A genomic dissection of travel associated ESBL producing *Salmonella Typhi* originating from the Philippines –

A one-off occurrence or threat to the effective treatment of typhoid fever?

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Antimicrobial treatment of S. Typhi

• The mortality of typhoid fever declined considerably due to antimicrobial treatment
  – Chloramphenicol (1948)
  – Ampicillin (1961)
  – Co-trimoxazole (1970s)
  – 3rd generation cephalosporins and fluoroquinolones (1980s)

• MDR emerged to all three “first line” antimicrobials in the 1980s followed by resistance to the fluoroquinolones in the 1990’s

• Cephalosporin resistance has been slower to emerge

• Should ESBL producing S. Typhi become widespread, treatment options may become severely limited
Global distribution of AMR in S. Typhi

Wain et al, accepted in Lancet
Late Breaker!
(from another study, Typhi in Zambia)

Chromosomal translocation of the classical MDR region from the *incHI1* plasmid of the global dominating *S. Typhi* haplotype H58

And what does that mean in terms of

- Virulence - lower fitness cost?
- Transmission of both the clones and region?
- AMR – rooms for a new AMR plasmid (ESBL)?
Global distribution of AMR in S. Typhi

Typhoid endemic countries with insufficient data available
Susceptible strains of S. Typhi only present
MDR S. Typhi present (H58, incHI1)
Combined MDR and decreased susceptibility to FQ
Extended spectrum β-lactam (ESBL) resistant S. Typhi

Wain et al, accepted in Lancet
ESBL resistance in S. Typhi – Myth or a reality?
Study objectives

- To identify potentially unreported cases of typhoid fever caused by ESBL producing Typhi at a global level

- To confirm ESBL production phenotypically and identify the responsible ESBL genes

- To investigate the genetic relatedness to other available ESBL producing Typhi isolates using
  - Whole genome sequence typing (WGST)
  - Variety of molecular and genomic analysis
  - To test the hypothesis - impaired restriction modification systems could be a factor for the development of ESBL resistance

Hendriksen et al, submitted JAC
Global Foodborne Infections Network

Electronic Discussion Group
Message #7_2012
Subject: ESBL Producing Typhi-Request for Information

Dear GFN Members:

Danish Technical University (DTU) and the US CDC are collaborating on an effort to study the genetic diversity of Salmonella Typhi.

There have been numerous anecdotal reports of ESBL producing Salmonella Typhi. However; there is currently only a single report of ESBL producing Typhi in the peer-reviewed literature.

Third generation cephalosporins (notably ceftriaxone) are considered among the first-line treatments for typhoid fever. We find these anecdotal reports of ESBL producing Typhi very concerning.

CDC and DTU are very interested in characterizing known or suspected ESBL producing isolates of Salmonella Typhi.

If you have any Typhi isolates in your collection which are resistant to 3rd generation cephalosporins (including: ceftriaxone, ceftazadime, cefixime, cefoperazone, or cefpodoxime); we would like to speak with you further.

If you have any confirmed or suspected ESBL producing Typhi, we would be grateful if you could email Matthew Mikoleit (euh1@cdc.gov) or Rene Hendriksen (rshe@food.dtu.dk).

Thank you in advance for your time.

Matt Mikoleit, CDC
Rene Hendriksen, DTU
#1107-3567 - SHV-12
- One isolate from Norway
- Suffered from gastroenteritis (stool)
- 27 year old female
- Travelled to the Philippines
- Mid-December 2007

Data resembled a previously published case from the Netherlands.

SHV-12
- Typhoid fever treated with CIP (blood and stool)
- 54 year old female
- Travelled to the Philippines
- Mid-November 2007 (two month visit)
Identical or random coincidence

Houston, we have a problem
Identical or random coincidence

Phenotypic approach
• MIC determination
• XbaI PFGE
• S1 nuclease to determine plasmid size
• Electroporation to determine plasmid transferability

Genotypic approach using online “plug and play” tools
• AMR genes by ResFinder
• Replicons by PlasmidFinder
• MLST by MLSTFinder
• pMLST by pMLSTFinder
• Typhi haplotype – bioinformatic tool by command-line
• InDels – bioinformatic tool by command-line
• Single Nucleotide Polymorphism (SNP) by SNPFinder
• Restriction Modification System by Restriction-ModificationFinder
Both strains exhibited resistance to:

- Ampicillin; \( \text{bla}_{\text{TEM}}^{-1} \)
- Cefotaxime, cefpodoxime, ceftazidime, ceftiofur
- Ceftriaxone; \( \text{bla}_{\text{SHV}}^{-12} \)
- Gentamicin; \( \text{aac}(6')\text{IIc} \)
- Streptomycin; \( \text{strA} / \text{strB} \)
- Tetracycline; \( \text{tetD} \)
- Trimethoprim; \( \text{dfrA18} \)
- Not the classic H58/\( \text{incHI1} \) MDR AMR gene profile.

Both were susceptible to; apramycin, azithromycin, cefepime, cefoxitin, chloramphenicol, ciprofloxacin, colistin, florfenicol, imipenem, meropenem, nalidixic acid, neomycin, spectinomycin, and sulfamethoxazole – incl. important antimicrobials for treatment!
Unique XbaI pattern,
Three bands difference to #1107-3567

Four identical profiles submitted PulseNet Int. 1995 and 1997,
susceptible isolates)

Two identical profiles submitted PulseNet Int. in 2007 Philippines,
(unknown susceptibility status)
MLST, Haplo-type and SNPs

The global dominating MDR H58 lineage

The H13 lineage, Suscep strains in Malaysia

No SNP difference between the two isolates (MLST ST1) from the Philippines
• Deletion of 144 kb in one of the genomes; from Norway
• Equivalent to *Salmonella* Pathogenicity Island, SPI-7
• Encoding the synthesis of the Vi capsule exopolysaccharide
  • Absence of
    • Conjugal DNA transfer region
    • The IVB pilus system
    • The DNA conjugational transfer region
• Partial absence of the *sopE* bacteriophage region
• Presence of the entire *viaB* operon
Plasmid characterization

- S1 nuclease revealed an app. 280 kb large plasmid
- The strains contained the two closely related *inc*HI2 and *inc*HI2A
- Both *inc*HI2 plasmids belonged to pMLST type ST1
- Mating experiments were successful only for strain; #TY5359 – the Dutch
  - *bla*SHV-12 was transferred and *str*A/B, *aac*(6')-IIc, *bla*TEM-1, *tet*D, and *dfra*18 co-transferred
- Conjugation of strain #1107-3567 was unsuccessful
- *de novo* assembly of the two strains were compared to pEC-IMPQ – no SNP differences
RMS analysis of the two Typhi genomes tested along with additional 11 genomes revealed:

- Three potential RM systems present in all strains
  - Type I system closely homologous to what is found in Paratyphi A and *E. coli*
  - Type III system homologues to what is found in Paratyphi A and Typhimurium
  - Type IV system closely related to what is in Typhimurium
- No genetic evidence for loss of integrity of the RM systems in the two ESBL-producing isolates

So, the hypothesis was rejected

- The RM systems may not be restricting this plasmid in Typhi......
- Or the introduction of the plasmid may have been initially transferred into an intermediate Typhi host with an impaired restriction gene but a functional methylation gene, and subsequently disseminated by horizontal transfer to the present Typhi host
Identical or random coincidence

Identical

- Same AST and AMR gene profiles
- Plasmids
  - Same plasmid size
  - Same pMLST
  - Same replicon
  - No SNP difference
- No chromosomal SNP difference
- Epidemiologically linked

“Differences”

- Same RMS
- Same MLST
- Same Haplotype – but rare!
  - found in same the region / time
- Similar PFGE profile – but rare!
  - 3 bands difference
  - found in same the region / time
- Conjugation
  - Only work for one
- InDel
  - Deletion of SPI7 in one genome
In summary

• The investigated strain was linked by WGST analysis, time, and geography to another Typhi isolate suggesting an undetected outbreak in the Philippines in 2007; to our knowledge - the first outbreak of ESBL producing Typhi

• It is uncertain if this was a one-off occurrence or is persisting as a threat to the effective treatment of typhoid fever with severe public health implications

• Capacity building needs to be implemented in the region to conduct reliable antimicrobial susceptibility testing /confirmation procedures

• There is a vital need to establish a well-functioning surveillance system in the Philippines and the region if these strains persist and to implement interventions should they re-emerge
Thank you for your attention

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