible plasmids [4], have been fully characterized on a molecular level [5].

LT is very close in its structure and antigenicity, to cholera toxin and and the impact of these molecules on cellular physiology are very similar. The molecular mass (84 kDa) and the subunit structure of the two toxins were essentially identical, with an active (A) subunit surrounded by five identical binding (B) subunits [1,6,7]. Following colonization of the small intestine by ETEC and release of LT, the LT-B subunits bind irreversibly to GM1 ganglioside on the surface of epithelial cells while the internalized A subunit activates adenylate cyclase, resulting in increases in intracellular cyclic AMP. Subsequent activation of protein kinases then stimulates chloride secretion through the cystic fibrosis transmembrane regulator channel (CFTR) and inhibits neutral sodium chloride in the villus tips, ultimately leading to a net loss of salt and water into the intestinal lumen. When these actions exceed the absorptive capacity of the bowel, purging of watery diarrhea results [1,6].

ST is a nonantigenic low-molecular-weight peptide, consisting of 18 to 19 amino acids. There are two variants, STp and STh, named from their initial discovery from pigs and humans, respectively, and which have identical mechanisms of action. Released in the small intestine, ST binds reversibly to the extracellular domain of guanylate cyclase C, resulting in increased intracellular levels of cyclic GMP [8]. Activation of protein kinase II also leads phosphorylation and stimulation of CFTR [9] and decreases in sodium-hydrogen ion exchange. Intriguingly, because ST can activate cGMP-dependent antiproliferative signaling pathways in intestinal epithelial cells, it has been suggested that exposure to this toxin may account for the dramatically lower prevalence of colon cancer in developing world relative to industrialized countries [10].

Production of any one of these enterotoxins is sufficient [11–13] to cause severe diarrheal illness. In its most severe form ETEC diarrhea is clinically indistinguishable from that caused by *V. cholerae* [14–17].

In the classical paradigm for ETEC pathogenesis, these organisms must first colonize the small intestine where they employ plasmid-encoded fimbrial colonization factors (CFs) to bind enterocytes in the small intestine. Here they must produce and effectively deliver their ST and/or LT enterotoxins [1]. CF-based adhesion to the mucosal epithelial cells of the small intestine promotes transfer of ETEC enterotoxins that stimulate the release of fluid and electrolytes from the intestinal epithelium resulting in watery diarrheal illness. These plasmid-encoded traits have been considered to be the key virulence factors, and have

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therefore been intensively studied over the last three decades. Interestingly, while over 25 unique CF types and putative colonization factors have been characterized so far, in some studies more than 50% of all ETEC clinical isolates have an identifiable CF [1,18]. This has stimulated interest in discovery of both plasmid and chromosomally encoded novel adhesins, as well as other virulence factors (Table 1) that may have gone undetected using classical molecular biology and immunological approaches. A comprehensive account of the recent information that is available on alternative virulence factors of ETEC is discussed in the review. Young children in developing countries, particularly those under the age of two years, are disproportionately susceptible to severe infections with ETEC [19], and would benefit from a safe and broadly protective vaccine. However, despite the global importance of ETEC, vaccines against these pathogens are still in development. After being pursued over the last decade, a number of candidate vaccines are currently in phase I-III clinical studies, but are not yet available [20]. The classical paradigm for ETEC pathogenesis has served as the basis of all ETEC vaccine development efforts to date. Nevertheless, emerging data from recent studies of ETEC suggest that both the molecular pathogenesis of these organisms [21] as well immune responses to these pathogens [22] are significantly more complex than previously appreciated, potentially affording novel antigenic targets and new approaches to vaccine development.

Challenges in enterotoxigenic *E. coli* in vaccine development

ETEC genomics and the identification of conserved antigenic targets

The genetic plasticity of *E. coli* poses a fundamental challenge in formulating vaccines against these heterogeneous pathogens. Whole genome sequencing projects of *E. coli* have revealed that pangenome (the collection of all genes identified to date) of these bacteria is “open” [23]. Remarkably, in effect this means that every time a new *E. coli* genome is sequenced hundreds of unique genes, not shared with the rest of the pangenome will be identified. This genetic diversity of ETEC is reflected in the wide variety of O (LPS) and H (flagellar) serotypes represented within this pathotype [24]. ETEC belong to a wide variety of O, H and K antigenic types, and contrary to other *E. coli* pathotypes causing diarrhea (*e.g*., EHEC), there is no clear relationship of virulence to specific serotypes [1].

Present data appear to support the concept that ETEC genomes are essentially mosaics and that a small number of pathotype-specific features tiled onto diverse array of *E. coli* host backgrounds could be sufficient to cause clinical illness [25]. Essentially, ETEC utilize these pathotype-specific features, *e.g*., the toxins in the context of core features, *e.g*., flagella, common to many *E. coli* (including commensal isolates), to successfully deliver their toxin payload to cognate receptors on the epithelial surface. No single pathotype-specific antigen common to all ETEC has been described to date, and the majority of pathotype-specific antigens identified are encoded on plasmids. A challenge at the center of ETEC vaccinology then lies in defining antigens that are sufficiently conserved to provide broad coverage against the diverse population of strains that cause diarrheal disease.

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immunologic correlates of protection are not yet defined

Importantly, prospective studies of young children in highly endemic areas demonstrate significant declines in symptomatic ETEC infections during the first 2 years of life suggesting that natural infections afford significant protective immunity [19,26]. However, the precise nature of this protection remains uncertain, and there are conflicting data regarding the correlation of immune responses to the classical virulence factors and protection [26–28]. Collectively, these studies seem to suggest that additional effort is required to elucidate mechanisms of protection [29] associated with natural infection to inform vaccine antigen selection.

Vaccines currently in development

Colonization factor and toxoid based vaccine concept

Vaccines focused on the classical paradigm of ETEC pathogenesis have attempted to achieve broad coverage by combining the most prevalent CF molecules. These CFs have typically been combined with either cholera toxin or LT toxoids, such as the B subunit of LT or genetically detoxified versions of LT, that retain both anti-toxin and the inherent adjuvant properties of these molecules. While there is significant regional variation in CF and toxin-expression profiles [30], epidemiological and natural history studies have suggested that the important CFs are CFA/I, CS1-CS7, CS14, CS17 and CS21 (Longus), expressed either alone or in combination, and it has been estimated that globally approximately 60% of isolates express LT, either alone or in combination with ST [30,31]. Therefore, most ETEC vaccine development to date has centered on inclusion of 4–5 CFs with LT-based toxoids.

current oral ETEC vaccines in development

The concept of delivering oral vaccines to protect against ETEC was initiated in the 1990s with a multivalent approach that incorporated combinations of CF expressed in killed whole cell ETEC preparations [32,33]. In each of these attempts, the design has been to formulate vaccines such that both anti-toxic and anti-adhesin immunity can be achieved. An inactivated whole cell vaccine expressing CFA/I, CS1-3, CS5 as well as recombinant B subunit of the cholera toxin was designed to give a broad range of protection. This vaccine progressed to Phase II/III trials in children in endemic countries but did not prove efficacious and needed additional improvement in the safety profile for infants and young children [32,34]. Interestingly, this vaccine did however show protection against more severe forms of diarrhea in traveller's to Guatemala and Mexico despite its lack of protective efficacy in children [35]. Since then, the vaccine has been reformulated with higher expression of CFs [36] to enhance safety and immunogenicity, and will soon be tested in young children in ETEC endemic countries.

A similar approach has been taken in the design of a live-attenuated oral ETEC vaccines currently in testing in the human volunteer challenge model. In this vaccine, ACE527, three different wild type ETEC strains expressing different CFs were genetically attenuated by making selected mutations (*aroC, ompC, ompF*) [37] and by deleting genes for enterotoxins. Genes encoding CS1, not present in the original parent strains, and the *eltB* gene encoding B subunit of LT were then inserted to arrive at a live-attenuated mixture expressing 6 CFs

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(CFA/I, CS1, CS2, CS3, CS5, CS6), and LT-B [38]. This vaccine was well-tolerated in human volunteers, elicited robust immune responses to both the CFs and LT-B [39], and on initial testing resulted in some reduction in moderate-severe diarrhea on challenge with ETEC [40]. Further studies with this vaccine, reformulated to include double mutant LT (see dmLT below) are currently underway.

colonization factor tip adhesin approach

The ETEC vaccines currently in advanced stages of clinical development rely on expression of the whole CF fimbrial structure. However, details of both the molecular structure and biogenesis of these fimbriae [41,42] have fostered the development of a prototype vaccine based on the CFA/I tip adhesin molecule, CfaE. Intradermal administration of this molecule was recently shown to be protective against ETEC challenge in a non-human primate *Aotus nancymaae* model [43]. This approach obviates the need to express entire CF operons, and potentially permits development of a multivalent recombinant subunit approach that incorporates the most relevant tip adhesin structures to achieve broad coverage.

Alternative ETEC antigen delivery approaches

heterologous bacterial expression systems

Potential alternatives to classical killed whole-cell or live-attenuated vaccines for oral delivery of ETEC antigens rely on expression of these proteins in heterologous vectors. Given the high prevalence of both ETEC and *Shigella* infections in developing countries [2], one attractive approach in development includes oral bivalent vaccines constructed to express ETEC antigens including CFs and mutant forms of LT in attenuated vector strains of *Shigella* [44–48]. Similarly attenuated *Salmonella* vectors [49,50], as well as the Ty21a typhoid vaccine strain [51] have been used for heterologous expression of a number of ETEC antigens. Recent stable expression of *Shigella O-polysaccharide genes in* Ty21a [52], a licensed, live-attenuated typhoid vaccine strain, which has been safely administered in hundreds of millions of doses worldwide [53], could provide an alternative multivalent platform to protect against multiple enteric pathogens including ETEC and *Shigella*.

transgenic plants

Another concept that has been explored is that of edible plant vaccines where LT-B has been expressed in a variety of transgenic plants [54,55]. Intriguingly, human volunteers who ingested transgenic potatoes [56] or corn [57] expressing LT-B mounted robust mucosal immune responses to this antigen. However, it is not yet clear whether a sufficient number of other ETEC antigens likely required for broad-based protection can be stably expressed in plants.

Transdermal delivery of LT

Delivery of LT via a transdermal route using skin-patches impregnated with holotoxin could provide an alternative approach to anti-toxin immunity. A recently published phase 3 safety and efficacy study ETEC diarrhea in travellers following administration of heat-labile toxin via skin patch demonstrated that by itself LT was not effective overall in preventing moderate to severe ETEC diarrhea [58]. Nevertheless, the LT-patch did elicit high titers of

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antibody against LT and afforded some protection against strains that produced only LT. Despite the fact that these studies failed to meet their primary endpoint, they should provide additional impetus for inclusion of LT as a protective immunogen, while highlighting the need to select additional antigens to enhance protective efficacy and broaden protection.

Novel antigens with potential utility in ETEC vaccines

novel plasmid-encoded ETEC antigens

ETEC vaccinology to date has focused primarily on engendering immune responses to colonization factors (CFs) and heat-labile toxin. However, data emerging from recent ETEC pathogenesis studies [21] suggest that the interactions of these pathogens with the gastrointestinal mucosal are considerably complex, and involve a variety of additional pathotype-specific and common *E. coli* antigens (table 1).

A search for novel ETEC secreted proteins with transposon-based methods led to the identification of two plasmid-encoded molecules currently in preclinical investigation as candidate ETEC vaccine antigens, EtpA [59] and EatA [60].

EtpA adhesin—EtpA, a 170 kD glycoprotein secreted by the etpBAC two-partner secretion system, is required for efficient colonization of the small intestine in murine models [61] and for both adhesion and toxin delivery [62] to target epithelial cells *in vitro*. EtpA appears to function as an adhesin in a unique fashion by forming a molecular bridge between highly conserved regions of flagelllin available at the tips of ETEC flagella, and the host cell surface [63], permitting ETEC to utilize the long flagellar appendages in interactions with host cells [63]. Antibodies directed at either EtpA or the conserved regions of flagellin inhibit toxin delivery in *vitro* [62] and prevent intestinal colonization of mice following gastrointestinal challenge with ETEC [64]. Interestingly, antibodies directed against EtpA demonstrate at least an additive effect in preventing delivery of heat-labile toxin by ETEC when combined with antibodies against either the A or B subunit of LT. Likewise, vaccination of mice using LT together with EtpA significantly impaired intestinal colonization. Collectively these studies suggest that EtpA could be a useful vaccine subunit [62], potentially expanding both valency and efficacy of ETEC toxoid approaches.

Importantly, data emerging from a number of molecular epidemiology studies suggest that EtpA may be reasonably conserved among the ETEC pathotype. It has been identified in a variety of strains from different phylogenetic groups [65] and from geographically disparate locations [66], where *etpA* genes were found in more than 70% of the strains examined. Because EtpA is expressed by strains from most CF-groups tested to date, it could complement CF-based anti-adhesin approaches. Still, further molecular epidemiology studies, including some currently under way, are needed to examine the conservation of this antigen in ETEC.

EatA protease—EatA is a member of the serine protease autotransporter family of virulence proteins [60]. Recent studies have shown that EatA has at least two known functions in ETEC. First, EatA degrades the EtpA adhesin thereby modulating bacterial adhesion, and accelerating delivery of heat-labile toxin [67]. The secreted passenger domain

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of EatA has also recently been shown to be highly active in degrading MUC2, the major mucin in the lumen of the intestine [68]. MUC2 normally serves as a significant barrier to prevent pathogen interaction with enterocytes and EatA significantly enhances toxin delivery by promoting bacterial access to cell surface receptors [68]. Early studies have demonstrated that antibodies against the secreted passenger domain of EatA protect against colonization in the murine model and prevent toxin delivery *in vitro*. Like the etpBAC locus, *eatA* genes are present in a diverse collection of ETEC strains [65,66,69]. In addition, the significant homology of EatA and SepA, an autotransporter identified in *Shigella flexneri* [70] suggests that EatA and similar proteins could represent important targets in hybrid ETEC-*Shigella* vaccines. Similar molecules are also expressed by other diarrheagenic *E. coli* including enteroaggregative strains associated with more severe forms of infection [71]. Therefore, targeting these mucin-degrading enzymes could find utility in protecting against a number of important enteric pathogens.

Highly conserved, chromosally encoded antigens

A number of surface-expressed or secreted proteins are encoded as core features on the chromosome of many *E. coli* strains including ETEC as well as some commensal strains. While these molecules are not specific to the ETEC pathotype, they do appear to function in concert with pathotype-specific molecules and theoretically could serve as putative targets for vaccine development.

YghJ metalloprotease—Another protein highlighted by immunoproteomic studies [22] of convalescent sera is YghJ, an effector molecule secreted by the same type II secretion system (T2SS) responsible for secretion of LT [72,73]. This molecule also appears to enhance delivery of LT, in part through its metalloprotease activity and degradation of MUC2 as well as cell-surface bound MUC3, and antibodies against YghJ inhibit toxin delivery *in vitro* [74]. Although this chromosomally-encoded molecule is widely conserved across many pathogenic *E. coli,* some commensal strains also carry the genes encoding YghJ and its requisite T2SS. Vaccination with YghJ has been shown to afford modest protection against extraintestinal pathogenic *E. coli* [73], however it is not yet clear whether it offers protection against ETEC.

EaeH—Originally identified by subtractive hybridization of ETEC [75], the *eaeH* gene is significantly up-regulated upon host cell contact [76], and encodes a putative adhesin consisting of a series of tandem bacterial immunoglobulin-like (Big) domains similar to those involved in eukaryotic cell surface adhesion proteins [77]. These features are shared with two established virulence proteins, invasin and intimin of *Yersinia pseudotuberculosis* and enteropathogenic *E. coli*, respectively. Molecular pathogenesis studies in our laboratory suggest that EaeH enhances ETEC interactions with intestinal epithelial cells, and promotes toxin delivery *in vitro*, as well as mucosal colonization *in vivo* (unpublished data). Interestingly vaccination with FdeC, an EaeH homolog inhibited kidney colonization in mice infected with uropathogenic *E. coli* [78], however demonstration of protection against ETEC remains outstanding.

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Chromosomally-encoded fimbriae

Embedded in the chromosomes of most *E. coli* strains, including ETEC are multiple regions that encode potential fimbriae or pili. Similar to the plasmid-encoded CFs, these are easily recognized by a series of genes encoding a chaperone, an outer membrane usher protein, and major and minor pilin structural subunits. While many *E. coli* strains possess 5–6 chaperone-usher-pilus (CUP) operons, neither the role of the respective fimbriae in ETEC pathogenesis or their utility as vaccine targets have been determined.

Type 1 fimbriae

In addition to the plasmid-encoded CFs, ETEC have long been known to possess chromosomally-encoded type 1 fimbriae (T1F) [79,80]. Recent demonstration that ETEC T1F genes are up-regulated with epithelial cell contact [76], has rekindled interest in these structures as potential vaccine targets. In addition, recent *in vitro* studies suggest T1F are required for optimal epithelial cell adhesion and toxin delivery (Sheikh, unpublished). Interestingly while vaccination with T1F does protect pigs against ETEC [81], and these structures appear to play some role in mediating binding of ETEC to human intestine [82] early attempts to employ preparations of whole ETEC T1F as vaccines yielded mixed results [83]. Nevertheless, current detailed knowledge of T1F biogenesis [84] and structure could permit improved approaches targeting the highly conserved FimH tip adhesin [85], a strategy currently being applied to uropathogenic *E. coli*.

E. coli common pili (ECP)

These pilus structures, originally identified in *E. coli* associated with neonatal meningitis and referred to as MAT fimbriae [86] were later identified in enterohemorrhagic *E. coli* (EHEC) [87], in a variety of other pathotypes, and commensal isolates. Sera from healthy individuals as well as EHEC patient convalescent sera recognize EcpA the major pilin subunit. A majority of ETEC strains also encode potential ECP chaperone-usher-pilus operons [88], however further study is need to define their role in ETEC pathogenesis and to determine whether the structural elements [89] of these conserved structures can be successfully targeted in vaccines.

autotransporter proteins

Autotransporter (AT) proteins are ubiquitous in *E. coli* genomes, with many strains encoding multiple putative AT molecules. Because passenger domains of these proteins are either surface-expressed or secreted they are often highly immunogenic and may serve as potential vaccine targets. Indeed two chromosomally-encoded proteins in ETEC, antigen 43, and pAT are highly immunogenic, recognized during convalescent immune responses to ETEC and protective against ETEC small intestinal colonization in mice [90]. These proteins deserve additional consideration as potential vaccine targets. (table 1).

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Emerging anti-toxin strategies

mutant LT

Both cholera toxin and heat-labile toxin exhibit potent adjuvant activity and antibodies against LT afford partial protection against LT-producing strains. While the B subunit of both toxins does have adjuvant activity, optimal benefit likely requires retention of the A subunit. However, substantial inherent toxicity limits the utility of LT holotoxin as orally administered mucosal adjuvants. To overcome this, mutant forms of LT were designed to retain adjuvant activity while eliminating the toxic activity. One such double mutant (dmLT) contains mutations in the A subunit (R192G, L211A) that prevent proteolytic activation of LT into its catalytically active form [91]. In phase 1 human volunteer challenge studies, dmLT was safe in doses up to 100 μg, with immune responses peaking after a single does of 50 μg [92]. These studies will likely set the stage for development of subunit or live-attenuated vaccines in which dmLT is included both as an immunogen and for its potent adjuvant activity.

ST toxoids

Strains of ETEC infecting humans frequently produce the ST-1 heat-stable toxins ST-1a (ST-P) and ST-1b (ST-H). With or without LT, these strains have the capacity to cause severe diarrhea and dehydration [12]. Since nearly half of all strains produce only ST [30], implementation of an ST-toxoid strategy could be critical for successful development of a broadly protective ETEC vaccine. ST toxoid-based vaccines face inherent challenges including the poor immunogenicity of these small molecules, and their similarity to endogenous human peptides guanylin, and uroguanylin [5]. However, emerging results suggest that it is possible to design ST molecules that are devoid of toxicity, which elicit neutralizing antibodies that do not cross-react with native guanylin and uroguanylin peptides [93], and that construction of non-toxic LT-ST toxoid fusions are feasible [94,95].

Expert Commentary and Five year view

In addition to the classical antigens presently targeted, a number of novel molecules highlighted in recent studies could contribute to development of ETEC vaccines. Of the ETEC pathotype-specific virulence molecules, EtpA and EatA appear to be among the most highly conserved antigens described to date. Preclinical studies suggest that these molecules participate in effective toxin delivery, and that they are protective antigens. Therefore, these secreted proteins could play an important role in complementing existing approaches to ETEC vaccines.

It is becoming increasingly clear that the plasmid-encoded pathotype specific virulence factors act in concert with more highly conserved chromosomally-encoded molecules that are common to many *E. coli*. While some core *E. coli* antigens contribute to ETEC pathogenesis and are also recognized during infection, their contribution to protective immunity that develops following infection has not been determined. Likewise, it is not known whether these can be safely targeted in ETEC vaccines without untoward effects on commensal *E. coli* organisms that share some of these antigens or the gastrointestinal microflora in general.

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A wealth of emerging immunoproteomic and genomic data will likely aid in defining the precise nature of protective immune responses that develop following natural infections, and focus selection of appropriate antigenic targets to accelerate development of future iterations of vaccines to prevent these infections of global importance.

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Key issues

- ETEC vaccine development faces a number of major challenges including an incomplete understanding of the nature or protective immunity, as well as heterogeneity and lack of conservation of known antigens.
- enhanced understanding of ETEC molecular pathogenesis resulting from discovery of novel virulence determinants has provided additional putative antigenic targets.
- novel antigens could expand valency and potentially efficacy of vaccines when combined with traditional ETEC vaccine targets (CFs and enterotoxins)
- new toxoids in development along with improved vector strategies for antigen delivery could accelerate delivery of effective vaccines

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Table 1

current landscape of potential ETEC vaccine targets

antigen/structure	role(s) in ETEC pathogenesis	required for optimal toxin delivery (*in vitro*)	protective?	estimated conservation in ETEC (%)	present in commensal strains
plasmid-encoded pathotype-specific antigens					
EtpA	secreted adhesin, intestinal colonization	yes	yes[a]	60–70	no
EatA	mucin-degrading serine protease	yes	yes[a]	60–70	no
LT	enterotoxin	-	yes[a,b]	60 [c]	no
ST(H/P)	enterotoxin	-	unknown	70 [c]	no
CFs	fimbrial adhesins, intestinal colonization	yes	yes[a,b]	60–70[d]	no
chromosomally-encoded conserved antigens					
flagellin	major structural flagellar subunit; motility	yes	yes[a]	>90%	yes
YghJ	type II secretion system effector, mucin-degrading metalloprotease	yes	?	>80%	yes
EaeH	outer membrane adhesin	yes	?	>80	yes
ECP	unknown; colonization?	unknown	unknown	>80	yes
type 1 fimbriae	unknown; colonization?	yes	unknown	>80	yes
autotransporters: antigen 43-like, pAT	unknown	unknown	yes[a]	>80%	yes

[a] in animal (mouse) intestinal colonization model of ETEC infection

[b] in human clinical studies

[c] includes LT/ST strains

[d] estimate based on detection of all presently known CF antigens (Isidean *et al*); actual proportion may be higher with identification of novel CFs.