Diarrheal Diseases in Asia: overview and update

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Research Policy and Cooperation
WHO, SEARO
Overview of the talk

- Global and Regional burden of diarrheal disease
- Emerging trends in the etiology of diarrheal pathogens
- New Frontiers
  - Asymptomatic infections
  - Intestinal microbiota and diarrhoel pathogens
  - Polymicrobial infections
- Interventions for diarrheal diseases
- Conclusion
Global and Regional burden of diarrheal diseases
Top 25 causes of Years of Life Lost due to premature mortality from 1990 to 2010

CAUSES OF PREMATURE DEATH
Years of life lost (YLLs) quantify premature mortality by weighting younger deaths more than older deaths.

Ranks for top 25 causes of YLLs 1990-2010, India

<table>
<thead>
<tr>
<th>Rank and disorder 1990</th>
<th>% YLLs in thousands (of total)</th>
<th>Rank and disorder 2010</th>
<th>% YLLs in thousands (of total)</th>
<th>% change</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>57,828 (12.4%)</td>
<td>1 Preterm birth complications</td>
<td>27,808 (7.4%)</td>
<td>-31</td>
</tr>
<tr>
<td>2</td>
<td>47,806 (10.3%)</td>
<td>2 Lower respiratory infections</td>
<td>26,127 (6.9%)</td>
<td>-45</td>
</tr>
<tr>
<td>3</td>
<td>40,134 (8.6%)</td>
<td>3 Diarrheal diseases</td>
<td>25,589 (6.8%)</td>
<td>-56</td>
</tr>
<tr>
<td>4</td>
<td>20,533 (4.4%)</td>
<td>4 Ischemic heart disease</td>
<td>25,253 (6.7%)</td>
<td>66</td>
</tr>
<tr>
<td>5</td>
<td>21,336 (4.6%)</td>
<td>5 COPD</td>
<td>17,761 (4.7%)</td>
<td>2</td>
</tr>
<tr>
<td>6</td>
<td>18,808 (4.1%)</td>
<td>6 Neonatal sepsis</td>
<td>16,594 (4.4%)</td>
<td>-23</td>
</tr>
<tr>
<td>7</td>
<td>17,426 (3.8%)</td>
<td>7 Tuberculosis</td>
<td>13,732 (3.6%)</td>
<td>-32</td>
</tr>
<tr>
<td>8</td>
<td>15,294 (3.3%)</td>
<td>8 Self-harm</td>
<td>12,981 (3.4%)</td>
<td>154</td>
</tr>
<tr>
<td>9</td>
<td>13,328 (2.9%)</td>
<td>9 Road injury</td>
<td>12,588 (3.3%)</td>
<td>63</td>
</tr>
<tr>
<td>10</td>
<td>16,651 (3.5%)</td>
<td>10 Stroke</td>
<td>11,726 (3.1%)</td>
<td>54</td>
</tr>
<tr>
<td>11</td>
<td>9,317 (2.0%)</td>
<td>11 Neonatal encephalopathy</td>
<td>11,099 (2.9%)</td>
<td>-17</td>
</tr>
<tr>
<td>12</td>
<td>9,031 (1.9%)</td>
<td>12 HIV/AIDS</td>
<td>8,696 (2.3%)</td>
<td>6,147</td>
</tr>
<tr>
<td>13</td>
<td>7,904 (1.7%)</td>
<td>13 Fire</td>
<td>8,172 (2.2%)</td>
<td>19</td>
</tr>
<tr>
<td>14</td>
<td>7,923 (1.7%)</td>
<td>14 Congenital anomalies</td>
<td>7,073 (1.9%)</td>
<td>4</td>
</tr>
<tr>
<td>15</td>
<td>7,399 (1.6%)</td>
<td>15 Road injury</td>
<td>6,528 (1.7%)</td>
<td>-66</td>
</tr>
<tr>
<td>16</td>
<td>7,057 (1.5%)</td>
<td>16 Cirrhosis</td>
<td>6,134 (1.6%)</td>
<td>84</td>
</tr>
<tr>
<td>17</td>
<td>6,949 (1.5%)</td>
<td>17 Meningitis</td>
<td>5,790 (1.5%)</td>
<td>-38</td>
</tr>
<tr>
<td>18</td>
<td>6,694 (1.4%)</td>
<td>18 Diabetes</td>
<td>5,056 (1.3%)</td>
<td>92</td>
</tr>
<tr>
<td>19</td>
<td>6,446 (1.4%)</td>
<td>19 Measles</td>
<td>5,861 (1.5%)</td>
<td>-63</td>
</tr>
<tr>
<td>20</td>
<td>5,699 (1.2%)</td>
<td>20 Self-harm</td>
<td>4,717 (1.2%)</td>
<td>1</td>
</tr>
<tr>
<td>21</td>
<td>4,578 (1.0%)</td>
<td>21 Drowning</td>
<td>4,214 (1.1%)</td>
<td>-35</td>
</tr>
<tr>
<td>22</td>
<td>4,082 (0.9%)</td>
<td>22 Peptic ulcer</td>
<td>4,281 (1.1%)</td>
<td>85</td>
</tr>
<tr>
<td>23</td>
<td>3,873 (0.8%)</td>
<td>23 Syphilis</td>
<td>3,627 (1.0%)</td>
<td>-54</td>
</tr>
<tr>
<td>24</td>
<td>3,911 (0.8%)</td>
<td>24 Asthma</td>
<td>4,336 (1.1%)</td>
<td>34</td>
</tr>
<tr>
<td>25</td>
<td>3,849 (0.8%)</td>
<td>25 Mechanical forces</td>
<td>3,130 (0.8%)</td>
<td>-20</td>
</tr>
<tr>
<td>26</td>
<td>27 Cirrhosis</td>
<td>27 Peptic ulcer</td>
<td>1,894 (0.5%)</td>
<td>-56</td>
</tr>
<tr>
<td>27</td>
<td>30 Typhoid fevers</td>
<td>30 Typhoid fevers</td>
<td>1,466 (0.3%)</td>
<td>-54</td>
</tr>
<tr>
<td>28</td>
<td>31 Diabetes</td>
<td>31 Diabetes</td>
<td>1,433 (0.3%)</td>
<td>-51</td>
</tr>
<tr>
<td>29</td>
<td>33 Falls</td>
<td>33 Falls</td>
<td>1,258 (0.3%)</td>
<td>-66</td>
</tr>
<tr>
<td>30</td>
<td>78 HIV/AIDS</td>
<td>78 HIV/AIDS</td>
<td>1,199 (0.3%)</td>
<td>-70</td>
</tr>
</tbody>
</table>
Diarrhea remains a leading killer of young children, despite the availability of simple treatment solutions.

Source: WHO and Maternal and Child Epidemiology Estimation Group (MCEE) provisional estimates 2015
Regional Burden of Diarrhea Mortality, Ages 0–4 Years, 2015

- Estimated number of deaths due to diarrhea 526,000
- 89% drop from 1980 and a striking 58% from 2000 to 2015
- Sub-Saharan Africa and South Asia account for 90% of the total
- 72% of the diarrhea deaths occur in the first two years of life

Keusch et al 2016; Liu, Hill, and others 2016
Annual number of deaths from diarrheal diseases among the 0–4 year age group in low- and middle-income countries (LMICs)
Deaths due to Diarrheal Diseases in WHO Regions

Deaths due to Diarrheal Diseases in SEAR (in millions)

Trend of mortality due to diarrheal diseases in SEAR (1990-2015)
Major interventions in Diarrheal diseases

• Early use of Oral rehydration solutions

• Appropriate use of antibiotics for bloody diarrhea and dysentery

• Continued breast feeding

• Nutritional interventions for persistent diarrhea

• Rapid restoration of nutritional status in all diarrhea patients
Major killers in India

1990

1. Ischemic heart disease
2. Lower respiratory infect
3. Diarrheal diseases
4. Tuberculosis
5. COPD
6. Neonatal preterm birth
7. Neonatal encephalopathy
8. Hemorrhagic stroke
9. Asthma
10. Measles
11. Ischemic stroke

2015

1. Ischemic heart disease
2. COPD
3. Lower respiratory infect
4. Diarrheal diseases
5. Tuberculosis
6. Hemorrhagic stroke
7. Ischemic stroke
8. Diabetes
9. Neonatal preterm birth
10. Neonatal encephalopathy
11. Self-harm
12. Hypertensive heart disease

Diarrheal diseases
Year: 1990
Rank: 3
Change: -56.11%
Rate: 84.94 deaths per 100,000 (77.64 – 90.53)

Year: 2015
Rank: 4
Change: -56.11%
Rate: 37.28 deaths per 100,000 (33.79 – 41.4)

Major killers in under 5 children in India

1990
1. Neonatal preterm birth
2. Lower respiratory infect
3. Neonatal encephalopathy
4. Diarrheal diseases
5. Measles
6. Tetanus
7. Other neonatal
8. Neonatal sepsis
9. Protein-energy malnutrition
10. Congenital heart

2015
1. Neonatal preterm birth
2. Neonatal encephalopathy
3. Lower respiratory infect
4. Diarrheal diseases
5. Other neonatal
6. Neonatal sepsis
7. Congenital heart
8. Other congenital
9. Encephalitis
10. Typhoid fever
11. Measles
12. Protein-energy malnutrition
13. Neonatal hemolytic
14. HIB meningitis
15. Tetanus

- Estimated global diarrhea incidence rates have not changed significantly since 1980
- Children in Sub-Saharan Africa and South Asia experience an average of 2.7 episodes of diarrhea per year
- Most mild to moderate lasting an avg. of 4.3 days
- Incidence rates vary but are higher in children in LIC and LMIC countries and highest in Sub-Saharan Africa (3.3 episodes per child per year)

Fischer Walker and others 2013; Keusch et al 2016;
Regional Burden of Severe Diarrhea Episodes, Ages 0–4 Years, 2010

- 5 to 15% of watery diarrhea cases progress to persistent diarrhea
- More than 50 percent of severe episodes occur in Sub Saharan Africa and South-East Asia

Fischer Walker and others 2013; Keusch et al 2016;
Emerging trends in the etiology of diarrheal pathogens

Many bacterial, viral and parasitic etiologies cause diarrheal disease but only a few account for a major portion of the burden.
Bacterial Viral and Parasitic diarrhoeal pathogens

**Bacteria**

Classical Serotyping and Molecular Analysis
- V. cholerae O1 and O139
- V. cholerae Non O1 and Non O139
- V. parahaemolyticus
- V. fluvialis
  - Conventional Microbiology, Serotyping and PCR
- Aeromonas spp.
  - Conventional Microbiology
- Campylobacter jejuni
  - Conventional Microbiology
- Campylobacter coli
  - Conventional Microbiology
- Shigella dysenteriae
  - S. flexneri
  - S. sonnei
  - S. Boydii
  - Classical Microbiology + Serotyping
- Salmonella
- EPEC
  - ETEC group (LT, ST, LT+ST)
  - EAEC, EIEC, STEC
  - Classical Microbiology + PCR

**Virus**

PAGE, RT-PCR and Kit
- Rotavirus
  - PAGE & Rota Adeno Kit
- Adenovirus
  - Rota Adeno Kit
- Norovirus GI
  - Norovirus GII
  - RT-PCR
- Sapovirus
  - RT-PCR
- Astrovirus
  - RT-PCR

**Parasites**

Microscopic and Molecular Analysis
- Giardia lamblia
  - Capture ELISA and PCR
- Cryptosporidium parvum
  - Capture ELISA and PCR
- Entamoeba histolytica
  - Capture ELISA and PCR
- Blastocystis hominis
  - Routine Microscopy
Diarrheal Etiologies

• In one study, 40 % of cause-specific attributable diarrhea mortality was due to two organisms: rotavirus (27.8%) and EPEC (11.1%) (Lanata and others 2013)

• Another large, multisite, clinic-based prospective case-control study of children under age five years with MSD identified four pathogens – rotavirus, Cryptosporidium, ETEC and Shigella responsible for most attributable episodes of MSD (Kotloff et al 2013)
Diarrheal Etiologies

- Certain pathogens such as Rotavirus, Shigella, *Vibrio cholerae* and Adenovirus serotypes 40/41 were more commonly isolated in children with moderate to severe illness.

- 72% of controls without diarrhoea also harbored one or more putative pathogens and 31% had two or more reflecting the fecally contaminated environment in which they live.
Attributable incidence of moderate to severe diarrhea per 100 child years, by pathogen and by age group, all 7 GEMS sites combined.
Rotavirus deaths estimates for 2008

India (22%) - 88,621
Nigeria (9%) - 41,057
Pakistan (9%) - 39,144
DR Congo (7%) - 32,653
Ethiopia (6%) - 28,218
Afghanistan (6%) - 25,423
Uganda (2%) - 10,637
Indonesia (2%) - 9,970
Bangladesh (2%) - 9,857
Angola (2%) - 8,788

Tate et al, Lancet Infect Dis 2012
Emerging trends in the etiology of enteric pathogens as evidenced from an active surveillance of hospitalized diarrhoeal patients in Kolkata, India

Gopinath Balakrish Nair*1, Thandavarayan Ramamurthy1, Mihir Kumar Bhattacharya1, Triveni Krishnan1, Sandipan Ganguly1, Dhira Rani Saha1, Krishnan Rajendran1, Byomkesh Manna1, Mrinmoy Ghosh2, Keinosuke Okamoto3 and Yoshifumi Takeda4
## Age wise isolation of enteric pathogens
(Nov 2007 to Oct 2009)

<table>
<thead>
<tr>
<th>Pathogen</th>
<th>0 - 11 month (n=245)</th>
<th>12 - 23 month (n=227)</th>
<th>24 - 59 month (n=176)</th>
<th>Total Age &lt;5 yr (n=648)</th>
<th>Age ≥ 5 yr (n=1871)</th>
<th>All Age Group (n=2519)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Bacteria</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Vibrio cholerae O1</em></td>
<td>22(9)</td>
<td>34(15)</td>
<td>50(28.4)</td>
<td>106(16.4)</td>
<td>548(29.3)</td>
<td>654(26)</td>
</tr>
<tr>
<td><em>Vibrio cholerae O139</em></td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>2(0.1)</td>
<td>2(0.1)</td>
</tr>
<tr>
<td><em>Vibrio cholerae Non O1 Non O139</em></td>
<td>2(0.8)</td>
<td>1(0.4)</td>
<td>1(0.6)</td>
<td>4(0.6)</td>
<td>51(2.7)</td>
<td>55(2.2)</td>
</tr>
<tr>
<td><em>V. parahaemolyticus</em></td>
<td>1(0.4)</td>
<td>0</td>
<td>2(1.1)</td>
<td>3(0.5)</td>
<td>71(3.8)</td>
<td>74(2.9)</td>
</tr>
<tr>
<td><em>Vibrio fluvialis</em></td>
<td>3(1.2)</td>
<td>7(3.1)</td>
<td>1(0.6)</td>
<td>11(1.7)</td>
<td>44(2.4)</td>
<td>55(2.2)</td>
</tr>
<tr>
<td><em>Aeromonas spp.</em></td>
<td>1(0.4)</td>
<td>2(0.9)</td>
<td>1(0.6)</td>
<td>4(0.6)</td>
<td>21(1.1)</td>
<td>25(1)</td>
</tr>
<tr>
<td><em>Campylobacter jejuni</em></td>
<td>18(7.3)</td>
<td>22(9.7)</td>
<td>20(11.4)</td>
<td>60(9.3)</td>
<td>58(3.1)</td>
<td>118(4.7)</td>
</tr>
<tr>
<td><em>C. coli</em></td>
<td>1(0.4)</td>
<td>0</td>
<td>1(0.6)</td>
<td>2(0.3)</td>
<td>20(1.1)</td>
<td>22(0.9)</td>
</tr>
<tr>
<td><em>Shigellae</em></td>
<td>8(3.3)</td>
<td>21(9.3)</td>
<td>22(12.5)</td>
<td>51(7.9)</td>
<td>103(5.5)</td>
<td>154(6.1)</td>
</tr>
<tr>
<td><em>Salmonella</em></td>
<td>0</td>
<td>1(0.4)</td>
<td>1(0.6)</td>
<td>2(0.3)</td>
<td>21(1.1)</td>
<td>23(0.9)</td>
</tr>
<tr>
<td><em>EPEC</em></td>
<td>11(4.5)</td>
<td>8(3.5)</td>
<td>2(1.1)</td>
<td>21(3.2)</td>
<td>24(1.3)</td>
<td>45(1.8)</td>
</tr>
<tr>
<td><em>ETEC Group</em></td>
<td>9(3.7)</td>
<td>13(5.7)</td>
<td>5(2.8)</td>
<td>27(4.2)</td>
<td>87(4.6)</td>
<td>114(4.5)</td>
</tr>
<tr>
<td><em>EAEC</em></td>
<td>32(13.1)</td>
<td>28(12.3)</td>
<td>18(10.2)</td>
<td>78(12)</td>
<td>81(4.3)</td>
<td>159(6.3)</td>
</tr>
<tr>
<td><strong>Virus</strong></td>
<td></td>
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<td></td>
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</tr>
<tr>
<td><em>Rotavirus</em></td>
<td><strong>115</strong>(46.9)</td>
<td><strong>124</strong>(54.6)</td>
<td><strong>39</strong>(22.2)</td>
<td><strong>278</strong>(42.9)</td>
<td><strong>124</strong>(6.6)</td>
<td><strong>402</strong>(16)</td>
</tr>
<tr>
<td><em>Adenovirus</em></td>
<td>24(9.8)</td>
<td>22(9.7)</td>
<td>8(4.5)</td>
<td>54(8.3)</td>
<td>44(2.4)</td>
<td>98(3.9)</td>
</tr>
<tr>
<td><em>Norovirus G1</em></td>
<td>3(1.2)</td>
<td>5(2.2)</td>
<td>3(1.7)</td>
<td>11(1.7)</td>
<td>21(1.1)</td>
<td>32(1.3)</td>
</tr>
<tr>
<td><em>Norovirus G2</em></td>
<td>10(4.1)</td>
<td>5(2.2)</td>
<td>2(1.1)</td>
<td>17(2.6)</td>
<td>25(1.3)</td>
<td>42(1.7)</td>
</tr>
<tr>
<td><em>Sapovirus</em></td>
<td>10(4.1)</td>
<td>4(1.8)</td>
<td>4(2.3)</td>
<td>18(2.8)</td>
<td>13(0.7)</td>
<td>31(1.2)</td>
</tr>
<tr>
<td><em>Astrovirus</em></td>
<td>5(2)</td>
<td>7(3.1)</td>
<td>5(2.8)</td>
<td>17(2.6)</td>
<td>38(2)</td>
<td>55(2.2)</td>
</tr>
<tr>
<td><strong>Parasite</strong></td>
<td></td>
<td></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td><em>Blastocystis hominis</em></td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>11(0.6)</td>
<td>11(0.4)</td>
</tr>
<tr>
<td><em>Entamaeba histolytica</em></td>
<td>8(3.3)</td>
<td>13(5.7)</td>
<td>5(2.8)</td>
<td>26(4)</td>
<td>56(3)</td>
<td>82(3.3)</td>
</tr>
<tr>
<td><em>Giardia lamblia</em></td>
<td>25(10.2)</td>
<td>34(15)</td>
<td>33(18.8)</td>
<td>92(14.2)</td>
<td>189(10.1)</td>
<td>281(11.2)</td>
</tr>
<tr>
<td><em>Cryptosporidium spp.</em></td>
<td>37(15.1)</td>
<td>22(9.7)</td>
<td>12(6.8)</td>
<td>71(11)</td>
<td>87(4.6)</td>
<td>158(6.3)</td>
</tr>
</tbody>
</table>
New Frontiers in Diarrhoeal Diseases

Subclinical or Asymptomatic infections
The GEMS study showed that 72% of controls without diarrhoea also harbored one or more putative pathogens and 31% had two or more reflecting the fecally contaminated environment in which they live.
Case control study on diarrhoea in urban slums of Kolkata

Principal Investigators
Dr. Dipika Sur
Dr. T. Ramamurthy
Global Enteric Multi Center Study

Study site: Map of Kolkata Municipal Corporation Area
**STUDY OVERVIEW**

**Survey health centers for diarrhea cases**
- [CRF 02: Case Registration log]

**Enroll CASES** (8-9 cases every 2 weeks per age group: 0-11 mo., 12-23 mo., 24-59 mo.)
- Follow CASES until they are discharged from clinic or hospital
- Determine eligibility
  - [CRF 03: Case eligibility]
- Interview caretaker
  - [CRF 04A: Case enrollment, non-medical]
- Record medical information
  - [CRF 04B: Case enrollment, medical]
- Measure height, weight, mid-upper arm circumference
  - [CRF 04B: Case enrollment, medical]
- Collect a stool sample & send to lab
  - [CRF 11: Stool collection form]

**Enroll CONTROL, matched to case**
- Determine eligibility
  - [CRF 06: Control eligibility]
- Interview caretaker
  - [CRF 07: Control enrollment]
- Measure height, weight, mid-upper arm circumference
  - [CRF 07: Control enrollment]
- Collect a stool sample & send to lab
  - [CRF 11: Stool collection form]

**Parent completes memory aid describing the child’s stools for 14 days**

**If child dies during enrollment encounter**
- [CRF 10: Health Center Information]

**If child death is reported at 60 day follow-up visit**
- Inform demographic surveillance team
  - [Verbal autopsy CRF]

**60-day follow-up visit at child’s home**
- Interview caretaker; record required observations
  - [CRF 05: 60 day follow-up questionnaire for cases & controls]
- Measure height, weight, mid-upper arm circumference
  - [CRF 05: 60 day follow-up questionnaire for cases & controls]
- Review and collect memory aid
  - [CRF 09: Memory aid score sheet]

**Select potential controls from census**
- [CRF 08: Control Registration log]

**Yellow box indicates health center based activities**
**Blue box indicated community-based activities**
Community Diarrhoea in an urban slum in Kolkata

CASE [N=1408]

CONTROL [N=1790]
Role of probiotic in preventing acute diarrhoea in children: a community-based, randomized, double-blind placebo-controlled field trial in an urban slum

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\textsuperscript{4} Indian Council of Medical Research, New Delhi, India

(Accepted 29 June 2010; first published online 30 July 2010)
The plan of study

3758 children (1 to 5 years)

Double-blind Controlled Trial

Probiotic Group 1894 children

12/07/07

12 week

Probiotic drink (65 ml/day)

13/10/07

12 week

Follow up

5/01/08

Determine & compare incidence, duration, outcome and pathogens of acute diarrhea

Nutrient Group 1864 children

12 week

Nutrient (65 ml/day)

Follow up

Nutritional assessment (height, weight and mid-arm circumference) were done at three points (①, ② and ③, beginning, after 12 weeks, and at the end of follow-up)
Periodical analysis of fecal microbiota

Feces could be collected from 131 of the 200 subjects.

Study group (n=100)
- Probiotic drink (65 ml/day)

Control group (n=100)
- Nutrient drink (65 ml/day)

Follow up

- 0
- 6
- 12
- 18
- 24

Collection of fecal sample for analysis of microbiota, pH and organic acid.
Pathogens detected in the gut of apparently healthy children who participated in the probiotic trial.
Detection of *Vibrio cholerae/mimicus* in faeces collected from healthy children

<table>
<thead>
<tr>
<th>Detection frequency</th>
<th>Detection rate</th>
<th>(%)</th>
<th>Bacterial counts</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>42/133&lt;sup&gt;a&lt;/sup&gt;</td>
<td>31.6</td>
<td>4.2 ± 1.5&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>2</td>
<td>21/133</td>
<td>15.8</td>
<td>3.8 ± 1.0</td>
</tr>
<tr>
<td>3</td>
<td>6/133</td>
<td>4.5</td>
<td>4.5 ± 1.3</td>
</tr>
<tr>
<td>4</td>
<td>0/133</td>
<td>0.0</td>
<td>-</td>
</tr>
<tr>
<td>5</td>
<td>1/133</td>
<td>0.8</td>
<td>4.3 ± 0.9</td>
</tr>
<tr>
<td>Total</td>
<td>70/133</td>
<td>52.6</td>
<td>4.1 ± 1.3</td>
</tr>
<tr>
<td>Twice or more</td>
<td>28/133</td>
<td>21.1</td>
<td>4.0 ± 1.1</td>
</tr>
<tr>
<td>Twice or more in a row</td>
<td>17/133</td>
<td>12.8</td>
<td>4.2 ± 1.1</td>
</tr>
</tbody>
</table>

<sup>a</sup> No. of positive subjects/No. of subjects tested

<sup>b</sup> Mean ±SD, log<sub>10</sub> cells/g feces
Comparison of diarrheal and non-diarrheal stool

Proportional abundance of genera in non-diarrheal controls and MSD cases in different age categories

Each color represents a different group. The order and color for each group is the same for controls and MSD cases.

Mihai Pop et al., Genome Biology 2014, 15:R76
Subclinical (asymptomatic) infections
Environmental Enteric Dysfunction

• Mounting and diverse evidence suggests that subclinical infections with diarrhoea pathogens can cause physiological and structural alterations of the gut with diverse consequences on child nutrition and growth.

• Subclinical infections reduce nutrient absorption and impair growth by many of the same mechanisms present during clinical episodes.

• *Giardia intestinalis* causes diarrhoea with growth retardation in infants; often identified in the stools of asymptomatic children in endemic areas.
Because asymptomatic infections were twice as common as diarrhoea their ultimate effects might exceed those of clinical diarrhoea.
New Frontiers in Diarrhoeal Diseases

Diarrhoeal Diseases and Intestinal microbiota
A total of 4,788 specimens from 242 screened and phenotyped adults (129 males, 113 females) were used for this study, from a cohort of 300 individuals. Women were sampled at 18 body habitats, men at 15.
Gut Microbiota

- Bacteroidetes and Firmicutes make up around 90% of the gut microbiota
- Each individual harbors his/her own distinctive pattern of gut microbial communities
- For a given individual, the fecal microbiota remains remarkably stable over a person’s lifetime
Gut Microbiota - Functions

- Prevents colonization by pathogens
- “Educates the immune system”
- Metabolic role
  - Caloric salvage
  - Produces
- SCFA
- Vitamin K and folate
- Participates in drug metabolism
- Activates 5-ASA
- Deconjugates bile acids
Members of the human gut microbiota involved in recovery from *Vibrio cholerae* infection

Ansel Hsiao¹, A. M. Shamsir Ahmed²,₃, Sathish Subramanian¹, Nicholas W. Griffin¹, Lisa L. Drewry¹, William A. Petri Jr⁴,₅,₆, Rashidul Haque³, Tahmeed Ahmed³ & Jeffrey I. Gordon¹

Given the global burden of diarrhoeal diseases¹, it is important to understand how members of the gut microbiota affect the risk for, course of, and recovery from disease in children and adults. The acute, voluminous diarrhoea caused by *Vibrio cholerae* represents a dramatic example of enteropathogen invasion and gut microbial community disruption. Here we conduct a detailed time-series metagenomic study of faecal microbiota collected during the acute diarrhoeal and recovery phases of cholera in a cohort of Bangladeshi adults living in an area with a high burden of disease². We find that recovery is characterized by a pattern of accumulation of bacterial taxa that shows similarities to the pattern of assembly/maturation of the gut microbiota in healthy Bangladeshi children³. To define the underlying mechanisms, we introduce into gnotobiotic mice an artificial community composed of human gut bacterial species that directly correlate with recovery from cholera in adults and are indicative of normal microbiota maturation in healthy Bangladeshi children³. One of the species, *Ruminococcus obeum*, exhibits consistent increases in its relative abundance upon *V. cholerae* infection of the mice. Follow-up analyses, to D-Ph4. Every diarrhoeal stool was collected from every participant. Faecal samples were also collected every day for the first week after discharge (recovery phase 1, R-Ph1), weekly during the next 3 weeks (R-Ph2), and monthly for the next 2 months (R-Ph3). For each individual, we selected a subset of samples from D-Ph1 to D-Ph3 (Methods), plus all samples from D-Ph4 to R-Ph3, for analysis of bacterial composition by sequencing PCR amplicons generated from variable region 4 (V4) of the 16S ribosomal RNA (rRNA) gene (Supplementary Information, Extended Data Fig. 1a and Supplementary Table 3). Reads sharing 97% nucleotide sequence identity were grouped into operational taxonomic units (97%-identity OTUs; Methods).

We identified a total of 1,733 97%-identity OTUs assigned to 343 different species after filtering and rarefaction (Methods). *V. cholerae* dominated the microbiota of the seven patients with cholera during D-Ph1 (mean maximum relative abundance 55.6%), declining markedly within hours after initiation of oral rehydration therapy. The microbiota then became dominated by either an unidentified *Streptococcus* species (maximum relative abundance 56.2–98.6%) or by *Fusobacterium* species (19.4–
Bifidobacterium can repress V. cholerae virulence by modifying bile acids

Microbiota modify bile acids to inhibit T6SS-mediated killing of commensal bacteria. This interplay is novel interaction between commensal bacteria, host factors, and Vc.

Bachmann et al., 2015, PNTD
Variation of Microbiota across nutritional status

Gut Microbiomes of Indian Children of Varying Nutritional Status

Tarini Shankar Ghosh¹, Sourav Sen Gupta², Tanudeep Bhattacharya¹, Deepak Yadav¹, Anamitra Barik³, Abhijit Chowdhury³,⁴, Bhabatosh Das², Sharmila S. Mande¹*, G. Balakrish Nair²*
Our Research Questions

• What are pathogens doing in the gut of apparently healthy children and adults?
• Why are they present in low numbers?
• How does commensals restrain pathogen growth?
• Why are the pathogens not eliminated from the gut microbiota?
• Does the presence of pathogens induce inflammation and...
• What is the role of pathogens?
New Frontiers in Diarrhoeal Diseases

Polymicrobial Infections
Diarrhoea at the Infectious Diseases Hospital, Kolkata

November 2007 and October 2009

Patients Admitted (45,004)

Patient Enrolled
2,536 (5.6%)

Samples taken
2,519 (99.3%)

Samples not taken
17 (0.7%)

Sole Pathogen
1,080 (42.9%)

Mixed Pathogen
736 (29.2%)

No Pathogen
703 (27.9%)

2 pathogens
528 (71.7%)

3 pathogens
155 (21.1%)

4 pathogens
41 (5.6%)

5 pathogens
8 (1.1%)

6 pathogens
4 (0.5%)
What is the relationship between pathogens associated with mixed infections?
Testing Possible Associations

To test for possible associations, we used the Fisher Exact test to compare pairs of pathogens (1, both or neither) with an independent assortment based on the overall frequency with which pathogens were detected. To establish criteria for statistical significance, we calculated p values, odds ratios and 95% confidence intervals.
Odds ratios (ORs) showing odds of rotavirus co-occurring with various other pathogens.
Odds ratios (ORs) showing odds of *Vibrio cholerae* co-occurring with various other pathogens.
Polymicrobial infections associated with *Vibrio cholerae* and Rotavirus cases was non random
## Interventions for Diarrheal Diseases

<table>
<thead>
<tr>
<th>Category</th>
<th>Options</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Therapeutic</strong></td>
<td>• Oral rehydration solutions</td>
</tr>
<tr>
<td></td>
<td>• Antimicrobials for bloody diarrhea or dysentery</td>
</tr>
<tr>
<td></td>
<td>• Nutritional treatment of persistent diarrhea</td>
</tr>
<tr>
<td></td>
<td>• Zinc supplementation</td>
</tr>
<tr>
<td><strong>Preventive</strong></td>
<td>• Protected safe water</td>
</tr>
<tr>
<td></td>
<td>• Handwashing sanitary disposal of fecal waste</td>
</tr>
<tr>
<td></td>
<td>• Vaccines</td>
</tr>
<tr>
<td></td>
<td>• Improved nutrition, vitamin A and Zinc.</td>
</tr>
</tbody>
</table>
Vaccines against diarrheal diseases

• Rotavirus – Two vaccines – Merck and GSK are widely used

• A less expensive Indian manufactured vaccine named Rotavac has been pre qualified by WHO and is approved for use in India

• Cholera vaccine – Dukoral and Shancol
Summary

• The burden of DD in children under age five years in LIMCs reduced dramatically
• Although there are no magic bullets to control the incidence of DD the following are highly effective:
  – Improved nutrition of young children
  – Water and sanitation improvement
  – Hand washing and implementation of simple but highly effective interventions such as ORS
  – Appropriate use of antibiotics
  – The role and cause of EED
Thank you for your attention