The next steps in typhoid diagnostics
(from a personal standpoint)

Stephen Baker 01/02/13
Searching for the elusive typhoid diagnostic

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Abstract
Typhoid (enteric) fever is still a common disease in many developing countries but current diagnostic tests are inadequate. Studies on pathogenesis and genomics have provided new insight into the organisms that cause enteric fever. Better understanding of the are limited in their diagnostic information. Here we discuss the current position of and suggest potential ways of advancing.

The utility of diagnostic tests for enteric fever in endemic locations

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Enteric fever, an infection caused by Salmonella enterica serovar Typhi and serovar Paratyphi A, is common and endemic in many areas of the Asian and African continents. In endemic areas, diagnostic tests are needed to diagnose acute cases for clinical management, to detect convalescent and chronic fecal carriage and for contact tracing. A suitable test may also allow an assessment of disease burden in a community to determine the need for vaccination programs.
The Salmonella Typhi genome project
**Salmonella Typhi Genome sequencing project**

- **Initiated in 1998 by the Sanger Centre**
  - 18 months to sequence and assemble
  - 10 months to analyze
  - Estimated cost of 600,000 GBP
  - Published in October 2001
  - Improvements in sequencing technology

- **Heralded as a landmark in studying typhoid fever**
  - Opportunity for new insight
  - Vaccines
  - Treatment
  - Diagnostics

- **These are yet to materialise – but are coming slowly**
Typhoid is a disease that can be eliminated

- This is tangible target
- Should we invest in and improve
  - Diagnostics?
  - Treatment?
  - Vaccination?
  - Education?
  - Sanitation?
- How do we push research findings into policy?
- What are the long term perspectives for typhoid?
- Are there other infectious diseases that we benefit from particular approaches?
- Can we use current knowledge to reach local elimination?
- Can we apply genomics to tackle elimination?
Why do we need new Diagnostics?

- Current diagnostic tests are poor
- Since the genome sequence new diagnostics have entered the market place
- Yet the technology is pre-genome and rely on non-specific markers

- Important for patient and clinician
- Important for assessing burden and effect of intervention
- If elimination is a realistic target we have to calculate the nature of the problem

- Clinical, microbiological, serological, molecular
- Different advantages / disadvantages
- Practicality, sensitivity, specificity, time, cost
- No agreement on standard
- Confusion on interpretation of data and methods
Can we do better than this?
Blood culture is a gold standard......

Result of Blood Culture from Patients with Clinically Diagnosed Enteric Fever

- No Growth: 712 (57.7%)
- Salmonella Typhi: 381 (42.3%)
- Salmonella Paratyphi A: 142
Median 1 CFU/mL

Higher counts in children

Counts decline with illness duration

Obvious effects of antimicrobials

FIG. 1. Distribution of blood bacterial counts in acute typhoid fever.

George Fernand Widal 1862-1929
The Widal

- However………
  - Unspecific
    - Due to the nature of the bacterial antigens
    - Difficult to know if the patient is in the acute phase
    - Patients rarely demonstrate a 4 fold increase
    - Often no increase or only 2 fold increase
    - Not rapid (takes 14 days)
    - Not standardized
    - Negative in 30% of cultured confirmed cases
    - Often done in a single tube
    - Lack of seroprevalence data

- Still commonly performed
  - Fraught with issues
  - Lacks sensitivity and specificity
Rapid diagnostic kits

- A number of rapid serological tests are available
- Range in cost and specificity
- Different performance
The sensitivity of real-time PCR amplification targeting invasive Salmonella serovars in blood (BMC infect dis 2010, 10:125)

- 100 blood samples culture positive for S. Typhi (54) or S. Paratyphi A (46)
- 23/54 Typhi positive samples also positive by RT-PCR
- 18/46 Paratyphi A samples also positive by RT-PCR
- Sensitivity 42 % and 39 %
- Specificity 100 %

- 50 blood samples from patients with ‘clinical’ enteric fever – all negative
- 28 bone marrow samples positive for S. Typhi – 28 (100 %) positive
- Final sample volume in PCR 75 µL
- For 100 % need to extract DNA from 5 – 10 mls
Summary of current methods

- **Clinical**
  - Cheap, essential for treatment, further testing, rapid
  - Un-reliable without confirmation
  - Limited use for reporting

- **Serological**
  - Varying cost, useful when combined with other tests, rapid
  - High % of false negatives in endemic areas
  - Some use in reporting, lack of standardization
  - Rapid tests ….. Same issues

- **Molecular**
  - Can be cheap, not very useful in resource poor setting
  - Results appear good, but un-physiological bacterial loads
  - No use in reporting

- **Microbiological**
  - Blood or Bone marrow +/- Stool sample
  - Essential for reporting and burden assessment
Transcriptional response in the peripheral blood of patients infected with *Salmonella enterica* serovar Typhi


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We used microarrays and transcriptional profiling of peripheral blood to investigate the host response of 29 individuals who contracted typhoid fever in the Mekong Delta region of Vietnam. Samples were taken over a nine month period encompassing acute disease, convalescence, and recovery. We found that typhoid fever induced a distinct and highly reproducible signature in the peripheral blood that changed during treatment and convalescence, returning in the majority of cases to the "normal" profile as measured in healthy uninfected controls. Unexpectedly, there was a strong, distinct signature of convalescence present at day 9 after infection that remained virtually unchanged one month after acute infection and in some cases persisted as long as nine months despite a complete clinical recovery in all patients. Patients who retain the convalescent signature may be genetically or temporarily incapable of developing an effective immune response and may be more susceptible to reinfection, relapse, or the establishment of a carrier state.

The challenges

- The gap between academia and endemic locations
- Raising the Typhoid profile
- The difficulty of detecting the organism
- Diagnosing other febrile diseases
- Persuading groups to be involved
- Ensuring accuracy
- Validating tests
- Implementation
- Finances
- The people in this room working together!!
The technology

- Kit/assay development
- Rapid diagnosis with a simple yes/no test
- Serological
- Proteomics
- Biochemistry
- Metabolomics
- Mass spectrometry
- Molecular biology
Ongoing research….. And a way forward

- Typhoid diagnostics consortium
- A universal biobank
- Biomarkers
  - IVIAT
  - ALS
  - Metabolites
  - Enrichment PCR
  - LAMP-PCR
  - Immunochip screening

- We are at the point when some of these methods should have more in depth field testing
- Get a second opinion!
- Clear difference between control and Typhi.
- $p = 9.8 \times 10^{-12}$
GC-TOFMS analysis – Classification model; control vs Typhi
- Difference can be found between Typhi and Para A.
- $p = 0.048$
Serodominant IgM Antigens: uninfected vs acutely infected typhoid patients in Vietnam
Microarray ROC on normalized data for IgG antigens:
Uninfected vs infected typhoid patients in Vietnam

Microarray multiple IgG Antigens ROC

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<th>Sensitivity</th>
<th>Specificity</th>
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<tr>
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<td>85%</td>
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<tr>
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