Method optimisation for detection of *Salmonella* Typhi from the environment

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Malawi background & rationale

Malawi is an typhoid endemic country
- Outbreak of unknown origin in Blantyre, Malawi started 2012
- >16,000 cases per year, ~200 deaths associated with typhoid

Methods Development Project
Primary aim: culture & detect S. Typhi from the environment

Figure 1: Feasey et al., 2015, Monthly trends in bloodstream invasive Salmonella diagnosed at QECH from November 2010-October 2014.
Isolation of *Salmonella* Typhi from the environment is difficult but not impossible

Sampling from sewerage proved effective

- **≤1950s**
  - Moore’s swabs
  - Specific required culture methods

- **1980s**
  - Reinforced the use of Moore’s swabs
  - Selenite-F is the most effective for *S. Typhi*

- **Viable but non-culturable (VBNC)**

New approaches use molecular methods

- Strongly associated with water & indirect transmission in endemic regions

Culture remains important

*Figure 1: Electron micrograph of a salmonella taken by Matthew Hannah, PHE*
Twenty isolates from the PHE culture collection were used

Isolates from different regions selected for laboratory testing

The use of a novel chromogenic agar, mCASE was selected for the isolation of salmonella

Figure 2: Global distribution of strains used in optimisation & evaluation of environmental isolation (Satheesh Nair, PHE)

Figure 3: Pure S. Typhi on mCASE
Methods for the isolation of *Salmonella* Typhi were assessed

![Diagram showing the steps for isolation]

- **Pathways were narrowed to four options from original 15**
  - Selenite cysteine broth was preferred due to its selectivity but does not remove *S. Typhi* like alternatives (e.g. Rappaport Vassiliadis broth)
  - Bile broth: infection starts post exposure to bile
Too Many Plates

Preliminary working at PHE Food, Water & Environmental Laboratory

- Hundreds of plates a week processed
  - Under laboratory conditions with control strains, S. Typhi was reliably retrieved
  - Environmental testing required

Photo Credit: https://www.flickr.com/photos/erikaleef
Following narrowing of the pathways, challenges set up with blind, mixed cultures

Challenge Organisms
- *Salmonella* Nottingham – same colour as *S. Typhi* on mCASE
- *Bacillus cereus* – similar colour & overgrows
- Fungi - blind cultures grew relatively well in the broths

Immuno-magnetic bead separation ran in parallel
- Pan-*Salmonella* bead, developed by Ezzeddine Elmerhebi, Neogen LabM
- Further work required *in situ* as artificial mixtures showed no major difference in recovery rates

*Figure 5: Mixed culture on mCASE, where S. Typhi was successfully selected*
Molecular approaches utilised to reinforce culture methods developed

• The main aim of the project is to develop a method for culturing environmental *S. Typhi*

• Molecular methods are more cost effective & high-throughput

• Quantitative PCR also allows for screening of incoming samples
  - Inhibitors such as environmental, chemical & faecal contaminants

*Figure 6: Pathway emphasising confirmation step*

Note: Current DNA-based molecular methods cannot prove viability
Novel assay from PHE used for rapid diagnostics

Satheesh Nair, PHE, designed an assay for diagnostics
- The original assay included primer’s for Paratyphi A, B & C
- This project aims to multiplex the S. Typhi targets
- Multiplexed assay is currently being optimised
- Probes changed from original to minimise interference in multiplex

Table 1: Primer sequences of PHE assay and source publications

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High-Resolution Melt (HRM) PCR

The multiplex assay is being adapted to HRM

- Thomas Edwards, LSTM, consulted in assay conversion
  - Results consistent with S. Typhi & non-typhoidal salmonella control strains
  - Further optimisation & design changes

*Figure 7: HRM melt curves for positive Salmonella Typhi*
Role of the assay

As a tool for screening & confirmation, the blind cultures were processed with both the probe based q-PCR & the HRM PCR

HRM: <£0.40 (<$0.5)
Probe: <£0.50 (<$0.6)
Moving forwards, work to be done in Malawi

Final Culture Pathways

- Limit of detection (LOD) & limit of quantification (LOQ) work still to be performed
- To finalise culture pathways based on further evaluation in Malawi
Moving forwards, work to be done in Malawi

Sampling strategy – in collaboration with Jillian Gauld, University of Lancaster
- Pilot Study
- Challenges
  - Road conditions & water access
  - Logistics

Methodology
- Continued optimisation
- Immuno-magnetic separation & microfluidics
- Adapt to new challenges from environmental samples

*Figure 10: Cumulative map of Typhoid cases with highlighted sampling environmental areas*
Figure 11: Examples of water access on the Likhubula River
Figure 12: Examples of water access on the Likhubula River and walkway through village
Figure 13: Examples of water access on the Lunzu River
Thuchila River

Figure 14: Examples of water access on the Thuchila River
knowledgements

**Supervisors:**
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Dr Ezzeddine Elmerhebi, Neogen LabM
Dr Hywel Morgan, University of Southampton
Dr Marie Chattaway, PHE