Bacterial Enteric Pathogens: Enterotoxigenic E coli (ETEC) & Shigella

Duncan Steele
Bill & Melinda Gates Foundation

Acknowledgements:
Dick Walker, Sachin Mani and Tom Wierzba, PATH
Eric Houpt and James Platts-Mills, U VA
Evan Sturtevant and Lyou-Fu, BMGF
Outline of presentation

• Burden of ETEC and Shigella
• Status of ETEC vaccine development
• Status of Shigella vaccine development

• Norovirus: Burden and status of vaccine development
The challenge – diarrhoeal disease associated mortality is ~600,000* children each year

• Diarrhoea remains the second biggest infectious diseases killer after respiratory diseases of children under the age of five years

• Globally, there are nearly 1.7 billion cases of diarrhoeal disease every year, many with acute and chronic effects

• GEMS confirmed that ETEC and *Shigella* are two of the top four pathogens causing moderate-to-severe diarrhoea among children < 5 years old in Africa and South Asia

• Molecular re-analysis of the GEMS and MAL-ED data indicates that ETEC and *Shigella* burden is higher than previously thought

• CHERG estimates ETEC among top 4 pathogens causing diarrhoea-associated deaths among children < 5 years old

• ETEC is also the most common cause of travelers’ diarrhoea, the most frequent health problem among travelers visiting developing countries

*Total diarrhoea deaths derived from 2012-2013 CHERG and IHME estimates
GEMS identifies highest burden pathogens

Likely under-represents overall burden of enteric and diarrhoeal disease:

- Diseases from enteric infections can lead to malnutrition, stunted growth, and impaired cognitive development, leading to diminished productivity over a lifetime for millions of people
- 8-fold mortality identified in GEMS 60 days post diarrhoea episode would not be captured as diarrhoea-related death
- Typhoid - as enteric but not diarrhoeal - does not appear on chart, but represents an estimated ~200,000 mortality burden

Estimated diarrhoeal deaths for U-5 children* % of total deaths (~700K total**)

- **Rotavirus** 37%
- **ETEC** 15%
- **Crypto** 10%
- **Shigella** 7%
- **Cholera** 5%
- **Norovirus** 5%
- **Other/Unknown** 21%

Molecular re-analysis of the GEMS samples

~300 cases/controls 0-11 mo from all 7 sites (n=1929)

- Rotavirus
- Adenovirus 40/41
- Cryptosporidium
- Shigella/EIEC
- ST-ETEC
- C. jejuni/coli
- Norovirus GII
- Astrovirus
- Aeromonas
- V. cholerae
- Salmonella

~300 cases/controls 12-23 mo from all 7 sites (n=1880)

- Shigella/EIEC
- Rotavirus
- ST-ETEC
- Adenovirus 40/41
- Cryptosporidium
- Sapovirus
- Astrovirus
- Norovirus GII
- V. cholerae
- Salmonella
- Aeromonas

Confidential and proprietary information
Results of molecular re-analysis of GEMS

• Re-analyzing a random subset of GEMS samples with quantitative PCR
• Scaling the Odds Ratio for diarrhoea based on pathogen PCR quantity
• Then utilizing the same general AF methodology, reveals:
  – Robust, unchanged Rotavirus and Cryptosporidium estimates
  – Much greater attribution to *Shigella* and ETEC, in both age ranges, particularly *Shigella* in 12-23 month olds (~40%).
  – These infections are detected by qPCR, missed by culture, but are highly diarrhoea-associated. (Fastidious pathogens. Use of antibiotics)
  – Overall attribution greatly increased – pathogen identified in
    • ~75% in <12 month olds
    • ~93% in second year of life
• Mixed infections remain common:
  – Each case had 1.19 ± 0.96 (range 0-6) diarrhoea-associated pathogens at diarrhoea-associated quantities
Increasing coverage of treatment has impacted death rates, yet incidence has not been largely impacted.

- Mortality burden has declined, but the burden of morbidity is relatively unchanged.
- Rate of death declining at 6.5% annually (2000-2013) - before rotavirus vaccine introductions.
- Diarrhoeal incidence decline is not so dramatic - 2.9 episodes per child annually (down from 3.4 episodes per child in 1990).
- A focus on treatment alone will not sufficiently impact the significant morbidity of pathogens such as ETEC and *Shigella*.
- Key to decreasing incidence *and* accelerating mortality declines is to focus on both prevention & treatment strategies.

Fischer Walker C et al. *BMC Pub Health* 2013
ETEC and *Shigella* Diarrhoea - Annual DALYs by Age

**Total DALYs from ETEC and Shigella Diarrhea in populations <5 years and >5 years**

![Graph showing DALYs for different regions and age groups]

- ETEC DALYs: children <5
- Shigella DALYs: children <5
- ETEC DALYs: population >=5
- Shigella DALYs: population over 5

Rheingans R et al.

Capturing the full burden of ETEC/Shigella

Growing evidence of nutritional and long-term effects

The impoverished gut—a triple burden of diarrhoea, stunting and chronic disease

Figure 3 | Chronic consequences of early childhood enteric infections and stunting. The triple burden of enteric infections, impaired physical development (including low HAZ-2, or stunting and BMI-2) and cognitive development, and later life risk of obesity and its comorbidities are shown. Abbreviation: HAZ-2, height-for-age z-score at age 2 years.

Under-nutrition and infectious diseases

- Under-nutrition is caused by poor dietary intake that may not provide sufficient nutrients
- Under-nutrition is also a result of impaired absorptive function of the gut, due to repeated enteric infections and gut enteropathy
- Stunting at 24 months of age (a marker of under-nutrition and vulnerability to disease and death) increased by 5% with each diarrhoeal episode experienced by the child
- Enteric disease researchers estimate that poor gut health (the condition of its nutrient-absorbing villae as well as its microbial population, which break down and use or release nutrients) is responsible for 30-50% of the stunting burden in children
  - Under-nutrition leads to vulnerability to infectious diseases including diarrhoea
  - Diarrhoea reduces the absorptive ability of the gut, leading to under-nutrition

Potential Impact of ETEC and *Shigella* induced Stunting

- ETEC and *Shigella* episodes would result in an estimated
  - 2.6 million additional children with moderate stunting and
  - 2 million additional children with severe stunting
- Resulting in an additional 31,000 deaths annually from diarrhoea, pneumonia, malaria and measles
- Impact is greatest in Africa
- Stunting effects may be greater due to concentrated risks within countries (i.e., may be most likely among children already low height for age and also at greater risk of infectious disease)
- **New IHME Global Burden of Diseases DALY estimates will be available by end of the year**
Enterotoxigenic *E coli* (ETEC): Burden of disease

- ETEC is the most common cause of diarrhoeal episodes globally
- Repeat infections are associated with growth faltering

- ETEC estimated to cause -
  - ~157,000 deaths in young children U5 s
  - ~400 million cases of diarrhoea in the U5 s
  - 89,000 deaths in older children and adults
  - 8.5 million DALYs / 1 million YLDs
  - 44 million cases in older children and adults

- New GEMS & MAL-ED molecular analysis of the diarrhoea samples indicates substantial more data than previously recognized
- IHME and PATH developing new estimates of DALY burden
ETEC targets for vaccine development: Colonization Factors (CFs) and toxins

An ETEC vaccine formulated to induce anti-toxin immunity to LT and anti-CF immunity to CFA/I, CS1, CS2, CS3, CS5, and CS6 would potentially provide coverage for 80-90% of ETEC strains associated with illness in endemic areas.

**Fimbriae**  *Intestinal adherence*
- CFA/I
- CFA/II CS**1, CS2, CS3
- CFA/IV CS4, CS5, CS6
- Others (CS17, CS14, PCF071)

**Toxins**  *Cause diarrhoea*
- LT (Thermal labile)
- ST (Thermal stable)
- LT/ST

Current Status of ETEC Vaccine Development

• Whole cell approaches to ETEC vaccine development
  – Killed, whole cell strains (eg. ETVAX, Scandinavian BioPharma)
  – Live attenuated strains (eg. ACE 527, TD Vaccines and CNBG)

• Subunit/peptide approaches
  – Fimbrial tip adhesins (FTA) (US NMRC and PATH)
  – 7 CFA-based Multi-epitope Fusion Antigen (MEFA)

• Other innovative approaches in pipeline
  – ST toxoid (CIH, Norway; STOPENTERICS)
  – New conserved ETEC antigens
  – Vectored combination vaccines (ETEC-Shigella; ETEC-Typhoid)

Walker RI. Vaccine. 2015;33:954-965
### ETEC Vaccine development: TPP Summary

<table>
<thead>
<tr>
<th>High Level TPP$^1$</th>
<th>Minimum</th>
<th>Optimistic</th>
</tr>
</thead>
<tbody>
<tr>
<td>Indication</td>
<td>Immunization against ETEC</td>
<td>Immunization against ETEC</td>
</tr>
<tr>
<td>Target population</td>
<td>&lt;1 year of age</td>
<td>&lt;1 year of age</td>
</tr>
<tr>
<td>Efficacy</td>
<td>50% against severe diarrhoea</td>
<td>80% against severe diarrhoea</td>
</tr>
<tr>
<td>Duration of Protection</td>
<td>3 years</td>
<td>Lifelong</td>
</tr>
<tr>
<td>Safety</td>
<td>Similar to placebo</td>
<td>Similar to placebo</td>
</tr>
<tr>
<td>Route of administration</td>
<td>Oral</td>
<td>Oral</td>
</tr>
</tbody>
</table>

ETVAX – most advanced candidate in clinical development

Multivalent vaccine

CFA/I
CS3
CS5
CS6

Adjuvant

LCTBA
dmLT

Second generation ETEC vaccine – WHO recommendations
• Increased CF antigen content (over-expressing strains)
• CS6 and adjuvant added
• Clinical endpoints defined

WHO. Wkly Epidemiol Rec. 2006. 81:97-104
The ETVAX vaccine offers dual routes of protection

The vaccine composition offers two potential lines of defense:

- Protects against colonization of the intestine with bacteria
- Induces antibodies that neutralize the heat-labile toxin (LT) produced by the bacteria

This strategy has already shown to be very successful for the cholera vaccine Dukoral
Primary endpoints were met and exceeded in all vaccine groups

Toxin Neutralization Titers improved with dmLT

Highest frequency of responders to all antigens (83%) obtained with vaccine + low dose dmLT

Graph showing data from two different clinical trials
Highly significant mucosal (ALS) immune response to all vaccine antigens

CFA/I

CS3

CS5

CS6

Great improvements compared to previous vaccines
- Highly significant frequency of responders
- Highly significant magnitude of response to all antigens
- Additional highly significant response to CS6 (lowest antigen conc. plus low dose dmLT group)

* p = 0.01 greater than vaccine alone
** p = 0.01 greater than placebo
*** p = 0.001 greater than placebo
ETVAX: Clinical development for developing country vaccine in infants

**WHO roles (2016-2017)**
- Clinical trial endpoints
- Immunology
- Assay development

**Key decisions for phase 3 efficacy in infants in endemic country**
ETVAX: Criteria for Phase III Trial in Infants

- No safety “stopping rules” met in descending age trials (OEV-122)
  - Formulation, volume, and dosing regimen/interval optimized
- Immunological and safety criteria met for expanded descending age trial in target population on EPI schedule (OEV-124)
  - >50% of target age group mounting mucosal or serum IgA responses to at least four of the five predefined key antigens (LTB, CFA/I, CS6, CS3)
- Demonstrated efficacy >50% against moderate to severe ETEC diarrhoea in first of human trials in adult travelers (OEV-123)
- Successful manufacture to commercial scale
Summary of ETVAX development

- ETVAX can be platform for combined vaccine product
  - Readily lends itself to a combination vaccine strategy
  - Stable
  - Validated production system and vaccine analytics for standardization
  - Comparatively short development timeline
- Great improvements in immunogenicity over previous vaccine; dmLT adjuvanted vaccine extremely well tolerated
- dmLT improves multiple aspects of immune response to vaccine components; dose sparing will be investigated in Study OEV124, age-descending/dose-ranging study
- Study of protective efficacy in adult travelers in preparation
- Advancement of ETVAX to descending age studies in endemic area underway
ETVAX vaccine formulations under consideration for development

Travelers’ formulation

Vaccine in suspension

Buffer + dmLT

Add to 150 ml water in drinking vessel

Infant formulation under consideration

ETVAX dmLT + buffer

Frangible Seal
ACE527 – live, attenuated candidate in clinical development

• Vaccine characteristics
  • All toxin and antibiotic resistance genes deleted
  • *aroC; ompC; ompF* genes deleted
  • Recombinant CS1 and LTB expressed from chromosome

• Early Phase 1/2b studies of frozen preparation (10^{11} cfu in two doses):
  • Majority of subjects (>50%) mounted mucosal responses to key antigens: LTB; CFA/I; CS3; CS6
  • Did not meet primary endpoint of protection against moderate/severe diarrhoea
  • Significantly impacted secondary measures of incidence and severity of disease; 41% efficacious against severe disease (p = 0.03)
  • Significantly reduced shedding of challenge strain
ACE527 ± dmLT: Efficacy against severe diarrhoea

Results led to study with lyophilized preparation of ACE527 at a dose of $10^{10}$ cfu in three doses with and without 25 µg dmLT adjuvant

<table>
<thead>
<tr>
<th>Group</th>
<th>N</th>
<th>Severe Diarrhoea</th>
<th>Protective Efficacy vs. Controls (P-value)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Yes (%)</td>
<td>No (%)</td>
</tr>
<tr>
<td>Controls</td>
<td>31</td>
<td>21 (68%)</td>
<td>10 (32%)</td>
</tr>
<tr>
<td>ACE527</td>
<td>13</td>
<td>7 (54%)</td>
<td>6 (46%)</td>
</tr>
<tr>
<td>ACE527 + dmLT</td>
<td>13</td>
<td>3 (23%)</td>
<td>10 (77%)</td>
</tr>
</tbody>
</table>

1Primary Endpoint: Prevention of severe diarrhoea defined as cumulative passage of more than 800 grams of grade 3 to 5 diarrhoea stools for episodes beginning during the 120-hour observation period post-challenge

Fimbrial Tip Adhesins (FTA)

- FTA are important antigenic and functional component of the fimbria; the binding site domain
- Final vaccine will have 3-5 FTA types. May offer broader coverage for strains of ETEC relevant to developing countries
- Passive protection in humans; (active protection in non-human primate when given with mLT by ID route)

- Monovalent form (CfaE): 3 doses was safe and immunogenic in adult volunteers
- Challenge study with mLT showed value of protection against severe disease

Assessment of WHO role for ETEC vaccines

• Limited WHO engagement in this field although three previous meetings (2003, 2005, 2007) remain valuable guidance criteria for ETEC vaccine development

• Crucial priorities for 2016-2017:
  • Consensus on clinical trial design; clinical trial endpoints
  • Consensus on preferred product characteristics for ETEC vaccines
  • Immune responses and correlates of protection
  • Technical consultation on assay development
Shigella disease and burden

- Gram negative, facultative anaerobe, non-spore forming, and non-motile
- Four serogroups of *Shigella*
  - *Flexneri* (6 serotypes)
  - *Sonnei* (1 serotype)
  - *Bodyii* (19 serotypes)
  - *Dysenteriae* (15 serotypes)
- Symptoms
  - Diarrhoea (often containing blood or mucous)
  - Abdominal cramps; and Fever
- Burden
  - 123,000 deaths, mostly in children less than five years old; 8.5 percent of all childhood diarrhea deaths
  - 40,000 deaths among children older than five years
  - 88.4 million cases per year, with school age children at highest risk
  - 7 million DALYs and 750,000 YLDs

Lancet, 2012. 380(9859)2095-2128
### Postulated coverage of *Shigella* strains required for a *Shigella* vaccine

<table>
<thead>
<tr>
<th>Vaccine products</th>
<th>% <em>Shigella strains</em> Covered</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Pentavalent</strong></td>
<td></td>
</tr>
<tr>
<td>– <em>S. sonnei</em></td>
<td>17%</td>
</tr>
<tr>
<td>– <em>S. flexneri</em> 2a, 3a, 6</td>
<td>36%</td>
</tr>
<tr>
<td>– Other <em>S. flexneri</em> via cross-protection*</td>
<td>35%</td>
</tr>
<tr>
<td>– <em>S. dysenteriae</em> 1</td>
<td>pandemics</td>
</tr>
<tr>
<td><strong>Trivalent</strong></td>
<td></td>
</tr>
<tr>
<td>– <em>S. sonnei</em></td>
<td>17%</td>
</tr>
<tr>
<td>– <em>S. flexneri</em> 2a and 3a</td>
<td>29%</td>
</tr>
<tr>
<td>– Other <em>S. flexneri</em> via cross-protection*</td>
<td>35%</td>
</tr>
</tbody>
</table>

*Assumes that *S. flexneri* 2a and 3a provide cross-protection against all other *S. flexneri* serotypes and sub-serotypes except 6.
Current Landscape of *Shigella* Vaccine Candidates

• **Cellular candidates**
  – CVD1208 (live, attenuated)
  – WRSS1 (live, attenuated)
  – Ty21a + *Shigella* LPS
  – *Shigella* whole cell (inactivated)
  – Truncated whole cells

• **Subunit**
  – Conjugates: chemical, recombinant, synthetic
  – Invaplex
  – Generalized modules of membrane antigens (GMMA)
  – Outer membrane vesicle (OMV)
  – DB Fusion
  – 34 kDa OMP

# Shigella Vaccine development: TPP Summary

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<td>Safety</td>
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<td>Oral</td>
</tr>
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</table>
# Sf2aSWC Phase 1 Immunogenicity

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Serum IgA &gt;2-fold (%)</th>
<th>Serum IgG &gt;2-fold (%)</th>
<th>ALS IgA &gt;4-fold (%)</th>
<th>ALS IgG &gt;4-fold (%)</th>
<th>Fecal IgA &gt;4-fold (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Placebo N=10</td>
<td>1/10 (10)</td>
<td>3/10 (30)</td>
<td>0/10 (0)</td>
<td>0/10 (0)</td>
<td>1/10 (10)</td>
</tr>
<tr>
<td>Vaccine Cohort 3 N=20</td>
<td>10/18 (56)</td>
<td>6/18 (33)</td>
<td>8/18 (44)</td>
<td>ND</td>
<td>8/18 (44)</td>
</tr>
<tr>
<td>Vaccine Cohort 4 N=18</td>
<td>15/20 (75)</td>
<td>14/20 (70)</td>
<td>19/20 (95)</td>
<td>16/20 (80)</td>
<td>11/20 (55)</td>
</tr>
</tbody>
</table>

Cohort 3 = $2.5 \times 10^{10}$ vp/dose X 3.
Cohort 4 = $2.5 \times 10^{11}$ vp/dose X 3.

Van De Verg L, unpublished data.
Live, attenuated *Shigella* vaccine

- WRSS1 is the *S. sonnei* component of a multivalent vaccine being developed at Walter Reed Army Institute of Research (WRAIR)
- Attenuated by VirG deletion which limits cell-to-cell spread of bacteria
- Safe and immunogenic in adult volunteers
- Currently being evaluated at the *iccdrb* in adults, 5- to 9-year olds and 12- to 23-month old children
- Attenuated strains with this or other attenuation strategies could vector other antigens to make combo vaccine formulations

Generalized Module for Membrane Antigens

- Pure outer membrane buds by genetic engineering → efficacy & affordability of whole cell vaccine without the side effects
- Increase GMMA release
- Remove / modify toxic components
  - Lipid A of LOS or LPS
- Delete unwanted antigens
  - surface polysaccharides, capsule
- Add new antigens
  - over express homologous antigens
  - add heterologous antigens
## Shigella candidate pipeline

<table>
<thead>
<tr>
<th>Discovery &amp; preclinical</th>
<th>Phase 1</th>
<th>Phase 2</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Live</strong></td>
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<tr>
<td>Shigetec (O)</td>
<td>WRSs2 &amp; WRSs3 (O)</td>
<td>CVD1208S (O)</td>
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<td><em>EveliQure</em>¹</td>
<td><em>NIAID, Walter Reed</em></td>
<td><em>U. Maryland, PATH</em></td>
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<tr>
<td>Ty21a expressing LPS (O)</td>
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<tr>
<td><em>Protein Potential</em></td>
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<tr>
<td>WRSf2a2 &amp; WRSf2a3 (O)</td>
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<tr>
<td><em>NIAID, Walter Reed</em></td>
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<tr>
<td><strong>Killed</strong></td>
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<tr>
<td>Inactivated trivalent (O)</td>
<td>Sf2aWC (O)</td>
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<tr>
<td><em>Walter Reed, PATH</em></td>
<td><em>Walter Reed</em>²</td>
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<tr>
<td><strong>Subunit</strong></td>
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<tr>
<td>SPS-Sf2a</td>
<td>O-SPC/rBU, OSPC-rDT</td>
<td>GVXN SD 133</td>
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<tr>
<td><em>Pasteur Institute</em></td>
<td><em>Shriver Institute</em></td>
<td><em>GlycoVaxyn</em></td>
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<tr>
<td>GMMA</td>
<td>DB Fusion</td>
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<tr>
<td><em>NVGH</em></td>
<td><em>PATH</em></td>
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<tr>
<td>InvaplexAR</td>
<td>34 kDa OMP</td>
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<tr>
<td><em>Walter Reed</em></td>
<td><em>NICED</em></td>
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<tr>
<td>Invaplex 50 (O)</td>
<td></td>
<td></td>
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<tr>
<td><em>Walter Reed</em>³</td>
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</tbody>
</table>

(O) Indicates oral vaccine

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1. Shigetec is a combination ETEC-Shigella vaccine currently in development by a private vaccine manufacturer in Austria.
2. 2,3,4. Vaccines currently on hold/no longer in development.
Assessment of WHO role for Shigella vaccines 2016-2017

• Clinical trial design and endpoints of trials
• Preferred Product Characteristics; TPP
• Assessment of laboratory assays
  – qPCR vs. culture
  – Immunology assays; correlates of protection
THANK YOU
Taqman Array Card Molecular Analysis
300 matched cases/controls, each site, each age stratum ~12,600

GEMS TAC

<table>
<thead>
<tr>
<th>Pathogen</th>
<th>L</th>
<th>R</th>
<th>Port</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
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<tbody>
<tr>
<td>Rotavirus (NSP3)</td>
<td>VP7 G1 &amp; G8</td>
<td>24</td>
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<td>MS2</td>
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<tr>
<td>Astrovirus (capsid)</td>
<td>Sapovirus (RdRp)</td>
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<tr>
<td>Norovirus GI (ORF1-ORF2)</td>
<td>Norovirus GII (ORF1-ORF2)</td>
<td>18</td>
<td></td>
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<tr>
<td>Adenovirus 40/41 (fiber gene)</td>
<td>Adenovirus pan (hexon)</td>
<td>17</td>
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<tr>
<td>Aeromonas (Aerolysin)</td>
<td>B. fragilis (EGBF)</td>
<td>16</td>
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<tr>
<td>C. difficile (tcdA &amp; B)</td>
<td>H. pylori (ureC)</td>
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<tr>
<td>C. jejuni/coli (cadF)</td>
<td>Campylobacter pan (cpn60)</td>
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<tr>
<td>Salmonella (ttr)</td>
<td>Shigella/EIEC (ipaH)</td>
<td>13</td>
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<tr>
<td>V. cholerae (hlyA)</td>
<td>STEC_stx1 &amp; stx2</td>
<td>12</td>
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<tr>
<td>18S</td>
<td>Bacterial 16S</td>
<td>11</td>
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<tr>
<td>EAEC_aaiC &amp; aatA</td>
<td>EAEC_aar &amp; aggR</td>
<td>10</td>
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<tr>
<td>ETEC_ST (Sth &amp; STp)</td>
<td>ETEC_LT</td>
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<td>EPEC_eae</td>
<td>EPEC_bfpA</td>
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<tr>
<td>Cryptosporidium (18S)</td>
<td>Cryptosporidium_LIB13</td>
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<tr>
<td>Giardia (18S)</td>
<td>Giardia_TPI</td>
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<tr>
<td>E. histolytica (18S)</td>
<td>Strongyloides (18S)</td>
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<td>PhHV</td>
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<tr>
<td>E. bieneusi (ITS) &amp; E. intestinalis (18S)</td>
<td>Cyclospora (18S) &amp; Isospora (ITS2)</td>
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<tr>
<td>Ascaris (ITS1)</td>
<td>Trichuris (18S)</td>
<td>2</td>
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<tr>
<td>Ancyclostoma (ITS2)</td>
<td>Necator (ITS2)</td>
<td>1</td>
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</tbody>
</table>

[Legend: ☐ GI pathogen assay ☒ Clinical Sample Control ☐ Manufacture Positive Control]
Double Mutant Heat Labile Toxin (dmLT) – May Facilitate Enteric Immunization

• Potent antigen and mucosal adjuvant
• Increases humoral and cellular immune responses
• Induces antibodies that neutralize LT enterotoxin activity
• Effective by many routes: oral, sublingual, intramuscular, intradermal, TCI, etc.
• Can increase number of responders and facilitate dose sparing

**Primary Efficacy Hypothesis:** The incidence of severe diarrhoea will be lower in the ACE527 alone or ACE527 + dmLT recipients compared to unvaccinated controls.

**Vaccine Dose:** $10^{10}$ cfu of reconstituted lyophilized formulation (~3x$10^9$ each strain)

---

# ETEC Vaccines in development

## Inactivated whole-cell

<table>
<thead>
<tr>
<th>Candidate</th>
<th>Producer</th>
<th>Strain</th>
<th>Characteristics</th>
<th>Route</th>
<th>Recent Findings</th>
</tr>
</thead>
<tbody>
<tr>
<td>ETVAX Killed Whole Cell</td>
<td>Scandinavian Biopharma</td>
<td></td>
<td>Inactivated tetravalent whole cell supplemented with LTB-CTB hybrid toxoid; may include dmLT adjuvant (ETVAX) [PATH; SBH]</td>
<td>Oral</td>
<td>P1</td>
</tr>
</tbody>
</table>

## Live attenuated

<table>
<thead>
<tr>
<th>Candidate</th>
<th>Producer</th>
<th>Strain</th>
<th>Characteristics</th>
<th>Route</th>
<th>Recent Findings</th>
</tr>
</thead>
<tbody>
<tr>
<td>(ACE527)</td>
<td>PATH</td>
<td></td>
<td><em>aroC, omp F, and Omp C</em>-based live attenuated; may include dmLT adjuvant</td>
<td>Oral</td>
<td>P2b</td>
</tr>
</tbody>
</table>
## ETEC Vaccines in Development

### Sub unit vaccine

<table>
<thead>
<tr>
<th>Candidate</th>
<th>Producer</th>
<th>Strain</th>
<th>Characteristics</th>
<th>Route</th>
<th>Recent Findings</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fimbrial Tip Adhesin (FTA)</td>
<td>NMRC PATH</td>
<td></td>
<td>Anti-adhesin based subunit vaccine</td>
<td>Parenteral</td>
<td></td>
</tr>
<tr>
<td>Anti-adhesin-toxoid fusion (MEFA)</td>
<td>[KSU; JHBSPH]</td>
<td></td>
<td>Anti-adhesin-toxoid fusion (MEFA) [KSU; JHBSPH]</td>
<td></td>
<td>Pre-Clinical</td>
</tr>
<tr>
<td>ST Toxoid</td>
<td></td>
<td></td>
<td>LT-ST fusion/LTB-ST conjugate [EntVac Consortium; GLOBVAC; STOPENTERICS; PATH]</td>
<td></td>
<td>Pre-Clinical</td>
</tr>
<tr>
<td>ZH9 attenuated</td>
<td></td>
<td></td>
<td>ZH9 attenuated typhoid vaccine expressing LT-ST toxoid (Typhetec)</td>
<td></td>
<td>Pre-Clinical</td>
</tr>
</tbody>
</table>
# Shigella vaccines in development

## Live attenuated genetic mutants

<table>
<thead>
<tr>
<th>Candidate</th>
<th>Producer</th>
<th>Strain</th>
<th>Characteristics</th>
<th>Route</th>
<th>Recent Findings</th>
</tr>
</thead>
<tbody>
<tr>
<td>CVD1208S</td>
<td>CVD, Univ. of Maryland</td>
<td><em>S. flexneri</em> 2a</td>
<td>Mutation: <strong>guaBA</strong> (purine biosynthesis), <strong>sen</strong> (ShET1), and <strong>set</strong> (ShET2 enterotoxin)</td>
<td>Oral</td>
<td>Phase 1 completed: Safe in adults; 75 percent excretion at $10^8$ CFU; strong ASC responses</td>
</tr>
<tr>
<td></td>
<td>Baltimore</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>WRSS1</td>
<td>WRAIR</td>
<td><em>S. sonnei</em></td>
<td>Mutation: <strong>virG</strong> (icsA)</td>
<td>Oral</td>
<td>Phase 1: age descending, dose escalation trial at icddrb, Bangladesh</td>
</tr>
<tr>
<td>WRSs2-WRSs3</td>
<td>WRAIR</td>
<td><em>S. sonnei</em></td>
<td>Mutations: <strong>virG</strong>, <strong>senA</strong>, <strong>senB</strong>, <strong>set</strong>, <strong>msbB2</strong></td>
<td>Oral</td>
<td>Preclinical</td>
</tr>
<tr>
<td>ShigETEC™</td>
<td>EveliQure Biotechnologies</td>
<td><em>S. flexneri</em> 2a</td>
<td>deletion of the rfbF, ipaB and/or ipaC genes, with mutated Shigella invasion plasmid encoding ETEC antigens</td>
<td>Oral</td>
<td>Preclinical</td>
</tr>
<tr>
<td></td>
<td>GmbH</td>
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</tr>
</tbody>
</table>

## Inactivated whole-cell

<table>
<thead>
<tr>
<th>Candidate</th>
<th>Producer</th>
<th>Strain</th>
<th>Characteristics</th>
<th>Route</th>
<th>Recent Findings</th>
</tr>
</thead>
<tbody>
<tr>
<td>TSWC</td>
<td>PATH; WRAIR</td>
<td><em>S. flexneri</em> 2a, <em>S. flexneri</em> 3a, <em>S. sonnei</em></td>
<td>Formalin inactivated strains; Will be co-administered + dmLT</td>
<td>Oral</td>
<td><em>S. flexneri</em> 2a prototype has completed Phase I; safe/immunogenic</td>
</tr>
</tbody>
</table>
### Vectored

<table>
<thead>
<tr>
<th>Candidate</th>
<th>Producer</th>
<th>Strain</th>
<th>Characteristics</th>
<th>Route</th>
<th>Recent Findings</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ty21a-vectored</td>
<td>Protein Potential, LLC</td>
<td><em>S. sonnei</em></td>
<td>Construct incorporates the <em>S. sonnei</em> LPS genes into the Ty21a chromosome</td>
<td>Oral</td>
<td>New stable hybrid that expresses both homologous <em>S. Typhi</em> and heterologous <em>S. sonnei</em> O-SP</td>
</tr>
</tbody>
</table>

### Conjugates

<table>
<thead>
<tr>
<th>Candidate</th>
<th>Producer</th>
<th>Strain</th>
<th>Characteristics</th>
<th>Route</th>
<th>Recent Findings</th>
</tr>
</thead>
<tbody>
<tr>
<td>GVXN (Recombinant glycoconjugate*)</td>
<td>GlycoVaxyn AD, Switzerland</td>
<td><em>S. dysenteriae</em> 1</td>
<td>Produced <em>in vivo</em> using E. coli; linked to <em>P. aeruginosa</em> exoprotein carrier (sugar antigen to a carrier protein)</td>
<td>IM</td>
<td>Robust immune response in phase 1. IgG and IgA seroconversion (four-fold increase) was 80 percent after 2(^{nd}) dose. The IgG GMT rose ~16-fold,</td>
</tr>
<tr>
<td></td>
<td></td>
<td><em>S. flexneri</em></td>
<td></td>
<td></td>
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<tr>
<td></td>
<td></td>
<td><em>S. sonnei</em> and <em>flexneri</em> 2a</td>
<td>O-SP are conjugated to rEPA</td>
<td>IM</td>
<td>Phase III: Safe, immunogenic in one to four year old Israeli children; 21 percent <em>sonnei</em> protection overall; 71 percent in 3-4 year olds</td>
</tr>
<tr>
<td></td>
<td>NIH</td>
<td><em>S. sonnei</em> and <em>flexneri</em> 2a</td>
<td></td>
<td>IM</td>
<td>Preclinical</td>
</tr>
<tr>
<td></td>
<td>Institute Pasteur</td>
<td><em>S. flexneri</em> 2a</td>
<td><em>In vitro</em>, synthetically produced <em>S. flexneri</em> 2a oligosaccharide “mimics” conjugated to protein carriers</td>
<td>IM</td>
<td>Preclinical</td>
</tr>
<tr>
<td>Candidate</td>
<td>Developer</td>
<td>Strain</td>
<td>Characteristics</td>
<td>Route</td>
<td>Recent Findings</td>
</tr>
<tr>
<td>-------------------</td>
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<td>--------------------------------------------------------------------------------</td>
</tr>
<tr>
<td>DB Fusion</td>
<td>Oklahoma State; PATH</td>
<td>Serotype Independent</td>
<td>IpaB/IpaD proteins are expressed as a single recombinant polypeptide chain</td>
<td>ID</td>
<td>Intranasal mouse challenge given with dmLT provides reasonable protection against <em>S. flexneri</em> and <em>sonnei</em> challenge</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Serotype non-specific</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>Co-administered with dmLT</td>
<td></td>
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</tr>
<tr>
<td>Invaplex&lt;sub&gt;AR&lt;/sub&gt;</td>
<td>WRAIR</td>
<td><em>S. flexneri</em> 2a</td>
<td>IpaB, IpaC, IpaD, and LPS of <em>S. flexneri</em> 2a (Serotype specific)</td>
<td>Nasal</td>
<td>Early version was immunogenic but did not protect in Phase 2b</td>
</tr>
<tr>
<td></td>
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<td></td>
<td>May be co-administered with dmLT</td>
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<tr>
<td>GMMA</td>
<td>NVGH</td>
<td><em>S. sonnei</em></td>
<td>Outer Membrane Vesicles (Serotype specific)</td>
<td>IM</td>
<td>Phase 1</td>
</tr>
<tr>
<td>Outer Membrane Vesicles (OMV)</td>
<td>University of Navarra, Spain</td>
<td><em>S. flexneri</em> 2a</td>
<td>Serotype specific, outer membrane vesicles naturally secreted into bacterial culture medium of wild-type strains</td>
<td>Oral, nasal, or ID</td>
<td>Preclinical: Challenge in mice model with OMV nanoparticles by oral, nasal or ID showed 100 percent (n=6) protection</td>
</tr>
<tr>
<td>OmpA</td>
<td>National Institute of Cholera and Enteric Diseases, Kolkata, India</td>
<td><em>S. flexneri</em> 2a</td>
<td>Cross-reactive, conserved, and surface exposed protein (serotype non-specific)</td>
<td>Nasal</td>
<td>Preclinical: 100 percent protection in mice challenged with virulent <em>S. flexneri</em> 2a</td>
</tr>
</tbody>
</table>