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Multiplex PCR Assay to Detect High Risk Lineages of Salmonella Typhi


*Department of Medicine, University of Cambridge, UK

Introduction

- Typhoid fever infections remain a significant public health issue with up to 21 million infections and 161,000 deaths per year
- Current methods of ID require culture and sequencing to determine the specific lineage
- H58 (4.3.1) is the main circulating lineage of S. Typhi in many South Asian countries and associated with high levels of antibiotic resistance
- XDR Typhi strains (4.3.1.1.P1) are additionally resistant to ceftriaxone, further limiting antibiotic treatment options
- There is an increased need for rapid molecular tests to identify and track these high-risk lineages for treatment decisions, surveillance and vaccine prioritisation

Methods

- **Figure 1.** Our SNP-based multiplex PCR assay detects Salmonella species down to lineage level:

<table>
<thead>
<tr>
<th>Subspecies</th>
<th>Salmonella enterica subsp. enterica</th>
<th>Serovar</th>
</tr>
</thead>
<tbody>
<tr>
<td>S. Typhi</td>
<td>S. Paratyphi A</td>
<td></td>
</tr>
<tr>
<td>Non-XDR/Non-H58</td>
<td>4.3.1.1.P1 (XDR)</td>
<td>Low risk</td>
</tr>
<tr>
<td>H58</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

- Building on previous work identifying SNPs specific for particular lineages
- STY0307 and SSPA0850 conserved genes used for S. Typhi and S. Paratyphi A targets
- For H58/XDR lineages, primers designed with SNP plus additional mutations to confer specificity to target

Results

- **Figure 2.** Pairwise alignment of CT18 reference against H58 (A) and XDR (B):

<table>
<thead>
<tr>
<th></th>
<th>A</th>
<th>2,348,880</th>
<th>2,348,888</th>
<th>2,348,902</th>
<th>2,348,914</th>
</tr>
</thead>
<tbody>
<tr>
<td>S. Typhi CT18</td>
<td>ACGGGGTTAACGCGGGG</td>
<td>ACGGGGTTAACGCGGGG</td>
<td>ACGGGGTTAACGCGGGG</td>
<td>ACGGGGTTAACGCGGGG</td>
<td></td>
</tr>
<tr>
<td>H58 509F</td>
<td>ACGGGGTTAACGCGGGG</td>
<td>ACGGGGTTAACGCGGGG</td>
<td>ACGGGGTTAACGCGGGG</td>
<td>ACGGGGTTAACGCGGGG</td>
<td></td>
</tr>
<tr>
<td>S. Typhi H58</td>
<td>ACGGGGTTAACGCGGGG</td>
<td>ACGGGGTTAACGCGGGG</td>
<td>ACGGGGTTAACGCGGGG</td>
<td>ACGGGGTTAACGCGGGG</td>
<td></td>
</tr>
</tbody>
</table>

- **Figure 3.** Gel image of positive PCR reactions (S. Typhi 227bp, S. Paratyphi A 374bp):

- A total of 36 bacterial DNA including non-Typhi Salmonella and non-Salmonella species → 100% specificity with no false positives
- Additional 75 DNA samples originally identified as S. Typhi by MALDI-TOF MS → 13/75 PCR positive for only S. Paratyphi A → confirmed by WGS

Conclusions

- PCR assay to distinguish S. Paratyphi A / S. Typhi + and H58 / XDR lineages
- Clinical and environmental sample testing planned
- Potential low-cost alternative to WGS for lineage level detection for rapid diagnostics and environmental surveillance

References