Lethal invasive non-typhoidal *Salmonella* infections in young children in sub-Saharan Africa

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Baltimore, MD

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Dhaka, Bangladesh, March 1, 2013
Invasive bacterial infections among children < 5 years of age in sub-Saharan Africa

Blood-borne pathogens on the radar screen

- *Haemophilus influenzae* type b (Hib)
- *Streptococcus pneumoniae* (“pneumo”)
- *Neisseria meningitidis* (interest in Group A)

A blood-borne pathogen not on the radar

- Non-typhoidal *Salmonella* (“NTS”)
## Annual incidence of invasive pneumo & NTS disease in 3 African sites

<table>
<thead>
<tr>
<th>Site</th>
<th>Age group</th>
<th>Inc. inv. pneumo/10^5</th>
<th>Inc. inv. NTS/10^5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kilifi, Kenya (1998-02)</td>
<td>0-11 mos.</td>
<td>241</td>
<td>170</td>
</tr>
<tr>
<td>J Berkley et al, 2005</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(~7% HIV prevalence)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Basse, Gambia (2000-4)</td>
<td>2-5 mos.</td>
<td>363</td>
<td>408</td>
</tr>
<tr>
<td>G Enwere et al, 2006</td>
<td>6-11 mos.</td>
<td>576</td>
<td>360</td>
</tr>
<tr>
<td>(~1% HIV prevalence)</td>
<td>12-17 mos.</td>
<td>526</td>
<td>334</td>
</tr>
<tr>
<td>Manhica, Mozambique (2001-6)</td>
<td>0-11 mos.</td>
<td>403</td>
<td>388</td>
</tr>
<tr>
<td>B Sigauque et al, 2009</td>
<td>12-59 mos.</td>
<td>187</td>
<td>262</td>
</tr>
<tr>
<td>(~15% HIV prevalence)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Incidence of NTS sepsis disease during the controlled field trial of RTS,S malaria vaccine in 7 African countries

<table>
<thead>
<tr>
<th>Age group on enrollment</th>
<th>Salmonella sepsis (cases/10^3)</th>
<th>RTS,S malaria vaccine (N=5949)</th>
<th>Rabies vaccine (N=2974)</th>
</tr>
</thead>
<tbody>
<tr>
<td>5-17 mos.</td>
<td>6.9/10^3 /18 mos fu</td>
<td></td>
<td>7.7/10^3 /9 mos fu</td>
</tr>
<tr>
<td>6-12 wks.</td>
<td>3.7/10^3/9 mos fu</td>
<td></td>
<td>4.6/10^3/9 mos fu</td>
</tr>
</tbody>
</table>

RTS,S Clinical Trials Partnership. NEJM 2011; 365:1863-1875
Annualized incidence of NTS sepsis disease during the controlled field trial of RTS,S malaria vaccine in 7 African countries

<table>
<thead>
<tr>
<th>Age group</th>
<th>Salmonella sepsis (cases/10^5/12 mos of follow-up)</th>
<th>RTS,S malaria vaccine (N=5949)</th>
<th>Rabies vaccine (N=2974)</th>
</tr>
</thead>
<tbody>
<tr>
<td>5-17 mos.</td>
<td>460</td>
<td></td>
<td>770</td>
</tr>
<tr>
<td>6-12 wks.</td>
<td>493</td>
<td></td>
<td>613</td>
</tr>
</tbody>
</table>

RTS,S Clinical Trials Partnership. NEJM 2011; 365:1863-1875
Invasive NTS disease

Industrialized countries

- Invasive disease as a complication of gastroenteritis
- Severe invasive disease:
  - Infants < age 3 months
  - The elderly
  - Immunocompromised
- Incidence is increasing
- Typhimurium (ST19), Enteritidis, Heidelberg, Dublin, Schwarzengrund
- Animal reservoir
- Promiscuous host range
Clinical features among young children in sub-Saharan Africa with invasive NTS disease

- Most cases do not present with gastroenteritis, nor do they have a history of recent gastroenteritis!!

- Children with invasive NTS disease are clinically indistinguishable from young children with invasive pneumococcal infections
Clinical features of invasive NTS infections among children < age 2 years in Gambia

- ~ 90% non-focal – bacteremia/septicemia
- ~ 10% focal – meningitis, septic arthritis, etc.

<table>
<thead>
<tr>
<th>Symptom</th>
<th>Pneumo (N=74)</th>
<th>NTS (N=92)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Very sick</td>
<td>88%</td>
<td>79%</td>
</tr>
<tr>
<td>Diarrhea</td>
<td>16%</td>
<td>38%</td>
</tr>
<tr>
<td>Vomiting</td>
<td>58%</td>
<td>62%</td>
</tr>
<tr>
<td>Resp. sx</td>
<td>95%</td>
<td>67%</td>
</tr>
<tr>
<td>® Resp. rate</td>
<td>96%</td>
<td>77%</td>
</tr>
</tbody>
</table>
What non-typhoidal *Salmonella* (NTS) serovars are causing invasive NTS disease in sub-Saharan Africa?
Invasive NTS infection serovars among children < age 3 yrs in sub-Saharan Africa – early studies

- *Salmonella* Typhimurium
- *Salmonella* Enteritidis

These two serovars account for ~ 75-95% of the cases of invasive pediatric NTS disease in Kenya, Malawi, Gambia, Mozambique & Mali
Surveillance at l’Hôpital Gabriel Touré (HGT), Mali
Surveillance at l’Hôpital Gabriel Touré (HGT), Mali

- 71% of pediatric admissions had presumed infections (J Campbell, S Sow, et al 2004)
- 50% had a clinical dx of malaria
- 21% of admissions died in hospital
- Clinical Bacteriology Laboratory established by CVD in 2002
- Malian personnel were trained at CVD in Baltimore and locally in Mali in clinical microbiology, GCP & data management
- Blood cultures or body fluid cultures (e.g., CSF) systematically (7 days/week) obtained from hospital admissions with:
  - Age: 0-15 years
  - Fever: ≥39°C and/or
  - Clinical syndrome compatible with invasive bacterial disease (e.g., sepsis, meningitis, septic arthritis, etc.)
Hospital admissions and disposition

- July 2002 – June 2012
- Children < 15 years of age admitted to l’Hôpital Gabriel Touré

Admissions
N = 41,478

Eligible for Bld Cx
N = 24,472 (59%)

Not Eligible
N = 17,006 (41%)

Enrolled
N = 23,151 (94.6%)

Cx (+) w/pathogen
N = 4,606 (19.9%)

Cx (-) for pathogen
N = 18,545 (80.1%)

Non-Typhoidal Salmonella
N = 566 (12.3%)

Typhi + Paratyphi A
N = 124 (2.7%)

S. pneumoniae
N = 1399 (30.4%)

Other Pathogen
N = 2517 (54.6%)
Typhimurium and monophasic variants (I 4,[5],12:i:-) are mostly ST 313.
Total NTS and Typhimurium*/Enteritidis cases by age groups

- 80% of all cases
- 74% of cases in children < age 5 yrs

* Includes Typhimurium and monophasic variants

M Tapia et al 2012
Total NTS and Typhimurium*/Enteritidis cases < 60 months of age

* Includes Typhimurium and monophasic variants

M Tapia et al 2012
Total NTS and S. Typhimurium* & S. Enteritidis cases < 12 months of age

* Includes Typhimurium and monophasic variants

M Tapia et al 2012
Clinical presentation of hospitalized NTS cases, 7/02 - 6/12, Mali

<table>
<thead>
<tr>
<th></th>
<th>Bacteremia/Septicemia</th>
<th>Meningitis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of cases</td>
<td>509 (90%)</td>
<td>47* (8.3%)</td>
</tr>
<tr>
<td>% of cases due to Typhimurium or Enteritidis</td>
<td>74.7%</td>
<td>83%</td>
</tr>
<tr>
<td>Median age</td>
<td>23 months</td>
<td>11 months</td>
</tr>
<tr>
<td>Case Fatality Rate (CFR)</td>
<td>22%</td>
<td>23%**</td>
</tr>
</tbody>
</table>

- 1 case also had (+) culture of soft tissue
- ** 7 of 10 deaths were < 12 months of age
- Remaining cases included 1 septic arthritis, 1 peritonitis & 8 soft tissue infections.
- Total = 566 cases
## Case fatality rate (CFR) by serovar of hospitalized cases of invasive NTS, Mali, 7/02 - 6/12

<table>
<thead>
<tr>
<th>Serovar</th>
<th>Cases</th>
<th>CFR</th>
</tr>
</thead>
<tbody>
<tr>
<td>Typhimurium</td>
<td>202</td>
<td>16%*</td>
</tr>
<tr>
<td>Enteritidis</td>
<td>182</td>
<td>28%*</td>
</tr>
<tr>
<td>I 4,5,12:i:-</td>
<td>41</td>
<td>19%</td>
</tr>
<tr>
<td>Dublin</td>
<td>68</td>
<td>19%</td>
</tr>
<tr>
<td>Stanleyville</td>
<td>20</td>
<td>20%</td>
</tr>
<tr>
<td>Group C</td>
<td>20</td>
<td>13%</td>
</tr>
<tr>
<td>All others</td>
<td>33</td>
<td>41%</td>
</tr>
<tr>
<td><strong>TOTAL</strong></td>
<td>566</td>
<td>22%</td>
</tr>
</tbody>
</table>

* p=0.005

M Tapia et al 2012
## Case fatality rate by age among invasive NTS inpatients, Mali, 7/02-6/12

<table>
<thead>
<tr>
<th>Age (mos.)</th>
<th>Typhimurium*</th>
<th>Enteritidis</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Cases</td>
<td>CFR</td>
</tr>
<tr>
<td>0-11</td>
<td>52</td>
<td>19%</td>
</tr>
<tr>
<td>12-23</td>
<td>66</td>
<td>14%</td>
</tr>
<tr>
<td>24-35</td>
<td>28</td>
<td>22%</td>
</tr>
<tr>
<td>36-47</td>
<td>25</td>
<td>17%</td>
</tr>
<tr>
<td>48-59</td>
<td>15</td>
<td>17%</td>
</tr>
<tr>
<td>&lt; 60</td>
<td>186</td>
<td>17%</td>
</tr>
</tbody>
</table>

* Includes S. Typhimurium and monophasic variants

M Tapia et al 2012
# Antibiotic resistance of NTS isolates

<table>
<thead>
<tr>
<th>Serovar</th>
<th>N</th>
<th>Amp</th>
<th>Chlor</th>
<th>Ceftr</th>
<th>TMP/SMZ</th>
</tr>
</thead>
<tbody>
<tr>
<td>Typhimurium</td>
<td>191</td>
<td>96%</td>
<td>100%</td>
<td>5%</td>
<td>94%</td>
</tr>
<tr>
<td>Enteritidis</td>
<td>70</td>
<td>97%</td>
<td>39%</td>
<td>17%</td>
<td>61%</td>
</tr>
<tr>
<td>Dublin</td>
<td>54</td>
<td>4%</td>
<td>0%</td>
<td>2%</td>
<td>4%</td>
</tr>
<tr>
<td>Stanleyville</td>
<td>28</td>
<td>0%</td>
<td>0%</td>
<td>0%</td>
<td>0%</td>
</tr>
</tbody>
</table>
Severe invasive NTS disease in Africa

Host risk factors

• Reticuloendothelial system blockade
  – Severe anemia of malaria
  – Hemolytic anemias (e.g., sickle cell hemoglobinopathy, etc.)
• HIV (where prevalent)
• Malnutrition
Seasonality - NTS cases, Mali, 7/02 – 6/12

Blue - Cool dry months
Red - Hot months
Green - Rainy months

54% of cases
Year-to-year variability of invasive NTS infections & malaria smear positivity (blue), Mali, 7/02 – 6/12
Year to year variability of serovar composition, Mali, 7/02 – 6/12

* Includes Typhimurium and monophasic variants
Are the S. Typhimurium and S. Enteritidis isolates from pediatric invasive NTS patients distinctive?
Invasive MDR NTS isolates from Malawi & Kenya are mainly of an unusual MLST type (ST313)

Malawian strain D23580 sequenced by R Kingsley & G Dougan at the Sanger Institute, UK

Compared to genome sequences of “classic” Typhimurium strain LT2 & Typhi, D23580 showed:

- Genome degradation & convergence- Typhi-like
  - 34 pseudogenes not in LT2
  - 17 kb deleted; total coding loss of 61 genes
  - 32 of 61 lost genes are also degraded in Typhi or Paratyphi A

R Kingsley et al 2009
Malian S. Typhimurium and monophasic mutants are also ST313 clade & closely resemble the Malawi prototype
Can we make a safe, effective & affordable vaccine to prevent invasive NTS disease among infants & toddlers in Africa?

Lessons learned from successful typhoid vaccines serve as a guide
Immune responses likely to mediate protection against NTS & strategies to elicit the responses

**SIgA mucosal ABs**
Prevent invasion from gut

**Serum antibodies**
*Targets for antibodies:*
OPS; flagella; core PS, OMP?

*Biological activity of ABs:*
Opsonophagocytic killing -
Bactericidal - complement-mediated killing

**Cell-mediated immunity**
Eliminates intracellular bugs

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**Best for eliciting gut SIgA**
Live oral vaccine

**Best for serum IgG ABs**
Parenteral conjugate or protein vaccines

**Best for stimulating CMI**
Live oral vaccines
Clinical development of the bivalent (S. Typhimurium/S. Enteritidis) conjugate NTS vaccine: a public-private partnership

- **CVD, University of Maryland, Baltimore**
  - Vaccine design, preclinical, process development, clinical trials, project coordination

- **Wellcome Trust, London UK**
  - Funding (Strategic Translation Award), advocacy

- **Bharat Biotech, Hyderabad, India**
  - GMP pilot lots of the 2 monovalent conjugates & the bivalent conjugate; commercial manufacture post-licensure
Deploying a vaccine against invasive NTS

**Target age by disease burden**
- Children < 36 months of age

**Target age population by practicality of vaccine delivery**
- Expanded Program on Immunization (EPI)
  - ~ Age 6, 10 and 14 weeks in most of sub-Saharan Africa
  - NTS vaccine must be compatible with other EPI vaccines

EPI Unit in Health Center, Kangaba, Mali
Impact of Hib vaccine introduction on invasive Hib disease in infants, Bamako, Mali

- **36-month Baseline Period**, 7-02 to 6-05
- **12-month Transition Period**, 7-05 to 6-06
- **23-month Intervention Period**, 7-06 to 5-08

Invasive Hib cases/10^5 infants per 6-month intervals

88% reduction

S Sow et al, AJTMH 2009
Sub-Saharan Africa

<table>
<thead>
<tr>
<th>Countries and territories</th>
<th>U5MR</th>
<th>U5MR rank</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sierra Leone</td>
<td>185</td>
<td>1</td>
</tr>
<tr>
<td>Somalia</td>
<td>180</td>
<td>2</td>
</tr>
<tr>
<td>Mali</td>
<td>176</td>
<td>3</td>
</tr>
<tr>
<td>Chad</td>
<td>169</td>
<td>4</td>
</tr>
<tr>
<td>Democratic Republic of the Congo</td>
<td>168</td>
<td>5</td>
</tr>
<tr>
<td>Central African Republic</td>
<td>164</td>
<td>6</td>
</tr>
<tr>
<td>Guinea-Bissau</td>
<td>161</td>
<td>7</td>
</tr>
<tr>
<td>Angola</td>
<td>158</td>
<td>8</td>
</tr>
<tr>
<td>Burkina Faso</td>
<td>146</td>
<td>9</td>
</tr>
<tr>
<td>Burundi</td>
<td>139</td>
<td>10</td>
</tr>
<tr>
<td>Cameroon</td>
<td>127</td>
<td>11</td>
</tr>
<tr>
<td>Guinea</td>
<td>126</td>
<td>12</td>
</tr>
<tr>
<td>Niger</td>
<td>125</td>
<td>13</td>
</tr>
<tr>
<td>Nigeria</td>
<td>124</td>
<td>14</td>
</tr>
<tr>
<td>South Sudan</td>
<td>121</td>
<td>15</td>
</tr>
</tbody>
</table>

Very-high-mortality countries (U5MR≥100)

- Pneumonia 18%
- Diarrhoea 12%
- Malaria 17%
- Neonatal* 25%
- Other 25%
- AIDS 3%

(*) For neonatal death, mortality rate attributed to birth complications.
Cases & deaths from bacterial invasive infections in children < 5 yrs of age in sub-Saharan Africa

Above the water – the cases & deaths that we detect among children seen at hospitals & health centers

Below the water – the cases & deaths among children in the community who do not access health care facilities
**Invasive NTS disease**

**Industrialized countries**
- Invasive disease as a complication of gastroenteritis.
- Severe invasive disease:
  - Infants < age 3 months
  - The elderly
  - Immunocompromised
- Incidence is increasing
- Typhimurium (ST19), Enteritidis, Heidelberg, Dublin, Schwarzengrund
- Animal reservoir

**Sub-Saharan Africa**
- Serendipitous discovery
- Children < age 3 years
- Most do not present with gastroenteritis
- High case fatality > 20%
- Novel strains:
  - ST313 (by MLST)
  - Genomic degradation
- 75-95% of invasive NTS
  - S. Typhimurium (including variants), S. Enteritidis
Field epidemiology & clinical research colleagues
Samba Sow, Milagritos Tapia, Karen Kotloff, James Campbell

Laboratory research colleagues
James Galen, Sharon Tennant, Rafi Simon, Marcelo Sztein, Marcela Pasetti, Souleymane Diallo, Sofie Livio, James Nataro, Haim Levy, Jin Wang, Boubou Tamboura, Andrew Lees, Patrick Murray, Mary Boyd

Collaborators:
Gordon Dougan, Robert Kingsley, Sanger Institute, UK
Patricia Fields & Matthew Mikoleit, CDC
Laura Martin & Calman MacLennan, NVGH
Thank you for your attention