Institut Pasteur

The use of genomics for understanding introduction and propagation routes of cholera in Africa during the seventh pandemic

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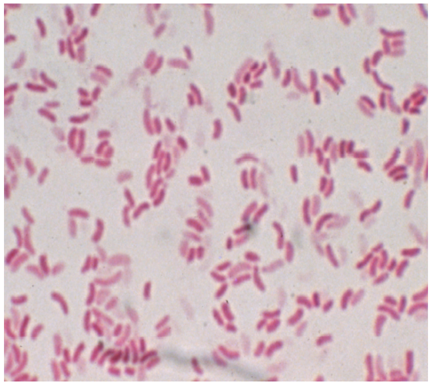
June 9th 2017

Cholera



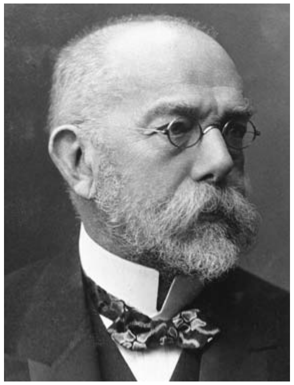
- Clinical-epidemiologic syndrome
- *Vibrio cholerae*, usually O1 with CTX toxin
- watery diarrhea (rice water) that rapidly lead to dehydration
- Explosive outbreaks/true pandemics
- Often in the wake of wars ,civil conflicts, climatic events, famine, refugees
- 1.03 billion people at risk (Asia and Africa)
- Estimated 2.86 million cases and 95,000 deaths/year (Ali et al. Plos NTD 2015)
- Treatment: rehydration +++ and antibiotics

History

1st-6th pandemic

Classical

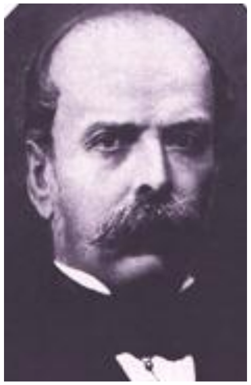
- 1817-1823 : Asia, Middle East, East Africa
- 1829-1851 : Global
- 1852-1859 : Global
- 1863-1879 : Global
- 1881-1896: Global
- 1899-1923 Asia, Middle East, Eastern Europe



R. Koch

1883, Egypt

Vibrio comma



F. Pacini

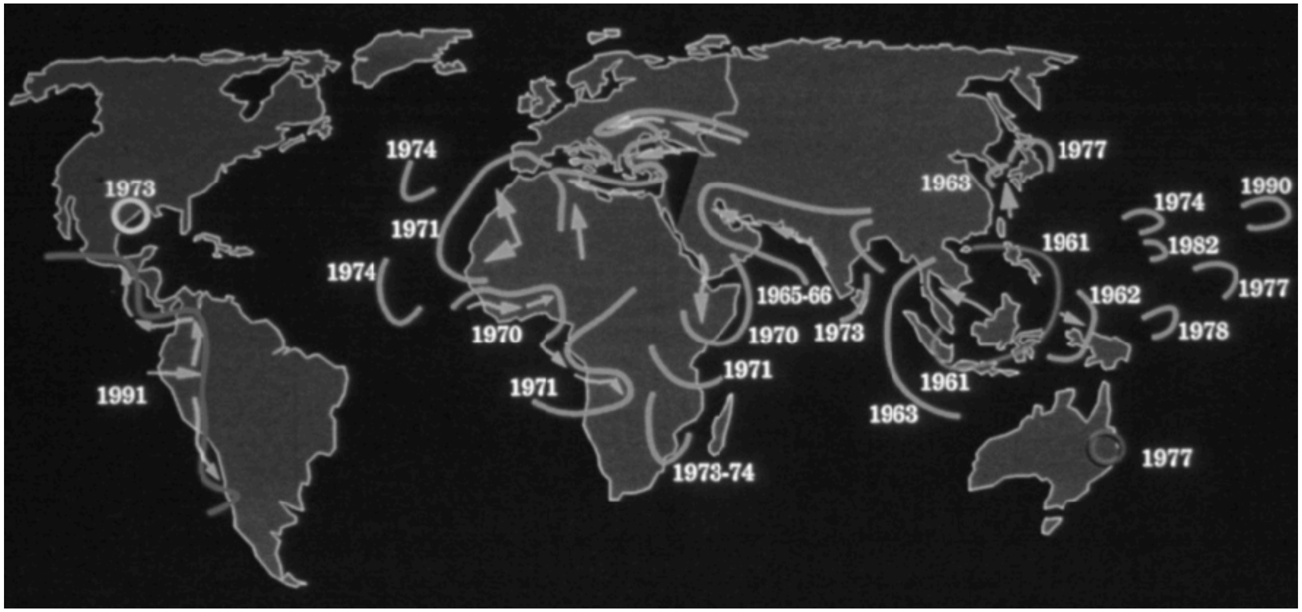
1854, Italy

Vibrio cholera

7th pandemic

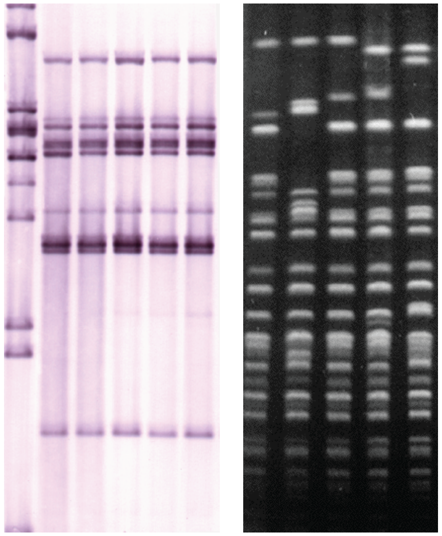
El Tor

1961, Indonesia



Classical laboratory methods

- O antigen serogrouping (O1, O139, O37, …)
- Biotyping (El Tor vs Classical)
- 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 !!!

Vc O1 El Tor (ET) genomes

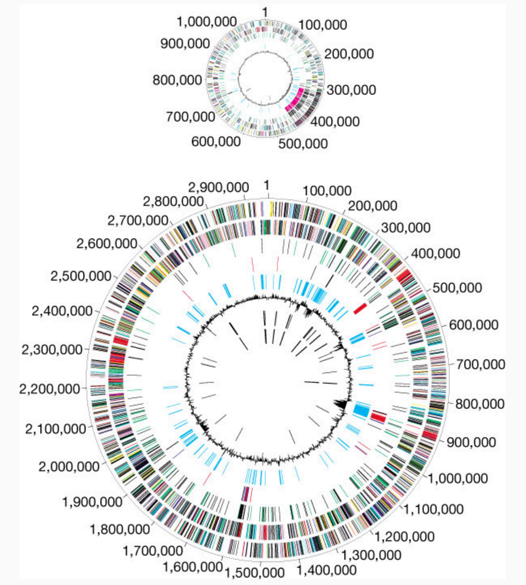
- N16961 (Heidelberg et al., Nature 2005)

    4.03 Mb, 3885 CDSs

    2 circular chromosomes (2.96 Mb, 1.07 Mb)

    CTXΦ phage on CHR1

    Large integron island (125 kb) on CHR2

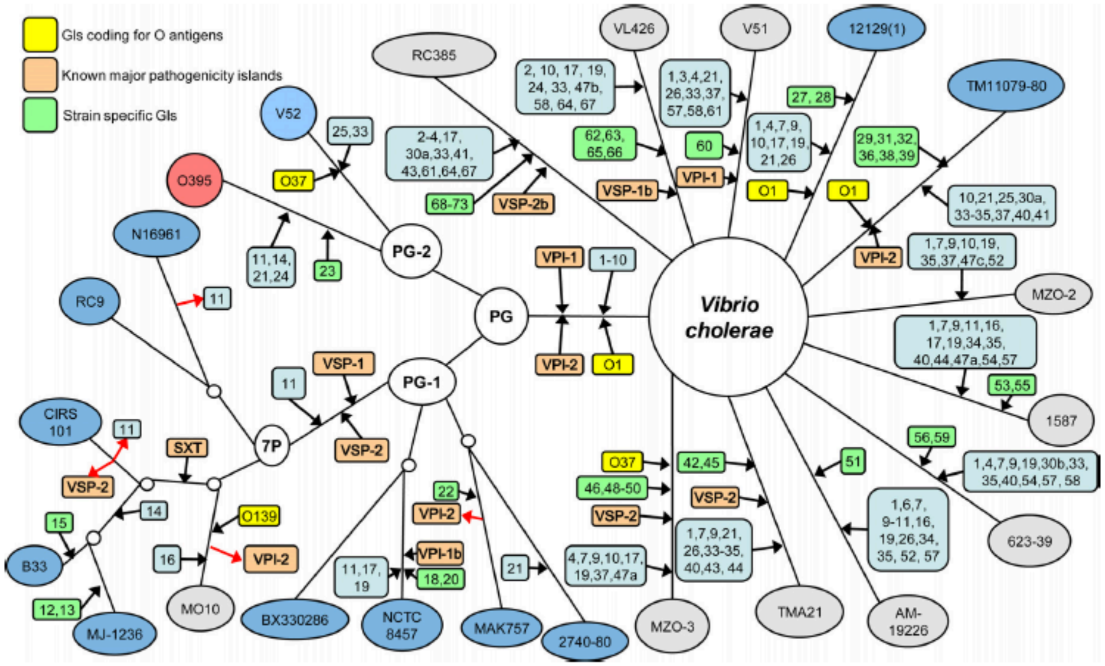


Bangladesh 1975

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

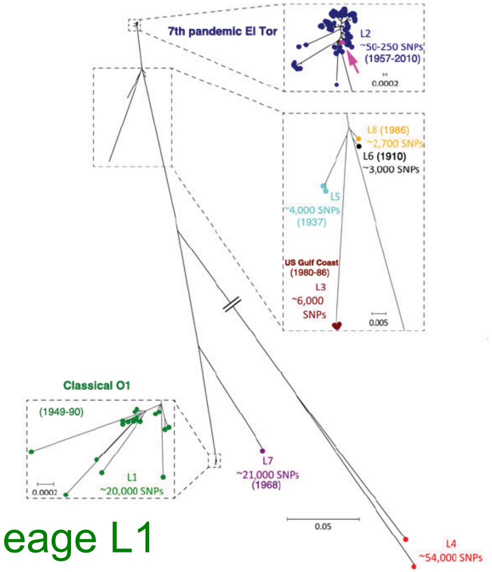
73 GI identified

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

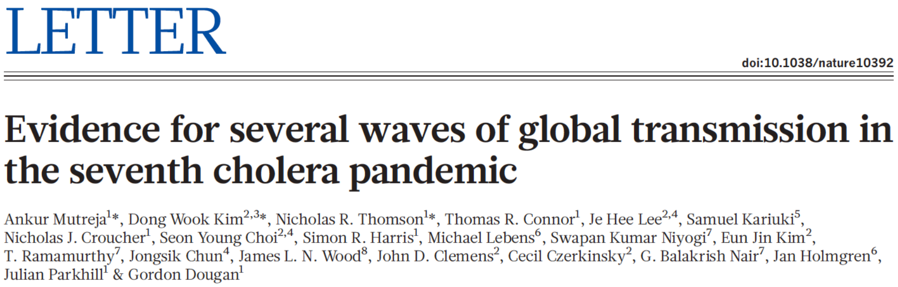


A pioneer genomic study

Lineage L2
El Tor (7th pandemic)



Lineage L1
Classical (1st to 6th pandemic)



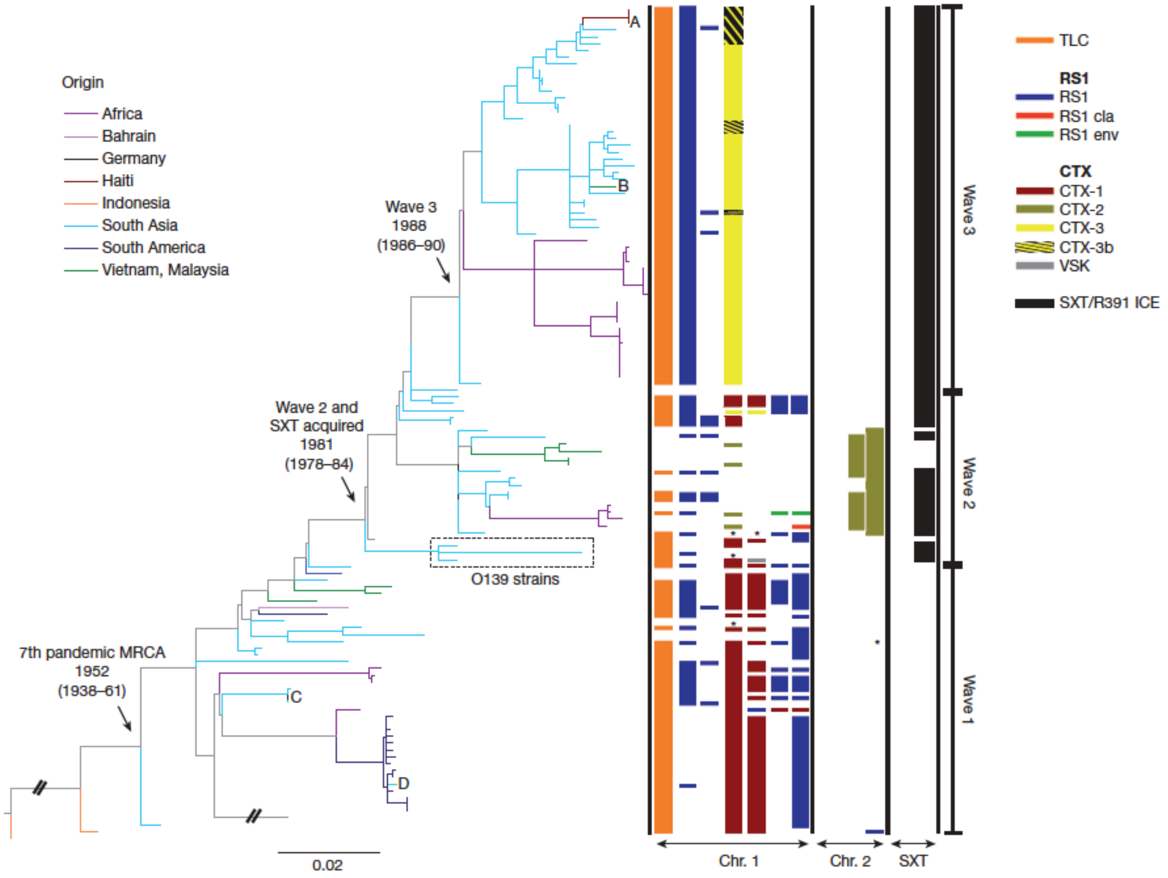
LETTER

doi:10.1038/nature10392

Evidence for several waves of global transmission in the seventh cholera pandemic

Ankur Mutreja[1]*, Dong Wook Kim[2,3]*, Nicholas R. Thomson[1]*, Thomas R. Connor[1], Je Hee Lee[2,4], Samuel Kariuki[5], Nicholas J. Croucher[1], Seon Young Choi[2,4], Simon R. Harris[1], Michael Lebens[6], Swapan Kumar Niyogi[7], Eun Jin Kim[2], T. Ramamurthy[7], Jongsik Chun[4], James L. N. Wood[8], John D. Clemens[2], Cecil Czerkinsky[2], G. Balakrish Nair[7], Jan Holmgren[6], Julian Parkhill[1] & Gordon Dougan[1]

136 global isolates sequenced including 123 O1 ET



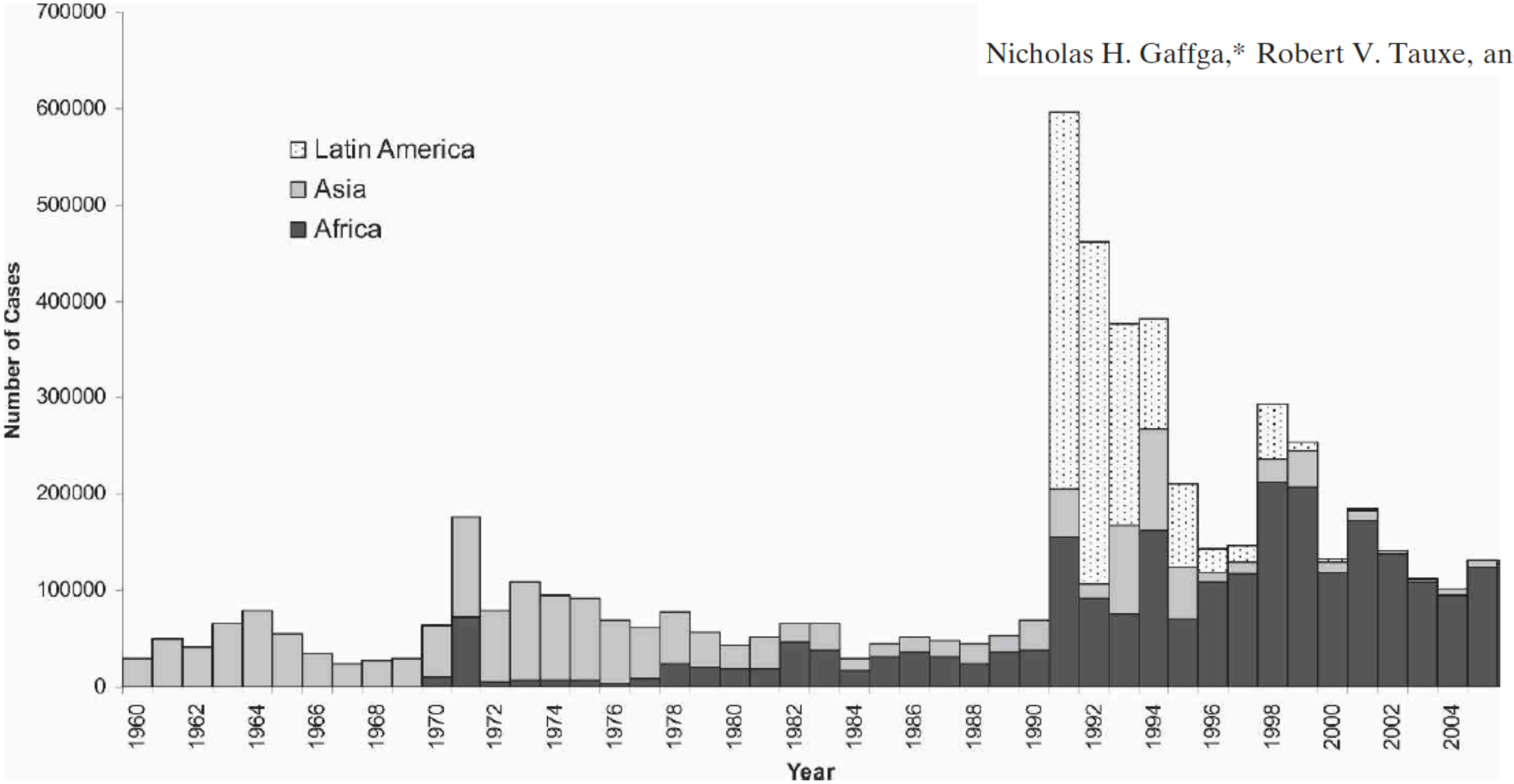
- 3 waves
- Role of the Bay of Bengal
- Identification of intercontinental transmission events

Why a genomic study on cholera in Africa ?

Am. J. Trop. Med. Hyg., 77(4), 2007, pp. 705–713

Cholera: A New Homeland in Africa?

Nicholas H. Gaffga,* Robert V. Tauxe, and Eric D. Mintz



- 2005 : incidence in sub-Saharan Africa was 95 and 16,600 times higher than that in Asia and Latin America, respectively
- 2005 : CFR in sub-Saharan Africa (1.8%) was 3 times higher than that in Asia (0.6%); no cholera deaths were reported in Latin America.
- 2007-2012, >95,000 cases/y notified by >20 countries in Africa

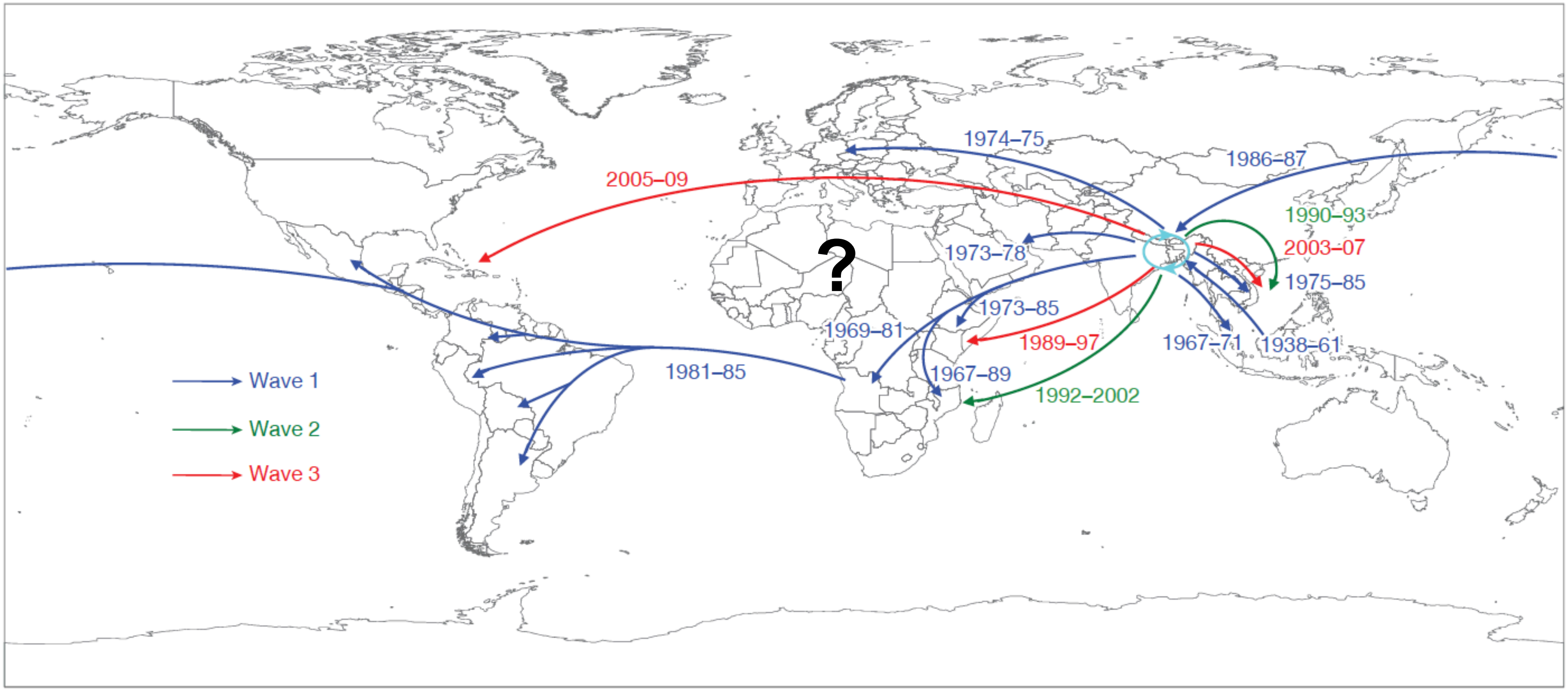


Figure 2 | Transmission events inferred for the seventh-pandemic phylogenetic tree, drawn on a global map. The date ranges shown for transmission events are taken from the BEAST analysis, and represent the median values for the MRCA of the transmitted strains (later bound), and the MRCA of the transmitted strains and their closest relative from the source location (earlier bound).

28 African isolates studied but all but one from East Africa :

Angola 1989 (n=1); Djibouti 2007 (n=3); Kenya 2005-2007 (n=17); Mozambique 1991, 2005 (n=6); Tanzania 2009 (n=1).

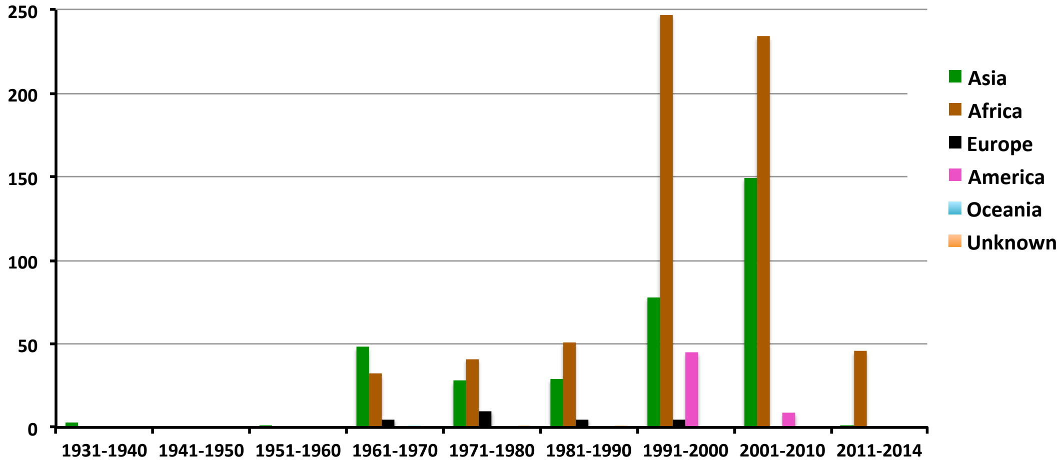
Objectives of our study

- Identify the introduction and transmission routes of *V. cholerae* O1 ET in Africa, 1970-2014
- 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)



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 estimatation of the divergence times of the different lineages/strains (RAxML after Gubbins, Path-O-Gen, BEAST)

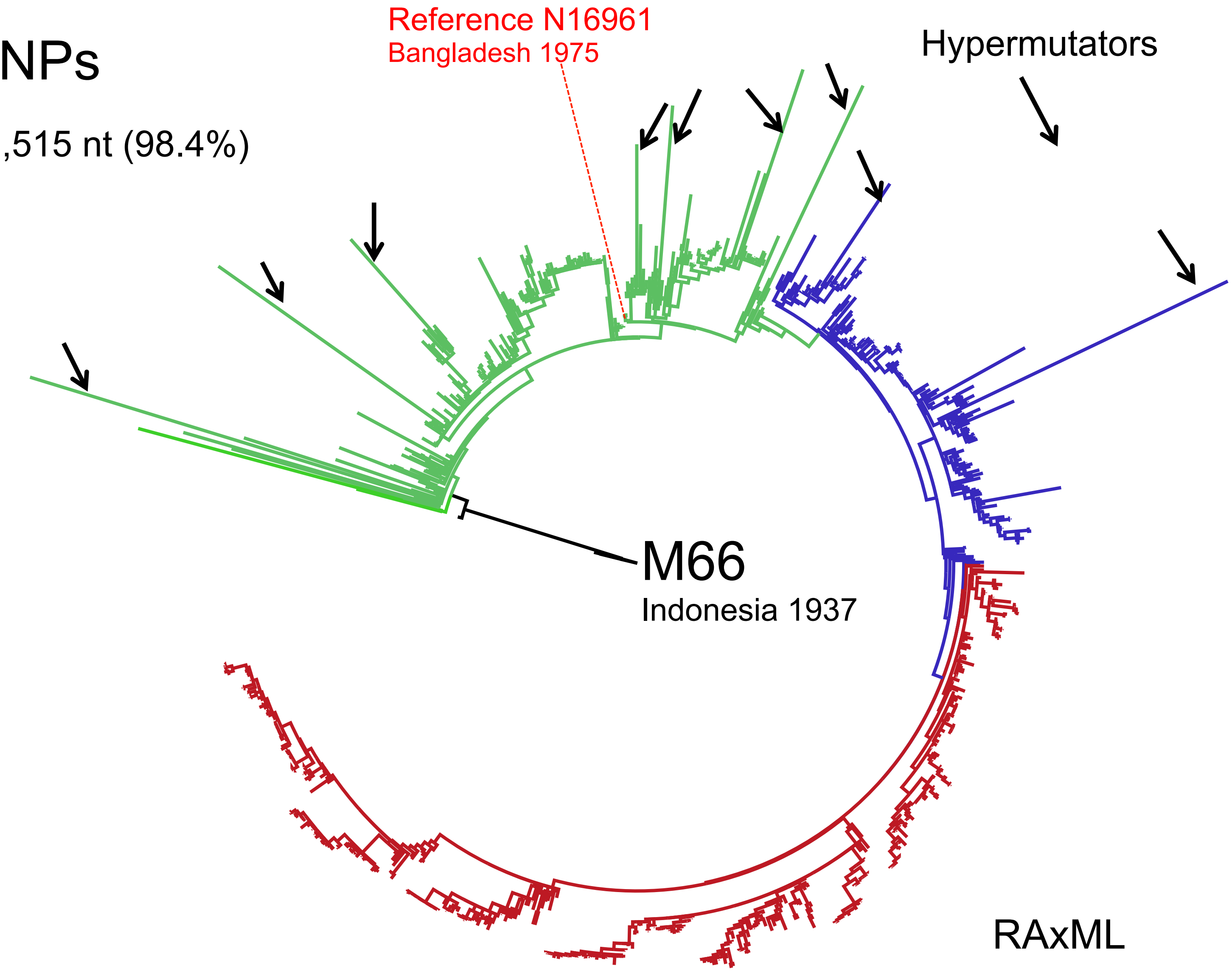
5. Extraction of antibiotic resistance genes and their genetic support (Resfinder on read assemblies, PacBio sequencing)

Maximum Likelihood phylogeny

1070 *Vibrio cholerae*, 1937-2014

9,300 SNPs

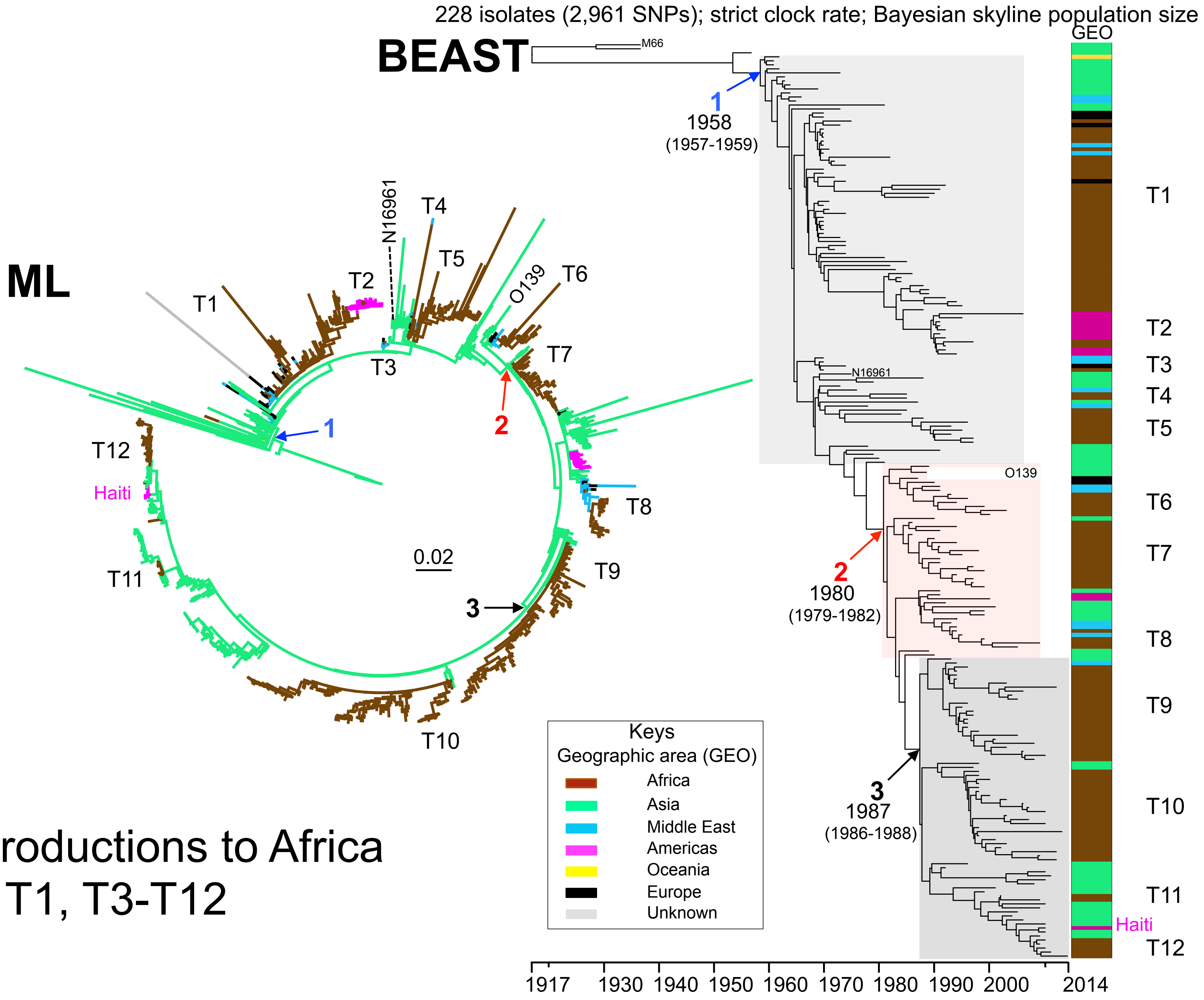
Analysis on 3,970,515 nt (98.4%)



Wave 1

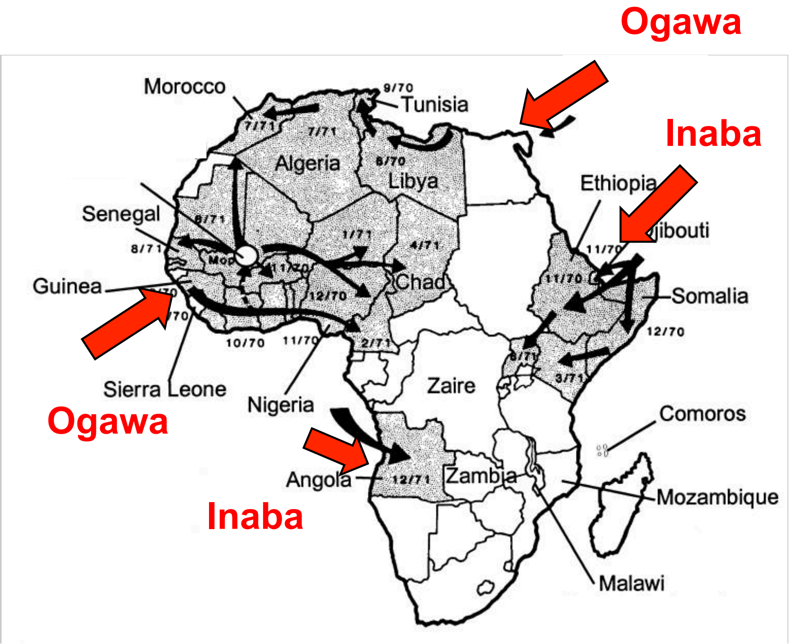
Wave 2

Wave 3

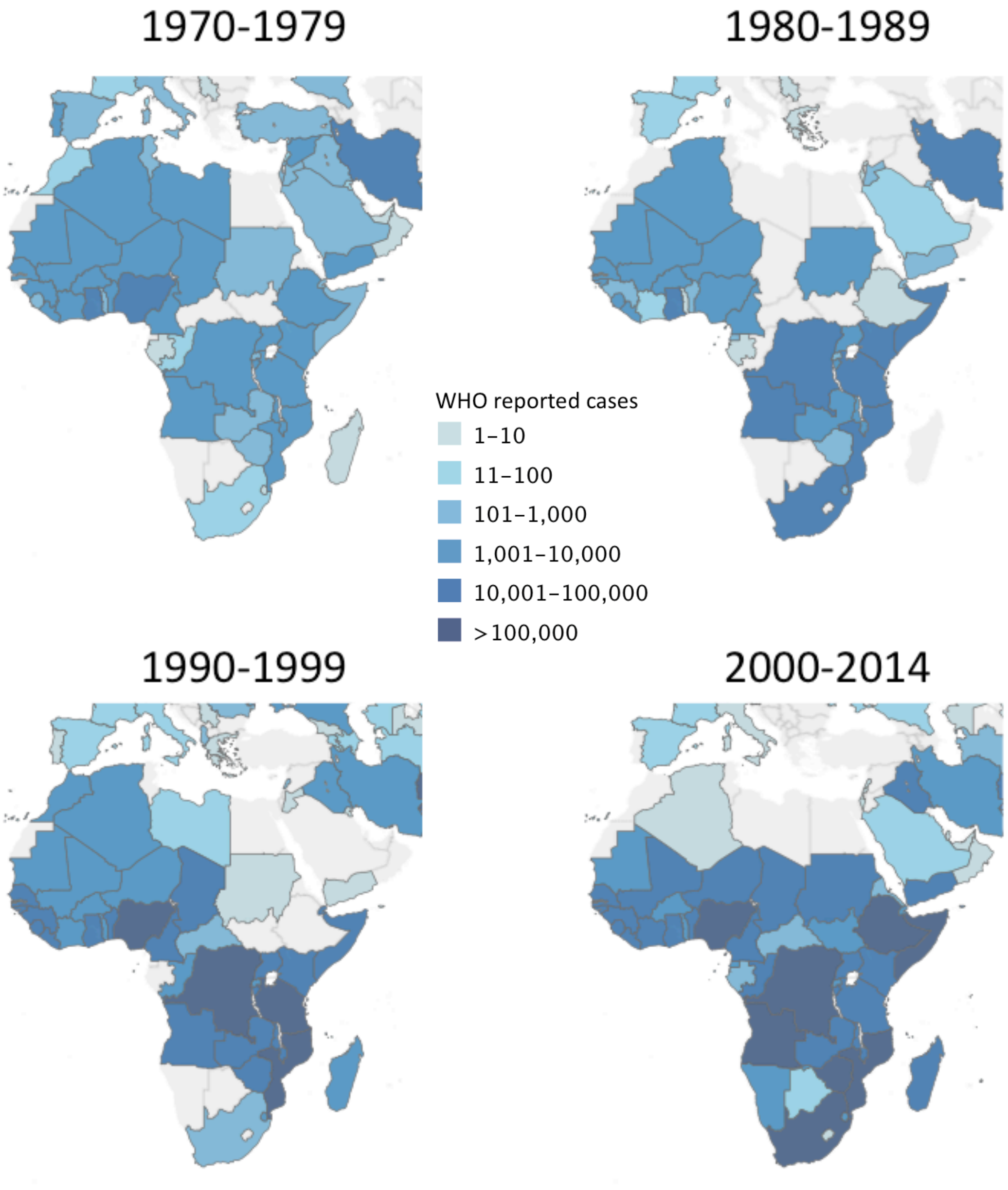


11 introductions to Africa
T1, T3-T12

1970



World Health Organization (WHO)



Guinea 1970, origin ?

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

Angola 1971 origin ?

-Portugal
West Africa

Africa, 1970-2014

11 introductions to Africa

Guinea 70 ←Middle East

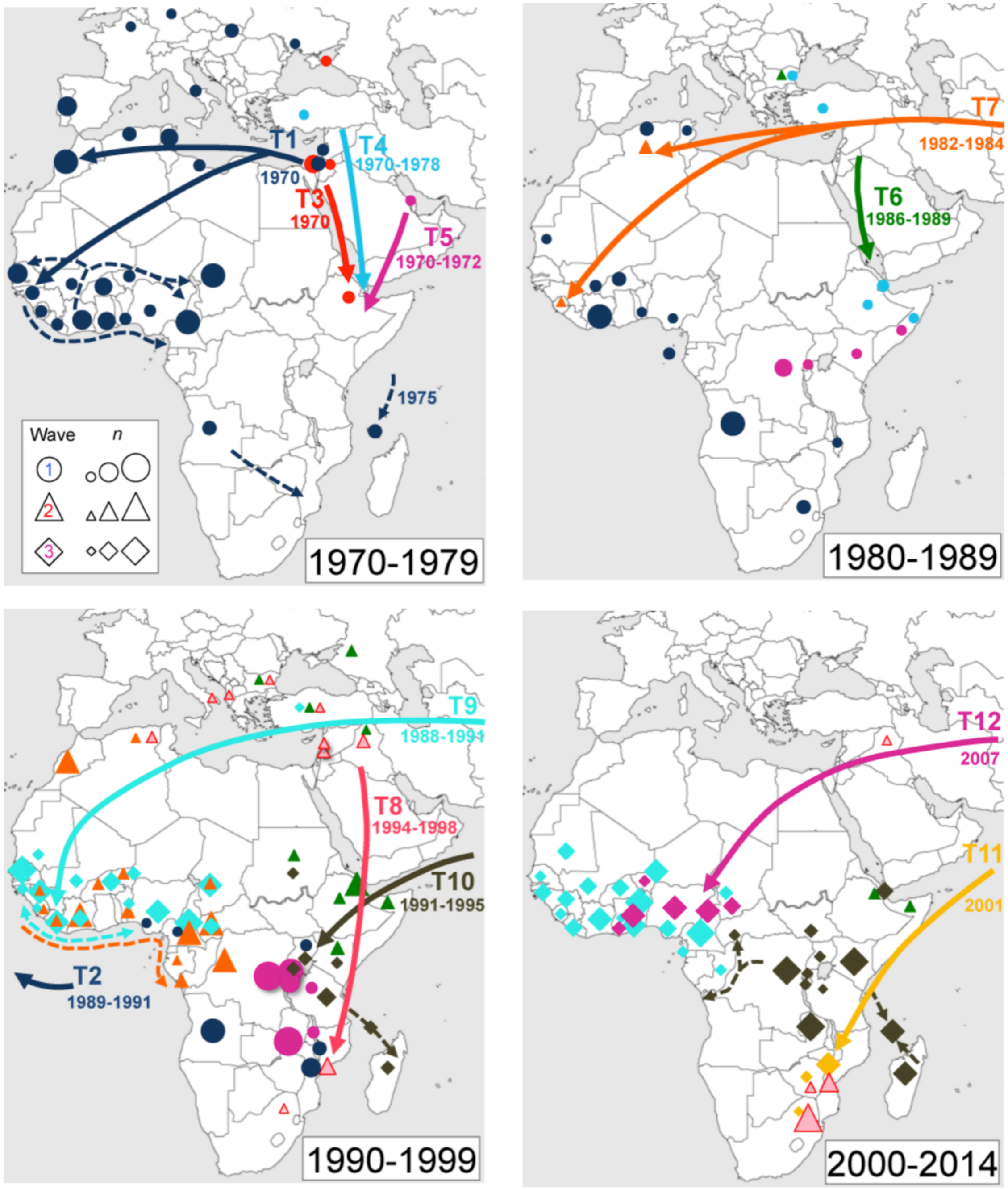
Angola 71 ←West Africa

Five introductions to West Africa and six to East Africa

Middle East acting as a springboard during six introductions.

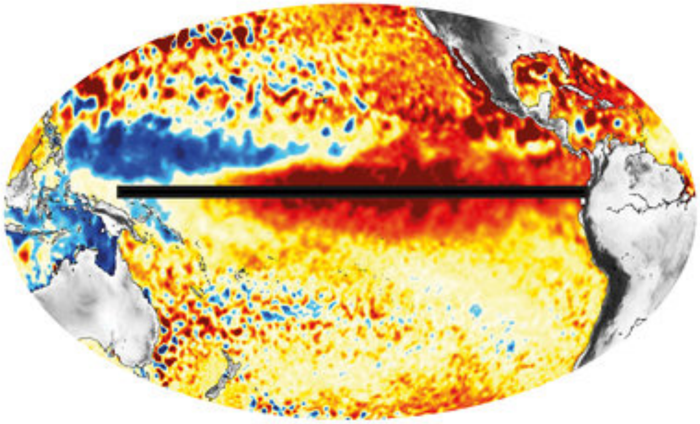
Followed by up to 28 years of regional circulation

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



Introduction of the cholera in America in 1991

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

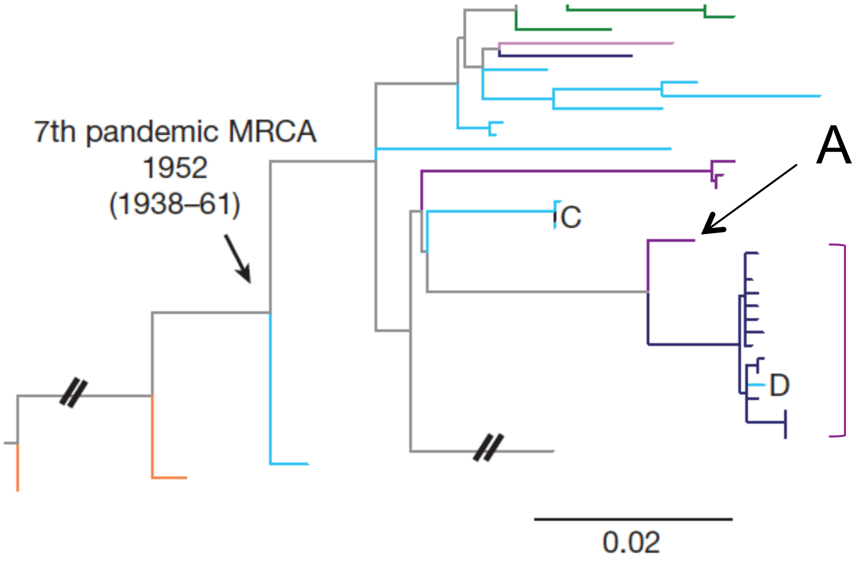


Martinez-Urtana et al. Nat Microbiol 2016

Different hypotheses:

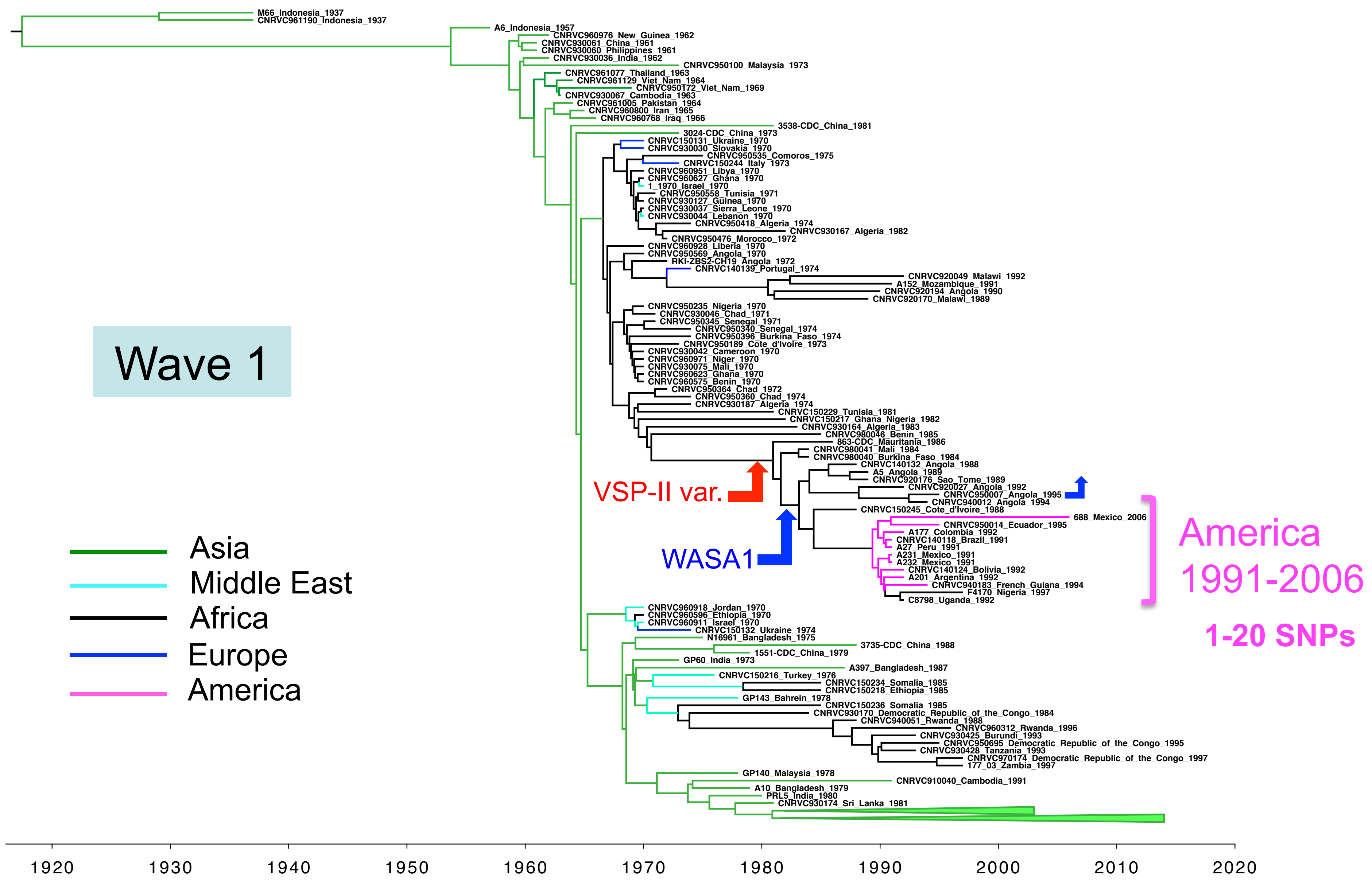
-a ship from China
-El Niño

West African origin ?

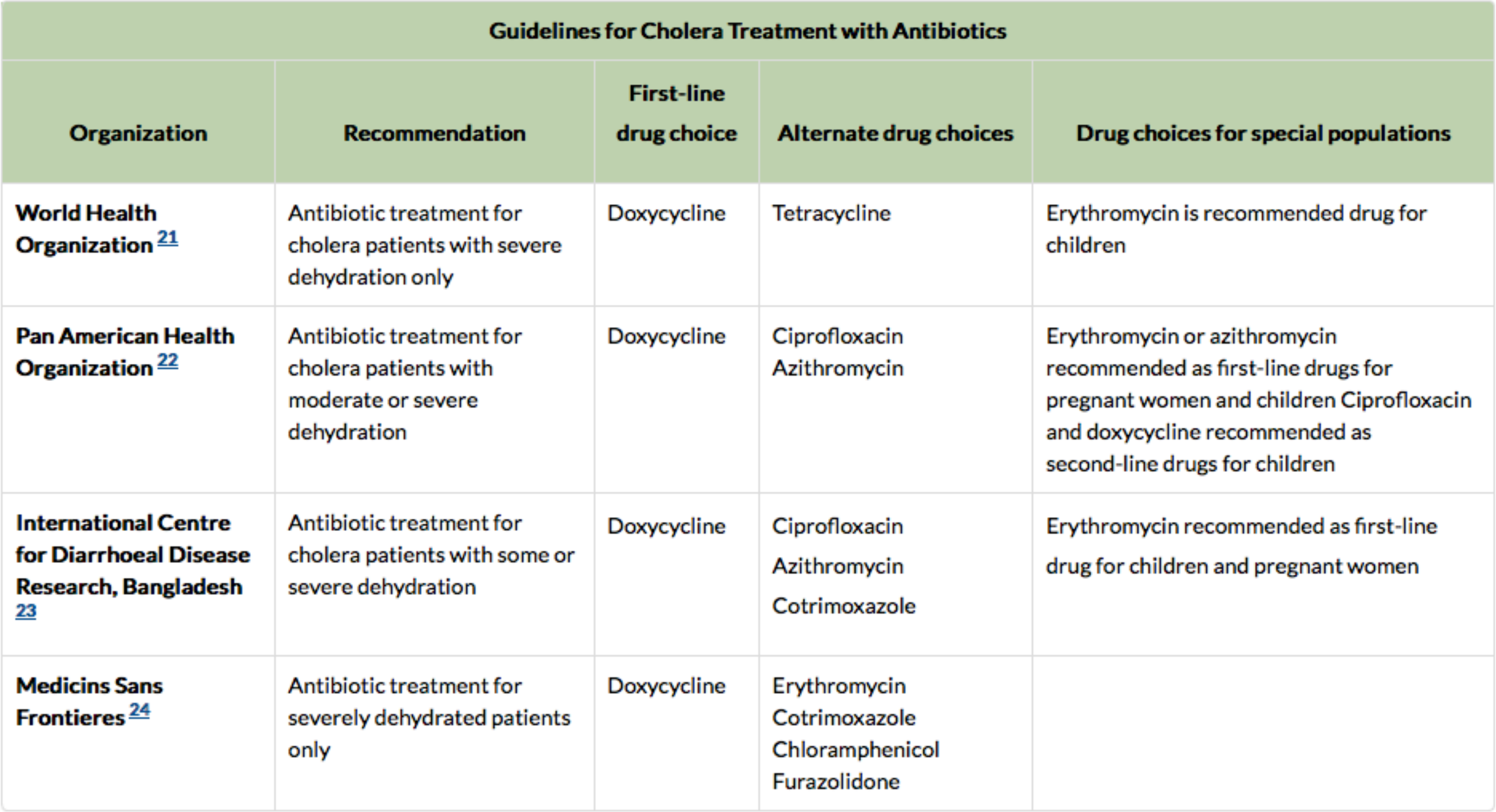


A5, Angola 1989

Latin America



The cholera was introduced to Peru from West Africa in 1991



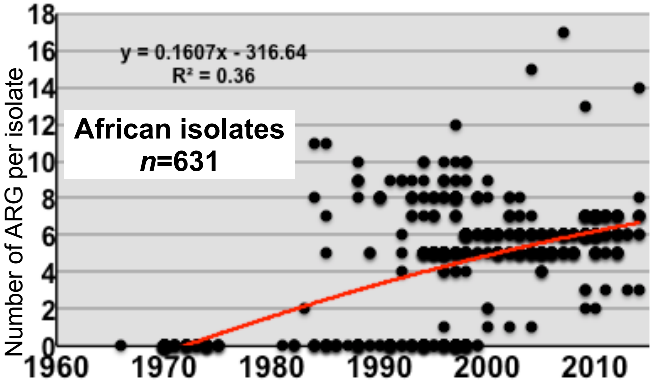
Guidelines for Cholera Treatment with Antibiotics

Organization	Recommendation	First-line drug choice	Alternate drug choices	Drug choices for special populations
World Health Organization [21]	Antibiotic treatment for cholera patients with severe dehydration only	Doxycycline	Tetracycline	Erythromycin is recommended drug for children
Pan American Health Organization [22]	Antibiotic treatment for cholera patients with moderate or severe dehydration	Doxycycline	Ciprofloxacin Azithromycin	Erythromycin or azithromycin recommended as first-line drugs for pregnant women and children Ciprofloxacin and doxycycline recommended as second-line drugs for children
International Centre for Diarrhoeal Disease Research, Bangladesh [23]	Antibiotic treatment for cholera patients with some or severe dehydration	Doxycycline	Ciprofloxacin Azithromycin Cotrimoxazole	Erythromycin recommended as first-line drug for children and pregnant women
Medicins Sans Frontieres [24]	Antibiotic treatment for severely dehydrated patients only	Doxycycline	Erythromycin Cotrimoxazole Chloramphenicol Furazolidone	

http://www.cdc.gov/

Antibiotic resistance

ARG, antibiotic resistance gene



Africa

1970-1984

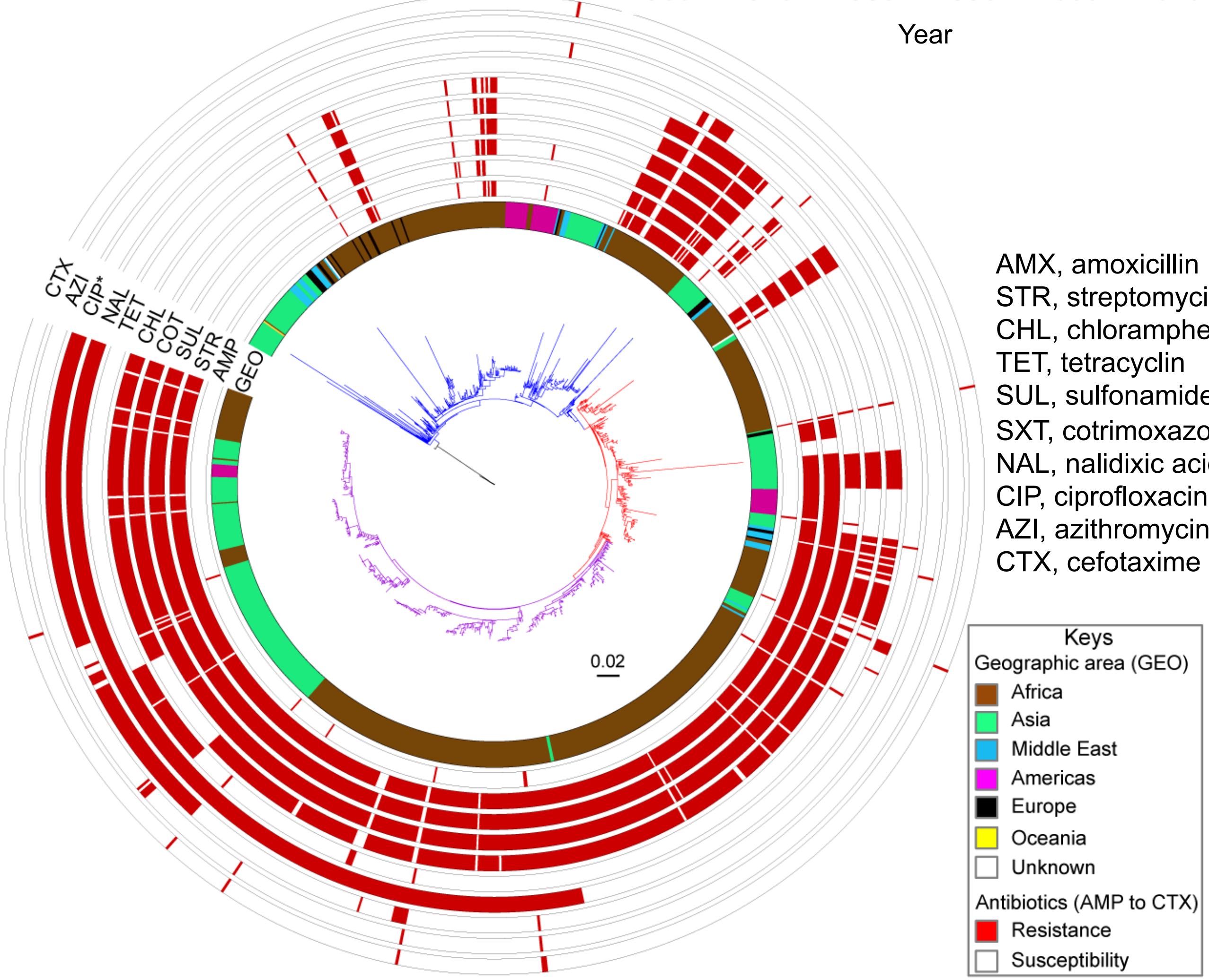
96.3% isolates wild-type (77/80)
0.2 ARG/isolate (0-11)

1985-1999

37.2% isolates wild-type (100/269)
4 ARG/isolate (0-12)

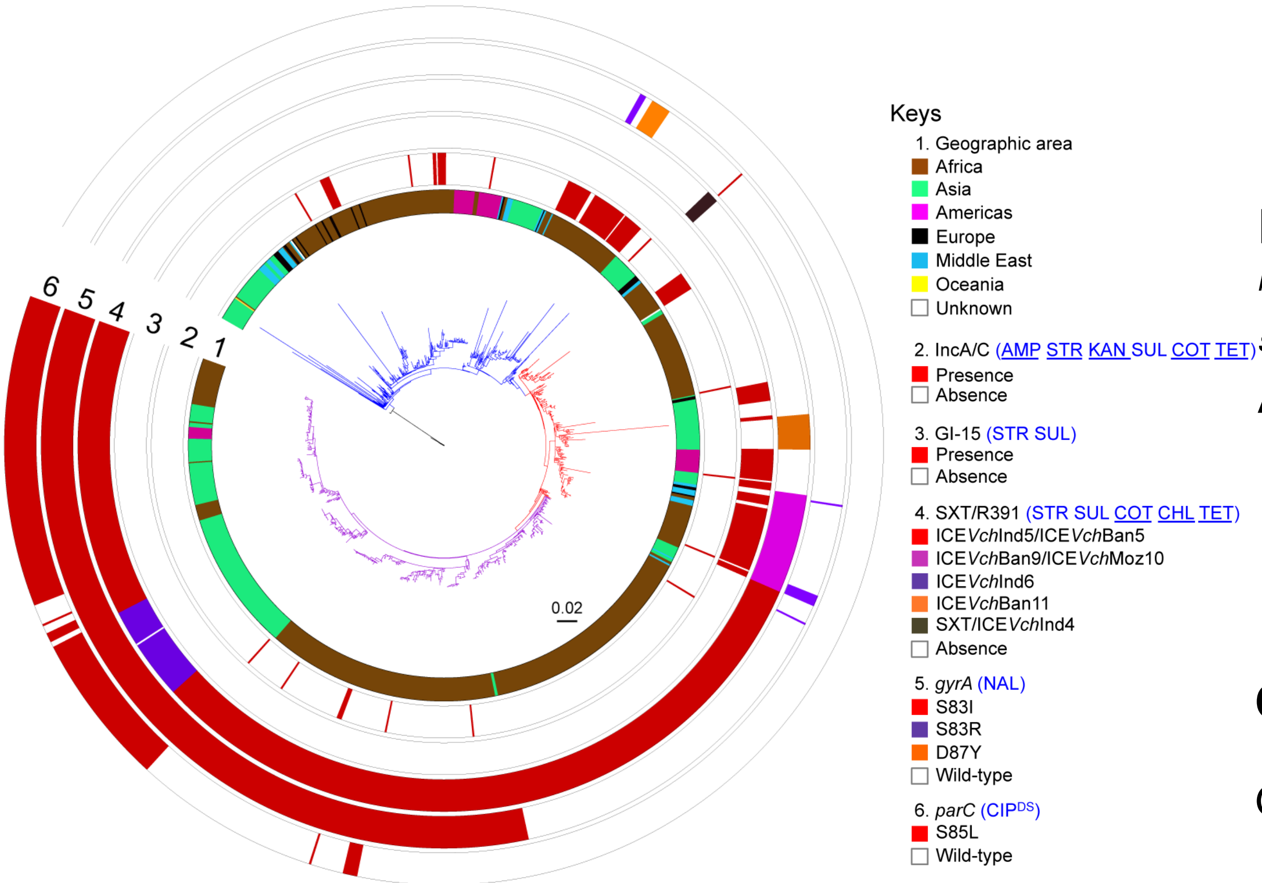
2000-2014

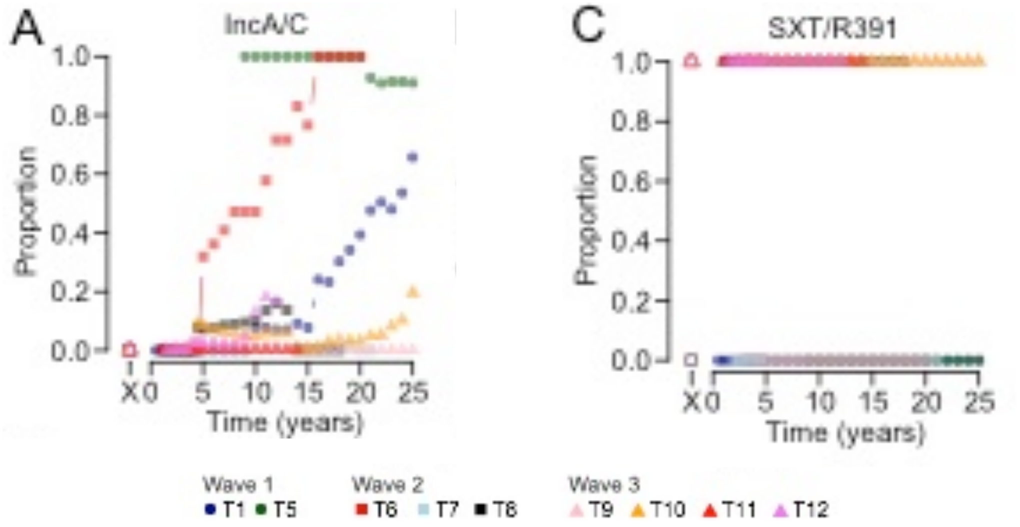
0% isolates wild-type (0/281)
5.9 ARG/isolate (1-17)



AMX, amoxicillin
STR, streptomycin
CHL, chloramphenicol
TET, tetracyclin
SUL, sulfonamides
SXT, cotrimoxazole
NAL, nalidixic acid
CIP, ciprofloxacin
AZI, azithromycin
CTX, cefotaxime

Only 16.6% (105/631) of the African isolates are inferred to be resistant to tetracyclins





Phase 1 (1970s-1980s)

Large incA/C plasmids

bla strAB aad aph(3')-I cat1 tetB tetC

sul1 sul2 dfrA15 dfrA15

Acquired in Africa

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*) (q*nrVC1*) (d*frA31*) 100kb

gyrA and ***parC*** mutations

Acquired in South Asia

Conclusions

- Iterative introductions of cholera to Africa from Asia (arguing against an environmental reservoir of *Vibrio cholerae* O1)
- 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
- Confirmation of the West African origin of the South American outbreak of 1991
- Accumulation of chromosomally-encoded multidrug resistance elements, as observed previously with human pathogens *Shigella dysenteriae* type 1 and *Salmonella* Typhi H58

Collaborators

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Thank you for your attention !