Pan genome clustering reveals the role of prophage elements in the evolution and adaptation of *Salmonella* Enteritidis

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Background

- S. Enteritidis is known to cause iNTS diseases in sub-Saharan African countries
- As of 2017, 79% of the 535,000 cases and 85% of the 77,500 deaths have been reported in sub-Saharan Africa (Stanaway et al., 2019)
- In South Asian countries, the prevalence of iNTS has been largely underestimated
- At our center, S. Enteritidis is the 2nd most common iNTS (30%) after S. Typhimurium (49%)
- The genomic insights, human adaptation and clinical features of this serovar are currently not known
Genomic insights: What we know so far!

- The dominant clone **ST11** carried diverse strains of *S. Enteritidis*
- Seven-gene MLST classification not robust
- Population Structure
  - East African clade
  - West African clade
  - Global Outlier/Atlantic
  - Global Epidemic
- AMR burden mostly on both the African clades
- Characteristic phages in different clades
- Variants of pSEN plasmids
Methods

Clinical Samples → Clinical isolates → Genomic DNA extraction → Whole Genome Sequencing → Phylogenetic tree construction → Pan-genome clustering

Clinical data

AST

Comparative genome analysis

Pan-genome

Clustering

Phage inserts

Plasmids

Pseudogene

Clinical WorkStation

ICMR

Pan-genome clustering

Comparative genome analysis
<table>
<thead>
<tr>
<th>Variable</th>
<th>Overall n (%)/median (IQR) (n = 101)</th>
<th>Immunosuppressive conditions n (%)/median (IQR)</th>
<th>p-value</th>
<th>OR</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Yes (n=58)</td>
<td>No (n=43)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sex (Male)</td>
<td>66 (65.34)</td>
<td>36 (62.1)</td>
<td>30 (69.76)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age</td>
<td>34.5 (9 – 54.5)</td>
<td>36 (25 – 50)</td>
<td>24.5 (0 – 55.75)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pediatric (&lt;16 years)</td>
<td>28 (27.72)</td>
<td>9 (15.52)</td>
<td>19 (44.18)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Duration of fever</td>
<td>6 (3 – 10)</td>
<td>7 (4 - 11)</td>
<td>5 (1 – 8)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Co-infection</td>
<td>18 (17.82)</td>
<td>11 (18.96)</td>
<td>7 (16.28)</td>
<td>0.797</td>
<td>1.2</td>
</tr>
<tr>
<td>Antibiotic combination therapy</td>
<td>66 (65.34)</td>
<td>38 (65.52)</td>
<td>28 (65.12)</td>
<td>1</td>
<td>1.02</td>
</tr>
<tr>
<td>Abdominal pain</td>
<td>27 (26.73)</td>
<td>16 (27.58)</td>
<td>11 (25.58)</td>
<td>1</td>
<td>1.11</td>
</tr>
<tr>
<td>Ascites</td>
<td>12 (11.88)</td>
<td>9 (15.51)</td>
<td>2 (4.65)</td>
<td>0.066</td>
<td>4.27</td>
</tr>
<tr>
<td>Cough</td>
<td>31 (30.69)</td>
<td>18 (31.03)</td>
<td>13 (30.23)</td>
<td>1</td>
<td>1.04</td>
</tr>
<tr>
<td>Diarrhoea</td>
<td>36 (35.64)</td>
<td>19 (32.76)</td>
<td>17 (39.53)</td>
<td>0.532</td>
<td>0.75</td>
</tr>
<tr>
<td>Fever</td>
<td>76 (75.25)</td>
<td>46 (79.31)</td>
<td>30 (69.77)</td>
<td>0.351</td>
<td>1.66</td>
</tr>
<tr>
<td>Pneumonia</td>
<td>22 (21.78)</td>
<td>14 (24.14)</td>
<td>8 (18.6)</td>
<td>0.627</td>
<td>1.39</td>
</tr>
<tr>
<td>Septic Shock</td>
<td>20 (19.8)</td>
<td>16 (27.59)</td>
<td>4 (9.3)</td>
<td>0.025</td>
<td>3.71</td>
</tr>
<tr>
<td>Unhealthy/ Anaemia</td>
<td>60 (59.41)</td>
<td>38 (65.52)</td>
<td>22 (51.16)</td>
<td>0.158</td>
<td>1.81</td>
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<tr>
<td>Recovery</td>
<td>84 (83.17)</td>
<td>47 (81.03)</td>
<td>36 (83.72)</td>
<td></td>
<td></td>
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<tr>
<td>Death</td>
<td>11 (10.89)</td>
<td>8 (13.79)</td>
<td>3 (6.98)</td>
<td>0.345</td>
<td>2.13</td>
</tr>
</tbody>
</table>
Population structure of SE (n=476)

Phenotypic characterization
✓ Isolates confirmed by serotyping & biochemical
✓ Susceptible to all tested antibiotics except FQ

Genomic characterization
✓ Study isolates (n=65); Clinical – 48, Poultry – 17
✓ Isolates belong to ST11 or SLVs
✓ New phylogroup predominantly of invasive isolates from India (Asian cluster)
✓ Phylogroup distribution - Clinical
  ✓ Asian cluster (61.9%)
  ✓ Global epidemic (33.3%)
✓ Phylogroup distribution – Poultry
  ✓ Global Outlier (82.3%)
Population structure (n=476)

- African cluster predominantly of clinical isolates
- Global epidemic and Outlier cluster consist of clinical, Environmental and livestock isolates
- Asian cluster was diverse but majorly composed of clinical isolates
Invasiveness Index & HDCS

- S. Enteritidis phylogroups remain to be gastrointestinal (DBS <0.5)
- Invasiveness index varied among the phylogroups:
  - West African (median = 0.253, SD = 0.25)
  - Global epidemic (median = 0.188, SD = 0.01)
  - Asian clade (median = 0.221, SD = 0.013)
- High genome degradation in African and Asian

<table>
<thead>
<tr>
<th>Phylogroup</th>
<th>No. of Characteristic HDCS</th>
</tr>
</thead>
<tbody>
<tr>
<td>East African</td>
<td>13</td>
</tr>
<tr>
<td>West African</td>
<td>9</td>
</tr>
<tr>
<td>Asian</td>
<td>8</td>
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</table>
Pangenome Clustering

- Evidence of gene flux associated with the phylogroups
- Lineage specific core genes were separated using Twilight
- Insertions were characterized as prophages by Phaster
Phage inserts

Asian Cluster

Felslike

ΦSE12

Novel Phage Insert

East Africa (D7795)

Gifsy1

ΦSE12

FelsBT

P88

West Africa

Gifsy1

ΦSE12

P88

Global Epidemic (P125109)

Gifsy1

ΦSE12

Felslike

ΦSE20

Novel Phage Insert

Inserts listed include:

- ΦSE12
- Gifsy1
- ΦSE
- Felslike
- East Africa (D7795)
- West Africa
- Global Epidemic (P125109)

Clusters mentioned include:

- Asian Cluster
- East Africa
- West Africa
- Global Epidemic

Other elements mentioned include:

- Felslike
- P88
- Novel Phage Insert

Additionally, diagrams and labels are present, depicting various phages and proteins involved in the mentioned clusters.
Summary

• Severely immunosuppressed individuals are at a higher risk of iNTS

• Majority of S. Enteritidis causing invasive infection from India belonged to an emerging lineage that we propose to refer to as the Asian cluster

• Genotypic differences observed in isolates within the Asian cluster compared to strains found in livestock (Global Outlier)

• Isolates belonging to the Asian cluster exhibited the second highest invasiveness index, following the West African clade

• Asian cluster was characterized a high level of HDCS and a distinctive prophage repertoire
Acknowledgements

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