Development and Challenges faced in Optimization of Sensitive Diagnostic Tests for Typhoid fever

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Disease burden of enteric fever in Bangladesh

Studies in urban slums in Dhaka indicate that the incidence of typhoid fever for all age groups is 3.9 episodes/1000 person-years (Brooks et al., 2005).

A significantly higher proportion of cases were found in patients younger than 5 years of age. The highest rate of isolation was in the second year of life, among them >70% of isolates were from children aged 9 to 24 months (Saha et al., 2001).

The incidence of typhoid fever was shown to be 2.0 episodes/1000 person-years, with a higher incidence in children aged < 5 years (10.5/1000 person-years) than in older persons (0.9/1000 person-years) [Naheed et al., 2010].
Existing methods for diagnosis of enteric fever
For measurement of true disease burden, sensitive diagnostic methods play a crucial role and the timing of the testing is also important.

Blood or Bone marrow culture, PCR - Week 1
Serological assays - Week 2
Stool antigen - Week 2
Urine - Week 4

Testing for typhoid fever is not always sought early on and often antibiotics are taken before diagnosis is carried out. There are limitations to existing methods of diagnosis.
Blood culture

Blood culture is ~40 to 60% sensitive. Various factors contribute to low sensitivity.

Bacteria mostly remain intracellular (median - 0.5 CFU/ml blood); large volume of blood is a critical factor.

The efficacy of blood culture decreases with the duration of illness.

Use of antibiotics reduces the sensitivity of blood culture.

Requires at least 3-5 days for diagnostic results.

Real Time Multiplex PCR has been used and being optimized for detection of *S. Typhi* in blood

Tennant et al. 2015
Stephane Pouzal et al. presentation at CAT on 4th April
Culture plus PCR in Typhoid challenge models

Blood culture-PCR to optimise typhoid fever diagnosis after controlled human infection identifies frequent asymptomatic cases and evidence of primary bacteraemia

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Commercially available diagnostic kits (Tubex, Typhidot).

None of these assays have reached widespread use because of lack of sensitivity and specificity.

The clinical value of Tubex™ and Typhidot® rapid diagnostic tests for typhoid fever in an urban community clinic in Bangladesh was low.
What are the attributes for a suitable test for diagnosis and for determining burden of enteric fever?

i. Can be tested using low volumes of blood (1-2 ml) for use in infants and young children

ii. Broad window of testing time after onset of disease

iv. Non-interference by antibiotic use prior to testing

v. No effect of pre-existing antibodies as in endemic areas to S. Typhi/S. Paratyphi

vi. Relatively rapid so as to be useful for patient treatment (~ 12-24 hours)

vii. Easily adaptable to laboratories in developing country settings

viii. Key antigens incorporated for specific diagnostic analysis
Use of antibodies in lymphocyte secretions as a measure of recent exposure as opposed to plasma/serum for diagnosis of enteric fever.

B cells activated in the mucosa migrate via peripheral blood back to the mucosa.

Antigen specific Antibody response due to a recent exposure.

Results within 14-36 hrs of processing.

TPTEST

Exposure to pathogen in the gut.

ELISA

ALS
Antibodies in Lymphocyte Supernatants

Supernatant

Density gradient centrifugation

PBMCs
(Peripheral Blood Mononuclear Cells)
Assessment of the lymphocyte secreted IgA antibodies to diagnose Typhoid fever

Sensitivity of 100% and Specificity of 78-97% for diagnosis of typhoid and paratyphoid fever
Response in lymphocyte secretion from bacteremic young children

Using low volumes of blood - 1 ml
Bayesian Latent class modeling was used for comparing TPTTest with other diagnostic tests.

Table 3. Estimated sensitivity and specificity of six diagnostic tests for enteric fever in 92 febrile patients (95% confidence intervals shown in parenthesis), under a Bayesian latent class modeling approach.

<table>
<thead>
<tr>
<th>Test</th>
<th>Sensitivity</th>
<th>Specificity</th>
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<tbody>
<tr>
<td>Culture</td>
<td>51.8% (41.2–62.9%)</td>
<td>100%</td>
</tr>
<tr>
<td>TPTTest</td>
<td>96.0% (87.1%-99.8%)</td>
<td>96.6% (90.7%-99.2%)</td>
</tr>
<tr>
<td>Tubex</td>
<td>60.2% (49.3%-71.2%)</td>
<td>89.9% (79.6%-96.8%)</td>
</tr>
<tr>
<td>Typhidot</td>
<td>59.6% (50.1%-69.3%)</td>
<td>80.0% (67.7%-89.7%)</td>
</tr>
<tr>
<td>Widal ≥ 1:320</td>
<td>14.9% (7.6%-24.7%)</td>
<td>86.3% (75.7%-93.6%)</td>
</tr>
<tr>
<td>Widal 4 fold titer rise</td>
<td>12.6% (6.2%-22.0%)</td>
<td>100.0% (99.9%-100.0%)</td>
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</table>
Enteric fever surveillance is being carried out using TPTEST in field sites in Bangladesh.
Identification of novel antigens

Using a high throughput immunoscreening technique- SCOTS, IVIAT, microarray techniques- subsets of immunogenic S.Typhi, S. Paratyphi A proteins have been identified using sera or ALS from patients as probes

35 proteins in S.Typhi and 20 proteins in S. Paratyphi A infections

Immunoproteomic analysis and Mass Spectrometry- 57 proteins detected

Important antigens undergoing evaluation include- LPS, HlyE, YncE, and CdtB and others as single antigen or combined testing

Richelle Charles, Ed Ryan et al. ongoing studies
Characterization of anti- YncE (STY1479) immune responses

YncE and other antigens may lead to development of an improved diagnostic assay and better understanding of the survival of S. Typhi within the biliary tract of carriers.
Conversion of TPTEST to a lateral flow device platform
Sensitivity of 98% compared to blood culture results and a specificity that ranged from 78 to 100% depending on the definition of a true negative.
Diagnostic Kit for Typhoid Fever - Typhokit for use in diagnostic facilities around Bangladesh and challenges

Changes made from TPTEST to TYPHOKIT
1. RBC lysis instead of Ficoll
2. Incubator without CO2
3. Tubes instead of cell culture plates
4. Modification of antigen
Typhokit is being tested in Different Diagnostic Laboratories

Instruments are used for making the kits

FURTHER OPTIMIZATION ONGOING BASED ON FEEDBACK FROM DIAGNOSTIC LABORATORIES IN BANGLADESH
Conclusions

• The TPTest shows good sensitivity and specificity and can be used as a diagnostic tool for complementing blood culture tests/ qPCR
• New antigens need to be evaluated for incorporation in the diagnostic assays
• Large scale production and wider field testing of Typhokit will be needed
• These methodologies can be used as a useful tool for evaluation of burden of disease as well as for testing effectiveness of TCVs in the near future
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