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Human Enteric Vaccines

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Abstract

Worldwide, enteric infections are the second commonest cause of disease burden due to all infectious diseases. It is estimated that they are responsible for 1.3 million deaths per year, mostly in children below 5 years of age in the developing world. Enteric infections are caused by a gamut of bacterial, viral and parasitic agents. These include viruses (rotaviruses, enteric adenoviruses, astroviruses, human caliciviruses), bacterial agents (*Vibrio cholerae*, *Shigella* spp., enterotoxigenic *Escherichia coli*, *Salmonella* spp. including *Salmonella Typhi*) and parasites. While suitable effective licensed vaccines are available against some of the enteric infections, many such diseases do not have a vaccine against them. Understanding the current scenario of vaccine development against these diseases is of paramount importance. This article reviews the current scenario in vaccine research and development against some of the common human viral and bacterial enteric pathogens of public health importance. Vaccines against parasitic diseases are not discussed.

Keywords: Enteric vaccines; Oral vaccines; Parenteral vaccines; Cholera; Rotavirus

Introduction

Enteric infections are major public health problem, especially affecting children in the developing world with an estimated 1.3 million deaths worldwide in 2008, which account for 15% of total global child death [1]. Diarrheal diseases are global killers, making diarrhea the second leading cause of death in infants and young children. It kills 2,195 children every day being higher than AIDS, malaria and measles combined [2]. Outbreaks of cholera, shigellosis, rotavirus diarrhea and typhoid fever occur frequently in resource-poor countries resulting in high disease burden, mortality and slow economic growth. World Health Organization (WHO) has given highest priority to the development of new or improved vaccines against rotavirus, Shigella spp., enterotoxigenic *Escherichia coli* (ETEC), *Vibrio cholerae* O1 and *Salmonella Typhi* [3].

It is believed that for enteric vaccines to be effective, mucosal immunity requires boosting up. Ideally, mucosal protection is best achieved by administration of a vaccine through the mucosa and oral route has been found to be the best choice [3,4]. Use of live, attenuated bacteria as protective vaccine antigens via the mucosal routes is effective against microbial infections [5]. Transgenic plants or fruits offer a new strategy for the delivery of safe, oral subunit vaccines against ETEC and cholera, which are likely to be suitable for use in developing countries [6]. This mode of vaccine delivery is yet to be fully established and the safety issues of such vaccines need to be addressed. This review focuses on licensed enteric vaccines (Table 1) as well as those that are under development in research mode (Table 2).

Vaccines against	Routes of immunization	Active component (s)	Doses	Names of Licensed products (manufacturers)	Target population (age of vaccinees)	References
Cholera						
Killed whole-cell/ recombinant cholera toxin B-subunit vaccine (WC/rBS)	Oral	Mixture of 1011 heat-killed or formalin-killed. CholeraeO1 of classical and El Tor biotypes and Inaba and Ogawa serotypes +1 mg CT B subunit	2	Dukoral™ (Crucell/SBL).	Children, adult	[10,11]
CVD 103-HgR recombinant live attenuated vaccine	Oral	A ∆ctxA derivative of classical Inaba strain *V. cholera* 569B (CVD 103) and an Hg++ resistance gene introduced into the	1	Berna, Swiss Serum and Vaccine Institute as Orachol (Europe), or Mutacol (North America)*	Children, adult	[13]

		Hemolysin A locus of the chromosome. 108–109 cfu				
Killed whole cell (WC) bivalent vaccine	Oral	Inactivated *V. cholerae* bacteria (Same O1 composition as above + 5 X 1010 formalin-killed O139 bacteria)	2	Shancol (in India) mORC-Vax (in Vietnam)	Children, adult	[15]
Typhoid						
Live attenuated Ty21a	Oral	Vi negative mutant strain of *S. Typhi*	3	Vivotif (Berna Biotech)	Children>2 yr, adults	[22,23]
Vi polysaccharide	Parenteral	Purified Vi capsular polysaccharide (CPS)	1	Typhim Vi (Sanofi Pasteur)Typherix (GSK) TypBar (Bharat Biotech); Shantyph (Shantha Biotech);Typho-Vi (BioMed)	Children>2 yr, adults	[24,25]
Conjugate vaccine (Vi-TT)	Parenteral	Vi antigen is coupled to tetanus toxoid protein	2	Peda-typhTM, (BioMed)	Children<2 yr	
Multivalent combination vaccines	Parenteral	Combined Vi CPS and inactivated hepatitis A virus grown in human diploid cells and adsorbed onto aluminum hydroxide.	2	HepatyrixTM, (GSK); ViatimTM, (Aventis Pasteur)	adults and adolescents>15 yrs	[36]
Rotavirus diarrhea						
Pentavalent human-bovine reassortant WC3 vaccine	Oral	Bovine WC 3 reassortant viruses carrying G1, G2, G3 and G4 of P(8) RNA segment of human rotavirus	3	RotaTeq™ (Merck Vaccines, USA)	Young infants	[50]
Monovalent RIX-4414 human rotavirus strain	Oral	A G1 P [8] rotavirus isolated from a human infant that evoked neutralizing antibodies to rotaviruses of G types 1-4.	2	Rotarix™ (GSK Biologicals)	Young infants	[53]

Table 1: Licensed vaccines against common enteric diseases of public health importance (adapted from reference 3). *Manufacturing was discontinued after 2004.

Cholera

Cholera is an acute watery diarrheal disease caused by *Vibrio cholerae* (*V. cholerae*). The emergence of a new serogroup of *V. cholerae* O139 during early 1990s showed no cross-protection with serogroup O1, but remained confined to Bangladesh, India and other Asian countries [7]. Some cases were reported from developed countries, mostly among travelers [8]. The global burden of cholera is huge, particularly in developing countries. Every year an estimated 2.8 million cases of cholera and about 91,000 deaths occur in endemic countries [9].

A whole-cell injectable cholera vaccine was developed by Haffkine in 1894 in India and widely used throughout the world. This vaccine provided 48% protection for 3-5 months only. The vaccine was highly reactogenic and required 2 doses for development of protective immunity. In view of this, the vaccine was not any further recommended and was withdrawn.

Two types of oral cholera vaccines (OCV) are available (Table 1). Both the vaccines have been shown to be safe, immunogenic and efficacious. One of them is an inactivated vaccine and the other one is a live attenuated vaccine. These two OCVs have been licensed in a few countries and are mainly used for travelers' from industrialized countries to cholera endemic areas [3]. The only inactivated oral vaccine that is currently recommended by WHO (DukoralTM, licensed by SBL Vaccine, Sweden) consists of heat- or formalin-killed whole-cell *V. cholerae* O1, representing both serotypes (Inaba and Ogawa) and both biotypes (classical and El Tor), and supplemented with purified recombinant cholera toxin B-subunit (CTB) [3]. The whole cell/recombinant B subunit (WC/rBS) oral vaccine, which is given with buffer to neutralize stomach acidity, conferred 80%-90% protection during 6 months in all age groups in Bangladesh and Peru [10,11]. This was also used for mass vaccination among risk population to protect from emergence of potential outbreaks [12]. The second type of oral cholera vaccine consists of a live attenuated

genetically modified *V. cholerae* O1 Inaba strain (CVD103-HgR), which has been constructed so as to produce CTB but not the A subunit of CT (Cholera Toxin) [3]. Efficacy trial of a single dose of live oral cholera vaccine in North Jakarta, Indonesia showed only 15-20% protection at the end of third year of surveillance [13]. The vaccine has been licensed in several industrialized countries. Since, these are genetically modified bacteria, specific regulatory issues regarding their safety for humans and environmental use need to be elucidated [14].

A bivalent O1 and O139 killed whole-cell, oral vaccine without CTB was recently developed in Vietnam (Table 1). It was found to be safe and immunogenic in both adults and children, generating 90% anti-O1 and 68% anti-O139 vibriocidal responses after administration of a two-dose regimen [15]. In a collaborative initiative, International Vaccine Institute (IVI), South Korea and Kolkata's National Institute of Cholera and enteric Diseases (NICED) conducted a Phase III, double-blind, placebo controlled, randomized clinical trial in Kolkata. This bivalent (O1 and O139) vaccine is currently the only potential vaccine against cholera caused by O139 serogroup and licensed for marketing following the documentation of 66% protection for two years post-vaccination and 65% cumulative protection at the end of 5 years in all age groups [16,17]. Recent findings showed that in addition to their direct vaccine-specific protection, OCVs also provide substantial indirect 'herd' protection to unvaccinated persons in the community due to minimization of the transmission of V. *cholerae* to unvaccinated people. The overall protection may approach 80% in settings with high coverage [18].

Among new generation unlicensed cholera vaccine, Peru 15 recombinant live oral vaccine was shown to be safe and immunogenic in Bangladeshi children [19].

Typhoid Fever

Typhoid fever is caused by *Salmonella enterica* serovar Typhi (S. Typhi). The case fatality rate has been reported to be as high as 10%–20% in the absence of appropriate antibiotic treatment. However, due to the global emergence of strains resistant to commonly used drugs (chloramphenicol, ampicillin and co-trimoxazole) as well as fluoroquinolones, a tremendous therapeutic problem was encountered which indicated the need for a suitable vaccine effective against typhoid [20]. Typhoid fever remains a major cause of morbidity with an estimated global incidence of 22 million cases and 200,000 deaths per year [20]. In a recent multi-centric study in 5 Asian countries (China, India, Indonesia, Pakistan and Vietnam), it was estimated that the incidence of typhoid ranged from 15.3 per 100,000 persons/year in China to 451.7 per 100,000 persons/year in Pakistan. Population-based studies have demonstrated a wide variation in the incidence of typhoid fever both globally and within the same country [21].

First heat-killed, phenol preserved whole-cell *S. typhi* was used as parenteral vaccine in 1896 in Germany and England, but it lost popularity due to high reactogenicity [22]. Two other typhoid vaccines that are commercially available are the attenuated Ty21a live oral typhoid vaccine and the purified Vi polysaccharide (PS) parenteral typhoid vaccine [3].

The attenuated Ty21a live oral typhoid vaccine (Table 1) was tested in Phase III trials, initially in Egypt and then in Santiago, Chile, Indonesia and other countries. Randomized, controlled field trials involving children were conducted in these countries on the recommendations of the WHO and the Pan American Health Organization (PAHO). The vaccine elicited both anti-Salmonella antibodies and strong cell-mediated immune response. Three doses of the enteric-coated vaccine provided 67% protection over 3 years, and 62% protection over 7 years [23]. The field studies confirmed the efficacy and tolerability of Ty21a, and provided evidence of indirect protection (herd immunity).The vaccine is now licensed in 56 countries in Asia, Africa, Europe and the Americas [22,23].

The subunit Vi vaccine (Table 1) contains purified PS and elicits serum anti-Vi antibody response and is protective in 85%–95% of adults and children above 2 years of age after a single parenteral injection. *S. typhi* is highly sensitive to both complement-assisted killing and opsonophagocytotic effects via Vi-specific antibodies. This vaccine has been found to have 72%–77% efficacy in trials in Nepal and South Africa and is licensed in more than 92 countries globally [24,25].

A locally produced Vi vaccine in Shanghai, China, demonstrated a 69% protective efficacy in a randomized double-blind, placebo-controlled trial amongst 5–19 years old children [26,27]. Single-dose mass vaccination campaigns targeting school children were carried out in Vietnam and Indonesia and were also conducted in China in both adults and children [28-30]. The impact of drug resistance may improve the cost effectiveness of mass vaccination programs in typhoid endemic countries [31]. Herd immunity was noted in a recent cluster-randomized effectiveness trial of Vi PS vaccine in Kolkata, India: a 44% reduction in typhoid among unvaccinated subjects was found that significantly contributed to the 61% overall vaccine protection [32].

The lack of immunogenicity of purified Vi PS in younger children has prompted the development of a conjugate Vi vaccine using Pseudomonas aeruginosa exotoxin A (Vi-rEPA) [33]. Persistent efficacy of Vi conjugate vaccine against typhoid fever in young children was observed following 3 years post vaccination in Vietnam and Cambodia [34]. Other carrier proteins like Tetanus toxoid, Corynebacterium diptheriae toxin were also used for conjugation [35]. Two multivalent combination vaccines (combination of Vi PS of *S. typhi* and inactivated Hepatitis A virus) marketed under the names Hepatyrix™ and Viatim™ are available (Table 1). A prospective, randomized, observer-blind comparative study in healthy adults showed that both vaccines were well tolerated and induced high levels of protective antibodies [36].

New attenuated *S. typhi* strains that could be used as live oral vaccines are currently at an advanced clinical stage of development: Ty800, live attenuated oral single-dose vaccine, a phoP/phoQ deletion mutant of Ty2 has been shown to induce vigorous serum anti O-antibody responses in Phase I trials [37]. The CVD908-htrA live attenuated oral vaccine, an aroC/aroD/htrA deletion mutant was tested in Phase II trials [35]. Attenuated *S. typhi* strain M01ZH09 oral vaccine developed by Emergent Biosolutions tested in Phase II trial induced serum and secretory IgA specific for surface antigens other than Vi (e.g., O and H) [38].

Rotavirus Diarrhea

Rotaviruses are the leading causes (25-55%) of acute severe dehydrating diarrhea in infants and young children in both industrialized and developing countries and account for about 40% of hospitalizations in children under 5 years of age. Almost all children suffer from rotavirus infection/diarrhea within first 2-3 years of their lives. Outbreaks in day-care centers and hospitals are common and can spread rapidly [39].

Rotavirus causes approximately 400,000 deaths each year, mostly in children below 2 years of age [40,41]. Up to 85% of these deaths occur in "low-income" countries. Rotaviruses were detected in 56% of stool specimens from hospitalized children with diarrhea in Vietnam, 41% in China, 56% in Myanmar and 29% in Hong Kong [42].

Rotaviruses are 70 nm icosahedral viruses that belong to the family Reoviridae. The virus is composed of three protein shells consisting of an outer and an inner capsids and an internal core with the 11 segments of the double-stranded RNA genome. Two structural outer capsid proteins, VP7 (G protein) and VP4 (P protein) define the G and P serotypes/genotypes of the virus, respectively. These are the major antigens involved in virus neutralization. Human rotaviruses bearing VP7 G serotypes G1-G4 and G9 and VP4 P genotypes P[4], P[6] and P[8] are predominant worldwide [43,44]. P[8]G1 is the globally predominant strain, accounting for over 70% of rotavirus infections in North America, Europe and Australia, whereas about 30% of the rotavirus infections in South America and Asia, and 23% of those in Africa. Other frequently isolated strains are P [8]G3, P[4]G2, and P[8]G4 [45]. G9 strains emerged during late 1990s and became the predominant strains in some parts of Asia and Africa. Similarly, the distribution of the VP4 P[6] antigen is also different according to regions: P[6] strains constitute over 50% of the circulating strains in Africa, whereas P[8] strain is common in the rest of the world [46]. When mixed infections with distinct rotavirus strains occur, the gene segments may reassort independently, producing progeny "reassortants", which are important for viral diversity. An effective rotavirus vaccine should take into account such variations of prevalent strains [45-47].

The first rotavirus vaccine tested in humans was the live bovine strain RIT4237 (P[1]G6). Efficacy trials with this vaccine and other animal rotavirus strain derived vaccines did not show encouraging results leading to the discontinuation of these vaccines. In view of the inconsistency of the results, efforts were made to either use naturally attenuated human rotavirus strains or to develop reassortant rotavirus strains bearing a human rotavirus gene for the VP7 protein and other genes from a simian or a bovine rotavirus strain [48,49].

The first live oral reassortant vaccine was developed by the National Institutes of Health (NIH, Bethesda) as a tetravalent mixture of the P[3]G3 rhesus RRV strain and human rotavirus strains of G types 1, 2, and 4, respectively forming three independent rhesus-human reassortants [48]. The vaccine (RotaShield[TM]) was introduced in 1998 by Wyeih-Lederle. But there was a major setback in 1999 leading to the withdrawal of RotaShield[TM] in less than a year after its introduction due to the reported intussusceptions in those who received the vaccines [49]. Subsequently, new live oral rotavirus vaccines have been developed [3].

More recently, a pentavalent human-bovine (WC3) reassortants (Gl, G2, G3, G4 with P[8] and G6 with P[7]) live-attenuated, 3-dose oral vaccine, has been developed (Table 1). The vaccine was administered at 6–12 weeks of age at 1–2 months intervals. This vaccine (RotaTeq[™]) was tested in a Phase III trial in several countries including the USA and Finland on more than 70,000 children and carefully monitored for risks of intussusception. The vaccine was 74% efficacious in preventing any rotavirus disease and provided 98% protection in case of severe rotavirus diseases. In developing countries like Bangladesh and Vietnam, pentavalent rotavirus vaccines prevented severe rotavirus diarrhea by more than 50 percent during the first year of life, when children are at greatest risk for having

rotavirus diarrhea [50]. Merck is marketing the licensed vaccine RotaTeq[TM] globally [3].

Another multivalent bovine-human reassortant vaccine has been independently developed by the National Institute of Allergy and Infectious Diseases (NIAID, NIH, Bethesda). Analysis of Phase II data revealed a good immune response and no adverse interference with concomitantly administered childhood vaccines were noted [51]. Two naturally occurring human-bovine, neonate derived, reassortant strains (116E and 1321) are under development in India in partnership with Centers for Disease Control and Prevention (CDC), USA and the Children's Vaccine Programme funded by the Program for Appropriate Technology in Health (PATH). These strains have P[10]G9 and P[ll]G10 antigens, respectively [52].

A monovalent (P[8]G1) live-attenuated, 2-dose oral vaccine has been developed from a human rotavirus strain RIX-4414 (Table 1). The vaccine (Rotatrix[™]) has been tested in Latin American and European countries in a phase III trial on more than 63,000 children. The vaccine was 85-100% efficacious in preventing severe rotavirus disease. No increased attributable risk of intussusception was reported. The vaccine has been licensed in several countries in Latin America, Asia, Africa and Europe [53]. Phase II clinical trials were conducted in developing countries like Bangladesh, Vietnam and Philippines to investigate the safety and immunogenicity of the vaccine when given concomitantly with the oral polio vaccine (OPV). The Rotatrix[™] vaccine was found to be immunogenic when co-administered with OPV and did not interfere with OPV sero-protection rates in the infants [54,55].

Based on the clinical trial data from Asia and Africa, in 2009, the WHO's Strategic Advisory Group of Experts (SAGE) recommended that all countries should include rotavirus vaccines in their national immunization programs. Significant declines in hospitalization and deaths due to rotavirus and all-cause diarrhea have been observed in many of the countries that have introduced rotavirus vaccines [56,57]. Unvaccinated children and adults were found to be protected due to "herd immunity" [58]. The Global Alliance for Vaccines and Immunizations (GAVI) is sponsoring a new Public-Private organization, the Rotavirus Vaccine Programme at PATH, whose role is to accelerate the development and introduction of rotavirus vaccines in developing countries. Post marketing surveillance is required to measure the extent of cross-protection of the existing vaccine against different rotavirus serotypes, including serotype G9, which is becoming increasingly important across Asia and Africa.

Shigellosis

Shigellosis remains an important cause of morbidity and mortality globally, particularly among children less than 5 years of age in developing countries. In 1999, it was estimated that *Shigellae* caused approximately 113 million episodes and 0.6 million deaths annually. In addition, about 500,000 cases of shigellosis are reported each year among travelers and military personnel from industrialized countries [59].

Since *Shigellae* invade and destroys intestinal mucosa, antimicrobial therapy is the cornerstone of treatment for shigellosis, but the option is gradually narrowed down due to widespread occurrences of multidrug resistant strains in Asia where even resistance to ciprofloxacin has been observed [60]. Resistance has increased also to the second line choices like pivmecillinam and azithromycin (30–50%) and to the third generation cephalosporin [60,61]. Increasing occurrences of

outbreaks and spread of multidrug resistant *Shigellae dysenteriae* type1 strains is a matter of great concern [60]. It is imperative that there is an urgent need for a safe and effective Shigella vaccine to control the disease, but unfortunately currently no such vaccine is available.

There are four serogroups of *Shigellae*: *S. sonnei*, *S. flexneri*, *S. dysenteriae*, and *S. boydii*. These serogroups are subdivided into serotypes on the basis of the O-polysaccharide antigen of their lipopolysaccharide (LPS). *S. sonnei* is the predominant serogroup in industrialized countries, where it accounts for 77% of cases compared to 15% in developing countries. It was also the commonly obtained isolate in Thailand in recent years, a phenomenon possibly linked to the level of economic development of the country and tourists from developed regions. *S. flexneri* is endemic in developing countries and is the most frequently (60%) isolated species worldwide. The predominant serotype of *S. flexneri* is serotype 2a, followed by lb, 3a, 4a and 6, although recent studies from Asian countries showed wide variations in the prevalence of these serotypes. *S. boydii* is the relatively uncommon serotype. *S. dysenteriae* serotype 1is notorious for being multidrug resistant and has caused large scale severe epidemics of dysentery [59]

Antibody directed to the O somatic antigen of *Shigellae* is protective and is type specific. In view of the large number of Shigella serotypes, vaccine development against all serotypes is complex, although it is observed that a vaccine could give cross-reactive protection. Candidate shigellosis vaccines that are currently under development include both killed and live vaccines and are mostly targeted against *S. flexneri* [3,62,63]. Two approaches to develop Shigella vaccines have demonstrated reasonable protection in field trials. The first approach is the development of conjugate vaccines in which Shigella O-polysaccharides are linked to carrier proteins. The second approach is the development of live oral vaccines after attenuation of wild-type *Shigellae* spp. [3,64]. These vaccines are at preclinical stage (Table 2). This approach includes:

- Parenteral conjugate vaccines comprising of purified *S. dysenteriae* type 1 LPS conjugated to tetanus toxoid; *S. flexneri* 2a and *S. sonnei* LPS conjugated to recombinant *Pseudomonas aeruginosa* exotoxin A [65]. These vaccines were developed at the NIH, were safe and immunogenic in children greater than 4 years of age and afforded 74% protection when tested in field trials with Israeli military volunteers, except in pre-school children [66,67].

- A nasally administered bivalent invasin complex vaccine (Invaplex) against *S. flexneri* 2a and *S. sonnei* developed by Walter Reed Army Institute of Research (WRAIR), US is under evaluation [68].

- A nasally administered proteosome vaccine consisting of *S. sonnei* and *S. flexneri* 2a LPS linked to the outer membrane protein of group B *Neisseria meningitides* [69].

Definite progress has been made with candidate live oral Shigella vaccines (Table 2), but the problem remains with them that under-attenuation causes excessive reactogenicity and over-attenuation leads to poor immunogenicity in human subjects. These approaches include:

- A live, attenuated *S. sonnei* WRSS1 was developed by WRAIR with a single deletion mutation of the virG gene as oral *Shigella* vaccine. The safety and immunogenicity of the vaccine was tested in Israeli volunteer in phase II trial and the vaccine was found to elicit a significant immune response [70].

- A live, attenuated *S. flexneri* 2a strain (SC602), carrying mutations in their icsA (virG), iuc, int and toxA (stxA) genes and a *S. dysenteriae* type 1 strain (SC599) carrying mutations in their icsA, ent, fep and stxA genes were developed at the Pasteur Institute, Paris [71-73]. SC602 was tested in adult volunteers in the USA and in both adults and children in Bangladesh, although the outcome was not so encouraging in Bangladesh study [71,72]. SC599 was well tolerated in their phase II trials. A single oral immunization of SC599 vaccine elicited a significantly higher circulating IgA-antibody secretory cells and serum antibody, when compared to phase I trials [73].

- A series of strains were made auxotrophic for aromatic amino acids synthesis (amA) and guanine synthesis (guaBA) with progressive deletions of virulence genes virG, set (ShET-1) and sen (ShET-2) resulting in construction of CVD1203, CVD 1204, etc. culminating in strain CVD1208S, which was safe and immunogenic in phase I studies [74].

Enterotoxigenic *Escherichia coli*

Enterotoxigenic *E. coli* (ETEC) remains the major cause of infantile diarrheas in the developing world and of traveler's diarrheas in the industrialized countries among the travelers visiting the third world countries [75].

ETEC infections are characterized by profuse watery diarrhea, generally clinically indistinguishable from cholera, leading to dehydration and malnutrition in young children. ETEC causes approximately 280 million diarrhea based episodes and more than 400,000 deaths annually [76,77]. Active community and hospital surveillance in Bangladesh has shown that the prevalence of ETEC infections were 14–23% in children with diarrhea and 8% in asymptomatic children. ETEC are globally responsible for about 25% of persistent diarrheas and 26% of severe diarrheas requiring hospitalization [78].

ETEC attaches to specific receptors of the enterocytes in the small intestinal lumen by the hair-like fimbriae, which function as adhesins and define strain-specific antigenicity. More than 25 types of fimbrial antigens, called coli surface antigens (CSs) or colonization factor antigens (CFAs) have been described, with seven types (CFA/I and CS1 through CS6) occurring most frequently. Antibodies targeted to fimbriae are protective but showed serotype-specificity. Once attached to the intestinal epithelium, ETEC elaborates a heat-labile toxin (LT) and/or a heat-stable toxin (ST), which induces the watery diarrhea. Approximately one half of ETEC strains secrete only ST, 25% secrete only LT, and the remaining 25% secrete both LT and ST. LT is highly homologous to cholera toxin (CT) comprising of an active A subunit surrounded by five B subunits for attachment. ST is a small peptide toxin of 18 or 19 amino acids. Since ST is not immunogenic, it is not a suitable candidate for vaccine [3,78].

ETEC infections in children in developing countries confer immunity against subsequent infection as reflected by declining rates of ETEC diarrhea with increasing age and lower ratios of symptomatic to asymptomatic ETEC infections in adults. The protection against subsequent infections occurs if the infecting strains have similar toxin and/or colonization factor phenotypes to that of initial strains. Thus immunization against ETEC early in life is expected to confer effective protection. Travelers from industrialized country and military troops on deployment are important potential target population for vaccination against ETEC [78,79].

The oral killed WC/rBS cholera vaccine (Dukoral™ as stated earlier) was found to prevent 23% of all diarrhea episodes and 52% of episodes due to ETEC in tourists which did not last more than a few months [80]. Several approaches have been pursued to develop specific ETEC vaccines (Table 2).

Vaccine	Immunization route	No. of doses	Developer	Status	References
Shigellosis					
Attenuated *S. sonnei* strain WRSS1	Oral	2	Walter Reed Army Institute of Research	Phase II	[70]
Attenuated *S. flexneri*2a strain CVD 1208S	Oral	2	Center for Vaccine Development, University of Maryland	Phase I	[74]
Attenuated *S. flexneri*2a strain SC602	Oral	1-2	Pasteur Institute	Phase II	[71]
Attenuated *S. dysenteriae* 1 strain SC599	Oral	2	Pasteur Institute	Phase II	[73]
Shigella glycoconjugates (O polysaccharide covalently linked to carrier protein)	Intra-muscular	2	National Institute of Health and Human Development	Phase III	[65]
Shigella invasion complex (Invaplex)	Nasal	3	Walter Reed Army Institute of Research	Phase I	[68]
Proteosomes (OMP of Group B meningitides) to which S. sonnei or *S. flexneri*2a LPS is adsorbed	Nasal	2	ID Biomedical*	Phase I	[69]
ETEC diarrhea					
B subunit-inactivated whole fimbriated ETEC combination	Oral	2	University of Goteborg and SBL	Phase III	[82]
Attenuated fimbriated non-toxigenic *E. coli* (derived from ETEC)	Oral	2	Cambridge Biostability Ltd.	Phase I	[88]
Attenuated Shigella strains expressing ETEC fimbrial colonization factors and B subunit of LTh**	Oral	2	Center for Vaccine Development, University of Maryland	Phase I	[86]

Table 2: New generation unlicensed vaccines against Shigella and ETEC (adapted from ref. 3). *Now GSK Biologicals. **LTh, LT from a human ETEC strain.

One of the most successful vaccine approach developed by the investigators at the University of Goteborg (Sweden) is based on recombinant CTB combined with 4 formalin-killed ETEC strains that collectively express the colonization factors of epidemiological importance in developing countries [78,81]. Phase II studies of 2-doses of this vaccine have been conducted in Bangladesh, Egypt, Israel, Nicaragua, the USA and Europe. This vaccine was safe and immunogenic by inducing mucosal antibody responses to CTB and to the CFA components of the vaccine. The vaccine was less than 20% effective against ETEC infections in the very young [82]. Several factors were involved for these observations. Studies in US travelers showed 60%–84% protection against severe incapacitating diarrhea [83]. CS6 protein antigens could induce systemic immune responses in the presence of an adjuvant such as LT [84]. ST is not immunogenic even if coupled to a protein carrier and was very reactogenic. Lastly, feeding volunteers with transgenic corn or potatoes expressing the LT B subunit resulted in significant serum and gut antibody responses [85]. At the Center for Vaccine Development (CVD), University of Maryland (USA), the vaccine approach being pursued is by using live attenuated Shigella vectors for expression of ETEC fimbrial and LT antigens. Such constructs protect against both Shigella and ETEC [86]. The same approach is being followed by Micro science using their spi-VEC oral live attenuated typhoid vaccine as a vector for the delivery of ETEC antigens [87].

Two non-toxigenic ETEC strains have been attenuated by mutagenesis of the aroC and ompR genes or of the aroC, ompC and ompF genes, respectively, to be used as candidate live, oral attenuated vaccines. The mutagenized strains were found to be well tolerated and immunogenic when fed to human volunteers [88]. But none of these ETEC candidate vaccines were found to be protective in infants and young children in the endemic areas. Intense efforts need to be made to improve the immunogenicity of the candidate vaccines. The strategy is to include toxin antigen alone or together with over-expressing colonizing factors antigens [89,90].

Conclusion

In recent years, there is an increased research interest for understanding the biology and development of enteric vaccines due to improved knowledge about the mucosal immune system together with the development of improved methodologies for measuring local immune responses, both humoral and cell-mediated immunity. Several new enteric vaccines are in different stages of clinical testing, including improved alternatives to existing vaccines. Oral route has been extensively studied for mucosal vaccination due to its many attractive features. But the tolerance is a crucial challenge in the development of effective oral vaccines. Other challenges, including antigen degradation by proteolytic enzymes, the low dose of antigen absorbed, a lack of potent mucosal adjuvants, and difficulty in

directing antigens to M cells, are also responsible for the non-availability of a potent oral vaccine. Two areas that could revolutionize enteric vaccine research are (i) the development of new well-tolerated mucosal adjuvants that could influence the innate immune system vis-à-vis enhance the adaptive immune response to oral vaccines and (ii) the use of lectins or other means to target vaccine antigens or use of delivery vehicles for direct delivery of the purified subunit antigens to intestinal M cells.

The ability of M cells in Peyer's patches to take up diverse numbers of microorganisms to antigen-presenting cells (APCs) have made M cells an ideal target for delivery of vaccine to the mucosal immune system. Targeting specific receptors on the apical surface of M cells may have the ability to specifically increase the uptake and presentation of antigens, consequently initiating higher immune response and inducing protection against infectious agents [91]. WHO prequalified safe and effective enteric vaccines are licensed and commercially available in several countries. Some countries have introduced them in their routine immunization programs. During implementation of an enteric vaccine in developing countries where the disease is endemic, it was observed that poor people living in the slum communities prefer to have safe water, improved sanitation, housing and not the vaccines. It is true that safe water supply and promotion of good sanitation practice are the permanent measures to prevent diseases, improve the health and quality of life of people in a country, but achieving this is a difficult proposition in resource poor countries in near future. It is imperative that researchers should not only contribute to the development of suitable vaccines, but, it is also their responsibility to sensitize the political leaders, bureaucrats, policymakers and the common people about the cost-effectiveness, overall benefits and their impact on economic growth by introduction of these vaccines in routine immunization programs of the countries, which would mitigate the suffering of poor people. Oral cholera vaccine is now ready to be introduced in endemic areas. The vaccine should be used before the cholera season so that, it will prevent the occurrence of outbreaks and minimize the number of cholera cases.

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