Recent evolution in invasive Salmonellosis

Professor Gordon Dougan

Wellcome Trust Sanger Institute and Cambridge University
How does evolution deal with dogma

Statements that have launched a thousand grant applications and thesis introductions:-

• ‘Salmonella typhimurium is a cause of gastroenteritis in humans whereas Salmonella typhi is responsible for the systemic disease typhoid……….’
Comparison of pathogenesis of infection associated with Salmonella typhimurium versus typhi

Some pathogens are genetically monophyletic and cannot be differentiated by MLST.
Comparison of the Salmonella Typhi genomes of Ty2 (1912) and CT18 (1992) illustrates a conserved genome.

Although variation in Typhi is limited it can be defined and the consequences assessed – a paradigm for other human restricted pathogens?
What is the evidence for vertical and horizontal accumulation of mutation and diversity in S. Typhi?
Variation discovery in Typhi using sequencing of 200 genes and over 100 strains

Found only 88 SNPs! In ~ 2% of the genome
Any new isolate can be unequivocally assigned to a position in the tree.

The position in the tree is irrefutable.

SNP analysis of *Salmonella* Typhi linked to gyrase SNPs

Expanding SE Asian Naladixic acid resistant clones are H58

**NalR** - gyrA mutations
Selection of strains for resequencing S. Typhi

Because we have a phylogenetic Tree we can truly sample diversity

Roumagnac et al., 2006. Science.
New tech re-sequencing of *Salmonella Typhi*

<table>
<thead>
<tr>
<th>Strain</th>
<th>Country</th>
<th>Year</th>
<th>Haplogroup</th>
<th>Haplotype</th>
<th>Relative %</th>
<th>Solexa Coverage</th>
<th>Plasmids</th>
</tr>
</thead>
<tbody>
<tr>
<td>E00-7866</td>
<td>Morocco</td>
<td>2000</td>
<td>H46</td>
<td>10.5</td>
<td>98.9%</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>E01-6750</td>
<td>Senegal</td>
<td>2001</td>
<td>H52</td>
<td>8.16</td>
<td>95.3%</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>E02-0018</td>
<td>India</td>
<td>2002</td>
<td>H45</td>
<td>13.1</td>
<td>98.8%</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>E98-0664</td>
<td>Kenya</td>
<td>1998</td>
<td>H55</td>
<td>10.8</td>
<td>97.4%</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>E98-2068</td>
<td>Bangladesh</td>
<td>1998</td>
<td>H42</td>
<td>10.9</td>
<td>98.4%</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>J-185SM</td>
<td>Indonesia</td>
<td>1998</td>
<td>H85</td>
<td>13.5</td>
<td>98.8%</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>M223</td>
<td>Indonesia</td>
<td>1939</td>
<td>H8</td>
<td>11.1</td>
<td>99.9%</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>404ty</td>
<td>Indonesia</td>
<td>1983</td>
<td>H2 (H59)</td>
<td>8.49</td>
<td>97.0%</td>
<td>24.6x</td>
<td>pBSSB1a</td>
</tr>
<tr>
<td>AG3</td>
<td>Vietnam</td>
<td>2004</td>
<td>H58</td>
<td>10.1</td>
<td>99.0%</td>
<td>13.1x</td>
<td>-</td>
</tr>
<tr>
<td>E98-3139</td>
<td>Mexico</td>
<td>1998</td>
<td>H50</td>
<td>11.1</td>
<td>95.8%</td>
<td>5.40x</td>
<td>-</td>
</tr>
<tr>
<td>150(98)S</td>
<td>Vietnam</td>
<td>1998</td>
<td>H63</td>
<td>-</td>
<td></td>
<td>8.60x</td>
<td>-</td>
</tr>
<tr>
<td>8(04)N</td>
<td>Vietnam</td>
<td>2004</td>
<td>H58</td>
<td>-</td>
<td></td>
<td>13.1x</td>
<td>-</td>
</tr>
<tr>
<td>CT18</td>
<td>Vietnam</td>
<td>1993</td>
<td>H1</td>
<td>-</td>
<td></td>
<td>9.80x</td>
<td>pHCM1, pHCM2</td>
</tr>
<tr>
<td>E02-2759</td>
<td>India</td>
<td>2002</td>
<td>H58</td>
<td>-</td>
<td></td>
<td>65.5x</td>
<td>pHCM2</td>
</tr>
<tr>
<td>E03-4983</td>
<td>Indonesia</td>
<td>2003</td>
<td>H59</td>
<td>-</td>
<td></td>
<td>7.42x</td>
<td>pBSSB1a</td>
</tr>
<tr>
<td>E03-9804</td>
<td>Nepal</td>
<td>2003</td>
<td>H58</td>
<td>-</td>
<td></td>
<td>8.19x</td>
<td>pAKU1</td>
</tr>
<tr>
<td>ISP-03-07467</td>
<td>Morocco</td>
<td>2003</td>
<td>H58</td>
<td>-</td>
<td></td>
<td>7.87x</td>
<td>pAKU1</td>
</tr>
<tr>
<td>ISP-04-06979</td>
<td>Central Afric</td>
<td>2004</td>
<td>H58</td>
<td>-</td>
<td></td>
<td>72.9x</td>
<td>pAKU1</td>
</tr>
<tr>
<td>Ty2-SI</td>
<td>Russia</td>
<td>1916</td>
<td>H10</td>
<td>-</td>
<td></td>
<td>8.60x</td>
<td>-</td>
</tr>
<tr>
<td>Ty2</td>
<td>Russia</td>
<td>1916</td>
<td>H10</td>
<td>-</td>
<td></td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

* = FLX

Kathryn Holt
S. Typhi phylogenetic tree after sequence analysis

(a) S. Typhi phylogenetic tree

2736 SNPs identified; 1815 SNPs used

Variation landscape in typhoid

Typhi is genetically isolated with no evidence for antigenic variation
Accumulation of plasmids and mutation on the tree

Plasmids entering the tree

We have different typhoid disease severity in our part of the world, is this due to the host or the pathogen genetics...???

The differential accumulation of ~92 pseudogenes in different arms of the tree is giving rise to different pathotypes?
Typhi genotyping

Can SNPs framework be used for association style genetics as in mammals?
Haplotypes circulating in the Jatinegara district of Jakarta over a two year period

1500 SNPs assayed at once for each sample (ugs) on Illumina GoldenGate genotyping platform
Distribution of invasive salmonellosis disease

Endemic disease
Multidrug-resistant strains reported
Nalidixic acid–resistant strains reported

Non-typhoidal Salmonellosis
Reports from tropical Africa of NTS as a major cause of pediatric bacteraemia
NTS are the commonest cause of bacteraemia in Malawi:

Blood isolates 0-14 years, 1996-2002

- **NTS**: 1,873
- **S.pneumoniae**: 557
- **H.influenzae**: 873
- **S.aureus**: 300
- **N.meningitidis**: 152
- **Gp B strep**: 356
- **S.typhi**: 189
- **Other strep**: 580
- **Other GNR**: 189
- **Other**: 580
**NTS in HIV-positive adults**

Non-typhoidal salmonella bacteraemia among HIV-infected Malawian adults: high mortality and frequent recrudescence


AIDS 2002, 16:1633–1641

- **77/78 HIV-infected**
- **47% inpatient mortality**
- **77% dead at 1 year**
- **43% recurrence rate**
- Recrudescence not reinfection

---

**Fig. 2.** (a) Kaplan–Meier estimate of overall survival (in days) after non-typhoidal salmonella bacteraemia (n = 100). (b) Kaplan–Meier estimate of time (in days) to first recurrence among survivors of non-typhoidal salmonella bacteraemia (n = 45).
NTS in HIV-negative children

Clinical presentation of non-typhoidal Salmonella bacteraemia in Malawian children

A diagnostic conundrum:

- 97% fever, 67% cough, 48% diarrhoea, 72% tachypnoea
- just ~18% of cases are associated with HIV infection
- 31% malaria slide positive
- 27% Anaemia
- 15% <60% weight-for-age, 51% 60-80% weight-for-age
- No diagnosis without blood culture facility
- Overall mortality 23%
The neglected role of antibody in protection against bacteremia caused by nontyphoidal strains of *Salmonella* in African children

Calman A. MacLennan,1,2,3,4 Esther N. Gondwe,1,5 Chisomo L. Msefula,1,4,5 Robert A. Kingsley,6 Nicholas R. Thomson,6 Sarah A. White,1,7 Margaret Goodall,2 Derek J. Pickard,8 Stephen M. Graham,1,5 Gordon Dougan,8 C. Anthony Hart,3 Malcolm E. Molyneux,1,5 and Mark T. Drayson2

[Graphs and tables showing data related to Salmonella bacteremia and antibody titers.]
In vitro models of Salmonella Bacteraemia

Whole blood assay
8 salmonella strains
Healthy adult donor blood

Heat-inactivated serum

Washed blood cells resuspended in RMPI

Serum

C9 deficient serum

C9 deficient serum + purified C9

Minutes exposure

Log10 change in Salmonella cfu/ml

A

B

C

D
Complement-mediated killing of salmonella is dependent on classical pathway activity
The emergence of drug resistant invasive salmonellosis in Malawi as monitored by Wellcome Trust Laboratories, Blantyre
Many NTS strains circulating in Africa (Kenya/Malawi) have a novel MLST type

ST313 NTS

Classic gastroenteritis (DT104, LT2, SL1344)

Root
Complete sequence of a Malawian MDR NTS S. Typhimurium ST313

Rob Kingsley and Nick Thomson
S. Typhimurium D23580 has a unique phage repertoire compared to other sequenced strains
Most S. Typhimurium NTS isolates from Malawi between 2003-2004 have identical pulse field and plasmid pattern to D23580 V517.

Indistinguishable strains of S. typhimurium NTS in HIV-positive adults and HIV-negative children.
Antibiotic resistance genes located on an integron situated in the virulence plasmid of *S. Typhimurium D23580*
The invasion associated plasmid harbours a sophisticated mobile cassette encoding antibiotic and disinfect resistance.

- β-lactamase
- aminoglycoside
- sulphonamide
- trimethoprim
- streptomycin
- chloramphenicol
- quaternary ammonium
- 2004
Plasmid, Pulse Field Gel Electrophoresis and Multi Locus Sequence Typing Profiles

MDR, Cm-S

Chloramphenicol treatment

Fluoroquinolone treatment

MDR, Cm-R

Cm-S MDR, Cm-R Chloramphenicol treatment Fluoroquinolone treatment
Salmonella typhimurium isolated before 2004 have a different element encoding MDR
The pressure for the use of prophylactic antibiotics in HIV positive children

Scaling-up co-trimoxazole prophylaxis in HIV-exposed and HIV-infected children in high HIV-prevalence countries

Rony Zachariah, Anthony D Harries, Chewu Luo, Gretchen Bachman, Stephen M Graham

Co-trimoxazole (trimethoprim-sulfamethoxazole) is a widely available antibiotic that substantially reduces HIV-related morbidity and mortality in both adults and children. Prophylaxis with co-trimoxazole is a recommended intervention of proven benefit that could serve not only as an initial step towards improving paediatric care in young children with limited access to antiretroviral treatment, but also as an important complement to antiretroviral therapy in resource-limited settings. Despite co-trimoxazole’s known clinical benefits, the potential operational benefits, and favourable recommendations by WHO, UNAIDS, and UNICEF, its routine use in developing countries—particularly sub-Saharan Africa—has remained limited. Out of an estimated 4 million children in need of co-trimoxazole prophylaxis (HIV-exposed and HIV-infected), only 4% are currently receiving this intervention. We discuss some of the major barriers preventing the scale-up of co-trimoxazole prophylaxis for children in countries with a high prevalence of HIV and propose specific actions required to tackle these challenges.

Lancet Infectious Disease 2007, 7:686-93
Acknowledgements

Julian Parkhill
Nick Thomson
Many in the PSU

Stephen Baker
Kathryn Holt
Tim Perkins
Robert Kingsley
Derek Pickard
Others in Team 15

Jeremy Farrar
Christiane Dolecek

Duncan Maskell

Bruce Stocker

Sam Kariuki
Chisomo Msefula
Malcolm Molyneux
Melita Gordon
Robert Heyderman
Cal MacLennnan
The Non-typhoidal Salmonellosis problem

- Multi-drug resistance (chloramphenicol, ampicillin, cotrimoxazole) >90% in Malawi
- Diagnostic difficulty. Non-specific presentation
- 23% case fatality rate with blood-culturing facility and appropriate antibiotic therapy
- Rapid progression of clinical course
- Currently no vaccine against

Epidemics of Invasive *Salmonella enterica* Serovar Enteritidis and *S. enterica* Serovar Typhimurium Infection Associated with Multidrug Resistance among Adults and Children in Malawi

Melita A. Gordon,1,2 Stephen M. Graham,1,3 Amanda L. Walsh,1 Lorna Wilson,1 Amos Phiri,1 Elizabeth Molyneux,2 Eduard E. Zijlstra,3 Robert S. Heydenman,1,4 C. Anthony Hart,1,4 and Malcolm E. Molyneux1,4

1Malawi–Liverpool Wellcome Trust Clinical Research Programme and Departments of Medicine and Paediatrics, College of Medicine, University of Malawi, Blantyre, Malawi; and 2University of Liverpool and 3Liverpool School of Tropical Medicine, Liverpool, United Kingdom

Clinical Infectious Diseases 2008; 46:963–9