Vaccines against human enteric bacterial pathogens

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The development of vaccines against enteric bacterial pathogens presents a challenge because of the large number of pathogens capable of causing disease and the requirement to induce immunity that is effective in the gut. A new generation of enteric vaccines based either on live or non-living antigens delivered orally or by injection are reaching the clinic in the early phases of evaluation. However, considerable technical barriers have to be overcome before these vaccines reach the general population.

Enteric infections are still extremely common in all parts of the world. They are caused by different viruses, bacteria, parasites and even fungi and symptoms such as diarrhoea can also be associated with toxin ingestion. Although common globally, enteric infections associated with individual pathogens differ dramatically in their geographical distribution and epidemiology. In industrialised countries, enteric infections are normally relatively infrequent on an individual basis, are usually non-life threatening and are sporadic (or occur in small outbreaks). In non-industrialised countries, individuals are often chronically infected (for example with helminths) and may suffer simultaneously from infection by more than one infectious agent. In addition, in the poorer nations of the world, enteric infections are responsible for a great deal of chronic morbidity and mortality that can be regarded as life threatening. This general description of the current state of affairs has consequences for enteric vaccine development. The key drivers for vaccine development are often economic considerations and, consequently, the development of vaccines to target rare disease in industrialised countries is often neglected. For example, we have made very little progress in vaccine development for eukaryotic pathogens. The problem of effective vaccine development is compounded by the site associated with the initial phases of the establishment of the infection, the intestine. The induction of predominantly systemic immune responses by classical parenteral injection methods may not be the optimal way to induce immunity to a pathogen colonising the intestine.
The induction of specific gut-associated mucosal responses may favour more effective protection. This presents a dilemma to vaccine developers, who often have two options for vaccine development. One involves optimising classical parenteral vaccines delivered by injection. The other involves the development of vaccines that can be delivered directly to mucosal surfaces\(^1\). The second option usually involves a vaccine formulation suitable for oral delivery. The oral delivery route presents significant demands on a vaccine. Most antigens are poorly immunogenic when delivered mucosally. This is due to a combination of factors including antigen dilution or denaturation and tight immune regulation at mucosal surfaces\(^2\). Mucosal immune regulation is designed to prevent inadvertent immune responses to dietary or environmental antigens while favouring active immunity to dangerous antigens from pathogens\(^3\). As we still know relatively little about how this discrimination process works, we cannot easily create highly immunogenic mucosal vaccines from pure, non-living antigens. Therefore, historically, live vaccines have been favoured for oral vaccine development, although novel mucosal adjuvants may play an important role in the future development of mucosal, non-living vaccine formulations. Here we will review some recent developments in enteric vaccine development with an emphasis on bacterial vaccines.

**Early vaccine development**

Table 1 lists some of the main bacterial pathogens that cause enteric diseases in humans and provides examples of vaccines that have recently been licensed for human use or have been evaluated in the clinic. Many of the listed pathogens are members of the Enterobacteriaceae including *Escherichia coli*, *Shigella* and *Salmonella enterica*. Enteric infection-associated diseases can be caused by live agents (such as bacterial dysentery), by the toxins they release (such as staphylococcal food poisoning), or by a combination of these factors (such as cholera). Some of these pathogens act mainly through infections at the mucosal surfaces of the body (such as *Vibrio cholerae*) where as others are able to cause systemic infections (such as *S. enterica* subspecies *enterica* serovar Typhi or *S. Typhi*). Thus, the pathogenesis, aetiology and consequently immunity of these infections are diverse. One point to take from Table 1 is that vaccine development has been relatively slow in this area. This becomes more obvious when vaccine efficacy and uptake is taken into consideration. Many of the early vaccines, such as the whole cell, inactivated cholera vaccine were of low efficacy (often inducing less than a 50% chance of protection). The injected vaccines were also often significantly reactogenic and required multiple and regular doses. Consequently, vaccine
compliance was poor. Mainly as a consequence of these factors, none of these vaccines are currently commonly used in children’s vaccination programmes or EPI (Extended Programme of Immunisation) run through the World Health Organization. This means that most enteric vaccines are used sporadically, either to protect travellers or individual groups at severe risk.

A further complicating factor for enteric vaccine development is economic. Many potential vaccinees are located in the poorer nations and consequently cannot afford to buy vaccines. In addition, even in

### Table 1 Some of the main bacterial pathogens that cause enteric diseases in humans with examples of vaccines that have recently been licensed for human use or evaluated in the clinic

<table>
<thead>
<tr>
<th>Pathogen</th>
<th>Vaccine type</th>
<th>Comments</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>S. Typhi</td>
<td>Whole cell</td>
<td>Whole cell heat-inactivated phenol-preserved typhoid vaccine given with</td>
<td>Kaistha et al\cite{34}</td>
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<tr>
<td></td>
<td></td>
<td>tetanus toxoid or DEAE-dextran as adjuvants</td>
<td></td>
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<tr>
<td>Vi</td>
<td>Field trial using locally produced Vi: 130,000 people enrolled and protective efficacy 69% after 18 months</td>
<td>Yang et al\cite{32}</td>
<td></td>
</tr>
<tr>
<td>Live</td>
<td>80 healthy volunteers given CVD908 htrA. Good seroconversion achieved</td>
<td>Tacket et al\cite{31}</td>
<td></td>
</tr>
<tr>
<td>Heterologous</td>
<td>S. Typhi Ty800 (\textit{phoPQ} deleted) expressing \textit{H. pylori} urease. No immune responses to urease detected,</td>
<td>DiPetrillo et al\cite{32}</td>
<td></td>
</tr>
<tr>
<td>pathogens with</td>
<td>despite boosting</td>
<td></td>
<td></td>
</tr>
<tr>
<td>S. Typhi as a carrier</td>
<td>with urease plus heat labile toxin (LT)</td>
<td></td>
<td></td>
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<tr>
<td>Cholera</td>
<td>Whole cell</td>
<td>15,000 people given two dose regimen gained 66% protection</td>
<td>Trach et al\cite{37}</td>
</tr>
<tr>
<td>Whole cell + B subunit</td>
<td>14,000 children and adults. Vaccine efficacy up to 82% following three doses. Only 4% after first dose</td>
<td>Taylor et al\cite{38}</td>
<td></td>
</tr>
<tr>
<td>Live</td>
<td>Healthy volunteers given combined CVD103 and Ty21a vaccines. Serum and mucosal antibody responses, good seroconversion</td>
<td>Kollaritsch et al\cite{39}</td>
<td></td>
</tr>
<tr>
<td>Live</td>
<td>Live cholera vaccine administered to 67,000 people. No long-term protection seen</td>
<td>Richie et al\cite{40}</td>
<td></td>
</tr>
<tr>
<td>Live</td>
<td>Thymidine auxotrophic strain of \textit{V. cholerae} cholera toxin negative, haemagglutinin/protease negative</td>
<td>Valle et al\cite{41}</td>
<td></td>
</tr>
<tr>
<td>Shigella</td>
<td>Live</td>
<td>Single-dose of aroD knockout \textit{Shigella flexneri} vaccine strain gave mucosal immune responses for 1 year in a small (30) Vietnamese cohort</td>
<td>Li et al\cite{42}</td>
</tr>
<tr>
<td>LPS-based</td>
<td>O-specific polysaccharide bound to mutant \textit{Pseudomonas aeruginosa} exotoxin A, or mutant diphtheria toxin and given intramuscularly (single dose)</td>
<td>Passwell et al\cite{43}</td>
<td></td>
</tr>
<tr>
<td>LPS-based</td>
<td>Two intranasal doses yielded similar antibody titres to those considered protective when elicited by live vaccines</td>
<td>Fries et al\cite{44}</td>
<td></td>
</tr>
<tr>
<td>Campylobacter</td>
<td>Live, non-colonising</td>
<td>Intramuscular dose with or without adjuvant and with or without oral dose of non-colonising strain failed to give immunity to colonising strain</td>
<td>Ziprin et al\cite{45}</td>
</tr>
</tbody>
</table>
industrialised countries, vaccine companies often fail to see a potential market for vaccine development against diseases such as food poisoning where the disease is sporadic and the aetiological agent is often unknown. Finally, as mentioned above, there are severe technical hurdles to be overcome for effective vaccines to be developed against certain pathogens, for example Campylobacter where systems for genetic manipulation of the pathogen are only just becoming available.

Despite these problems, in recent years efforts to develop enteric vaccines have been intense. The field has been significantly stimulated by our improved understanding of the molecular basis of enteric infections. We now understand much more about how these infections are established and how the immune system controls them. We have been able to identify novel candidate vaccine antigens, develop candidate attenuated strains for live vaccines, and produce better systems for delivery and enhancing the immunogenicity of vaccines against enteric pathogens. To consider recent enteric vaccine development in a logical way, it is worth discussing the development of live vaccines separately from non-living vaccines.

**Recent advances in live enteric vaccine development**

Many investigators have favoured the use of live vaccines to tackle enteric infections. This approach is driven by several factors including the generally poor immunogenicity of dead antigen preparations. Live enteric vaccines usually involve oral formulations and these are increasingly attractive for use in non-industrialised countries where needle contamination with disease agents such as hepatitis B and HIV is common (see http://www.vaccinealliance.org). Historically, our ability to develop live vaccine candidates was limited by the availability of safe, attenuated, bacterial strains that could form the basis of such vaccines. The isolation of attenuated strains used to rely on empirical approaches involving spontaneous or chemically induced mutation accumulation in individual bacterial isolates. Examples of such candidates included S. Typhi strain Ty21a and V. cholerae strain Texas Star. Both these strains were generated by chemical mutagenesis of the bacteria involving the induction of multiple undefined mutations in the genome. Although rigorous safety testing established that the vaccines did not readily revert to virulence, they suffered the disadvantage that they were difficult to quality control, as the stability of individual mutations could not easily be monitored. Nevertheless, Ty21a was successfully developed as a human oral typhoid vaccine and is now licensed in many countries. Both vaccines were initially tested for safety and immunogenicity in volunteers. V. cholerae Texas Star, which harboured a mutation in the
cholera enterotoxin genes, was found to be highly immunogenic but was not developed further. Ty21a was thoroughly evaluated in volunteers and in successive field studies in different parts of the world using different formulations. This vaccine was consistently demonstrated to induce about 60% protection against typhoid if 3–4 doses of vaccine were employed.

The perceived limitations of the early vaccines has stimulated a scramble to produce live attenuated vaccine candidates harbouring fully defined attenuating mutations constructed using modern genetic manipulation techniques. The Holy Grail in this area is to develop a vaccine that can induce protection in a single dose while not inducing any significant clinical reaction in vaccinees. This has proved a difficult end-point to achieve. A number of clinical centres have been established around the world to test live vaccine candidates in controlled and contained clinical environments, and many vaccine candidates have now been administered to volunteers in such centres. Perhaps the most work has been undertaken on S. Typhi and Shigella with some significant work on V. cholerae.

Live typhoid vaccine development has focused on the generation of mutant S. Typhi harbouring two or more genetically defined and stable attenuating mutations. Several candidates have been shown to be relatively non-reactogenic and significantly immunogenic. Perhaps the most extensively characterised S. Typhi harbours mutations in aroC and aroD (effecting metabolism of the aromatic ring and effectively starving the bacteria in the host) and htrA (encoding a protein involved in macrophage survival). This strain, known as CVD908 HtrA, has been used in volunteers both as a typhoid vaccine candidate and also as a vector for delivering antigens from other pathogens (such as Plasmodium or hepatitis B) to the mucosal immune system. Oral administration of a single dose induces significant anti-S. Typhi mucosal and systemic humoral and cellular immunity.

Many research groups have attempted to develop genetically defined live Shigella vaccines suitable for use to induce oral protection against dysentery. Many have achieved the goal of generating immunogenic vaccines that show great promise, but a consistent problem with live Shigella vaccine development has been reactogenicity. Shigellosis is normally a highly invasive disease that induces considerable immune pathology in the intestine. The ability of vaccines to invade may be a prerequisite for the induction of effective immunity and striking the balance between immunogenicity and reactogenicity will determine the fate of any candidate live Shigella vaccines. Nevertheless, some candidates are showing great promise, particularly one developed in China.

Attempts to develop live oral cholera vaccines have focused on the generation of V. cholerae mutants harbouring defined mutations in the
cholera enterotoxin genes. Enterotoxin mutations are often combined with other mutations that either further attenuate the strain or act as genetic markers for monitoring shedding. The early volunteer studies using enterotoxin mutant *V. cholerae* were very encouraging. Volunteers tolerated oral doses of the live vaccine well, and the strains were highly immunogenic in terms of their ability to induce local and system vibriocidal antibodies. These early successes encouraged field efficacy studies with the candidate, but a major study in Indonesia provided little evidence for the induction of protective immunity against cholera. One major factor highlighted during these trials was that significantly higher doses of vaccine were needed in individuals in non-industrialised countries to induce similar levels of immunity to those observed in western volunteers. This has been attributed, in part, to physiological and architectural differences between the bowel walls of individuals from the two areas. These differences may be a consequence of the diet or type of antigens encountered in the different environment that consequently stimulate different gut development patterns. This difference is now perceived as a real barrier to oral vaccine development and merits more detailed investigation.

Few other attempts to generate live attenuated pathogens made with other enteric bacteria have reached the volunteer stage. Work on *E. coli* has been hampered by the multitude of serologically distinct *E. coli* strains and the perceived need to develop multivalent vaccines. Several volunteer studies have been performed to assess the role of different putative virulence factors in enteropathogenic *E. coli*, but these so far have not been followed up with detailed safety/immunogenicity studies. Work on non-typhoidal *S. enterica* has been limited by the perceived lack of a market for such vaccines in humans and work on *Campylobacter* has been limited by the lack of candidate vaccines.

Another alternative approach to live enteric vaccine development would be to use genetic manipulation to construct vector strains expressing heterologous antigens from other enteric pathogens. This has been investigated at an experimental level using vectors as carriers for fimbrial or toxin-based antigens, but, although it is an attractive idea, progress has been slow.

**Non-living oral vaccines**

Historically, few non-living vaccines have been developed that can routinely be administered via the oral route. This is because of the poor mucosal immunogenicity of most non-living antigens, combined with the lack of effective mucosal adjuvants and delivery systems. Volunteers have been fed a number of antigens, such as fimbriae/pili, but generally
immunogenicity has either been poor or inconsistent. This is in spite of the fact that hundreds of papers are published each year showing effective oral immunisation in model systems. A few observations have been made that might be used to improve this field in the future. Antigens that actively bind mucosal or immune cells and/or are able to resist degradation by gut enzymes often have enhanced mucosal immunogenicity. Encapsulation of antigen into materials that offer such protection (and also form particles) can also enhance mucosal immunogenicity. Finally, some molecules (e.g. cholera toxin) have been shown to have mucosal adjuvanting properties, activating immune responses to mucosally co-administered antigens. At the moment, cholera toxin is regarded as being too toxic for oral use as an adjuvant in humans, although non-toxic mutant derivatives of cholera toxin and the related E. coli heat-labile toxin are under evaluation. Oral vaccination with non-living antigens is a field in desperate need of a significant breakthrough in human studies. In spite of these problems, some slow but significant progress has been made. An oral cholera vaccine has been developed based on chemically inactivated whole V. cholerae cells supplemented with the B subunit protein of cholera toxin. This vaccine has been shown to induce some protection against cholera in field studies in Bangladesh. Indeed, this vaccine also offers some short-term protection against E. coli. The vaccine design takes advantage of the cell binding activity of the B subunit and the particulate and stable nature of the inactivated whole V. cholerae cells. However, immunogenicity is still not optimal as the administration of several doses is required to induce moderate protection. A similar vaccine is now under development for use against E. coli. This simple approach would appear to have a limited future.

A future breakthrough in this area will probably rely on a combination of technological developments. Cheap and effective mucosal adjuvants that can also improve the efficiency of antigen delivery to mucosal surfaces are urgently needed. One problem is that many mucosal vaccines that prove effective in small animals show poor immunogenicity in humans. Thus, the screening of molecules with mucosal potential is difficult without effective human immune correlates.

Non-living parenteral vaccines

Attempts to develop non-living parenteral vaccines against enteric bacteria have met with mixed success. Early work with inactivated whole bacterial cells ran into the problem of reactogenicity (due to lipopolysaccharide contamination) and poor efficacy. One solution is to
move to more defined vaccine antigens. This presents the problem of identifying non-toxic protective antigens. Early work with inactivated parenteral cholera toxin proved ineffective, but work with acellular typhoid vaccines based on the Vi polysaccharide has proved more successful. Vi (virulence) polysaccharide is a capsular material produced by *S. Typhi*. It can be readily purified and is immunogenic in adults but not infants. Vi polysaccharide has been shown to be an effective single dose vaccine against typhoid, inducing up to 70% protection in some field studies\(^3\). Indeed, Vi-based vaccines have now replaced whole cell typhoid vaccines in many parts of the world. Work is now under way to generate a conjugated form of Vi vaccine that is immunogenic in children under 2 years of age. Studies in older children in Vietnam have shown considerable promise\(^3\). Further studies should be performed over the next few years.

**Other enteric vaccines**

This short review has not been comprehensive in coverage of vaccines that could be defined as enteric. Diseases such as botulism could be considered to be of enteric origin and vaccine development is active in this area. Also, vaccine research on *Helicobacter pylori*, a cause of gastritis, has been intensive with a number of recent volunteer studies. However, as *H. pylori* is mainly associated with colonisation of the stomach, it has not been considered here.

**The gaps**

Progress in this area of research has been slow but sure over the past 20 years. However, there is no room for complacency as we still have many areas of disease that have not been effectively covered. Indeed, most of the existing vaccines against enteric pathogens could be significantly improved. Both existing live and non-living (Vi) vaccines against typhoid are showing promise. There is probably a clinical need for both types of vaccine in different settings as the two have complementary routes of delivery and mechanisms of inducing protection. There is an interest in introducing typhoid vaccines into the EPI programme in certain countries, but this will have to wait until the appropriate vaccines are available. *Shigella* vaccines are still held up in the early phases of human testing except in China where much more extensive field work has been performed. Much of the focus has been on live vaccines, but recent work on lipopolysaccharide conjugate vaccines is showing promise. Efforts to develop a live cholera vaccine are still suffering the impact of the
disappointing field results obtained in Indonesia. However, work on reformulation of this vaccine may provide a breakthrough. Efforts to develop an *E. coli* vaccine, particularly for use in travellers, are still compromised by the fact that multiple antigenic types are likely to be required to obtain proper vaccine coverage. More research in volunteers with experimental challenge systems could shed light in this area. Efforts to develop vaccines against other enteric bacteria such as non-typhoidal *Salmonella* and *Campylobacter* are more likely to be held up by economic considerations, as at present there is no obvious market or clear demand for such vaccines.

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**References**

Vaccination