



WHO PREFERRED PRODUCT
CHARACTERISTICS FOR

**vaccines against
enterotoxigenic
*Escherichia coli***



**World Health
Organization**

**WHO preferred product characteristics for vaccines
against enterotoxigenic *Escherichia coli***

WHO preferred product characteristics for vaccines against enterotoxigenic *Escherichia coli* (ETEC)

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Abbreviations and glossary

ALS	antibodies in lymphocyte secretions/ supernatants	IVB	immunization, vaccines & biologicals
AMR	antimicrobial resistance	LMIC	low- and middle-income country
ASC	antibody secreting cell	LT	heat-labile toxin
CF	colonization factor	LTB	B subunit of LT
CFA	colonization factor antigen	MCEE	maternal child epidemiology estimation
CHIM	controlled human infection model	PCR	polymerase chain reaction
CoP	correlates of protection	PDVAC	Product Development for Vaccines Advisory Committee
CS	coli surface antigen	PPCs	preferred product characteristics
DALYs	disability-adjusted-life-years	PQ	pre-qualification
EED	environmental enteric dysfunction	R&D	research and development
ELISpot	enzyme-linked immunosorbent spot	SAGE	Strategic Advisory Group of Experts (on immunization)
EPI	expanded programme on immunization	ST	heat-stable toxin
ETEC	enterotoxigenic <i>Escherichia coli</i>	TPP	target product profile
FVVA	full value of vaccine assessment	VVM	vaccine vial monitor
GBD	global burden of disease	WHO	World Health Organization
HIC	high-income country		
IHME	Institute for Health Metrics and Evaluation		

Executive summary

Diarrhoeal diseases are among the leading causes of morbidity and mortality worldwide. Among children younger than 5 years, it is estimated that diarrhoea is responsible for about 446 000 deaths (390 894–504 613), which are geographically concentrated in sub-Saharan Africa and South Asia.

Enterotoxigenic *Escherichia coli* (ETEC) is one of the leading bacterial causes of diarrhoea, especially among children in low-resource settings, and travellers and military personnel from high-income countries. It is estimated that ETEC causes about 220 million diarrhoea episodes globally, with about 75 million episodes in children under 5 years of age, resulting in between 18 700 deaths (Institute for Health Metrics and Evaluation (IHME) estimates), and 42 000 deaths (maternal child epidemiology (MCEE) estimates) in children younger than 5 years. Diarrhoeal mortality rates for ETEC and other pathogens are declining due to improvements in economic development and availability of safe water and sanitation; however, these reductions have not been paralleled by significant declines in diarrhoea-associated morbidity, which continues to impact negatively on infant and child health in many low- and middle-income countries (LMICs). In the 0–5 year age group, an intervention

that is able to effectively reduce the mortality, as well as the morbidity burden of ETEC diarrhoea, will impact the long-term consequences of infection related to malnutrition that lead to poor physical and cognitive development, as well as increasing the risk of death due to other infectious diseases. Such an intervention could avert 4.2–6.0% of under-5 diarrhoea deaths and offer significant, but currently under-recognized, public health value to older children, adolescents and adults by contributing to improved social and economic development.

ETEC may be the first enteric illness encountered by many infants, so full protection is needed by the age of 9 months to cover peak incidence and mortality through the first 24 months of life. The widespread use of antibiotics, which are often prescribed empirically to treat diarrhoea, or used without prescription in some LMICs, contributes to the increased spread of anti-microbial resistant (AMR) strains of ETEC and other bacteria. In addition to potential direct, individual effects on ETEC mortality and morbidity, an ETEC vaccine is also likely to have significant indirect effects, such as decreasing antibiotic use and prevalence of AMR bacteria; increasing herd protection at the community level; healthcare cost savings through prevention of malnutrition; and improving child physical and cognitive development. Protection from all-cause diarrhoea may also be observed, a phenomenon that has been seen with use of rotavirus vaccines. Although several ETEC vaccine candidates have been tested and are in the pipeline at different stages of product development, currently no licensed vaccines against ETEC diarrhoea exist.

Prevention and treatment options to address diarrhoeal illness from ETEC are available and are important for averting and reducing the high ETEC disease burden; however, their implementation and sustainability is not always practical in low-resource settings. Consequently, the need to develop better prevention and control measures for diarrhoeal diseases, such as vaccines with equitable access, remains a public health priority for the World Health Organization (WHO), particularly for young children in LMICs. An ETEC vaccine would also benefit international travellers and military personnel based in endemic areas, as well as age groups above 5 years of age in LMICs that bear a significant burden of ETEC-associated illness.

“Enterotoxigenic *Escherichia coli* (ETEC) is one of the leading bacterial causes of diarrhoea, especially among children in low-resource settings, and travellers and military personnel from high-income countries.”



Photograph courtesy of Gagandeep Kang

An investment case for ETEC vaccine development suggested that younger age groups in emerging middle-income countries may be an additional target for ETEC vaccine use.

WHO preferred product characteristics (PPCs) provide strategic guidance on WHO preferences for new vaccines, particularly from a LMIC perspective. The intent of this PPC guidance is to help advance development of an ETEC vaccine that is suitable for use in the primary target population, in contexts where it is most needed, and to raise awareness of potential considerations for future policy

recommendations. To frame the development of ETEC vaccine PPCs, WHO convened global stakeholders to assess the priority public health needs, particularly in endemic areas. The outcome of this consultation was a consensus statement that **the primary strategic goal is to develop a safe, effective and affordable ETEC vaccine that reduces mortality and morbidity due to moderate-to-severe diarrhoeal disease in infants and children under 5 years of age in LMICs.** Participants considered critical vaccine attributes in the context of this strategic goal. These discussions are the foundation for this guidance on PPCs for an ETEC vaccine.



Photographer Asem Ansari, photograph courtesy of icddr,b through Alejandro Cravioto

1. Background and purpose of the World Health Organization's preferred product characteristics (PPCs)

The mission of the World Health Organization's (WHO's) department of Immunization, Vaccines and Biologicals (IVB) is to accelerate the development and uptake of safe and effective vaccines and related technologies that could have global public health impact. Priority areas for IVB include developing guidance and coordinating activities that enable: 1) prioritization and acceleration of vaccine candidates towards licensure; and 2) identification and generation of evidence to inform policy recommendations for candidate vaccines as they progress to advanced stages of development, in order to avoid a delay between licensure and vaccine implementation.

Vaccine preferred product characteristics (PPCs), published by WHO's IVB, are intended to encourage innovation and promote the development of vaccines for use in settings most relevant to the global, unmet public health needs. They describe preferred parameters pertaining to vaccine indications, target populations and immunization strategies, as well as data that should be collected for safety and efficacy evaluation and policy consideration (1). PPCs are pathogen-specific and do not specify minimally acceptable product characteristics; they are intended to provide early guidance to inform optimal characteristics for candidate-specific target product profiles (TPPs).

Disease areas for vaccine PPC development are identified by WHO's Product Development for

Vaccines Advisory Committee (PDVAC), based on the unmet public health need for a vaccine, interest and demand for a vaccine from LMIC stakeholders, and technical feasibility. They may be updated in the event of product or technology innovations, or other changes in the identified need or research and development (R&D) landscape.

The primary target audience for WHO PPCs is any entity intending to eventually seek WHO policy recommendation and pre-qualification (PQ) for their products. Communication of WHO preferences can be useful to all those involved in vaccine development, including academic groups, funders and manufacturers. As such, the various ETEC vaccine candidates will likely benefit from guidance regarding WHO and LMIC preferences as they approach upcoming stage gates for future investment and strategic decisions, particularly regarding field efficacy testing and the recommendations for introduction by the Strategic Advisory Group of Experts (SAGE) on immunization policy. However, it is important to note that a vaccine that offers the preferred characteristics and is intended for use in LMICs will also undergo evidence-based assessment by WHO's SAGE (2). As such, WHO PPCs offer early guidance intended to complement, but not to supersede, existing WHO processes for vaccine development and evaluation for a particular vaccine class or product.

2. Development of an ETEC vaccine for LMICs – a strategic priority for WHO

The immunization agenda 2030 (IA2030) is a global stakeholder strategy for the decade of 2021–2030, to Leave No-one Behind (3). It includes the primary goals to: 1) reduce mortality and morbidity from vaccine-preventable diseases across the life course, and 2) decrease disease burden by increasing access to and uptake of new vaccines.

ETEC vaccine development has been a WHO priority for the last 20 years, and a guidance document published in 2006 has helped to guide development efforts (4). The WHO priority strategic objective for ETEC vaccine development is to develop a safe, effective and affordable ETEC vaccine that reduces mortality and morbidity due to moderate-to-severe diarrhoeal disease in infants and children under 5 years of age in LMICs. This priority goal is reflective of the public health stakeholder input and the scientific community's understanding of the predominant burden of ETEC infections, as well as their adverse long-term sequelae; they decrease the potential socioeconomic prosperity of future generations in some of the most impoverished areas of the world.

Other target groups that would benefit from the availability of an effective vaccine include: infants and young children in emerging middle-income countries (5); older children, adolescents, adults – including older adults – in ETEC endemic LMICs (6); as well as international travellers and military personnel deployed to endemic areas (7, 8).

The development of vaccines against ETEC infections has been hampered by technical challenges, insufficient support for coordination of R&D efforts, and a poorly defined market to motivate investment in product development. In response to the urgent need for a

vaccine in LMICs, and to provide guidance for the numerous candidates in product development, IVB's PDVAC recommended the development of PPCs for ETEC vaccines. The development of PPCs is particularly pertinent, considering the characteristics and status of vaccine candidates in development. At this time, the most advanced candidates are based on a combination of inactivated ETEC strains or live attenuated strains. Both oral and parenteral candidates are in clinical development (9).

Any approach that supports the development of a combination vaccine could improve cost-effectiveness due to simplified delivery, particularly in low-resource settings. This is especially relevant in the case of parenteral candidates, given the increasingly congested vaccination schedule for infants and young children, and the availability of co-formulation options, such as the parenteral typhoid vaccines and *Shigella* vaccine candidates (10, 11, 12). For oral candidates the coformulation options include rotavirus, cholera vaccines or *Shigella* candidates.

Any enteric vaccine may have a significant impact on antibiotic use and subsequent development of antimicrobial resistance (AMR). Antimicrobial therapy is usually given to address serious syndromic presentations (namely, watery diarrhoea or dysentery), independent of a specific diagnosis. Antibiotic exposure among children under 5 years of age in LMICs is relatively high, with prescription levels estimated to be five times higher than those observed in high-income countries (13). Diarrhoea and enteric diseases are among the leading drivers of antibiotic use (14). In a recent study in six African countries, plus Nepal and Haiti, 50% of children presenting with diarrhoea to a healthcare facility were prescribed an antibiotic (13).

3. Background of ETEC diarrhoea

3.1 ETEC infection and diarrhoea

Among the six recognized diarrhoeagenic pathotypes of *Escherichia coli* (15), ETEC is the most common, particularly in LMICs (16). ETEC is one of the first symptomatic enteric illnesses encountered by children, causing several million cases of diarrhoea each year, mostly in under 5-year-olds (17, 18). Infection with ETEC can cause profuse watery diarrhoea and abdominal cramping. Fever, nausea with or without vomiting, chills, loss of appetite, headache, muscle aches and bloating can also occur, but are less common. Illness develops 1–3 days after exposure and usually lasts 3–4 days. Some infections may take a week or longer to resolve. Without adequate treatment this can lead to severe dehydration, electrolyte imbalance and eventually death (19).

Repeated ETEC infections – with or without symptomatic episodes – are common among children in LMICs, in part because of the multiple pathotypes (enterotoxin and colonization factor combinations) associated with the disease. However, the decrease in incidence of symptomatic illness with increasing exposure and age indicates that protective immunity develops (15, 20, 21), suggesting biological plausibility for protection by vaccination. The incidence of ETEC diarrhoea in low-income countries rises rapidly in the first 6–9 months of life, and peaks during the first 2 years of life. Given the antigenic diversity among the pathotypes, ETEC can also be a significant cause of diarrhoeal illness in older children and adults, particularly in South Asia and Africa, and may contribute

to periodic outbreaks or epidemics of watery diarrhoea affecting a broad range of age groups (6, 22).

ETEC is also the most common cause of diarrhoea in travellers, affecting individuals from high-income countries who visit endemic areas in LMICs (7, 8, 23). A systematic review suggests that diarrhoeal disease among long-term travellers remains a frequent occurrence, and the associated morbidity is significant, even though a high percentage of cases are not brought to medical attention (23). ETEC was detected in up to 30% of cases of diarrhoea in travellers, with the highest rates seen in those travelling to areas with a high prevalence of ETEC, such as Latin America, the Caribbean and the Middle East (8).

Beyond its potentially devastating and immediate impacts on health, repeated ETEC infections can induce or exacerbate stunting and other forms of malnutrition, reduce immune function, and increase the propensity for subsequent irritable bowel syndrome (24, 25, 26, 27). This results in adverse consequences on growth and cognitive development, as well as increased risk of death due to other infectious diseases, such as pneumonia, measles, and malaria (28). Collectively, these factors detrimentally impact school attendance and performance on an individual level, and economic status at a population level (29, 30, 31, 32).

There are three genotypes of ETEC based on the presence of toxin genes responsible for production of heat-stable toxin (ST-ETEC), heat-labile toxin (LT-ETEC), or both LT/ST-ETEC. The relative proportions of LT, ST and LT/ST toxin-producing ETEC vary from one geographic area to another in patients with ETEC diarrhoea or asymptomatic carriers. ETEC strains expressing only LT may be considered less important as pathogens in some populations, especially since they are more frequently isolated than the other two toxin types from healthy persons than from those infected (20). However, the multicentre, community-based Malnutrition and Enteric Disease Study (MAL-ED) cohort study showed an association between infection with LT-only ETEC strains with persistent diarrhoea. The role of LT needs to be further examined, given that persistent diarrhoea can frequently be a prelude to malnutrition, stunting and Enteric Enteropathy Dysfunction (EED) (33). In addition, recent controlled human infection models (CHIMs) studies have shown

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“ETEC is also the most common cause of diarrhoea in travellers, affecting individuals from high-income countries who visit endemic areas in LMICs.”

that LT-only strains that also express a colonization factor may be more efficient pathogens (34).

3.2 Prevention and treatment of ETEC diarrhoea

Proven, lifesaving interventions to prevent and treat diarrhoeal disease, including illness caused by ETEC, already exist (35). They include prevention methods such as improved sanitation and hygiene, access to safe drinking water, exclusive breastfeeding, optimal nutrition and vaccines against other pathogens (for example,

rotavirus and measles). While it is likely that living conditions in LMICs will improve with economic progress, the timelines are unpredictable, and are likely too slow to achieve the United Nations Sustainable Development Goals (SDG) (36) and the immunization agenda 2030 (3). While the available treatment strategies have been increasingly and successfully used over the past decades, there are notable limitations and issues with coverage and sustainability. Therefore, vaccination is considered one of the most equitable preventive interventions.

A three-day oral course of fluoroquinolones is the common treatment regimen for ETEC. Other treatment regimens are also recommended (ampicillin-sulbactam, doxycycline, azithromycin, ciprofloxacin, and others); however, the treatment regimen differs by country and reflects local resistance patterns. AMR ETEC strains are on the rise, rendering the prevention of infectious diarrhoea and the need for an effective vaccine an even greater public health priority (37). The Wellcome Trust conveyed its concerns about the increasing levels of multiple antibiotic resistant organisms among enteric pathogens in a recent report (14), and recommended that the development of vaccines against enteric *E. coli* pathogens such as ETEC be accelerated. As such, WHO, together with partners, is developing a value-attribution framework to evaluate the impact of vaccines (including ETEC) against AMR.

4. Full value of vaccines

assessment for ETEC vaccines

The value proposition of a vaccine candidate defines its epidemiologic, product development, economic, market, policy, financing, delivery and regulatory environments to guide investments in that product. Value propositions seek to identify, engage, and seek alignment of the major stakeholders and beneficiaries who may value the product differently, and articulate how the envisaged product will address their unmet need, as well as identify gaps in evidence to justify the product's development and uptake (38).

One of the fundamental elements that will inform the ETEC vaccine value assessment is a robust assessment of the current and future mortality, impact on AMR, and morbidity-related economic burden of disease. Therefore, it is imperative to capture the entire burden resulting from ETEC illness. To date, ETEC burden estimates have not included comprehensive estimates of disability-adjusted life-years (DALYs), which are the sum of the number of years of life lost due to premature mortality (YLL) and the number of years lived with disability (YLD) (39). DALYs are now widely used in public health practice to assess and monitor population health and to set health priorities in a given country. The Institute For Health Metrics and Evaluation (IHME) has

"The value proposition of a vaccine candidate defines its epidemiologic, product development, economic, market, policy, financing, delivery and regulatory environments to guide investments in that product. "

conducted a study to quantify the long-term sequelae from diarrhoea due to growth faltering, suggesting that the global burden is substantially underestimated when only incidence and mortality are considered (25). Accounting for long-term sequelae associated with growth impairment increased the number of diarrhoea DALYs lost among children younger than 5 years by about 40%.

In many settings, diarrhoea diagnosis and case detection are inadequate or not possible. This creates significant data gaps in many geographies; therefore data from surrounding regions are extrapolated to generate regional and global burden estimates. An additional contributor to uncertainty intervals in the mortality estimates is the geographic heterogeneity that exists for ETEC disease burden. A recent study explored how accounting for subnational and economic heterogeneity in *Shigella* and ETEC disease burden affects both projected vaccine impact and cost-effectiveness of ETEC and *Shigella* vaccines, after introduction in four sub-Saharan African countries, using dynamic models for provincial areas and socioeconomic status (40). It concluded that cost-effectiveness would be more favourable if vaccinations reach the most vulnerable children in under-served provinces, suggesting that an ETEC vaccine may have greater impact if introduction in high-burden areas at sub-regional or sub-national levels is prioritized.

To inform investments in ETEC vaccine development, as well as to determine the potential market size and implementation strategy, the epidemiology of ETEC needs to be characterized at regional and national levels therefore surveillance data are essential. Modelling of indicators for high ETEC prevalence from existing longitudinal cohorts can be helpful, as the most vulnerable populations are not likely linked to centres of excellence in diarrhoeal disease research.

While both travellers and military populations represent substantial market segments that contribute to the value proposition for ETEC vaccines, the target product profiles for vaccines that are developed for these predominantly high-income populations may not be compatible with the programmatic requirements



Photograph courtesy of Ibrahim Khalil

“Surveillance data will be essential to inform the distribution and magnitude of burden, and are needed to guide the implementation strategy, either as a single ETEC vaccine, or co-administered, co-formulated or combined with other vaccines.”

for a vaccine to be suitable for paediatric use in LMICs. The constraints in LMICs relate to attributes such as storage and volume requirements, ease of administration, number of doses and duration of protection, and must fit within the established immunization schedule. Infants (1–2 mo–<2 yr) have a

continuum of exposure to ETEC based on age, and the protective immune responses in infants have been observed to decline, with age de-escalation for many orally administered enteric vaccines (41, 42, 43, 44). So, while the value proposition for an ETEC vaccine in travellers and military populations may appear more lucrative, vaccines that are optimized for use in infants and young children in LMICs will likely also be effective travellers’ vaccines, and therefore have a larger market. Therefore, to achieve potential global reach and impact on reducing disease and transmission, travellers’ vaccines must be developed with the endemic use indication in consideration (45, 46).

The endemic–country awareness of the true impact that ETEC disease has or may have on a country’s population is fundamental to informing health–policy decisions. Policymakers in some endemic nations may be unaware of the significance of ETEC and its burden in the context of diarrhoeal illness (17, 18). Surveillance data will be essential to inform the distribution and magnitude of burden, and are needed to guide the implementation strategy, either as a single ETEC vaccine, or co-administered, co-formulated or combined with other vaccines. Considering that the first vaccine may be within 5–10 years from licensure, the level of awareness must improve in the near-term to create a pull for these vaccines, otherwise the potential impact of an ETEC vaccine may be limited because of low uptake, due to inadequate information and advocacy.

5. Burden of ETEC diarrhoea

Current mortality burden estimates for enteric pathogens are, in some cases, inconsistent and divergent, and incidence data are incomplete and vary widely by region and season. Coinfecting enteric pathogens, subclinical infections, antigenic diversity and the variability of diagnostic methods can complicate the determination of diarrhoeal aetiology for children in LMICs (47, 20, 3). Epidemiologic studies are hampered by methodological limitations and narrowly focused study populations. Furthermore, diagnostic and modelling methods are continually undergoing optimization, resulting in variation of the mortality estimates for each iteration.

The global burden of enteric diseases, including ETEC estimates, are currently being modelled by two groups – IHME and maternal child epidemiology estimates (MCEE). Each disease burden model has its strengths and limitations. Factors such as inclusion/exclusion criteria, model inputs and adjustments, assessment of pathogenicity, geographical representativeness and country or regional extrapolation affect conclusions about the attributable burden. Neither the MCEE or IHME burden estimates accounted for differences in the ST, LT, ST/LT toxin genotypes, despite observations that strains that produce ST either alone or in combination with LT produce more severe disease.

The limitations and divergence in the IHME and MCEE mortality estimates pose challenges for vaccine developers, funders and policy makers in prioritizing the relative importance of intervention strategies against ETEC. The drivers for these different estimates are being investigated by a WHO working group on the burden of enteric diseases (48,49).

“Epidemiologic studies are hampered by methodological limitations and narrowly focused study populations.”

5.1 IHME Global Burden of Disease study mortality estimates

According to the IHME's Global Burden of Disease (GBD) study estimates, diarrhoea accounts for more than 1 million deaths and about 4% of the total global DALYs per year across all age groups (17). ETEC was the eighth leading cause of diarrhoea mortality in 2016 among all age groups, accounting for 51186 (26 757–83 064) deaths – about 3.2% (1.8–4.7) of diarrhoea deaths. Among children younger than 5 years of age, ETEC was responsible for an estimated 18 700 deaths (9 900–30 659) – about 4.2% (2.2–6.8) of diarrhoea deaths (18). The greatest estimated number of under-5 deaths due to ETEC was in eastern sub-Saharan Africa with 5 485 deaths (2 889–8 941).

5.2 MCEE group mortality estimates

The MCEE group, previously known as the Child Health Epidemiology Group (CHERG), published estimates of pathogen-specific, global mortality for children under 5 years of age for the year 2011, using aetiologic data from hospital inpatient studies as a proxy for the pathogen distribution (50). MCEE estimated 712 200 diarrhoea deaths in children under 5 and 42 000 ETEC diarrhoea deaths in children younger than 5 years of age.

5.3 Diarrhoeal diseases and ETEC morbidity burden estimates

The potential value of an ETEC vaccine should incorporate the benefits that such a vaccine could provide in reducing long-term effects, as well as in reducing the use of antibiotics for treatment, and in reducing the prevalence of AMR ETEC strains (51). Therefore, the likely impact of ETEC on individual health, cognitive function and economic productivity in endemic countries would also benefit from further study (52).

Frequent episodes of diarrhoea can lead to malnutrition, and the chance for “catch-up” growth is linearly ablated (53). Infection with specific enteric pathogens, such as ETEC, can affect growth even in the absence of overt diarrhoea (54). It has been suggested these repeated episodes in the first 2 years of life can lead to a loss of up to 10 IQ points and absence of up to 12 months of school attendance by the age of 9 years (55, 56).



Photograph courtesy of Shutterstock/Riccardo Mayer

“Infection with specific enteric pathogens, such as ETEC, can affect growth even in the absence of overt diarrhoea ”

The cost of the vicious cycle of enteric infections and malnutrition (and their potential lasting impact) is so great that multiple, likely synergistic, approaches to interrupt the cycle must be taken (57). Not taking these consequences into account would thus be a

serious oversight in accruing clinical and epidemiologic evidence to address this substantial burden.

After inclusion of these long-term sequelae in IHME's burden estimates, diarrhoea moves from the fifth-leading to the third-leading cause of DALYs among children younger than 5 years, surpassing malaria and neonatal encephalopathy in the number of DALYs in this age group (25). However, ETEC-specific analysis to quantify the additional DALYs burden due to long-term sequelae is urgently needed. If available, this would help to refine the pathogen-specific burden estimates and the full value of a vaccine for ETEC. Indeed, as noted above about the importance of DALYs estimates, the blinded controlled study of a vaccine for ETEC, with height-for-age z-scores (HAZ) measurements, can uniquely help assess and document the magnitude of ETEC's role in this common additional DALY burden, with or without overt diarrhoea.

6. ETEC vaccine development

6.1 ETEC vaccine feasibility

Evidence from field studies and CHIMs indicates that protective immunity to ETEC develops after natural or experimental infection, suggesting that vaccine-induced ETEC immunity should be feasible (58, 59, 60, 61, 62, 63, 64, 65). In ETEC-endemic areas, age-specific attack rates for symptomatic ETEC infection decline after 3 years of age (60, 61, 62). In human challenge studies, subjects who recovered from ETEC diarrhoea were protected against disease when challenged a second time with the same strain (66, 63, 89). Both field studies and human challenge studies indicate that antibodies against colonization factors and LT toxin can play a role in protection (66, 67, 68, 69).

To provide effective strain coverage, ETEC vaccines are expected to be multicomponent formulations or combinations. Recent genotyping studies on ETEC strain collections, from various geographic locations, indicate that vaccines providing coverage for the most common colonization factors – CFA/I, CS3, CS5 and CS6, along with related antigens like CS7 and other class 5 fimbriae – should cover 80–90% of the ETEC strains associated with diarrhoea in LMICs and among travellers (20, 70). Recent studies have also suggested that including conserved ETEC antigens in vaccine formulations may reduce vaccine cost and also help improve strain coverage, particularly for those strains

“Evidence from field studies and CHIMs indicates that protective immunity to ETEC develops after natural or experimental infection, suggesting that vaccine-induced ETEC immunity should be feasible.”

that may lack identifiable colonization factors (71, 51). The application of new “omics” technologies has identified a number of novel conserved proteins that may contribute to toxin delivery or colonization, and thus may also have vaccine potential, since they tend to be shared across ETEC pathotypes (71, 72).

Current ETEC vaccine development efforts have focused on inducing anti-toxin and anti-colonization immunity, as studies indicate that antibodies against both antigen types can contribute to protection (85). Efforts to improve vaccine immunogenicity are ongoing, and include formulation with adjuvants or investigation of new delivery routes that may potentially facilitate vaccine dose sparing and improve efficacy (73, 21, 62, 74). Coadministration or coformulation of enteric vaccines that can target the same age group – for example, ETEC–cholera or ETEC–cholera–typhoid – could be very beneficial, if their delivery strategies are also compatible.

Vaccines intended for use in paediatric populations in LMICs must be formulated and delivered in such a way that their costs are reasonable, and their tolerability and immunogenicity are assured (75, 45, 46). The latter criterion is a particular challenge for vaccines used in low-resource settings, as suboptimal performance in terms of efficacy and effectiveness, especially with oral vaccines, has been demonstrated in many countries in Asia and Africa (42, 43, 44). Several reasons for this phenomenon have been suggested, including the underlying gut enteropathy, coinfections and malnutrition (24, 44). These aspects are likely to be less of a challenge in the development of a travellers’ vaccine or, potentially, with the use of parenterally administered vaccines (76).

6.2 ETEC vaccine clinical development considerations

Controlled human infection (or ‘challenge’) models for ETEC are well developed (66, 63) and provide a tool to demonstrate early clinical proof-of-concept, to potentially compare relative performance of different candidates and to investigate correlates of protection. However, CHIM studies are unlikely to be sufficient to support policy recommendations or to inform introduction decisions for a paediatric vaccine intended for use in LMICs, so vaccine developers will need to



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undertake field efficacy studies. Archiving specimens from field studies to for future analysis would enable discovery of correlates of protection.

A major challenge is the lack of consensus on how to define ETEC diarrhoea severity in community-based studies in LMICs. The lack of a consistent severity score means that case definitions for the chosen clinical endpoints may vary, and this limits comparability between candidates and studies in the endemic settings. The Vesikari score (77) was designed for use in rotavirus vaccine trials, and although useful in that context (78), it may be less so in community-based studies where there are multiple aetiologies. Other scores have been proposed (79, 80, 81), and one of the scores, Community Diarrhea (CODA) (82), was validated in a large multisite study (MAL-ED), providing more confidence than scores that have not been validated, or only validated in a single geographical site. However, CODA has not been broadly implemented.

Given the desire to demonstrate vaccine impact across the spectrum of ETEC disease, there is a need to reach consensus on a severity score that is validated and is amenable for field use. Human challenge model data can help to develop a scoring system; however, the criteria may not be suitable for field trials of both travellers and young children in LMICs. A scoring system has been proposed in a recent clinical trial involving infants in Zambia (83); however, the most promising scoring systems need to be comparatively evaluated in clinical and epidemiological studies that are being planned and

"Given the desire to demonstrate vaccine impact across the spectrum of ETEC disease, there is a need to reach consensus on a severity score that is validated and is amenable for field use."

tested, with the goal of validating 1–2 scores for use in future Phase III efficacy trials. In the event that it is practical to develop a standardized ETEC-specific severity score, consensus on a definition for moderate-to-severe diarrhoea attributable to ETEC may be more realistic.

No clear efficacy threshold has been defined for achieving a minimal public health benefit for ETEC vaccines. Acceptable thresholds for efficacy can be informed by updated vaccine impact models (40, 84), inclusive of those that demonstrate indirect effects,

and by market research with key stakeholders. A cost-effectiveness model (84) suggested that introducing ETEC or *Shigella* vaccines, each with 60% efficacy, could prevent a substantial number of direct ETEC and *Shigella* diarrhoea and stunting deaths, in addition to a favourable, incremental, cost-effectiveness ratio.

6.3 ETEC vaccine formulation and delivery considerations for use in LMICs

The target age group for an ETEC vaccine in LMICs, namely, infants from 6 months and young children under 5, has proven difficult to immunize effectively against enteric pathogens via the oral route (73). For this reason, other routes of administration and a variety of vaccine types are being evaluated. Oral vaccines avoid many of the delivery challenges associated with injectable vaccines in LMICs; they are relatively easy to administer, have the capacity to induce local mucosal immunity in the intestinal mucosa, and potentially can be produced at a relatively low cost (85). However, for policy recommendation, procurement and widespread use of oral vaccines in LMICs, it will be crucial to develop vaccine formulations and presentations that are both efficacious and facilitate use in the target population (62, 86).

Preclinical and human data suggest that alternative delivery approaches, such as intradermal and sublingual routes, may improve the mucosal response as an alternative option to intramuscular delivery for inducing mucosal immunity (87, 88). Although the intradermal route is considered problematic to implement in mass vaccination campaigns or in the expanded programme on immunization (EPI) schedule, novel delivery devices may render this vaccination route more practical and attractive, given its potential for improved immunogenicity and dose sparing (89).

It is imperative to consider the vaccine presentation requirements for programmatic delivery early in product development, so that a suitable presentation can be included in pivotal clinical trials that will support

"It is imperative to consider the vaccine presentation requirements for programmatic delivery early in product development"

licensure. For oral formulations, considerations should be given to protection from gastric acidity to prevent antigen degradation during passage through the stomach (74, 89). Dose-volume optimization is also an important consideration for administration to infants and young children. While minimizing dose volumes reduces the storage footprint for the vaccine and facilitates delivery, it may impact the osmolarity, which may have detrimental effects on vaccine stability and palatability of the final formulation (74).

Packaging technologies that improve product shelf-life, and that also allow for packaging of dry and liquid vaccine components in one container, would help to address some of the delivery challenges (90, 91, 92). Several manufacturers are developing innovative designs for dry and liquid vaccine presentations (93, 94).

The number of doses, vaccination schedule, and the possible need for booster doses should be carefully considered, based on the safety, efficacy and ability of the vaccine and regimen to induce immunological memory. In addition, cost of the final vaccine presentation, as well as its compatibility within the immunization programme, will impact the cost effectiveness of this vaccine and needs to be optimized.



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7. PPCs for ETEC vaccines

Parameter	Preferred characteristic	Notes
Indication	Prevention of moderate-to-severe diarrhoea (MSD) due to ETEC infection.	<p>Primarily, prevention of moderate-to-severe diarrhoea (MSD) due to ETEC is considered the optimal clinical endpoint to provide a measurable impact.</p> <p>Improved consensus and standardization of case definition for MSD is required.</p> <p>Prevention of MSD does not imply induction of sterilizing immunity that prevents infection, but prevention of severe and moderate disease. Prevention of mild disease is also considered important but, if measured in trials, should be a secondary endpoint.</p> <p>Other anticipated direct effects include reduction of stunting, prevention of malnutrition, risk reduction of subclinical ETEC infections, prevention of all-cause diarrhoea. Indirect effects include decrease in antibiotic use, decrease in ETEC AMR, induction of herd protection and financial risk protection. While these are important outcomes that will contribute to the full value of vaccine assessment (FVVA) for ETEC vaccines, they are challenging to assess as primary clinical endpoints pre-licensure. Where feasible, exploratory endpoints related to these effects should be collected during clinical studies.</p> <p>Measurement of the impact of an ETEC vaccine on strains associated with AMR is unlikely to be feasible in the context of a vaccine clinical trial. Reduction in the total use of antibiotics as a result of diarrhoea could serve as a proxy to measure the vaccine impact on AMR. WHO encourages efforts to measure, analyse and widely report data on pathogen-associated antibiotic use in vaccine trials and vaccine impact studies.</p>
Target population	<p>Infants from 6 months and children up to 24 months of age.</p> <p>Longer-term effectiveness data in children up to 5 years of age will be of interest for decision-making.</p>	<p>The immunization goal is full protection of infants by the end of 9 months of age, to cover peak ETEC incidence and mortality through the first 24 months of life.</p> <p>Some country and regional variation (+/- 6 months) in peak incidence is expected.</p> <p>Ideally, protection would extend up to 5 years of age. Prevention of MSD up to this age would significantly reduce death and morbidity due to both immediate and long-term sequelae, such as growth stunting associated with infection.</p> <p>Other target populations that would likely benefit from an efficacious vaccine are older children, adolescents, adults and older adults in LMICs and emerging market countries, as well as military personnel and others travelling to endemic areas.</p>

Parameter	Preferred characteristic	Notes
Dose regimen & schedule	<p>At least two doses are expected to be needed for primary immunization, between the ages of 6 and 9 months.</p> <p>An additional booster dose may be required to maintain effective, long-lasting immunity through the first 5 years of age.</p>	<p>The schedule should provide protection prior to the peak of infection to prevent the majority of ETEC infections and disease, and thus prevent the initiation of the environmental enteric dysfunction (EED) pathogenic process.</p> <p>This vaccine is expected to be delivered through the routine immunization schedule, although it may be implemented on a sub-regional or sub-national level in areas of heterogenous endemicity. Every effort should be made to align the dose schedule with existing EPI vaccination schedules.</p> <p>Depending on the vaccine platform and formulation, two or three doses might be needed for primary immunization, with the first dose at 6 months, concomitantly with other EPI vaccines, and the final dose in the primary series potentially to be given with measles-containing vaccine (MCV) at 9 months.</p> <p>A booster dose after the primary series may be needed. If this is in the second year of life, it could be given with the second MCV dose at 15 months. No more than one booster dose in the first 5 years of life is preferred.</p> <p>The optimal delivery schedule will be determined by assessment of clinical efficacy and cost effectiveness. Consideration for coformulation with EPI vaccines or other pipeline vaccines that have a compatible route of administration, immunization schedule and delivery requirements would be advantageous. In some situations, such as outbreak, the ETEC vaccine may be delivered through special immunization campaigns. It could be also delivered pre-emptively with cholera vaccines and/or typhoid vaccines.</p>
Safety	<p>A safety and reactogenicity profile at least as favourable as current WHO-recommended routine vaccines in the comparable age group.</p>	<p>A favourable safety profile will need to be demonstrated in adults before progressing to younger ages and the target population. Contraindications should be restricted to known hypersensitivity to any of the vaccine components.</p>

Parameter	Preferred characteristic	Notes
Clinical endpoints	<p>Primary: Reduction of MSD caused by ETEC, according to the case definition for MSD.</p> <p>Secondary and exploratory: Reduction in acute, less-severe diarrhoea (LSD) – diarrhoea leading to care-seeking but without dehydration.</p>	<p>Although there is alignment on the need to prevent MSD due to ETEC in the target population, there is a lack of consensus on the case definition (and associated severity score) for MSD and LSD in community settings. This consensus is needed to compare studies and candidates. Alternatively, trials could assess vaccine impact on medically-attended MSD using a passive surveillance study design.</p> <p>To facilitate policy consideration, secondary endpoints should include initial and follow-up HAZ scores to measure potential impact on growth stunting, with or without overt diarrhoea.</p> <p>Direct effects: On vaccine preventable ETEC strains (however, cross-protection against colonization factors (CFs) not in the vaccine or other defined conserved putative protective antigens may be beneficial), protection against LSD, immune correlates of protection, microbiological correlates of protection (PCR vs culture), mortality.</p> <p>Indirect effects: On all ETEC diarrhoea, reduction in shedding, herd protection, reduction in antibiotic use, reduction in ETEC AMR or other bacteria, reduction in all-cause hospitalization, improved linear growth and other nutritional parameters, cost-effectiveness.</p>
Efficacy	<p>Efficacy of 60% (point estimate) or more against moderate-to-severe ETEC diarrhoea.</p> <p>Assessment of field efficacy in response to all circulating serotypes would inform vaccine effectiveness.</p>	<p>Moderate efficacy (50-60%) is considered clinically meaningful and would be comparable to rotavirus vaccine in some LMICs with high ETEC burden. Efficacy should be based on laboratory-confirmed cases in addition to clinical symptoms for robust assessment of vaccine impact.</p> <p>Value proposed is based on observed lower performance of enteric vaccines in endemic paediatric settings. Efficacy should be based on protection against vaccine-preventable outcomes (VPOs), defined as other strains that have the same putative protective antigens as those in the vaccine.</p> <p>Vaccine impact models should evaluate and guide the efficacy targets.</p>
Duration	<p>Protection to at least 2 years of age starting 14 days after the last dose in the primary series, with protection up to age 5 years desirable.</p>	<p>Protective immunity should be present as early as 9 months of age. Duration of protection from the primary vaccine course up to 5 years of age is optimal; however, a booster dose in the second year or later may be required.</p>
Adjuvant requirement	<p>Preference for the absence of an adjuvant, unless there is clinical evidence of immunological benefit in the target population of 6 months to 5 years.</p>	<p>An adjuvant could be included if proven enhancement of vaccine immunogenicity and efficacy is observed with vaccines in the primary target population in endemic settings.</p>

Parameter	Preferred characteristic	Notes
Immunogenicity	Seek to establish correlate or surrogate of protection based on a validated assay measuring immune effector levels and functionality, which have been directly related to efficacy in the target population.	<p>A correlate of protection would provide an immunological benchmark for the evaluation of ETEC vaccines and immunization regimens; however, these correlates are not well defined. Field and controlled human infection model (CHIM) studies suggest that ELISA-based assays, measuring the level of serum IgG or IgA against key colonization factor and LTB or LT-derived components in the vaccine, may provide a practical correlate of protection (CoP). However, further studies, including sero-epidemiology and field efficacy studies, are needed to better establish and validate threshold levels that best correlate with protection. Correlates of risk could also be helpful.</p> <p>The longevity of the immune response should be characterized, and the relationship to the duration of protection should be investigated.</p> <p>ETEC infections are confined to the mucosal surfaces in the gut, therefore induction of local mucosal immunity is expected to play an important role in protection against ETEC. Immune protection is most likely provided by locally produced secretory IgA antibodies. Accordingly, it has been assumed that assessment of the relative immunogenicity of vaccine candidates should focus on antigen-specific antibody responses induced at the intestinal mucosa, or on surrogate antibody measures of intestinally derived antibody responses such as the ASC, -ELISPOT or ALS responses.</p>
Non-interference	Demonstration of favourable safety and immunologic non-interference upon coadministration with other vaccines recommended for use within the EPI schedule.	<p>There should be no significant interference in relation to safety and immunogenicity with concurrently administered or co-formulated vaccines.</p> <p>To accelerate development of a combined vaccine, it is advised to assess the potential interference between vaccine components, including adjuvants and excipients.</p>
Route of administration	Oral or injectable (IM, ID or SC), using standard volumes for injection, as specified in programmatic suitability for prequalification (PQ), or needle-free delivery.	Presentation and route must be suitable for use in the primary target population of 6 to 24 months of age.
Product stability and storage	<p>Two years at 2 to 8°C.</p> <p>For a powder formulation: Vaccine vial monitor (VVM) for at least 30 days at 37°C.</p> <p>For a liquid formulation: VVM for at least 14 days at 37°C.</p>	<p>If some components need to be kept separate from the vaccine until administration, i.e. buffer or diluent, it would be critical that these are not required to be stored in the cold chain.</p> <p>Data on controlled temperature chain (CTC) stability would be desirable.</p>

Parameter	Preferred characteristic	Notes
Vaccine presentation	Low-dose vials or blow-fill-seal multi-mono-dose containers to reduce missed opportunities for vaccination and vaccine wastage.	<p>Presentations with minimal components and cold chain footprint that ease preparation/administration and reduce disposal waste are encouraged.</p> <p>Novel delivery technologies and packaging presentations may help to optimize and overcome the delivery challenges and increase vaccine effectiveness.</p>
Registration, PQ and programmatic suitability	The vaccine should be prequalified according to the process outlined.	WHO-defined criteria for programmatic suitability of vaccines should be met.
Access and affordability	<p>The vaccine should be cost-effective and price should not be a barrier to access, including in LMICs.</p> <p>Dosage, regimen and cost of goods amenable to affordable supply.</p>	<p>It is imperative to capture the full burden of ETEC diarrhoea, including the full morbidity burden, in determining an acceptable price. In addition to the direct and indirect effects of infection, herd protection and assessment of the broader societal and economic benefits of vaccination are important factors when articulating the value of an ETEC vaccine from an LMIC prospective.</p> <p>The vaccine's impact on health systems and other aspects of implementation science should be evaluated pre- or post-approval, as this will also contribute to assessment of vaccine value.</p>

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