Status of vaccine research and development for Shigella

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Abstract

Shigella are gram-negative bacteria that cause severe diarrhea and dysentery. In 2013, Shigella infections caused an estimated 34,400 deaths in children less than five years old and, in 2010, an estimated 40,000 deaths in persons older than five years globally. New disease burden estimates from newly deployed molecular diagnostic assays with increased sensitivity suggest that Shigella-associated morbidity may be much greater than previous disease estimates from culture-based methods. Primary prevention of this disease should be based on universal provision of potable water and sanitation methods and improved personal and food hygiene. However, an efficacious and low-cost vaccine would complement and accelerate disease reduction while waiting for universal access to water, sanitation, and hygiene improvements. This review article provides a landscape of Shigella vaccine development efforts. No vaccine is yet available, but human and animal challenge–rechallenge trials with virulent Shigella as well as observational studies in Shigella-endemic areas have shown that the incidence of disease decreases following Shigella infection, pointing to biological feasibility of a vaccine. Immunity to Shigella appears to be strain-specific, so a vaccine that covers the most commonly detected strains (i.e., S. flexneri 2a, 3a, 6, and S. sonnei) or a vaccine using cross-species conserved antigens would likely be most effective. Vaccine development and testing may be accelerated by use of animal models, such as the guinea pig keratoconjunctivitis or murine pneumonia models. Because there is no correlate of protection, however, human studies will be necessary to evaluate vaccine efficacy prior to deployment. A diversity of Shigella vaccine constructs are under development, including live attenuated, formalin-killed whole-cell, glycoconjugate, subunit, and novel antigen vaccines (e.g., Type III secretion system and outer membrane proteins).

1. About the disease and pathogen

Shigellosis is caused by the ingestion of bacteria of the genus Shigella. Three species of Shigella are responsible for the majority of infections: S. flexneri is the most frequently isolated species worldwide, accounting for most cases in the least-developed countries; S. sonnei is more common in low- and middle-income countries; and S. dysenteriae has historically caused epidemics of dysentery, particularly in confined populations such as refugee camps. A fourth species, S. boydii, a cause of infection in less-developed countries, accounts for 6 percent or less of Shigella cases [1].

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disease [5]. In addition, a meta-analysis of hospitalization and stool culture data projected that Shigella may contribute to an additional 40,000 deaths per year among age groups older than five years in Africa and South Asia [6]. This analysis also estimated that, in 2010, shigellosis was more common in these older age groups than cholera and typhoid combined, with 88.4 million shigellosis cases versus 9 million typhoid and cholera cases (approximately 6 million and 3 million, respectively), with school-age children being at the highest risk for all illnesses. Supporting these findings, IHME also estimated 40,500 deaths in 2013 from Shigellosa infection in persons older than five years of age [7]. In addition to mortality, IHME found that, in 2010, shigellosis disability-adjusted life years (DALYs) were estimated at 7 million (7.9 percent of all diarrhea DALYs) and years lived with disability (YLDs) due to shigellosis were estimated at 744,000 (9 percent of all diarrhea YLDs) [8,9]. Travelers and deployed military service members visiting Shigella-endemic areas also frequently suffer from shigellosis and contribute to the overall disease burden.

A recent evaluation of a new quantitative polymerase chain reaction (qPCR) assay for Shigella diagnosis confirms that traditional culture methods may seriously underestimate the global burden of Shigellosa-associated illness [10]. Using samples from GEMS, investigators found that use of qPCR almost doubled the percentage of MSD cases attributable to Shigella, from 9.6 percent by traditional culture methods to 17.4 percent by qPCR [10].

There is no animal reservoir for Shigella, and infection is transmitted person-to-person, via fomites, and from ingestion of contaminated food or water. Shigellosis is therefore associated with poor sanitation and hygiene and limited access to clean drinking water. Transmission control under these circumstances is made more difficult by the relatively low infectious dose of this pathogen [11]. Furthermore, the variety of species and serotypes associated with shigellosis makes it possible for reinfections to occur locally or during travel to areas where other serotypes predominate. For example, the heterogeneous distribution of Shigella serotypes found in cases from urban and rural areas of Bangladesh suggest that multivalent vaccines will be needed to prevent shigellosis in these settings [12]. Healthy individuals with mild infections usually recover without specific treatment, but because Shigella invades the mucosal lining of the colon, it often causes dysentry, which is not amenable to oral rehydration. Antibiotic treatment is recommended for dysentry, severe shigellosis, and individuals with compromised immune systems. However, the emergence of multi-drug-resistant strains of Shigella further complicates antibiotic treatment, making prevention of infection critical.

Based on the emerging qPCR data mentioned above that indicate a higher Shigella disease burden than previously estimated, the introduction of a cost-effective, broadly protective Shigella vaccine could have a significant public health impact [13]. An effective Shigella vaccine could substantially reduce the global burden of shigellosis and also reduce Shigella-associated mortality and complications associated with diarrhea and dysentry due to this pathogen. In low- and middle-income endemic countries with inadequate access to proper sanitation, safe water, and treatment options for severe diarrhea that may be resistant to common antibiotics, a Shigella vaccine would become an ideal choice in diarrheal disease management. It is important to note that Shigella infections are rare during the first six months of life, possibly due to the presence of maternal immunity and the relatively low direct interaction with the environment. Incidence increases after this age, peaking at 12–23 months and decreasing moderately afterwards [2]. Therefore, any potential Shigella vaccine would need to be safe and effective in children at least up to five years of age and administered within the current Expanded Programme on Immunization vaccination schedule (at 6, 10, and 14 weeks of age, possibly with a later booster dose given at the time of measles vaccination). In addition, due to the number of other enteric pathogens that also affect children in early life, another important goal for a Shigella vaccine is compatibility for combination with other enteric vaccines to be given by the same route.

2. Overview of current efforts

2.1. Biological feasibility for vaccine development

A successful strategy to control childhood diarrhea caused by Shigella would need to employ all effective diarrheal disease prevention and treatment interventions—including not only vaccines but also improved sanitation and hygiene, access to clean and potable water, and exclusive breastfeeding for the first six months of life—to help ensure long-term success and maximum impact. While a comprehensive approach to diarrhea prevention and control is the ideal solution, cost–benefit analyses show that water and sanitation infrastructure development can be cost-prohibitive and time-consuming, particularly for low-income countries. In the near term, many public health stakeholders view vaccination as a much more equitable and cost-effective preventive intervention.

At present, there are no licensed vaccines available for Shigella. However, studies in animals and humans have demonstrated that protection by vaccination is feasible. Among 12 non-human primates, a challenge/rechallenge study with virulent S. flexneri 2a demonstrated 100 percent protection [14]. Similarly, in controlled human challenge models, protection was suggested for adults given attenuated S. flexneri 2a strains and challenged with virulent S. flexneri 2a [15]. In one model using six adult volunteers, 100 percent protection was observed against fever and diarrhea associated with clinical S. sonnei infection in all volunteers who were rechallenged with a virulent S. sonnei strain, and 70 percent protection was observed in volunteers challenged and rechallenged with virulent S. flexneri 2a [16]. Field epidemiology studies suggest a chronological association of protection with age in younger individuals due to a decrease in age-specific incidence rates and the development of adaptive immunity through natural exposures [7,17,18]. Seropidemology studies also indicate that the presence of serum antibody correlates with protection from homologous strains [19,20].

Multiple factors affect the development of long-lasting protective immunity to Shigella infection. A key factor is that serum and mucosal antibody responses to Shigella are predominantly homologous, i.e., directed against a serotype-specific Shigella lipopolysaccharide (LPS)-associated O antigen [21]. While these responses are robust and lead to the induction of memory B-cell responses, evidence of their ability to cross-protect against diverse serotypes is inconclusive [13,22]. While other antigens such as the conserved invasion plasmid antigens (Ipas) do induce serum and mucosal antibody responses against Shigella, they are usually produced in lower quantities compared to anti-LPS antibodies. This could possibly be due to the sequestration of conserved epitopes in a way that evades T-cell recognition during natural Shigella infections.

With four major species and 50 different serotypes of Shigella, the task of developing an all-encompassing vaccine, while scientifically feasible, might become an impractical and expensive endeavor. Based on the serotype distribution from the seven sites of the GEMS study, Shigella flexneri and Shigella sonnei comprised nearly 90 percent of all Shigella isolates [13]. About 24 percent of all isolates were Shigella sonnei. Of the Shigella flexneri serotypes, 2a and 3a comprise nearly 30 percent of the isolates and serotype 6 comprises about 11 percent. S. flexneri 2a, S. flexneri 3a, and S. flexneri 6 strains also share O-antigen group determinants with the remaining 11 S. flexneri serotypes and its subserotypes [23–26]. Hence, an ideal multivalent vaccine that would provide maximal coverage would incorporate the three S. flexneri serotypes and S. sonnei.
Another approach that would provide broad protection with minimal antigen components is to use the conserved plasmid-encoded virulence proteins, such as virG and lpa proteins, that are expressed by all virulent *Shigella* regardless of serotype [21]. Such an approach induces serological and CMI responses to these (and perhaps to other common protein) antigens that hopefully can result in broad cross-protection against any wild-type *Shigella*.

2.2. General approaches to vaccine development for low- and middle-income markets

Virulent strains of *Shigella*, orally ingested through contaminated food and water, cross the epithelial barrier at the distal colon and rectum, entering through specialized M cells that express pathogen recognition receptors [27]. The M cells act to transcytose adherent bacteria from the lumen to the mucosal immune effector cells located within Peyer's patches as well as in isolated lymphoid follicles and intraepithelial lymphocytes [28,29]. *Shigella* induces cell death in macrophages, providing an escape route for the bacteria, which is believed to actively invade adjacent mucosal epithelial cells basolaterally in vivo with the help of the lpa proteins [27–29]. Within epithelial cells, *Shigella* multiplies intracellularly and spreads rapidly from cell to cell with the help of the bacterial VirG(lcsA) protein, resulting in the secretion of multiple proinflammatory cytokines and chemokines such as IL-8 that trigger innate immune defenses [29]. This results in inflammation, fever, and phagocytosis that provide an immediate response against the invading microorganism. In order to modulate the host response to infection and resulting inflammation, the pathogen secretes a number of effector proteins into the host cells through a needle-like structure that juts out of the bacterial membrane as part of the type III secretion system (TTSS) [27–29]. Later, the same cytokines and other secreted host factors released during the innate immune reactions drive B and T cell-based adaptive immune responses to *Shigella* antigens such as the LPS and the lpa proteins [29]. These adaptive responses can be measured during vaccination studies [24,25,30]. A precise correlation of a specific adaptive immune response or responses to protection remains unclear.

Most *Shigella* vaccine candidates, whether cellular, hybrid, or subunit, include the LPS-associated O-specific polysaccharide (O-SP) antigen. The use of this antigen in most candidates is based on the previously mentioned observation that *Shigella* immunity is serotype-specific. Although GEMS found the presence of all four *Shigella* species and their respective serotypes in the stools of children with moderate-to-severe diarrhea, only certain types are considered to be frequent causal agents of human shigellosis. Since the occurrence of diarrhea disease caused by *S. dysenteriae*—sporadically associated with epidemic outbreaks—and *S. boydii* is rare and infrequent, a *Shigella* vaccine targeting the O-SP antigens of *S. flexneri* 2a, 3a, and 6 as well as *S. sonnei* should cover the majority of all *Shigella* illnesses. Researchers from GEMS have estimated that such a vaccine construct would provide direct protection against 64 percent of *Shigella* strains and cross-protection for up to 88 percent of all strains [13]. Such coverage would be expected to be beneficial in many locations worldwide by covering the most common serotypes in each area.

3. Technical and regulatory assessment

While there are no established correlates of protection to ascertain the effectiveness of a vaccine response, there have been some observed immunological associations based on natural wild-type infection, animal models, and human challenge models [21]. Protection appears to be mediated by convergence of immune responses that include both systemic and mucosal responses to serotype-specific *Shigella* O antigens and conserved invasion plasmid antigens such as lpa B and lpa D. Protection also appears to be provided by antigen-specific B memory (B<sub>M</sub>) responses that correlate with antigen-specific systemic and mucosal responses and antigen-specific antibody–secreting plasma cells in peripheral circulation [21]. Positive correlations between antigen-specific anti-LPS B<sub>M</sub> cells and antigen-specific anti-LPS IgA/<sub>Total IgA</sub> have been observed [31]. A strong correlation between anti-LPS IgA B<sub>M</sub> cells and peak anti-LPS IgA antibody and antibody-secreting cell (ASC) responses further reinforces the contention that B<sub>M</sub> cells may be an important indicator for long-term humoral immunity and a possible surrogate of protection against shigellosis. The trigger of an early-stage Th1-type cytokine response mediated in concert with the above-mentioned antibody responses has been shown to lead to durable and protective mucosal responses [32].

Accurate measurement of these putative correlates of protection (i.e., serum IgG antibodies against *Shigella* serotype-specific LPS O antigen, serotype-specific peripheral blood IgA ASCs, and mucosal IgA secretions) requires the use of sensitive immunonasays and/or functional assays. While several immunonasays have been developed and established for discerning the mucosal response, functional assays that correlate with clinical severity and immunological status indicative of vaccine effectiveness are being developed. PATH is working with two groups, the Center for Vaccine Development (CVD) at the University of Maryland, Baltimore, and the University of Alabama, Birmingham, to develop an opsonophagocytic (OPA) assay and a serum bactericidal assay (SBA) that can be utilized to evaluate the efficacy of vaccine candidates in clinical field trials [33,34]. One of the anticipated outcomes of these assays is to be able to use immunological markers to predict the effectiveness of a vaccine in protecting against clinical outcomes. Preliminary data from the Pasetti laboratory at CVD have shown correlations between immunological status, disease severity, and clinical outcome using both a first-generation SBA and an OPA [35].

A number of animal challenge models are available to assess the potential of *Shigella* vaccine candidates. *Shigella* invade the corneal epithelia of guinea pigs and spread to adjacent cells, causing conjunctivitis. Thus, a guinea pig keratoconjunctivitis model can be used to explore *Shigella* vaccine immunogenicity and efficacy by varying doses, antigen forms, administration routes, dosing regimens, and boosting mechanisms [36–38]. A widely used murine pneumonia model employs mice inoculated intranasally with experimental *Shigella* vaccines to test for safety, immunogenicity, and efficacy [39]. Cynomolgus monkeys have been used to develop a *S. dysenteriae* 1 model [40], which resulted in an attack rate of 100 percent among six monkeys challenged intragastrically with 10<sup>11</sup> cfu of wild-type *S. dysenteriae*. Guinea pigs and piglets have also been successfully challenged via the intrarectal route and orally, respectively, to evaluate immunogenicity and protection [41,42]. In order to better understand the pathogenesis, prevention, and treatment of shigellosis, controlled human challenge models have been established [16,43]. Of the 19 published studies using either *S. sonnei* strain 53G or *S. flexneri* 2a strain 2457T, a majority (55 percent) were employed to evaluate vaccine efficacy [44].

Given that there is no established correlate of protection or functional assay to predict *Shigella* vaccine effectiveness through immunological markers, a pediatric field efficacy trial in a low- or middle-income country will be an important tool to evaluate efficacy of a standalone *Shigella* vaccine in children. Because *Shigella* vaccines, like rotavirus vaccines, are unlikely to prevent colonization and primary infection, a probable endpoint for such a trial is the measurement of the extent of MSD due to vaccine-preventable *Shigella* strains. To this end, a disease severity score similar to that of the Vesikari score [45] used for rotavirus trials will be required, but it will need to emphasize occult blood in stool and de-emphasize vomiting—a key clinical feature of rotavirus but not dysentery. If
such a vaccine is found to be efficacious, co-formulating this vaccine with other vaccines would seem warranted.

Like with other enteric pathogens such as *Salmonella* and *Campylobacter*, Shigella infection has been associated with reactive arthritis or post-infectious arthritis. This is not expected to be an issue with vaccines, which do not persist in the body for long periods of time [46]. Most modern Shigella vaccine candidates would be anticipated to meet few if any unusual regulatory hurdles. Those designed for injection would need to have preclinical pharmacology/toxicology studies performed before evaluations in humans. Additionally, trials in adults would precede safety and immunogenicity trials in children.

Some Shigella vaccines may benefit from co-administration with a mucosal adjuvant, which could present further regulatory considerations. The most extensively studied mucosal adjuvants are bacterial toxins such as cholera toxin (CT) and the *Escherichia coli* heat-labile toxin (LT), whose immunomodulatory properties are mediated by the binding of the toxin’s B subunit to GM1 ganglioside receptors on mucosal epithelial cells, M cells, DCS, and macrophages that are important for antigen presentation [47, 48]. Initially precluded from most mucosal vaccine development due to their toxicity, generation of non-toxic mutants have made CT and LT extremely attractive mucosal adjuvants for both oral as well as parental mucosal vaccines [49]. A newly developed double-mutant toxin (LTR192G/L211A; known as dmLT), which has significantly decreased enterotoxicity compared to the native toxin, remains effective in enhancing mucosal immune responses [50]. PATH is working with several partners to elucidate the mechanism of action of dmLT in potentiating a strong mucosal response as well as evaluate its use with a variety of vaccine types given via different routes. The use of dmLT in preclinical and clinical Phase 1 and 2 studies is creating a robust body of data-driven evidence showing that the maximum tolerable dose of dmLT is several thousand-fold higher than the immunomodulatory dose of dLT [51, 52]. It is anticipated that, with the extensive safety data available and a lowered administered dose, regulatory agencies would have few concerns related to the use of dmLT as an adjuvant [53, 54].

4. Status of vaccine R&D activities

*Shigella* vaccine research to date has been primarily focused on serotype-specific O-SPs, although some preclinical work has also evaluated protein antigens that could be more broadly conserved and still contribute to protection [26]. As noted above, if using serotype-specific immunity for protection, an optimal *Shigella* vaccine would include *S. flexneri* 2a, 3a, and possibly 6, as well as *S. sonnei*. The reduction in the total number of serotypes to these four for inclusion in a vaccine is based on the recent GEMS findings [2]. Table 1 provides a summary of the development status of current *Shigella* vaccine candidates.

4.1. Cellular candidates

Most research on the delivery of these antigens has involved live attenuated cells given orally, which have now been developed by selective genetic manipulations to be relatively safe and immunogenic. The two current approaches are a series of virG-based mutants under development by the Walter Reed Army Institute for Research (WRAIR) [25, 55] and guaBA-based mutants under development at CVD at the University of Maryland, Baltimore [56]. In contrast to earlier mutations of wild-type *Shigella*, these new constructs have mutations that limit their ability to spread between epithelial cells (virG) or replicate (guaBA) as well as deletion mutations for the *sen* and *set* genes associated with the *Shigella* enterotoxins. The inclusion of *msbB* deletions to reduce the pyrogenic potential of *Shigella* LPS is also being evaluated with the virG attenuation strategy [57]. These newer attenuated Shigella vaccine candidates induce robust immune responses in volunteers and have a superior safety profile (higher tolerability and decreased reactogenicity) compared to previous constructs. Studies with the VirG-based *S. sonnei* candidate WRRS1 are ongoing in a target population of infants and young children in Bangladesh.

Recent studies are evaluating *Shigella* whole cells not expressing LPS-O antigens due to a targeted deletion of the rfbF gene (Evelique’s ShigETEC) [58] as well as genetically modified bacteria (*dWZY*) (International Vaccine Institute, IVI) retaining one unit of O antigen, a shortened layer of LPS on the bacterial surface [59]. Both of these novel approaches, currently in preclinical development, result in increased exposure of broadly conserved outer membrane proteins. It is possible that vaccination using genetically modified bacteria with the enhanced exposure of common outer-membrane proteins could be an efficacious approach to develop universal *Shigella* vaccines and present new antigens that may improve protection, particularly in young children.

Another oral vaccine approach, under development by Protein Potential, LLC, is the use of the Ty21a vaccine for typhoid as a vector for *Shigella* LPS. Results from clinical studies using early iterations of this vaccine candidate were inconsistent due to plasmid-related genetic instabilities, but recent cloning improvements have resulted in a stable vaccine construct that may offer better results in future clinical trials [60, 61].

Safety and ease of formulation may be further improved by a trivalent formalin-killed whole cell vaccine being developed by PATH and WRAIR and containing *S. flexneri* 2a and 3a as well as *S. sonnei* [62, 63]. This vaccine candidate is in early clinical development and has been protective in animals. An *S. flexneri* 2a prototype for the trivalent vaccine was also safe and immunogenic in a Phase 1 trial. A heat-inactivated hexavalent *Shigella* vaccine is being developed by India’s National Institute of Cholera and Enteric Diseases (NICED) [64] that contains *S. flexneri* 2a, *S. flexneri* 3a, *S. flexneri* 6, *S. sonnei*, and *S. dysenteriae*. This vaccine is currently in preclinical development and has been shown to be immunogenic and protective against heterologous challenge in guinea pig models.

4.2. Glycoconjugate candidates

Research has also been conducted on subcellular approaches. The U.S. National Institutes of Health’s National Institute of Child Health and Human Development (NICHD) Laboratory of Developmental and Molecular Immunology (LDMI) proposed the concept of *Shigella* O-SP-protein conjugate vaccines for intramuscular injection [65]. Since purified O-SP is poorly immunogenic, NICHD/LDMI researchers covalently coupled O-SP purified from *Shigella* LPS with protein carriers in order to induce stronger and longer-lasting T-cell-dependent immune responses. Although some formulations using this approach have already undergone Phase 3 trials, a final formulation is still under development [66]. In addition, over the last decade, a research group at the Institute Pasteur has developed a glycoconjugate vaccine consisting of synthetically produced *S. flexneri* 2a oligosaccharide “mimics” conjugated to protein carriers (i.e., neoglycopeptides) which are in preclinical development [67]. Finally, a recombinantly produced glycoconjugate candidate currently in clinical trials is being developed by Limetach Biologics and has entered Phase 2b clinical evaluation [68]. These conjugates offer a safe approach and produce systemic immunity upon intramuscular administration. It remains to be determined, however, whether the benefits reported with conjugates are due to boosting previous mucosal exposure or to initiation of an IgG response. Recent observations that mutated heat-labile toxin of enterotoxigenic *E. coli* (ETEC) can be administered parenterally with a vaccine antigen to induce stronger
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<th>Candidate name/identifier platform</th>
<th>Developer</th>
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<td>guaBA-based live attenuated</td>
<td>CVD at the University of Maryland School of Medicine, Baltimore, Maryland USA</td>
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<td>(CVD 1208, CVD 1208S) Mutations in the guaBA operon (genes involved in guanine biosynthesis) leading to a guanine auxotroph and decrease in virulence. Further iterations included deletions of enterotoxin genes.</td>
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<td>virG-based live attenuated</td>
<td>WRAIR, Silver Spring, Maryland USA</td>
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<td>(WRSS1, WRSS3, WRSS3E) Primary attenuation by a 212bp deletion in the virG gene preventing intracellular spreading. Further iterations included deletions of enterotoxin genes and lipid A genes.</td>
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<td><strong>ShigETEC</strong></td>
<td>EveliQure Biotechnologies GmbH, Vienna, Austria</td>
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<td><em>Live, genetically attenuated Shigella vaccine strain that is amenable for the heterologous expression of diarrheal antigens and therefore can provide protective immunity against multiple pathogens</em></td>
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<td><strong>Truncated Shigella</strong></td>
<td>International Vaccine Institute, Seoul, Korea</td>
<td>X</td>
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<td>Mutant Shigella bacteria in which the gene encoding O-antigen polymerase is disrupted</td>
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<td><strong>Ty21a typhoid vaccine expressing Shigella LPS</strong></td>
<td>Protein Potential LLC, Rockville, Maryland USA</td>
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<td><em>Salmonella typhi Ty21a construct comprising a Shigella sonnei O-antigen biosynthetic gene region</em></td>
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<td><strong>Inactivated trivalent Shigella whole cell</strong></td>
<td>PATH, Washington DC and WRAIR, Silver Spring, Maryland USA</td>
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<td>Formalin inactivated Shigella whole cells</td>
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<td><strong>Heat Killed Multi Serotype Shigella (HKMS) vaccine</strong></td>
<td>NICED, Kolkata, India</td>
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<td><em>Heat killed preparation of 6 strains of Shigella that were subsequently combined to form an inactivated vaccine</em></td>
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<td>Chemically prepared glycoconjugate</td>
<td>LDMI at the NICHHD, NIH, Bethesda, Maryland USA</td>
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<td><em>O polysaccharide covalently linked to carrier protein</em></td>
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<td>Recombinant glycoconjugate</td>
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<td><em>O polysaccharide specific biconjugate vaccine</em></td>
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<td>Synthetic glycoconjugate</td>
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<td><em>use of synthetic oligosaccharides (Os), acting as efficient functional SF2a O-SP mimics, as the hapten for a conjugate vaccine</em></td>
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<td><em>2nd generation macromolecular complex composed of Shigella LPS and the Type 3 secretions system proteins (Ipa B, Ipa C, and IpaD)</em></td>
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<tr>
<td>GMMA</td>
<td>Selvo Behring Vaccines Institute for Global Health S.r.l [A GSK Company], Sienna, Italy</td>
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<td></td>
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<td>[71,77]</td>
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<td><em>Genetically derived outer membrane particles comprised of predicted Shigella outer membrane and periplasmic proteins without LPS using a novel protein vesicle technology</em></td>
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<td>OMV</td>
<td>University of Navarra, Navarra, Spain</td>
<td>X</td>
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<td><em>Shigella outer membrane vesicles encapsulated in nanoparticles</em></td>
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<td><strong>Subunit candidates</strong></td>
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<tr>
<td>DB Fusion</td>
<td>PATH, Washington, DC</td>
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<td><em>Fusion protein of two Type III secretion system antigens, invasion plasmid antigens B (Ipa B) and invasion plasmid antigen D (Ipa D)</em></td>
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<td>34 kDa OmpA</td>
<td>NICED, Kolkata, India</td>
<td>X</td>
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<td>[73]</td>
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<td><em>Conserved and cross reactive major outer membrane protein (MOMP) of Shigella flexneri 2a</em></td>
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mucosal and systemic response to ETEC antigens may improve the potential for using the conjugate approach in young children [69].

4.3. Novel antigen candidates

A team at WRAIR has continued development of second-generation TTSS-LPS complex vaccines by constituting artificial Invaplex$_{4.4}$ from recombinant IpaB and IpaC purified by IMAC technology with LPS purified by standard hot-phenol extraction [70]. This approach brings together important serotype-specific and conserved antigens of Shigella, and the artificial formulation may be more immunogenic than naturally produced Invaplex preparations studied previously [70].

Sclavo Behring Vaccines Institute for Global Health (SBVIGH) is developing outer membrane vesicles (OMVs) of Shigella, termed Generalized Modules for Membrane Antigens (GMMAs), as vaccines [71]. SBVIGH has described an industrially scalable, high-yielding manufacturing process for GMMAs using tangential flow filtration for purification and microfiltration for sterilization. Three intranasal immunizations using GMMAs derived from $S$. sonnei without O-SP/LipidA were protective against either $S$. sonnei or $S$. flexneri in the intranasal challenge model, but only GMMAs with homologous LPS were protective via the intradermal route. These vaccine candidates are now in early clinical trials. In addition, the University of Navarra in Spain is developing acellular Shigella vaccine candidates based on OMVs that are naturally secreted into the bacterial culture medium during the stationary phase of growth of wild-type strains [23]. OMVs are 40 percent LPS, and they contain major outer membrane protein antigens such as OmpA, OmpC/OmpF, IpaB, IpaC, and IpaD. Preclinical studies showed that OMVs protected mice from intranasal challenge with homologous $S$. flexneri 2a after a single immunization via the intranasal, ocular, or oral routes.

4.4. Subunit candidates

In contrast to the novel antigen candidates above, two protein-based subunit vaccine candidates may offer broad protection against all major serotypes, but have only been tested in animals. These include: the DB Fusion (being developed by PATH) [72], consisting of a genetic fusion of the TTSS proteins IpaB and IpaD; and a 34 kDa outer membrane protein (being developed by NICED) [73]. Both of these have provided some indication of protection in animal models. The DB Fusion may soon move to clinical trials, where it will be co-administered intradermally with dmLT to help induce both mucosal and systemic immunity.

The possibility for development of a successful vaccine against Shigella has never been better. Although the benefit of vaccinating with live attenuated organisms was recognized decades ago, an adequate understanding of pathogenesis has only recently made it possible to selectively construct safe and immunogenic mutants now undergoing clinical testing. To date, the oral delivery route used for these mutants has been the only successful means for protecting against disease in individuals not primed mucosally with Shigella. Parenteral immunization with conjugate vaccines has not been effective in inducing mucosal immunity against Shigella in unprimed individuals, but with the advent of new immunization techniques using intradermal administration of antigens admixed/formulated with adjuvants such as dmLT on the horizon, there is immense potential to enhance the efficacies of conjugates and other subunit antigens administered parenterally. As described above, major advances in recent years indicate that there are many promising new candidates for Shigella vaccines in the pipeline. These include the broadly conserved surface proteins of Shigella—although these candidates are still in the early stages of development and it is still unknown whether they can induce sufficient immune responses to be protective. Likewise, the inactivated Shigella whole cell is another approach that has only recently been given serious attention and, while immunogenic, it has yet to be shown to be protective in humans. Several excellent reviews provide further descriptions of the various approaches to Shigella vaccines currently under development [23–26].

5. Likelihood for financing

Gavi, the Vaccine Alliance has indicated an interest in enteric vaccines, including one for Shigella, though their strongest preference would be for a combined vaccine that also includes ETEC or another pathogen. Likewise, commercial interest in a standalone Shigella vaccine is weak but may be enhanced if it were part of a combined vaccine or if new qPCR data show the threat of Shigella infections to be much greater than previously supposed. As most development work on Shigella vaccines is conducted by nonprofit organizations, financing options are currently limited. However, expected new data on the Shigella disease burden could significantly increase the support for a standalone vaccine for Shigella.

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References


