Genomic insights into the epidemiology and surveillance of *Vibrio cholerae* O1 infections

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Foodborne pathogens & whole genome sequencing

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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 via water or food)
• 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>
</tr>
</thead>
<tbody>
<tr>
<td>Haemolysis</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Voges-Proskauer</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Chick red cell agglutination</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Phage IV sensitivity</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Polymyxin B sensitivity</td>
<td>+</td>
<td>-</td>
</tr>
</tbody>
</table>

Proportion of

- moderate to severe cases: 36% Classical, 6% El Tor
- asymptomatic cases: 59% Classical, 75% El Tor

Kaper et al., CMR, 1995
Occurrence of *Vibrio cholerae* Serotype O1 in Maryland and Louisiana Estuaries

RITA R. COLWELL,1,1 RAMON J. SEIDLER,2 JAMES KAPER,1 S. W. JOSEPH,3 SUE GARGES,1 HANK LOCKMAN,1 DAVID MANEVAL,1 HENRY BRADFORD,4 NELL ROBERTS,4 ELAINE REMMERS,1 IMDADUL HUQ,6 AND ANWARUL HUQ6

**Table 1. ***V. cholerae* O1 strains isolated from environmental sources in Louisiana and Maryland in 1977 to 1980

<table>
<thead>
<tr>
<th>No of strains</th>
<th>Source</th>
<th>ELISA&lt;sup&gt;a,b&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>7</td>
<td>Water</td>
<td>2/6</td>
</tr>
<tr>
<td>1</td>
<td>Crab</td>
<td>0/1</td>
</tr>
<tr>
<td>25</td>
<td>Sewer or canal</td>
<td>12/16</td>
</tr>
</tbody>
</table>

*Vibrio cholerae* serotype O1 has been isolated from Chesapeake Bay in Maryland and estuaries and sewers in Louisiana. The occurrence of *V. cholerae* O1 in the aquatic environment in the absence of human disease suggests that this organism survives and multiplies in the natural environment.
• Five symptomatic and three asymptomatic cases of cholera in Southwestern Louisiana between Sept and Oct 1978

• *Vibrio cholerae* O1 biotype El Tor serotype Inaba CTX+, particular phagetype

• Illness significantly associated with consumption of crabs ($p = 0.007$)

• Environmental investigation: same bacteria isolated from estuarine water, shrimps, crabs, sewage
Effects of Global Climate on Infectious Disease: the Cholera Model

Erin K. Lipp,1,2 Anwar Huq,1,3 and Rita R. Colwell1,3*

Global Climate and Infectious Disease: The Cholera Paradigm*

Rita R. Colwell

SCIENCE • VOL. 274 • 20 DECEMBER 1996

FIG. 1. Hierarchical model for environmental cholera transmission (modified from Colwell and Huq [30]).
Classical laboratory methods for *V. cholerae*

- O1 serotyping (Ogawa, Inaba, Hiwojima)
- 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
22 other genomes, including 12 O1 ET (Chun et al. PNAS 2009)

73 GIs identified

- 7P isolates contain VSP-1 and VSP-2
- CTXΦ can be carried on CHR2
Haiti, 2010

- January 2010, devastating earthquake
- October 2010, first cholera case
- 2017 (one million cases, 10,000 deaths)

**CONCLUSIONS**

The Haitian epidemic is probably the result of the introduction, through human activity, of a *V. cholerae* strain from a distant geographic source.
A new study has yielded the most solid evidence yet that **U.N. peace-keeping forces from Nepal inadvertently brought cholera to Haiti last year**, setting off an epidemic that has killed more than 6000 people so far. The paper, published today in the online open access journal *mBio*, is the first to compare the whole genomes of bacteria from Haitian cholera patients with those found in Nepal around the time in 2010 when the peacekeepers left their country. It found that the genomes from the two sets of bacteria are virtually identical.
Evidence for several waves of global transmission in the seventh cholera pandemic

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
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 epidemic potential of *V. cholerae* O1 CTX+.
The seventh pandemic of cholera in America

Three introductions into America (Peru 1991, Mexico 1991 and Haiti 2010)
Origins of the cholera epidemic strain, Peru 1991

Different hypotheses:
- a ship from China
- El Niño

Figure 1: El Niño events correlate with water surface temperature rises and emergence of new *Vibrio* infections in South America.

Martinez-Urtana et al. Nat Microbiol 2016

Wave 1

West Africa

America 1991-2006
1-20 SNPs

It was West Africa
The seventh pandemic of cholera in Africa

1970

Guinea 1970, origin?
- Guinean students returning from the Black Sea
- Pilgrims or soldiers from the Middle East

World Health Organization (WHO)
Objectives

- Identify the introduction and transmission routes of 7PET in Africa
- Linkage between the different outbreaks
- Emergence of antimicrobial resistance

Material

742 sequenced isolates (558 from IP)
328 published genomes

Analysis of 1,070 genomes, including 631 from Africa (45/54 countries)
Africa, 1970-2014

11 introductions to Africa
  Guinea 70 ← Middle East
  Angola 71 ← West Africa

Five introductions to West Africa and six to East Africa

Middle East acting as a springboard during six introductions

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

Followed by up to 28 years of regional circulation
**Phase 1 (1970s-1980s)**

Large IncA/C plasmids

- *bla strAB aad aph(3')-I cat1 tetB tetC sul1 sul2 dfrA15 dfrA15*

*Acquired in Africa* (Tanzania 1979, West Africa 1984, …)

**Phase 2 (after 1980s)**

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*
Civil war (March 2015)

Fatalities: 8757
Injuries: 50 000
3 000 000 displaced people

Massive disruption of basic infrastructures and health system

Two epidemic waves:
- Sept 28, 2016 to April 23, 2017
  25 839 suspected cases
  120 deaths (0.46%)
- April 24, 2017 to now
  >1 000 000 cases
  2265 deaths (0.2%)

October 6th 2016: Report of the first cholera cases

Camacho et al. Lancet Glob Health 2018
Cholera was reported in the Middle East (Iran, Iraq), East and Central Africa before September 2016

Origin:

Middle East ?
Horn of Africa ?
Indian subcontinent ?
**Objectives**

Confirmation of the pathogenic agent
Description of the genomic features (including AMR)
Linkage with the global radiation of 7PET

**Material**

Analysis of 1,203 7PET genomes, including 42 from Yemen
The Yemeni isolates were unexpectedly susceptible to antibiotics, including polymyxin B.

**TMP**<sup>R</sup>  **NAL**<sup>R</sup>  **FT**<sup>R</sup>

*TMP*, trimethoprim  
*NAL*, nalidixic acid  
*FT*, nitrofurantoin

**POLYMYXIN B**

7PET El Tor   Yemen

**VprA-VprB two component system**

*VprA* dependant lipid A modification confers polymyxin resistance and contributes to the intestinal colonization of the mammalian host.

Herrera et al, Mbio 2014

**Normal AMR phenotype of Wave 3 7PET isolates:**

<table>
<thead>
<tr>
<th>AMR Phenotype</th>
<th>Wave 3 7PET isolates</th>
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<tbody>
<tr>
<td>STR&lt;sup&gt;R&lt;/sup&gt;</td>
<td>SUL&lt;sup&gt;R&lt;/sup&gt;</td>
</tr>
<tr>
<td>TMP&lt;sup&gt;R&lt;/sup&gt;</td>
<td>SXT&lt;sup&gt;R&lt;/sup&gt;</td>
</tr>
<tr>
<td>TET&lt;sup&gt;R&lt;/sup&gt;</td>
<td>CHL&lt;sup&gt;R&lt;/sup&gt;</td>
</tr>
<tr>
<td>NAL&lt;sup&gt;R&lt;/sup&gt;</td>
<td>FT&lt;sup&gt;R&lt;/sup&gt;</td>
</tr>
<tr>
<td>POL&lt;sup&gt;R&lt;/sup&gt;</td>
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</tr>
</tbody>
</table>

**MIC (µg/ml)**

| MIC (µg/ml) | 128  | 2    |

vprA_D89N conserved aa

VprA dependant lipid A modification confers polymyxin resistance and contributes to the intestinal colonization of the mammalian host.
Recent sublineage \((ctxB7)\) Not linked with Middle Eastern isolates but with Eastern African isolates from a new introduction (T13)
Whole genome sequencing has revolutionized the epidemiology of *V. cholerae* O1

- Better prediction of epidemic potential (7PET vs others)
- New knowledge on global patterns of epidemic cholera transmission and reservoirs (i.e., no perennial aquatic reservoir of 7PET cholera in Africa or in America)
- Need of regional studies (propagation routes / main drivers)

Real-time whole-genome sequencing surveillance system and cross-border collaboration to enhance current surveillance effort

- Strain tracking
- AMR evolution
- Need of a global genomic database containing phylogenetic tools (i.e., cgMLST)
Collaborators

Paris:
ML Quilici
E Njamkepo
N Fawal
J Rauzier
JM Fournier
PAD Grimont
C Bouchier
H Salje
T Malliavin

Bangui:
S Breurec
R Bercion

Yaoundé:
A Ngandjio

Dakar:
B Garin

Niamey:
F Sidikou

Abidjan:
M Dosso

Iran
M Pourshafie
A Abubakar (EMRO)
J Wamala (South Sudan)
M Malik (EMRO)

A Azman

Yemen
A Almesbahi (NCPHL)
M Naji (NCPHL)
S Nasher (NCPHL)

India
N Sharma (MVIDH)
D Kumar (MVIDH)

Kenya
S Kariuki (KEMRI)
J Kiiru (KEMRI)
J Carter (Amref)
S Njoroge (KEMRI)

Iran
A Abulmaali (CPHL)

Iraq
B Bakhshi (TMU)

Saudi Arabia
A Assiri (MoH)

Mexico
A Cravioto (UNAM)
ML Lizzaraga-Partida (CICESE)

Thank you for your attention!