Association between enteric pathogens and diarrhea and child growth shortfalls: insights from the application of molecular diagnostics

James Platts-Mills, University of Virginia
Livestock, Sanitation, Hygiene, and Child Growth: Exploring the complex underlying causes of child stunting
March 1, 2018
Overview

1. Molecular diagnostics have substantially revised estimates of the etiology and burden of childhood diarrhea in low resource settings.
2. These same diagnostics have recently been applied to longitudinal studies evaluating the impact of early enteric infections on child growth.
3. Findings from these studies most clearly and consistently implicate inflammatory bacterial pathogens.
4. Extension of these diagnostics to interventional studies with child growth as the primary outcome may serve as an important secondary endpoint which can help elucidate the reasons for the impact (or lack thereof) of these interventions.
The standard work-up for diarrhea: expensive, complicated, and variably sensitive

Fecal sample

Selective media for bacteriology

- Salmonella
- Shigella
- Campylobacter
- Vibrios
- Aeromonas

Freeze stool for immunoassays

Pick 3 E. coli

- Multiplex PCR
  - ETEC
  - tEPEC
  - aEPEC
  - EAEC
  - STEC

- Rotavirus
- Adenovirus
- Cryptosporidium
- E. histolytica
- Giardia

Freeze stool for RT-PCR, and post hoc PCR

- Norovirus
- Astrovirus
- Sapovirus

Figure courtesy of Mike Levine
The TaqMan Array Card: simultaneous quantitative PCR for a broad range of enteropathogens

<table>
<thead>
<tr>
<th>Pathogen/Marker</th>
<th>Port</th>
<th>R</th>
<th>Sites</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rotavirus (NSP3)</td>
<td></td>
<td>VP7 G1 &amp; G8</td>
<td>24</td>
</tr>
<tr>
<td>MS2</td>
<td></td>
<td>MS2</td>
<td>20</td>
</tr>
<tr>
<td>Astrovirus (capsid)</td>
<td></td>
<td>Sapovirus (RdRp)</td>
<td>19</td>
</tr>
<tr>
<td>Norovirus GI (ORF1-ORF2)</td>
<td></td>
<td>Norovirus GII (ORF1-ORF2)</td>
<td>18</td>
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<tr>
<td>Adenovirus 40/41 (fiber gene)</td>
<td></td>
<td>Adenovirus pan (hexon)</td>
<td>17</td>
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<tr>
<td>Aeromonas (Aerolysin)</td>
<td></td>
<td>B. fragilis (EGBF)</td>
<td>16</td>
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<tr>
<td>C. difficile (tcdA &amp; B)</td>
<td></td>
<td>H. pylori (ureC)</td>
<td>15</td>
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<tr>
<td>C. jejuni/coli (cadF)</td>
<td></td>
<td>Campylobacter pan (cpx60)</td>
<td>14</td>
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<tr>
<td>Salmonella (ttr)</td>
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<td>Shigella/EIEC (ipaH)</td>
<td>13</td>
</tr>
<tr>
<td>V. cholerae (hlyA)</td>
<td></td>
<td>STEC_651 &amp; 652</td>
<td>12</td>
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<tr>
<td>18S</td>
<td></td>
<td>Bacterial 16S</td>
<td>11</td>
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<tr>
<td>EAEC_aaiC &amp; aatA</td>
<td></td>
<td>EAEC_aar &amp; aggR</td>
<td>10</td>
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<tr>
<td>ETEC_ST (Stx &amp; StT)</td>
<td></td>
<td>ETEC_LT</td>
<td>9</td>
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<tr>
<td>EPEC_eae</td>
<td></td>
<td>EPEC_bfpA</td>
<td>8</td>
</tr>
<tr>
<td>Cryptosporidium (18S)</td>
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<td>Cryptosporidium_LIB13</td>
<td>7</td>
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<tr>
<td>Giardia (18S)</td>
<td></td>
<td>Giardia_TPI</td>
<td>6</td>
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<tr>
<td>E. histolytica (18S)</td>
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<td>Strongylus (18S)</td>
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<td>PhHV</td>
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<td>PhHV</td>
<td>4</td>
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<tr>
<td>E. bieneusi (ITS) &amp; E. Intestinalis (18S)</td>
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<td>Cyclospora (18S) &amp; Isospora (ITS2)</td>
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<tr>
<td>Ascaris (ITS1)</td>
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<td>Trichuris (18S)</td>
<td>2</td>
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<tr>
<td>Ancyclostoma (ITS2)</td>
<td></td>
<td>Necator (ITS2)</td>
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</tbody>
</table>
The TaqMan Array Card: test characteristics vs. standard microbiologic studies
Sensitive molecular diagnostics yield many pathogens

Taniuchi M, et al. JID 2015
A common drinking water source in Haydom, Tanzania

Photo by Jean Gratz
GEMS: Etiology of moderate to severe childhood diarrhea in low-resource settings

<table>
<thead>
<tr>
<th>Location</th>
<th>Africa and Asia</th>
<th>Bangladesh</th>
<th>South America, Africa, Asia</th>
</tr>
</thead>
<tbody>
<tr>
<td>Study design</td>
<td>Case-control (moderate to severe diarrhea)</td>
<td>Birth cohort</td>
<td>Birth cohort</td>
</tr>
<tr>
<td>Sample size</td>
<td>5304 cases and matched controls</td>
<td>603 children from single site</td>
<td>1469 children from 7 sites</td>
</tr>
<tr>
<td>Age range</td>
<td>0-59 months</td>
<td>0-12 months</td>
<td>0 – 24 months</td>
</tr>
<tr>
<td>PCR testing</td>
<td>5304 diarrheal and 5304 non-diarrheal stools</td>
<td>1741 diarrhea episodes</td>
<td>4975 diarrheal and 30,647 non-diarrheal stools</td>
</tr>
</tbody>
</table>

**Tables:**

<table>
<thead>
<tr>
<th>EAEC</th>
<th>Shigella/EIEC</th>
<th>C. jejuni/C. coli</th>
<th>Adenovirus 40/41</th>
<th>Typical EPEC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Odds Ratio and 95% CIs</td>
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<td></td>
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<tr>
<td>LT–ETEC</td>
<td>Rotavirus</td>
<td>Cryptosporidium</td>
<td>ST–ETEC</td>
<td>Sapovirus</td>
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<tr>
<td></td>
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</tr>
</tbody>
</table>

**Graphs:**

- EAEC
- Shigella/EIEC
- C. jejuni/C. coli
- Adenovirus 40/41
- Typical EPEC
- LT–ETEC
- Rotavirus
- Cryptosporidium
- ST–ETEC
- Sapovirus

Kotloff K, et al. Lancet
GEMS: qPCR re-analysis of pathogen-specific burdens of diarrhea

89.0% of diarrheal incidence was attributable to a pathogen vs. 49.8% using the original microbiologic work-up.

Figure 3  Effect of diarrhoeal incidence prior to 24 months on stunting at 24 months of age. Point estimates of the effect of diarrhoeal incidence on stunting at 24 months are shown for each study. The size of the square around the point estimate is proportional to sample size. The lines represent 95% CI. In the pooled estimate, represented by a diamond, the odds of stunting at 24 months increased by 1.13 when diarrhoeal incidence prior to 24 months increased by five episodes (95% CI 1.07 to 1.19)
PROVIDE: Association between all-cause and etiology-specific diarrhea and linear growth

No relationship between all-cause diarrhea and length at 12 months (-0.01 decrease in 12-month LAZ per episode; 95% confidence interval: -0.06, 0.03)

Bacteria (-0.09 decrease in 12-month LAZ per attributable episode; 95% CI: -0.16, -0.01) and protozoa (-0.24; 95% CI: -0.49, 0.01) were more strongly associated with linear growth deficits than viruses (-0.01; 95% CI: -0.11, 0.08)
PROVIDE: Etiology-specific diarrhea and linear growth attainment in 603 Bangladeshi infants – effect for high (90th percentile) vs. low (10th percentile) attributable burden
Maternal and Child Health Clinic outside of Haydom
LAZ difference per diarrhea episode: -0.00, 95% CI: -0.02, 0.01.

Parasitic (LAZ difference: -0.12, 95% CI: -0.25, 0.01) and bacterial (-0.04, 95% CI: -0.08, 0.01) diarrhea were more strongly associated with decrements in LAZ than viral diarrhea (0.02, 95% CI: -0.02, 0.06).
MAL-ED: Subclinical enteropathogen burden and linear growth at 2 years of age

![Graph showing the LAZ difference at 2 years (95% CI) for various pathogens including bacteria, viruses, and protozoa. The graph includes symbols for LAZ difference between high and low pathogen prevalence and LAZ difference per one log increase in average quantity of pathogen.]
MAL-ED: Enteric pathogen detection in non-diarrheal stools and gut inflammation (MPO)
MAL-ED: Enteric pathogen detection in non-diarrheal stools and gut inflammation (MPO)

MAL-ED: Etiology of watery diarrhea vs. dysentery

The diagram illustrates the attributable incidence per 100 child-years (95% CI) for various causes of diarrhea, comparing those with and without blood present. The x-axis represents different causes, while the y-axis shows the attributable incidence. The proportion of attributable episodes with blood is also shown on the right y-axis.
2.6 Use of antimicrobials

*Antimicrobials should not be used routinely.* This is because, except as noted below, it is not possible to distinguish clinically episodes that *might* respond, such as diarrhoea caused by enterotoxigenic *E. coli*, from those caused by agents unresponsive to antimicrobials, such as rotavirus or *Cryptosporidium*. Moreover, even for potentially responsive infections, selecting an effective antimicrobial requires knowledge of the likely sensitivity of the causative agent, information that is usually unavailable. In addition, use of antimicrobials adds to the cost of treatment, risks adverse reactions and enhances the development of resistant bacteria.

Antimicrobials are reliably helpful *only* for children with bloody diarrhoea (probable shigellosis), suspected cholera with severe dehydration, and serious non-intestinal infections such as pneumonia. Anti/protozoal drugs are *rarely* indicated.
What is the role of antimicrobial therapy for non-dysenteric diarrhea?

**A Quiet Revolution in the Treatment of Childhood Diarrhea**

Global Health  
By DONALD G. McNEIL Jr.  
AUG. 10, 2015

WHO is rethinking the decades-old recommendation that only febrile, bloody diarrhea requires antibiotic therapy. A 15,000 child multisite mortality and growth co-primary endpoint clinical trial of azithromycin vs. placebo is planned (now underway).
Extending molecular diagnostics to interventional studies

1. **Water, Sanitation, and Hygiene**: the most comprehensive studies done to date (WASH Benefits, SHINE) have not shown clear growth benefits of WASH interventions. Did these interventions fail to reduce enteric pathogen exposure, carriage, and the development of EED? If so, why?

2. **Diagnosis and management of diarrhea**: does broadening the treatment indications for childhood diarrhea improve child growth? If so, who to treat: a) syndromic treatment that is broader than bloody diarrhea; b) POC test – either pathogen diagnostic or test for inflammation? c) based on child risk? (MAL-ED, ABCD)

3. **Treatment of subclinical enteropathogen burden**: does scheduled macrolide therapy improve child growth (and does it do so by reducing infection with invasive bacterial enteropathogens?) (ELICIT, CHAIN, MORDOR)

4. **Other therapies**: Micro/macronutrients, colostrum, microbiota-targeted therapeutics, novel gut therapeutics...
Acknowledgements

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PROVIDE: William Petri, Mami Taniuchi, Amanda Schnee, Beth Kirkpatrick, Rashidul Haque, Jashim Uddin


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MAL-ED: Comparison between original microbiology and qPCR
MAL-ED: Campylobacter burden


**Graph Description:**
- **Y-axis:** Cumulative incidence of Campylobacter infection
- **X-axis:** Age (months)
- The graph compares the cumulative incidence of Campylobacter infection across different locations:
  - Dhaka, Bangladesh
  - Vellore, India
  - Bhaktapur, Nepal
  - Naushero Feroze, Pakistan
  - Venda, South Africa
  - Haydom, Tanzania
  - Fortaleza, Brazil
  - Loreto, Peru

<table>
<thead>
<tr>
<th>Campylobacter species detected by ProSpecT EIA</th>
<th>Approximate frequency of detection by PCR/sequencing in TZH, BGD, PEL</th>
</tr>
</thead>
<tbody>
<tr>
<td>C. jejuni/coli</td>
<td>~70%</td>
</tr>
<tr>
<td>C. hyointestinalis</td>
<td>~5-15%</td>
</tr>
<tr>
<td>C. trogloidyts</td>
<td>~5-15%</td>
</tr>
<tr>
<td>C. upsaliensis</td>
<td>&lt;5%</td>
</tr>
<tr>
<td>C. concisus</td>
<td>&lt;5%</td>
</tr>
</tbody>
</table>