Unbiased metagenomics for determining etiologies of idiopathic meningitis

Senjuti Saha, PhD
Child Health Research Foundation, Bangladesh
Johns Hopkins Bloomberg School of Public Health, USA

Meningitis Research Foundation Conference 2019
London, UK
06 Nov 2019
To Proma of Bangladesh

• 13-year old Proma was admitted with fever, vomiting, and headache
• She was eventually diagnosed with meningitis by *Mycobacterium tuberculosis*
• After almost 2 years, Proma remains bedridden with persistent neurocognitive impairment.
Child Health Research Foundation, Bangladesh

“to improve child health in Bangladesh and around the world by facilitating appropriate policy decisions through research and advocacy”
Hib vaccine prevents deaths of 3,100 infants every year in Bangladesh

Introduction of Hib vaccine 17 years after UK
Reason: lack of evidence
Meningitis surveillance in Bangladesh, 1993 - present

The objectives of our semi-national surveillance are:

- to describe epidemiology and estimate burden of disease
- to establish a platform to measure vaccine needs and impact
- to characterize circulating bacterial types – AMR, serotypes, etc
- to determine long-term outcome of meningitis patients

World Health Organization IB-VPD sentinel sites

Dhaka Shishu Hospital
665 beds

Mirzapur

Dhaka

Chittagong
Addition of diagnostic/surveillance methods to detect etiology

Spn: *Streptococcus pneumoniae*; Nmen: *Neisseria meningitidis*; 
Hib: *Haemophilus influenzae* type b; GBS: Group B streptococcus
Meningitis surveillance in Bangladesh

Hospitalized suspected meningitis cases

CSF collected

- Culture
  - +ve
  - -ve
- Cytology
  - >9 WBC
  - ≤9 WBC

Antigen test for Nmen, Spn, Hib, GBS

- +ve
- -ve

qPCR for Nmen, Spn, Hib, GBS

- +ve
- -ve
Etiology remains unknown for >50% of cases with ≥ 10 WBC/cmm (8,125 of 24,000, 2003 - 2016)

No difference in outcome of solved vs mystery cases
Lack of evidence hinders policy decisions

We hypothesized that RNA metagenomics could solve the mystery, but we had no evidence to show we can conduct sequencing on site.
Breaking the vicious cycle...

• The Gates Foundation introduced us to the Chan-Zuckerberg Biohub, who had the precise expertise on solving cases of mysterious meningitis and we had the samples!

• Four important considerations:
  • All SOPs need to be open access
  • Local scientist needs to be trained
  • All technologies/pipelines need to be transferrable and sustainable
  • We will own the data

Dr. Farhad Imam, Gates Foundation
CHRF, Bangladesh; Feb 15, 2018
Elucidating the causative agents of meningitis using metagenomics: Phase I

96 samples and a Senjuti were shipped to the Biohub
Sample selection for Phase I

Hospitalized suspected meningitis cases

CSF collected

Culture
- +ve
- -ve

Cytology
- >9 WBC
- ≤9 WBC

Antigen test for Nmen, Spn, Hib, GBS
- +ve
- -ve

qPCR for Nmen, Spn, Hib, GBS
- +ve
- -ve

Positive-control, n = 36
Idiopathic, n = 25
Negative-control, n = 35
RNA metagenomics of CSF specimens

Extraction → Library preparation → Sequencing → Analysis

CSF

RNA

seq1
ATTGCCGTAG.....

seq2
ATTGCCGTAG.....

seq2
ATTGCCGTAG.....

Human
Bacteria 1
Bacteria 2
Virus 1
Fungus 1
Bacteria 1

Child Health Research Foundation
Prevent Infections, Save Lives
**Analysis: IDSeq**

### Taxon Analysis

<table>
<thead>
<tr>
<th>Taxon</th>
<th>Score</th>
<th>zA</th>
<th>rPA</th>
<th>rA</th>
<th>contig A</th>
<th>contig sA</th>
<th>read A</th>
<th>L A</th>
<th>log(10) A</th>
<th>NT</th>
<th>NR</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Alphavirus (9 viral species)</strong></td>
<td>2</td>
<td>151,781,606</td>
<td>99.0</td>
<td>7,998.5</td>
<td>255,911</td>
<td>3</td>
<td>235,348</td>
<td>111,784.8</td>
<td>307</td>
<td>368</td>
<td></td>
</tr>
<tr>
<td>Chikungunya virus</td>
<td>1,485</td>
<td>100.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td><strong>Ro Negro virus</strong></td>
<td>1,188</td>
<td>100.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td><strong>Semliki Forest virus</strong></td>
<td>891</td>
<td>100.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td><strong>Oropouche virus</strong></td>
<td>594</td>
<td>100.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
</tr>
</tbody>
</table>

**Note:** The table above shows analysis results from the IDSeq platform, specifically focusing on antimicrobial resistance and taxonomic classification. The data includes various metrics such as score, zA, rPA, rA, and others, indicating the presence and abundance of different viral species in the sample analyzed. The results highlight the importance of understanding viral species diversity and their potential impact on human health.
We found a diverse range of pathogens

Saha et al, 2019, Biorxiv
Clinical follow-up of 10 resolved mystery cases

<table>
<thead>
<tr>
<th>Suspect etiology</th>
<th>Age of child (m) at diagnosis / follow-up</th>
<th>Hospital duration (days)</th>
<th>Treatment</th>
<th>Clinical outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mumps</td>
<td>156 / 168</td>
<td>10</td>
<td>Ceftriaxone</td>
<td>Parents complain of lower IQ</td>
</tr>
<tr>
<td>Mumps</td>
<td>18 / 30</td>
<td>9</td>
<td>Ceftriaxone</td>
<td>Healthy</td>
</tr>
<tr>
<td>Enterovirus B</td>
<td>7 / 12</td>
<td>6</td>
<td>Ceftriaxone</td>
<td>Cannot eat solid food</td>
</tr>
<tr>
<td><em>Bacillus cereus</em></td>
<td>0 / 5</td>
<td>28</td>
<td>Ceftriaxone+</td>
<td>Needed vp shunt</td>
</tr>
<tr>
<td><em>M. tuberculosis</em></td>
<td>160 / 164</td>
<td>15</td>
<td>Ceftriaxone+</td>
<td>Bed-ridden; unresponsive to all stimuli</td>
</tr>
<tr>
<td><em>S. maltophilia</em></td>
<td>4 / NA</td>
<td>28</td>
<td>Ceftriaxone+</td>
<td>Not known</td>
</tr>
<tr>
<td><em>Salmonella enterica</em></td>
<td>4 / 8</td>
<td>23</td>
<td>Ceftriaxone+</td>
<td>Cannot sit yet</td>
</tr>
<tr>
<td>Chikungunya</td>
<td>0 / 5</td>
<td>10</td>
<td>Ceftriaxone+</td>
<td>Healthy</td>
</tr>
<tr>
<td>Chikungunya</td>
<td>1 / 13</td>
<td>6</td>
<td>Ceftriaxone+</td>
<td>Healthy</td>
</tr>
<tr>
<td>Chikungunya</td>
<td>86 / 86</td>
<td>45</td>
<td>Ceftriaxone+</td>
<td>Died</td>
</tr>
</tbody>
</table>

Saha et al, 2019, Biorxiv
We optimized a quick qPCR method to test CSF for CHIKV

<table>
<thead>
<tr>
<th>Month 2017</th>
<th>Total CSF tested</th>
<th>CHIKV positive</th>
<th>% CHIKV positive</th>
</tr>
</thead>
<tbody>
<tr>
<td>January</td>
<td>31</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>February</td>
<td>23</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>March</td>
<td>41</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>April</td>
<td>36</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>May</td>
<td>56</td>
<td>3</td>
<td>5</td>
</tr>
<tr>
<td>June</td>
<td>50</td>
<td>9</td>
<td>18</td>
</tr>
<tr>
<td>July</td>
<td>42</td>
<td>5</td>
<td>12</td>
</tr>
<tr>
<td>August</td>
<td>48</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>September</td>
<td>40</td>
<td>1</td>
<td>3</td>
</tr>
<tr>
<td>October</td>
<td>38</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>November</td>
<td>42</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>December</td>
<td>31</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>478</strong></td>
<td><strong>20</strong></td>
<td><strong>4</strong></td>
</tr>
</tbody>
</table>

12% CHIKV-positive

Saha et al, 2019, Biorxiv
CHIKV meningitis cases overlapped with CHIKV fever outbreak

Number of CHIKV fever cases

Month of 2017

Fever

PCR positive meningitis

Saha et al, 2019, Biorxiv
Addition of diagnostic/surveillance methods to detect etiology

Spn: *Streptococcus pneumoniae*; Nmen: *Neisseria meningitidis*; Hib: *Haemophilus influenzae* type b; GBS: Group B streptococcus
Elucidating the causative agents of meningitis using metagenomics: Phase II

We were funded to get our own sequencer!
Phase II: Received a grant for an iSeq100 Global Sequencer
Phase II: Received a grant for an iSeq100 Global Sequencer
Addition of diagnostic/surveillance methods to detect etiology

1993
2000
2004
2008
2013
2018

Culture

Latex
Nmen
Spn
Hib
GBS (<2 m)

ICT/
BinaxNow
Spn

cPCR
Nmen
Spn
Hib
GBS (<2 m)

qPCR
Nmen
Spn
Hib
GBS (<2 m)

Spn: *Streptococcus pneumoniae*; Nmen: *Neisseria meningitidis*; Hib: *Haemophilus influenzae* type b; GBS: Group B streptococcus

mNGS!
Empowering, educating, engaging local scientists at the frontlines of public health
Thank you