Genomics for tracing the cholera epidemic in Africa

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1st ICREID
Addis Ababa
12-14 March 2018
Cholera

- Clinical-epidemiologic syndrome

- Vibrio cholerae O1 (rarely O139) with CTX toxin

- Watery diarrhea that rapidly lead to dehydration

- Explosive outbreaks often in a context of wars, civil conflicts, climatic events leading to famine, human gatherings without clean water, decent sanitation and good hygiene

- Human-to-human transmission (direct or indirect)
• 1.03 billion people at risk

• Estimated 2.86 million cases and 95,000 deaths/year (Ali et al. Plos NTD 2015)

• Treatment: rehydration and antibiotics
History

1st-6th pandemic

*Vibrio cholerae* O1 Biotype Classical

Bay of Bengal

- 1817-1823: Asia, Middle East, East Africa
- 1829-1851: Global ✔
- 1852-1859: Global
- 1863-1879: Global
- 1881-1896: Global ✔
- 1899-1923: Asia, Middle East, Eastern Europe ✔

✔ confirmed
### 7th pandemic

**Vibrio cholerae O1 Biotype El Tor (7PET)**

1961, Indonesia

« Paracholera »

<table>
<thead>
<tr>
<th>Test</th>
<th>Classical</th>
<th>El Tor</th>
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<tbody>
<tr>
<td>Haemolysis</td>
<td>-</td>
<td>+</td>
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<tr>
<td>Voges-Proskauer</td>
<td>-</td>
<td>+</td>
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<tr>
<td>Chick red cell agglutination</td>
<td>-</td>
<td>+</td>
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<tr>
<td>Phage IV sensitivity</td>
<td>+</td>
<td>-</td>
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<tr>
<td>Polymyxin B sensitivity</td>
<td>+</td>
<td>-</td>
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Classical laboratory methods for 7PET V. cholerae

- O1 serotyping (Ogawa, Inaba, Hiwajima)
- Phage typing
- Multilocus enzyme electrophoresis (MLEE)
- Ribotyping
- Pulsed-field gel electrophoresis
- $ctxB$ (B subunit of cholera toxin) RFLP or sequencing
- tcpA (toxin coregulated pilus A) sequencing
- Sequencing of other virulence genes …..
- Multiple loci VNTR analysis (MLVA)

Confusion !!!
First Vc O1 7PET genome

N16961 (Heidelberg et al., Nature 2000)

4.03 Mb, 3885 CDSs
Two circular chromosomes (2.96 Mb, 1.07 Mb)
CTXΦ phage on CHR1
Large integron island (125 kb) on CHR2

Bangladesh 1975
Only two clones of *V. cholerae* O1 CTX+ are responsible of pandemic cholera

Others: sporadic cases or small outbreaks (w/o secondary cases) generally linked with an aquatic reservoir (seafood).

Genomics can predict the epidemiological potential of *V. cholerae* O1 CTX+
• Genetic homogeneity of 7PET (250 SNPs), different from Classical
• Three « waves » of dissemination of 7PET
• Role of the Bay of Bengal
• Identification of intercontinental transmission events
Guinea 1970, origin?

- Guinean students returning from the Black Sea
- Pilgrims or soldiers from the Middle East

World Health Organization (WHO)
Objectives of our study

- Identify the introduction and transmission routes of *V. cholerae* O1 ET in Africa, 1970-2014
- Linkage between the different outbreaks
- Monitoring antimicrobial resistance

Material

742 sequenced isolates (558 from IP)
328 published genomes

Analysis of 1,070 genomes, including 631 from Africa (45/54 countries)
Methodology

1. Illumina sequencing (75-250 bp PE short reads)

2. Mapping against reference genome N16961 (CHR1 and CHR2)

3. SNPs calling and filtering (SMALT), assembling (SPAdes)

4. Phylogenetic and phylogeographic analyses and estimation of the divergence times of the different lineages/strains (RAxML after Gubbins, Path-O-Gen, BEAST)

5. Extraction of antibiotic resistance genes and their genetic support (Resfinder on read assemblies, PacBio sequencing)
11 introductions of 7PET into Africa: T1, T3-T12

228 isolates (2,961 SNPs); strict clock rate; Bayesian skyline population size
1970-2014

11 introductions to Africa
Guinea 70 ↪ Middle East

Five introductions to West Africa and six to East Africa

Middle East acting as a springboard during six introductions

Followed by one to 28 years of regional circulation

Two separated and persistent foci (West Africa and the Great Lakes-Horn of Africa region).
Rare exceptions

T2 ↦ Latin America
However, only 16.6% (105/631) of the isolates contain genes encoding resistance to tetracyclines (first-line drug choice for WHO, Icddrb, MSF, PAHO)
Phase 1 (1970s-1980s, waves 1-2)

Large incA/C plasmids

*bla strAB aad aph(3’)-l cat1 tetB tetC sul1 sul2 dfrA15*

Acquired in Africa

Phase 2 (after 1980s, wave 3)

Chromosomal determinants

Genomic islands:

- **GI-15 aad_new sul1** 29kb
- **SXT/R391 (5 variants) strAB sul2 (floR) (dfrA1) (tetA) (tet_new) (qnrVC1) (dfrA31)** 100kb

**gyrA** and **parC** mutations

Acquired in South Asia
✓ Iterative introductions of cholera to Africa from Asia, with separated Western and Eastern African foci

✓ Role of the Middle East as a springboard (in particular in 1970) but recently direct transmission from South Asia

✓ Data do not support a long-term environmental reservoir of 7PET in Africa

✓ Accumulation of chromosomally-encoded multidrug resistance elements, as observed previously with human pathogens *Shigella dysenteriae* type 1 and *Salmonella Typhi* H58 (Njamkepo et al. Nat Microbiol 2016; Wong et al. Nat Genet 2014)
Future studies

Understanding the factors associated with the disappearance/replacement of 7PET lineages in Africa over time?

Herd immunity?
Lytic phages?
Others?

Building a global genomic database of 7PET isolates for a real-time genomic surveillance of cholera.
Genomes rewrite cholera’s global story

RESEARCH

CHOLERA

Genomic history of the seventh pandemic of cholera in Africa


Science 358, 785–789 (2017) 10 November 2017
Thank you for your attention!