Institut Pasteur

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

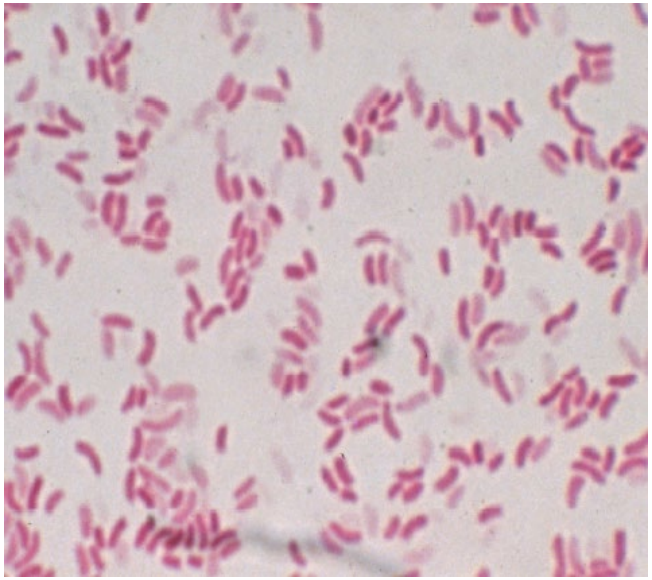
François-Xavier WEILL, MD, PhD
Enteric Bacterial Pathogens Unit
Infection & Epidemiology Department

Foodborne pathogens & whole genome sequencing

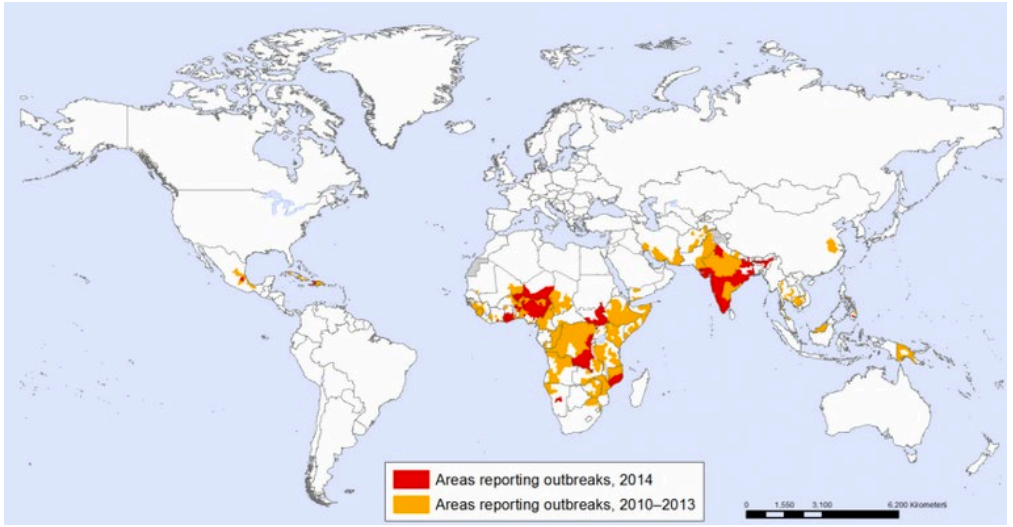
26-28 March 2019

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



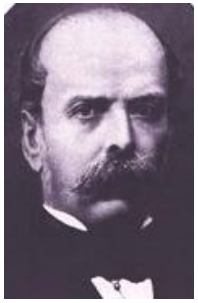
•Estimated 2.86 million cases and 95,000 deaths/year (Ali et al. Plos NTD 2015)

•Treatment: **rehydration** and antibiotics

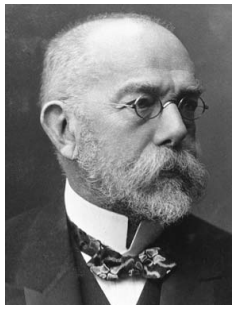
History

1st-6th pandemic

***Vibrio cholerae* O1 biotype Classical**



F. Pacini
1854, Italy
Vibrio cholera



R. Koch
1883, Egypt
Vibrio comma

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 ✔

Devault et al. N Eng J Med 2014

Philadelphia,
1849

✔ confirmed

7th pandemic

Vibrio cholerae O1 biotype El Tor (7PET)

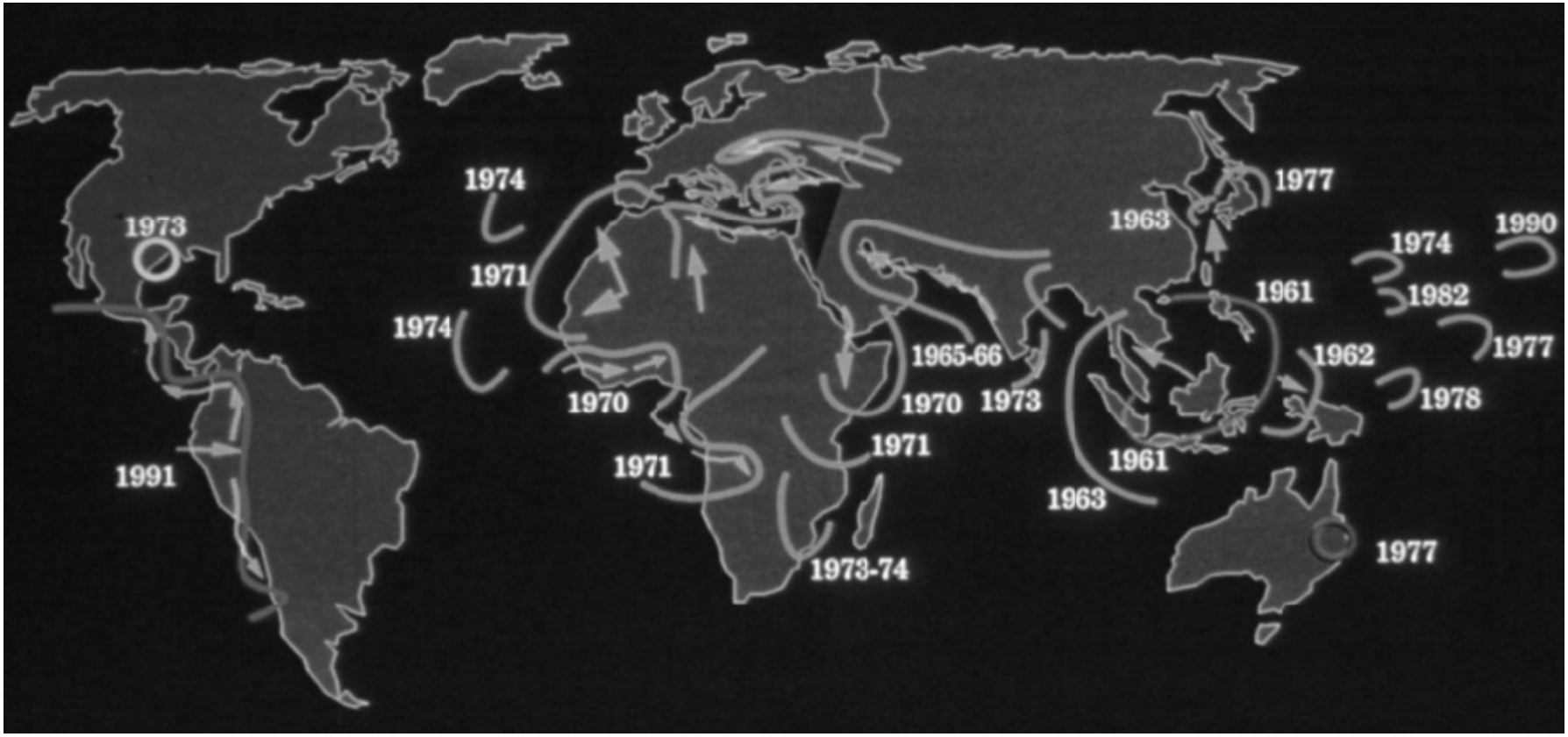
1961, Indonesia

« Paracholera »

Test	Classical	El Tor
Haemolysis	-	+
Voges-Proskauer	-	+
Chick red cell agglutination	-	+
Phage IV sensitivity	+	-
Polymyxin B sensitivity	+	-

Proportion of		
moderate to severe cases	36%	6%
asymptomatic cases.	59%	75%

Kaper et al., CMR,1995



The environmental theory

APPLIED AND ENVIRONMENTAL MICROBIOLOGY, Feb. 1981, p. 555–558
0099-2240/81/020555-04$02.00/0

Vol. 41, No. 2

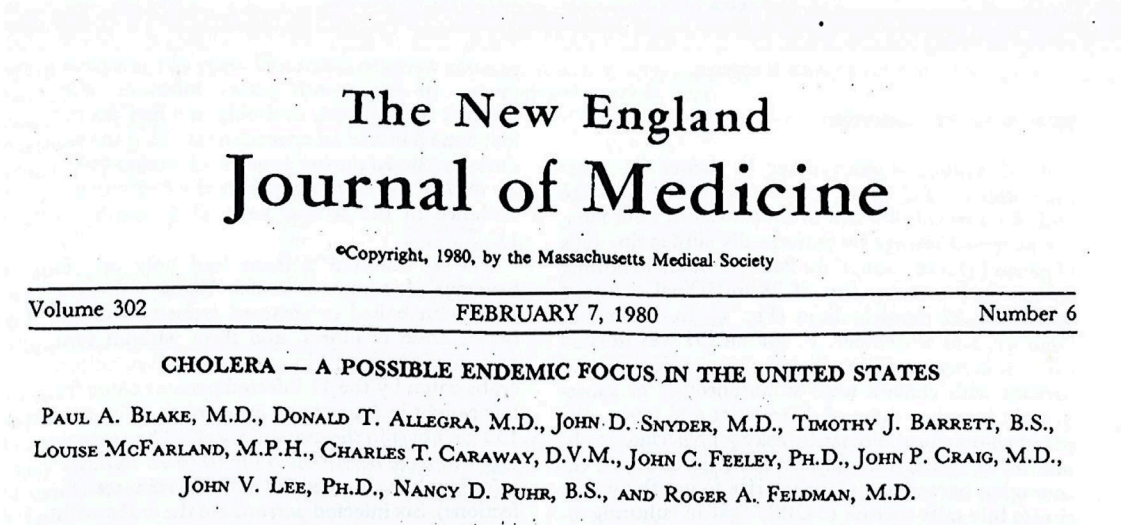
Occurrence of *Vibrio cholerae* Serotype O1 in Maryland and Louisiana Estuaries

RITA R. COLWELL,[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,[5] AND ANWARUL HUQ[5]

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

No of strains	Source	ELISA[a, b]
7	Water	2/6
1	Crab	0/1
25	Sewer or canal	12/16

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.



The New England
Journal of Medicine

Volume 302 FEBRUARY 7, 1980 Number 6

CHOLERA — A POSSIBLE ENDEMIC FOCUS IN THE UNITED STATES

PAUL A. BLAKE, M.D., DONALD T. ALLEGRA, M.D., JOHN D. SNYDER, M.D., TIMOTHY J. BARRETT, B.S., LOUISE MCFARLAND, M.P.H., CHARLES T. CARAWAY, D.V.M., JOHN C. FEELEY, PH.D., JOHN P. CRAIG, M.D., JOHN V. LEE, PH.D., NANCY D. PUHR, B.S., AND ROGER A. FELDMAN, M.D.

- 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

CLINICAL MICROBIOLOGY REVIEWS, Oct. 2002, p. 757–770 Vol. 15, No. 4
0893-8512/02/$04.00+0 DOI: 10.1128/CMR.15.4.757–770.2002

Effects of Global Climate on Infectious Disease: the Cholera Model

Erin K. Lipp,[1,2] Anwar Huq,[1,3] and Rita R. Colwell[1,3]*

ASSOCIATION AFFAIRS

Global Climate and Infectious Disease: The Cholera Paradigm*

Rita R. Colwell

SCIENCE • VOL. 274 • 20 DECEMBER 1996

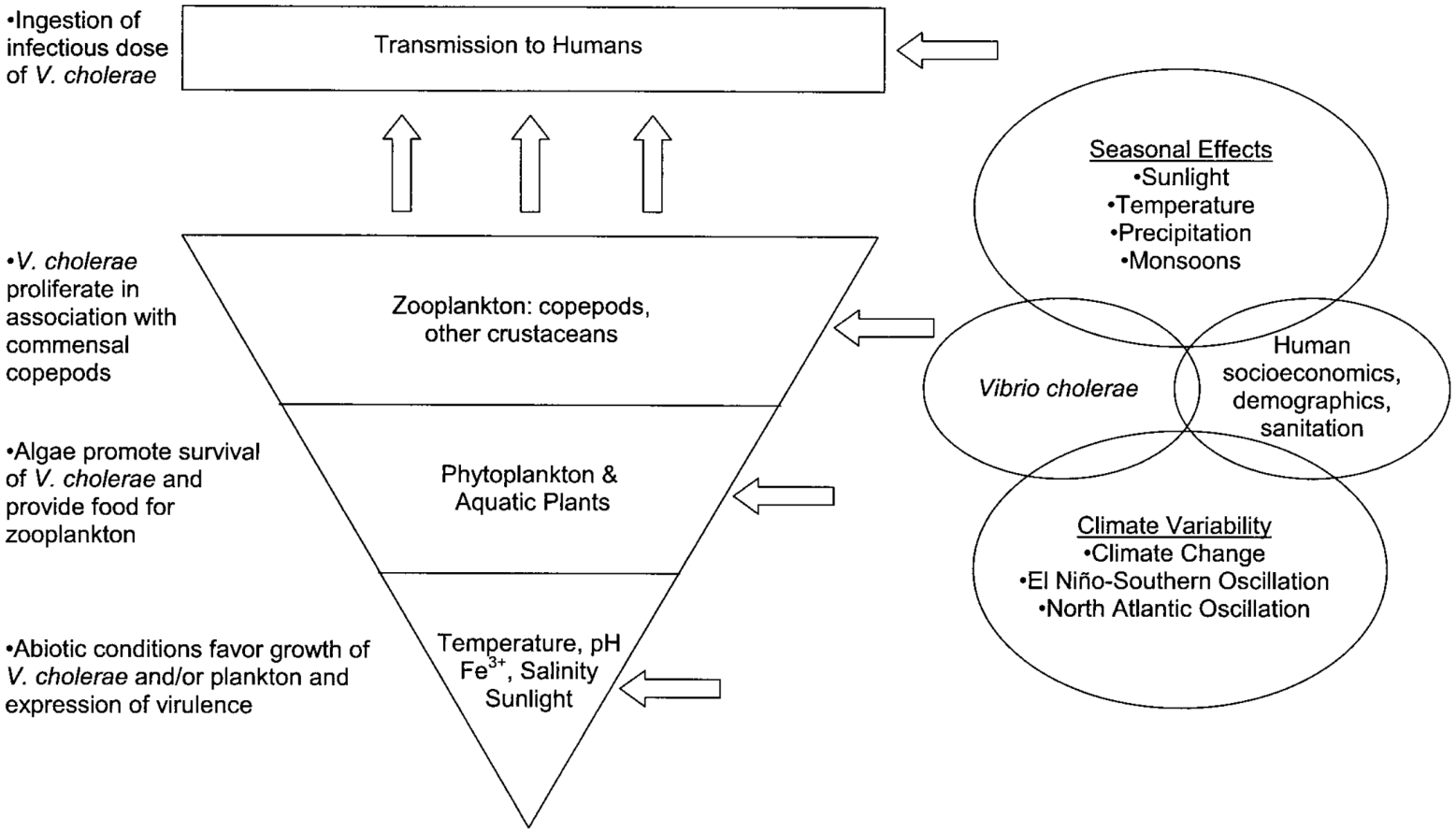
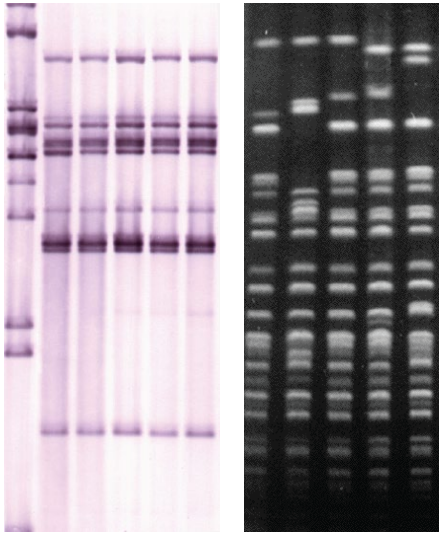


FIG. 1. Hierarchical model for environmental cholera transmission (modified from Colwell and Huq [30]).

Classical laboratory methods for 7PET *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)

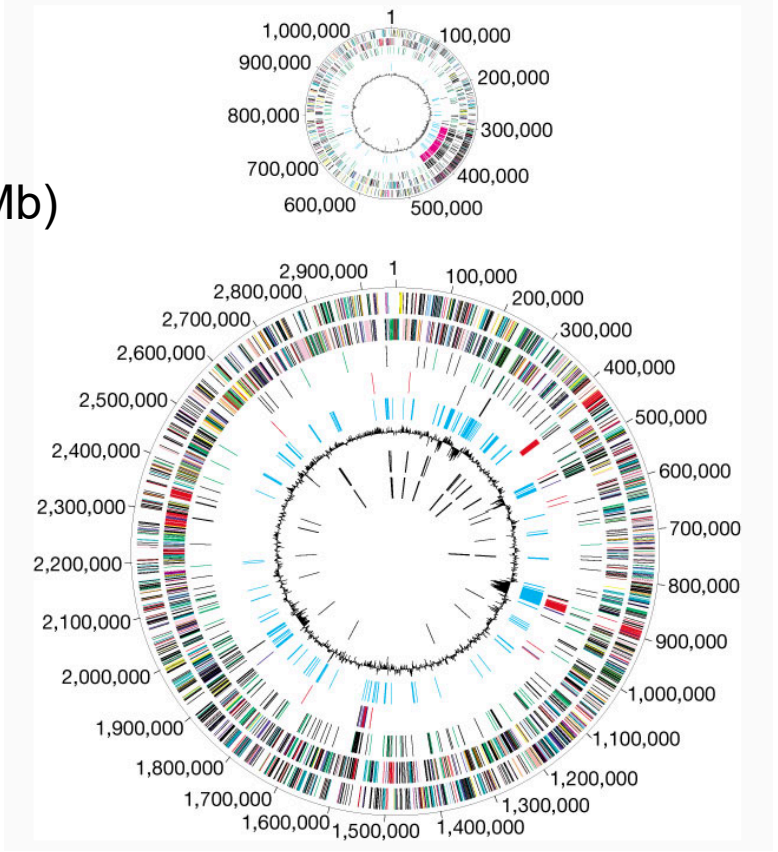


Confusion !!!

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

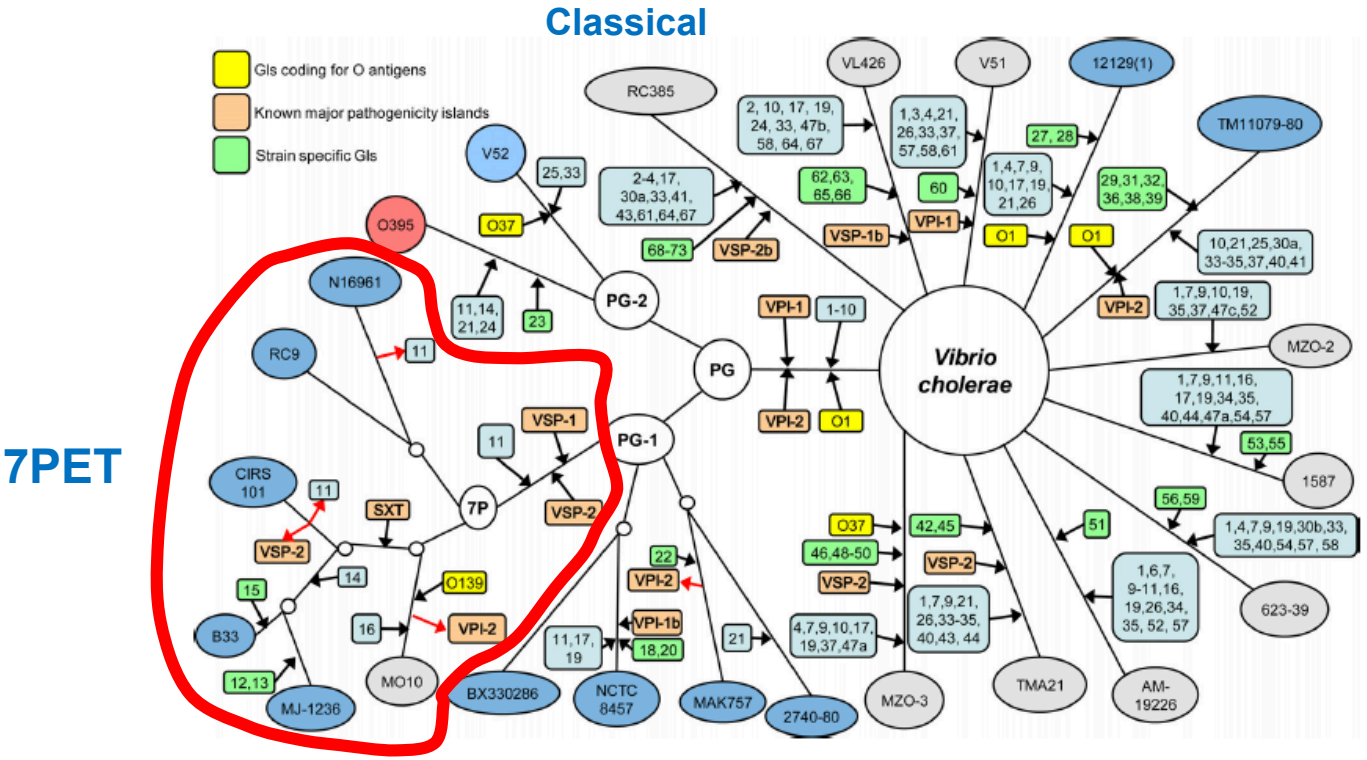
22 other genomes, including 12 O1 ET (Chun et al. PNAS 2009)

73 GI identified

- 7P isolates contain VSP-1

and VSP-2

- CTXΦ can be carried on CHR2



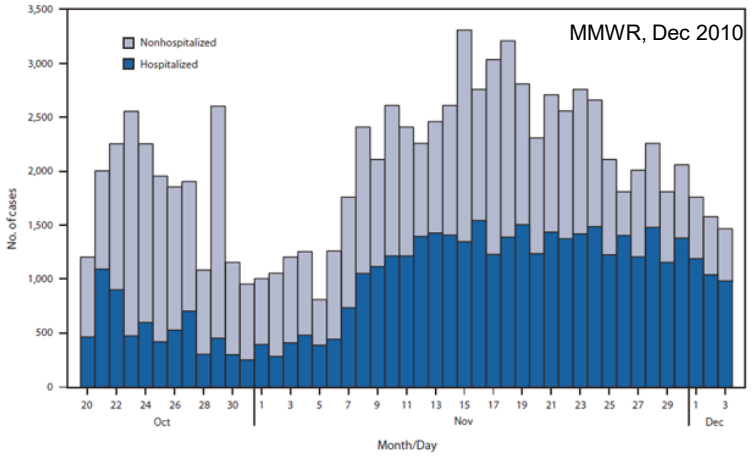
Haiti, 2010

January 2011

The NEW ENGLAND JOURNAL of MEDICINE

ORIGINAL ARTICLE

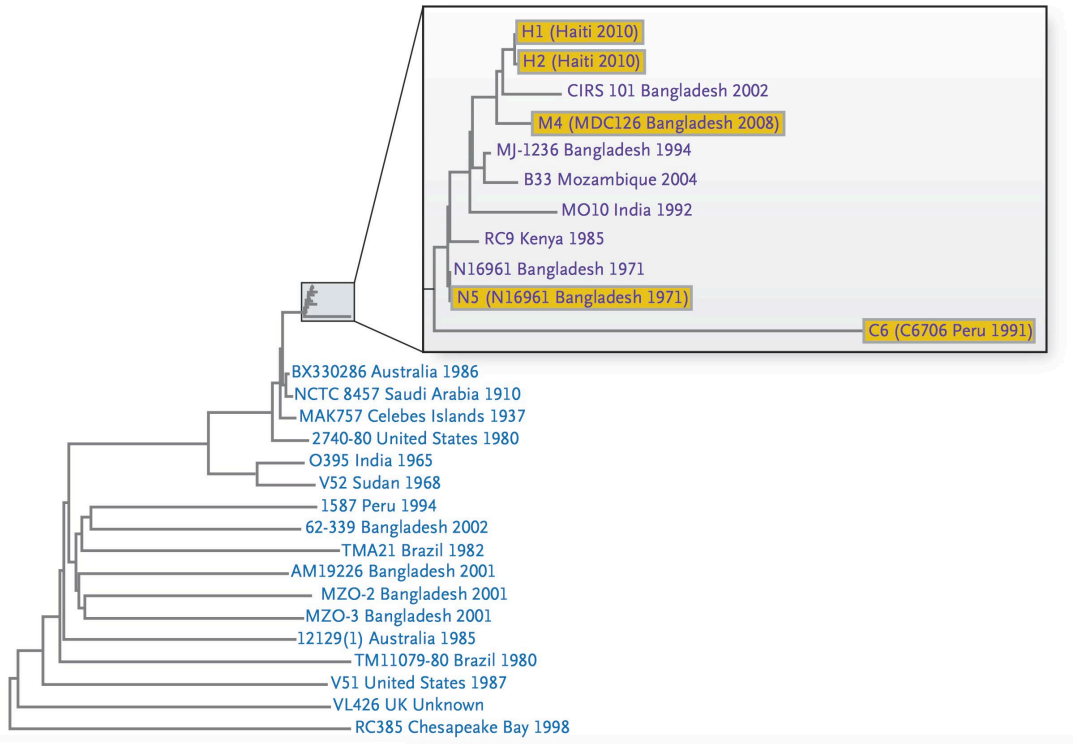
- January 2010, devastating earthquake
- October 2010, first cholera case



- 2017 (one million cases, 10,000 deaths)

The Origin of the Haitian Cholera Outbreak Strain

Chen-Shan Chin, Ph.D., Jon Sorenson, Ph.D., Jason B. Harris, M.D., William P. Robins, Ph.D., Richelle C. Charles, M.D., Roger R. Jean-Charles, M.D., James Bullard, Ph.D., Dale R. Webster, Ph.D., Andrew Kasarskis, Ph.D., Paul Peluso, Ph.D., Ellen E. Paxinos, Ph.D., Yoshiharu Yamaichi, Ph.D., Stephen B. Calderwood, M.D., John J. Mekalanos, Ph.D., Eric E. Schadt, Ph.D., and Matthew K. Waldor, M.D., Ph.D.



CONCLUSIONS

The Haitian epidemic is probably the result of the introduction, through human activity, of a *V. cholerae* strain from a distant geographic source.

August 2011

mBio mbio.asm.org

Population Genetics of *Vibrio cholerae* from Nepal in 2010: Evidence on the Origin of the Haitian Outbreak

Rene S. Hendriksen,[a] Lance B. Price,[b] James M. Schupp,[b] John D. Gillece,[b] Rolf S. Kaas,[a] David M. Engelthaler,[b] Valeria Bortolaia,[a] Talima Pearson,[c] Andrew E. Waters,[b] Bishnu Prasad Upadhyay,[d] Sirjana Devi Shrestha,[d] Shailaja Adhikari,[d] Geeta Shakya,[d] Paul S. Keim,[b,c] and Frank M. Aarestrup[a]

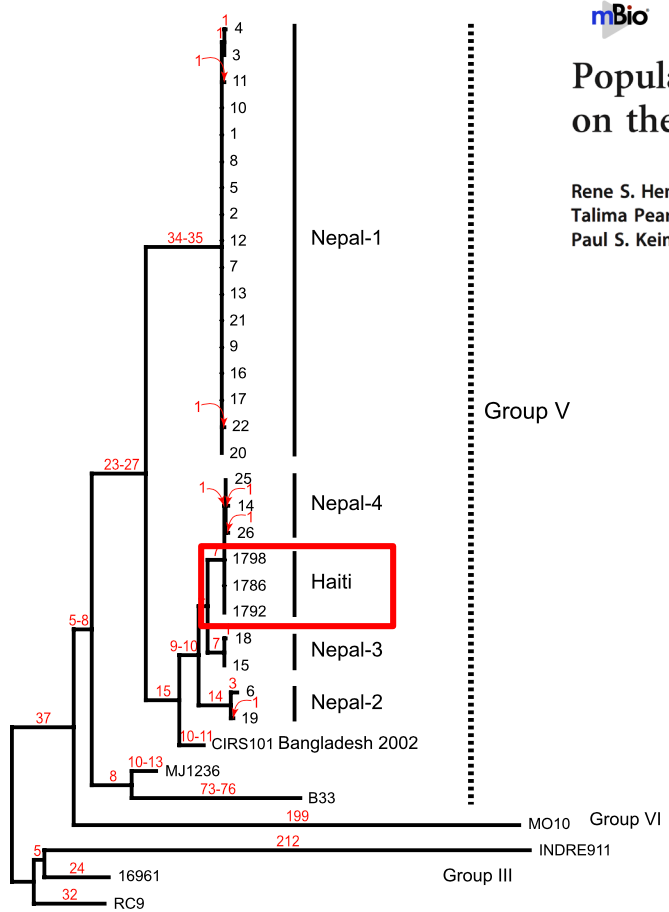


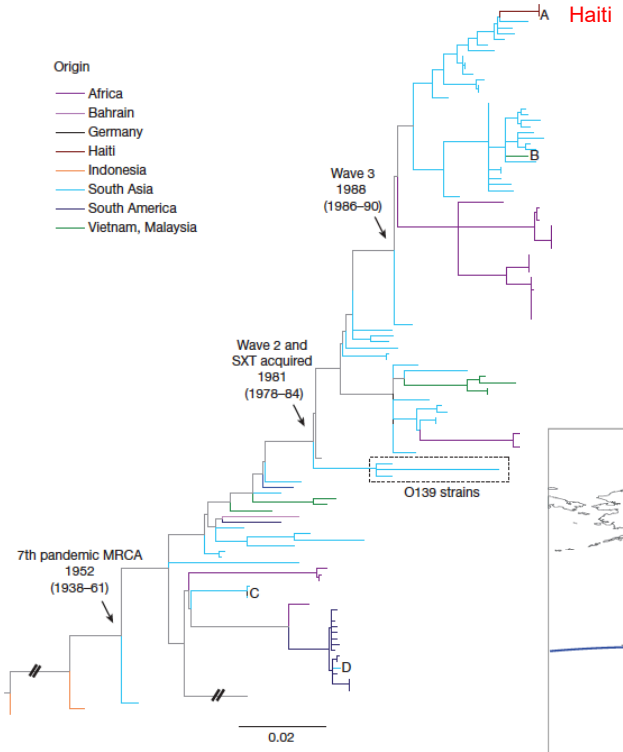
FIG 1 Genetic relationships among *V. cholerae* isolates from Nepal and Haiti. A single maximum parsimony tree was reconstructed using 752 SNPs from 34 whole-genome sequences. There were 184 parsimony-informative SNPs, of which 6 were homoplastic, resulting in a CI of 0.97 (excluding uninformative characters). The branch lengths are labeled in red, and for branches affected by homoplasy, minimum and maximum branch lengths are designated. Members of SNP genotypic group V (16) are indicated. SNP differences among the three most closely related Nepali groups and the Haitian group are shown and characterized in Table S1 in the supplemental material.

Science

Whole-Genome Study Nails Haiti-Nepal Cholera Link

By Martin Enserink | Aug. 23, 2011 , 5:36 PM

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.



LETTER

2011

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]

123 global 7PET genomes

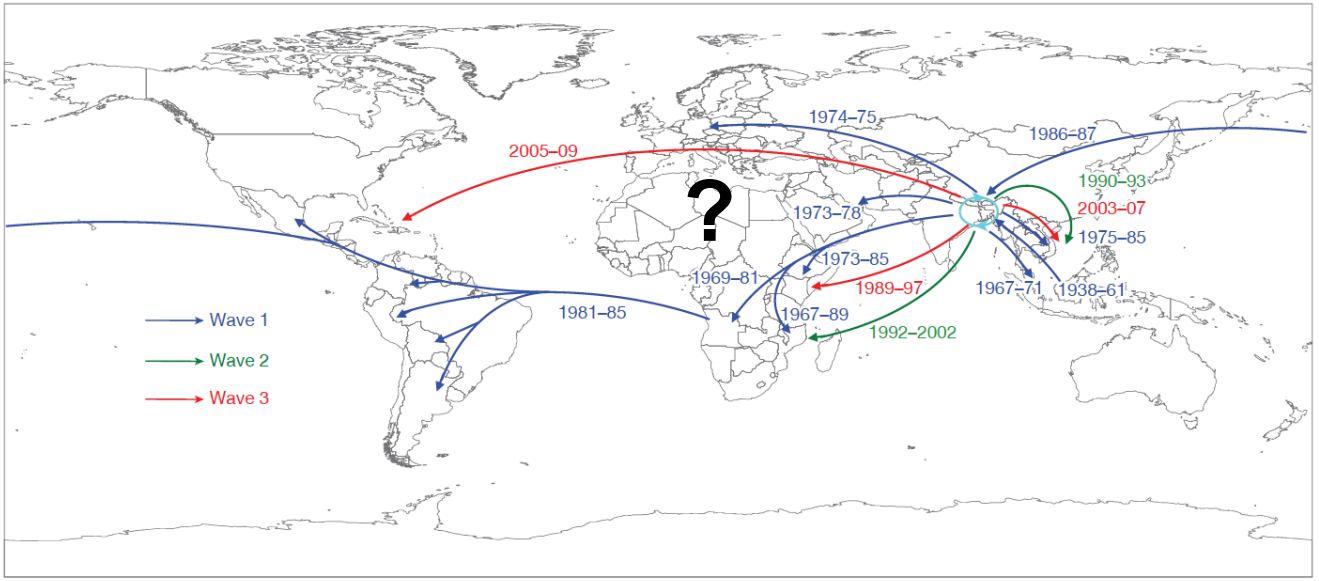
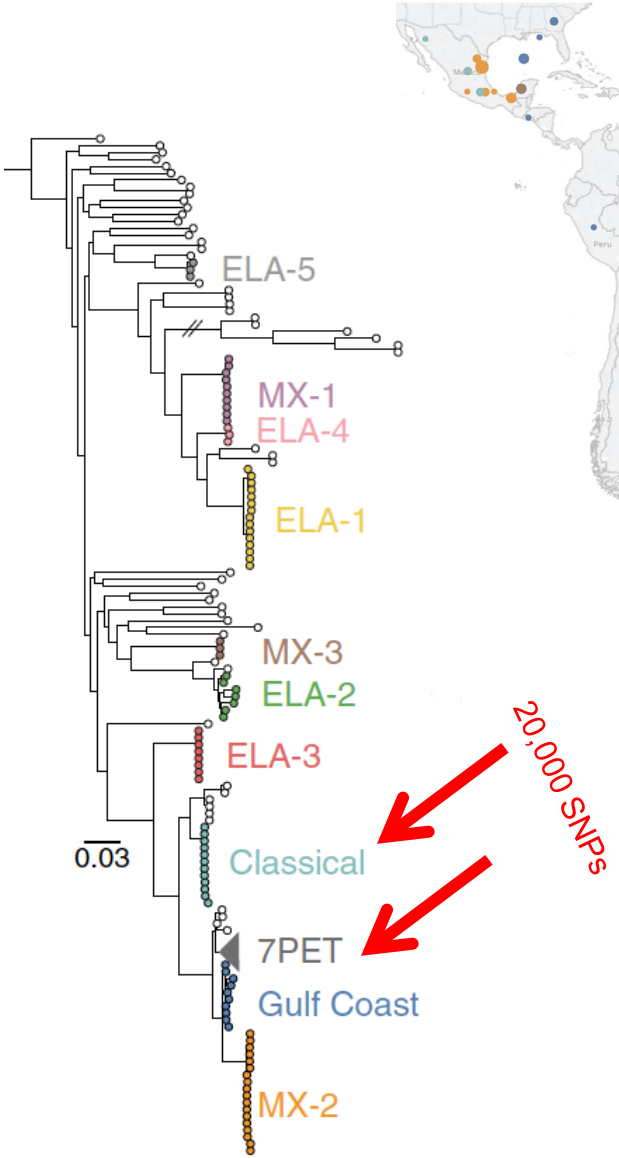


Figure 2 | Transmission events inferred for the seventh-pandemic phylogenetic tree, drawn on a global map. The date ranges shown for transmission events are taken from the BEAST analysis, and represent the median values for the MRCA of the transmitted strains (later bound), and the MRCA of the transmitted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

- 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

Cholera and *V. cholerae*



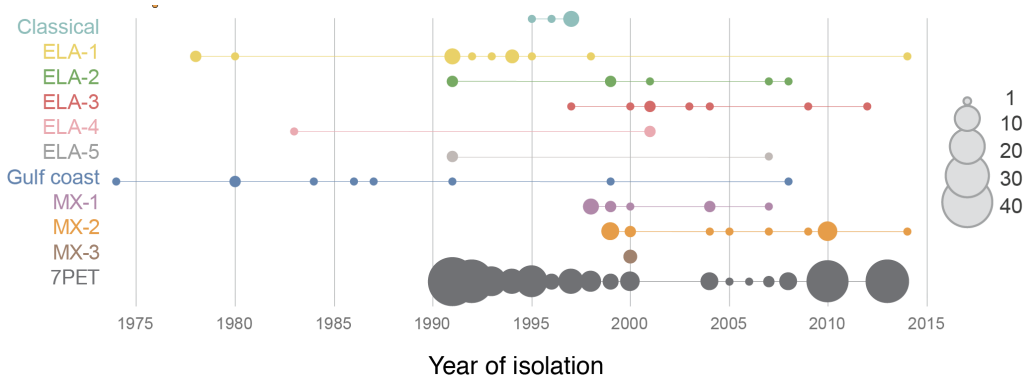
RESEARCH

CHOLERA

Integrated view of *Vibrio cholerae* in the Americas

Daryl Domman,[1*] Marie-Laure Quilici,[2] Matthew J. Dorman,[1] Elisabeth Njamkepo,[2] Ankur Mutreja,[1,3] Alison E. Mather,[1,4] Gabriella Delgado,[5] Rosario Morales-Espinosa,[5] Patrick A. D. Grimont,[6] Marcial Leonardo Lizárraga-Partida,[7] Christiane Bouchier,[8] David M. Aanensen,[9] Pablo Kuri-Morales,[10] Cheryl L. Tarr,[11] Gordon Dougan,[1,3] Julian Parkhill,[1] Josefina Campos,[12] Alejandro Cravioto,[13] François-Xavier Weill,[1,2†] Nicholas R. Thomson[1,14*†]

Domman *et al.*, *Science* **358**, 789–793 (2017) 10 November 2017

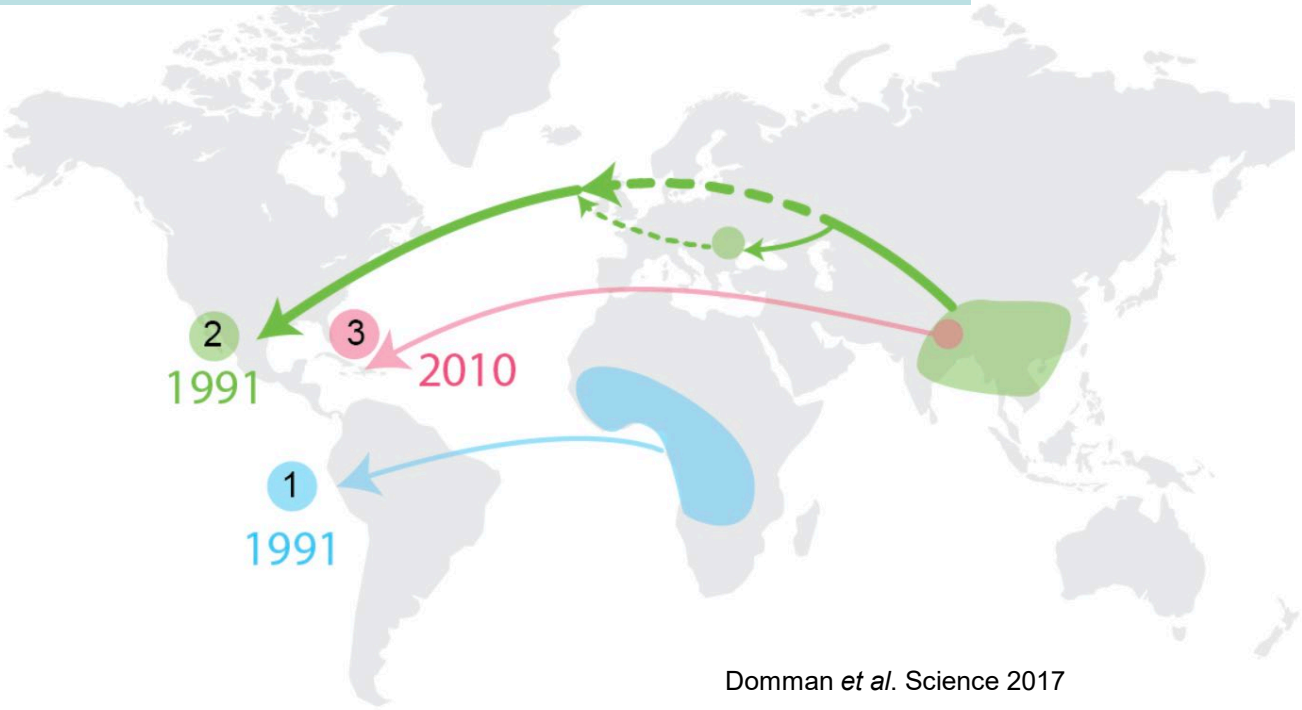


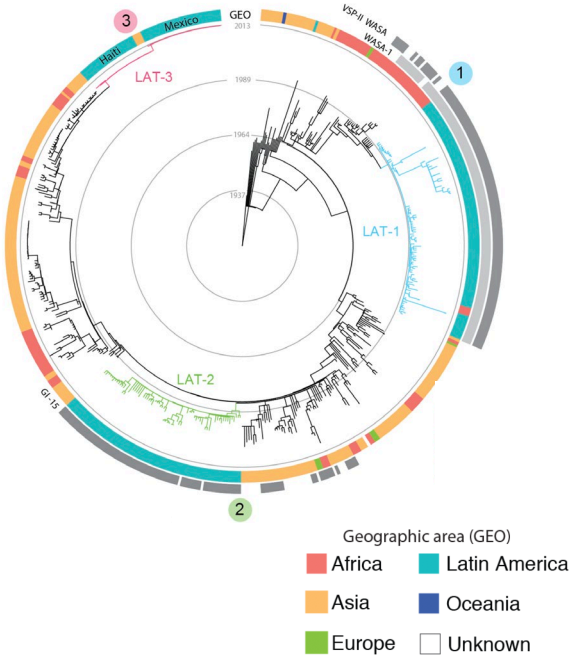
Only two clones of *V. cholerae* O1 CTX+ are responsible of pandemic cholera

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+

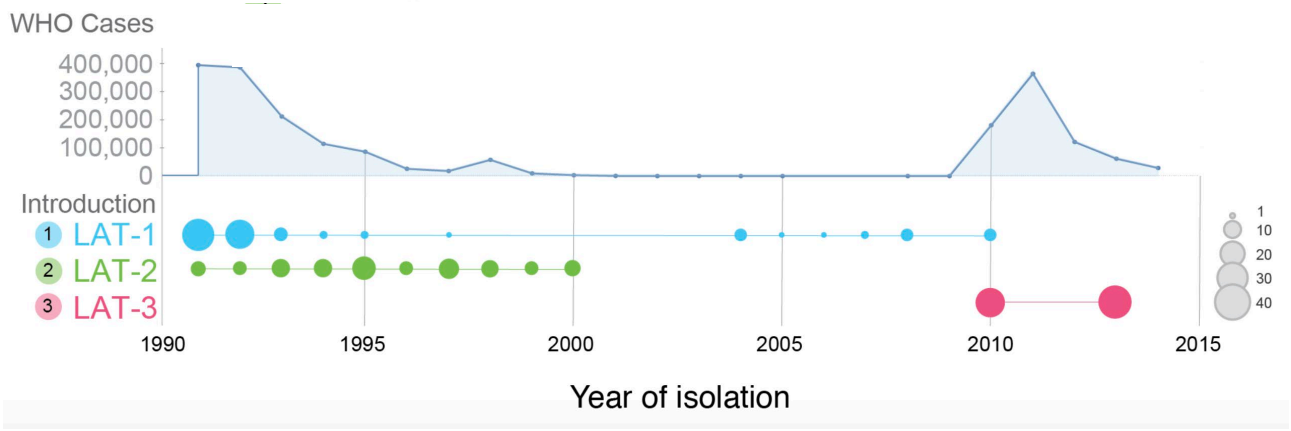
The seventh pandemic of cholera in America





Domman *et al*. Science 2017

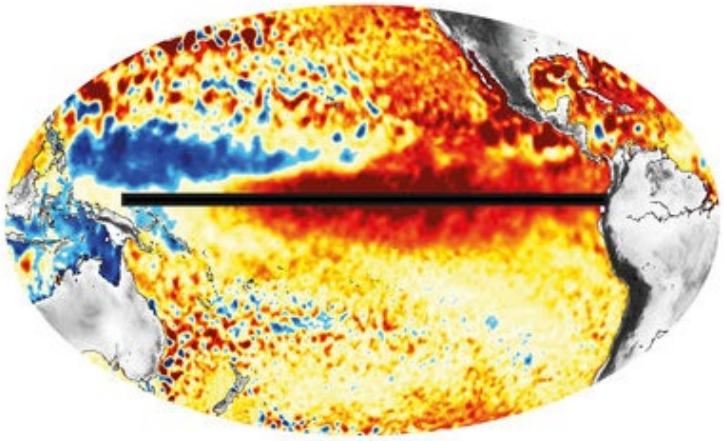
518 7PET genomes



Three introductions into America (Peru 1991, Mexico 1991 and Haiti 2010)

Origins of the cholera epidemic strain, Peru 1991

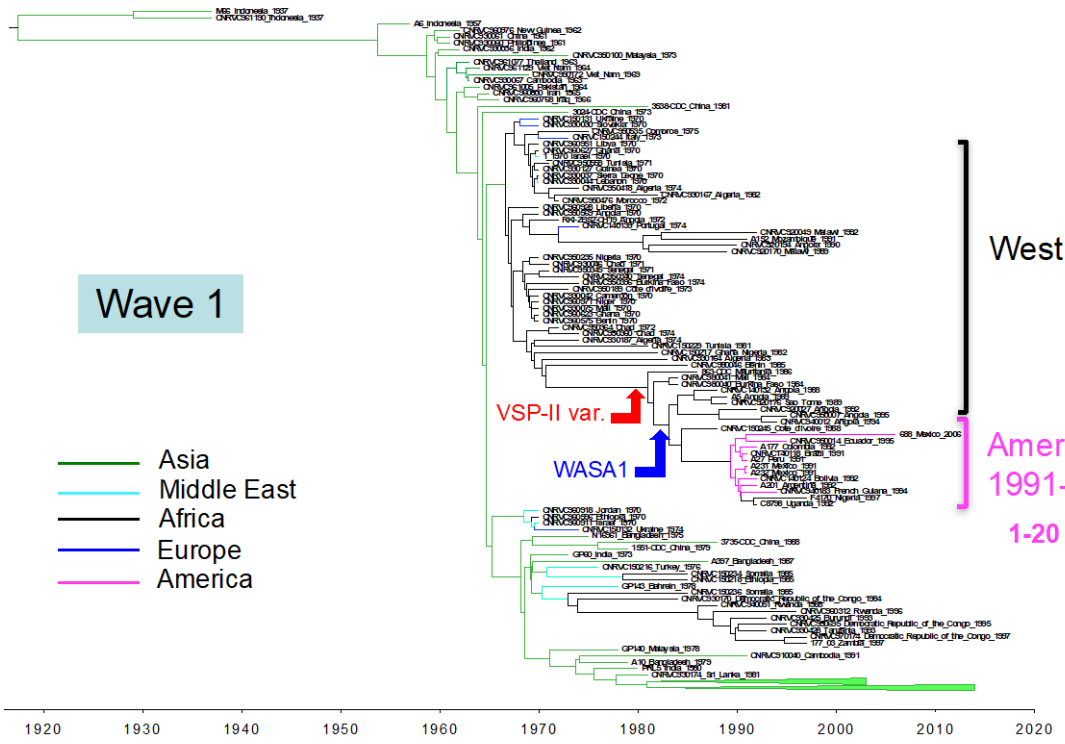
Figure 1: El Niño events correlate with water surface temperature rises and emergence of new *Vibrio* infections in South America.



Martinez-Urtana et al. Nat Microbiol 2016

Different hypotheses:

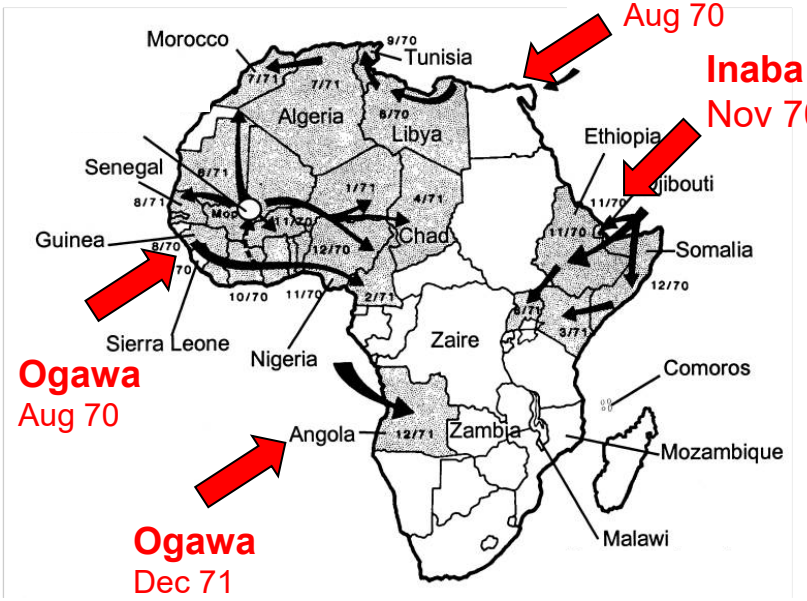
-a ship from China
-El Niño

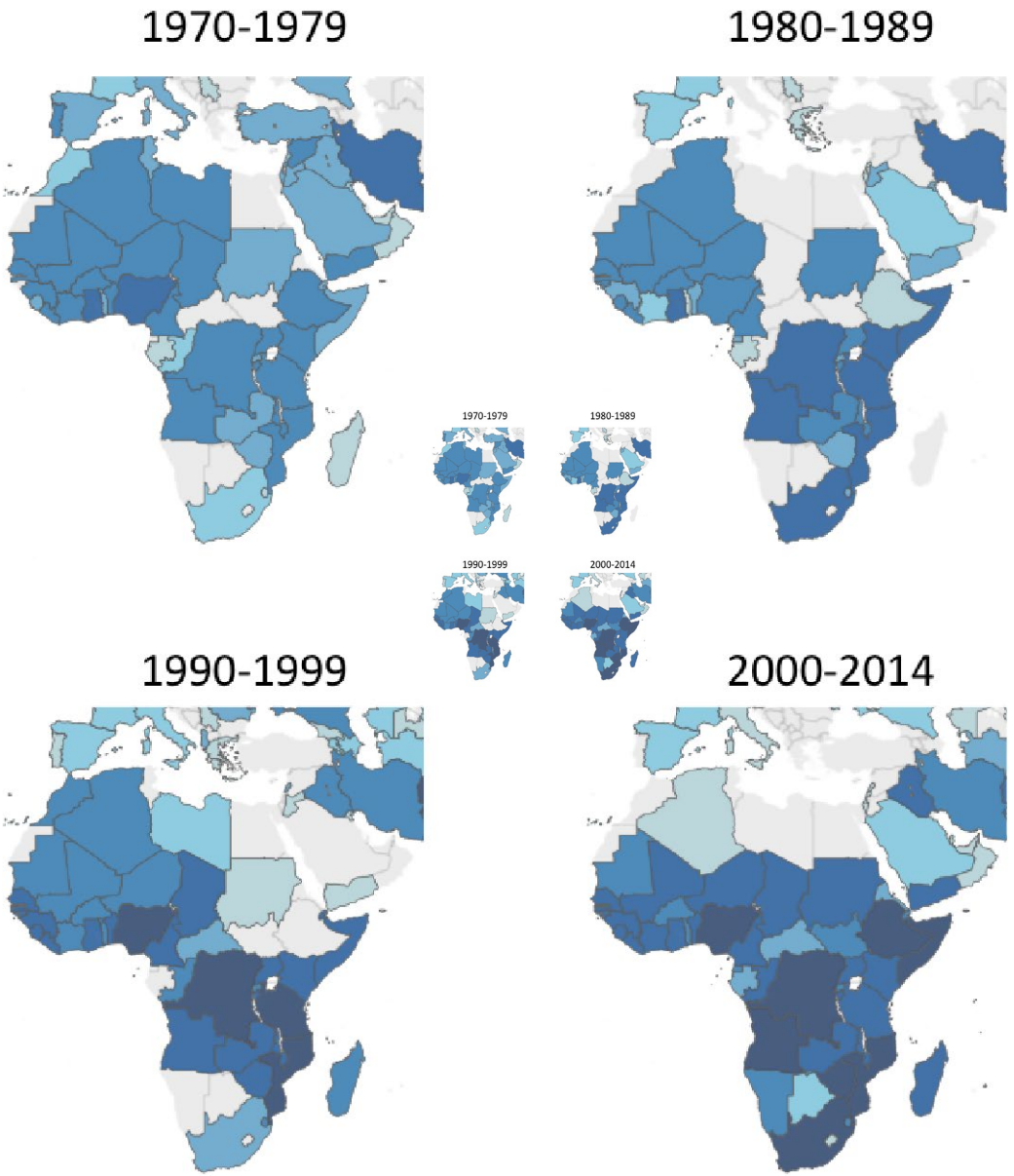


It was West Africa

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)

CHOLERA

Genomic history of the seventh pandemic of cholera in Africa

François-Xavier Weill,[1,2*] Daryl Domman,[2] Elisabeth Njamkepo,[1] Cheryl Tarr,[3] Jean Rauzier,[1] Nizar Fawal,[1] Karen H. Keddy,[4,5] Henrik Salje,[6,7] Sandra Moore,[8] Asish K. Mukhopadhyay,[9] Raymond Bercion,[10,11] Francisco J. Luquero,[12] Antoinette Ngandjio,[13] Mireille Dosso,[14] Elena Monakhova,[15] Benoit Garin,[11]† Christiane Bouchier,[16] Carlo Pazzani,[17] Ankur Mutreja,[18,19] Roland Grunow,[20] Fati Sidikou,[21] Laurence Bonte,[22]‡ Sébastien Breurec,[10]† Maria Damian,[23] Berthe-Marie Njanpop-Lafourcade,[24] Guillaume Sapriel,[25,26] Anne-Laure Page,[12] Monzer Hamze,[27] Myriam Henkens,[28]‡ Goutam Chowdhury,[9] Martin Mengel,[24] Jean-Louis Koeck,[29]§ Jean-Michel Fournier,[30] Gordon Dougan,[2,18] Patrick A. D. Grimont,[31] Julian Parkhill,[2] Kathryn E. Holt,[32] Renaud Piarroux,[8] Thandavarayan Ramamurthy,[19] Marie-Laure Quilici,[1,30]|| Nicholas R. Thomson[2,33]||

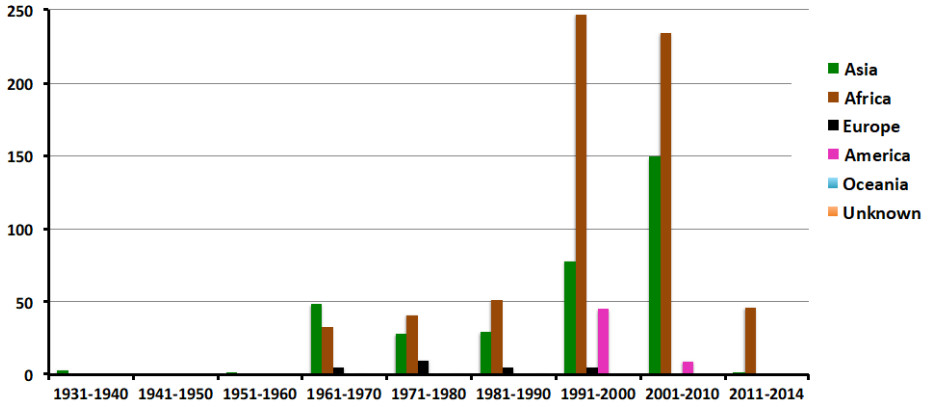
Science **358**, 785–789 (2017) 10 November 2017

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

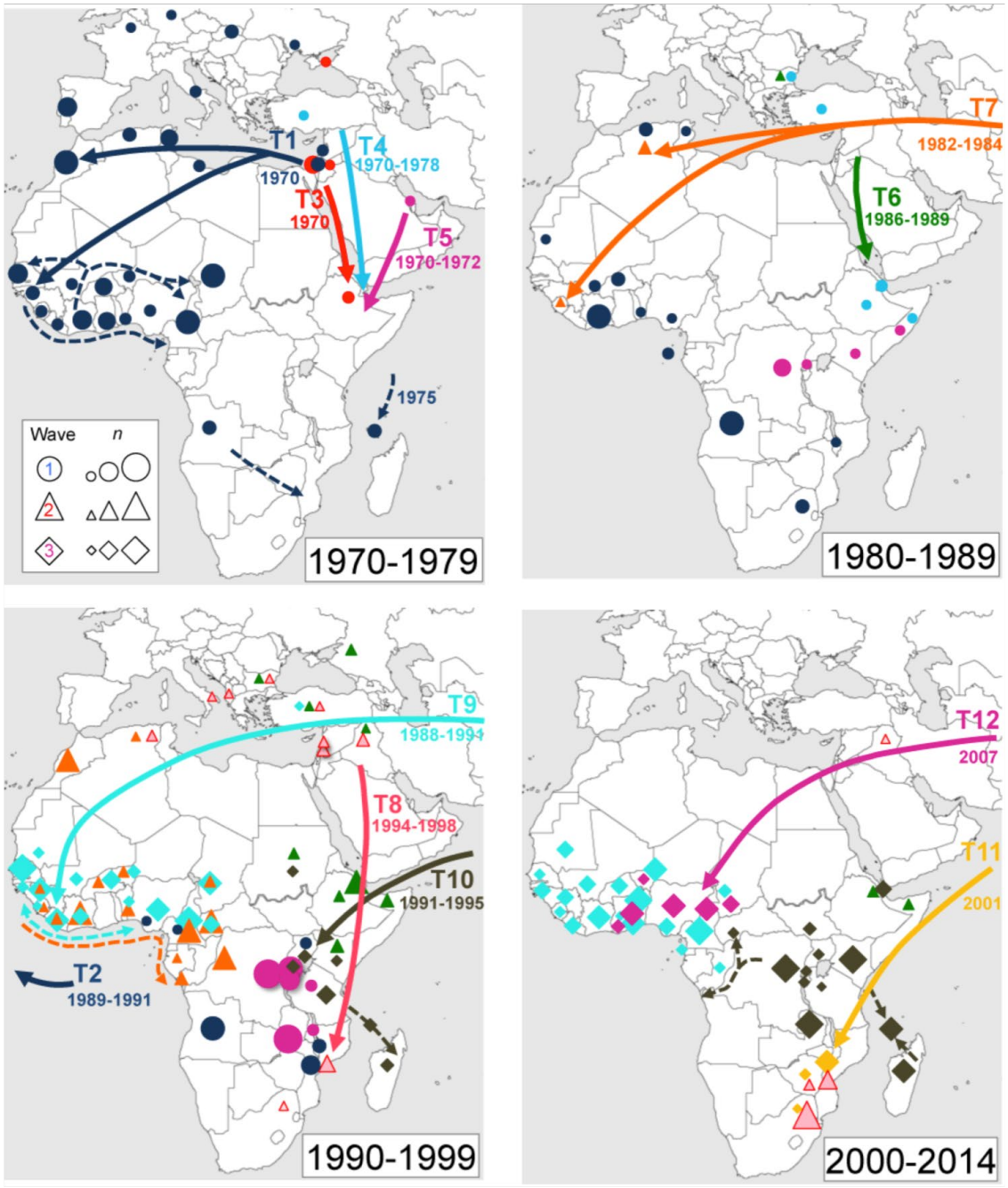
11 introductions to Africa
Guinea 70 ←Middle East
Angola 71 ←West Africa

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



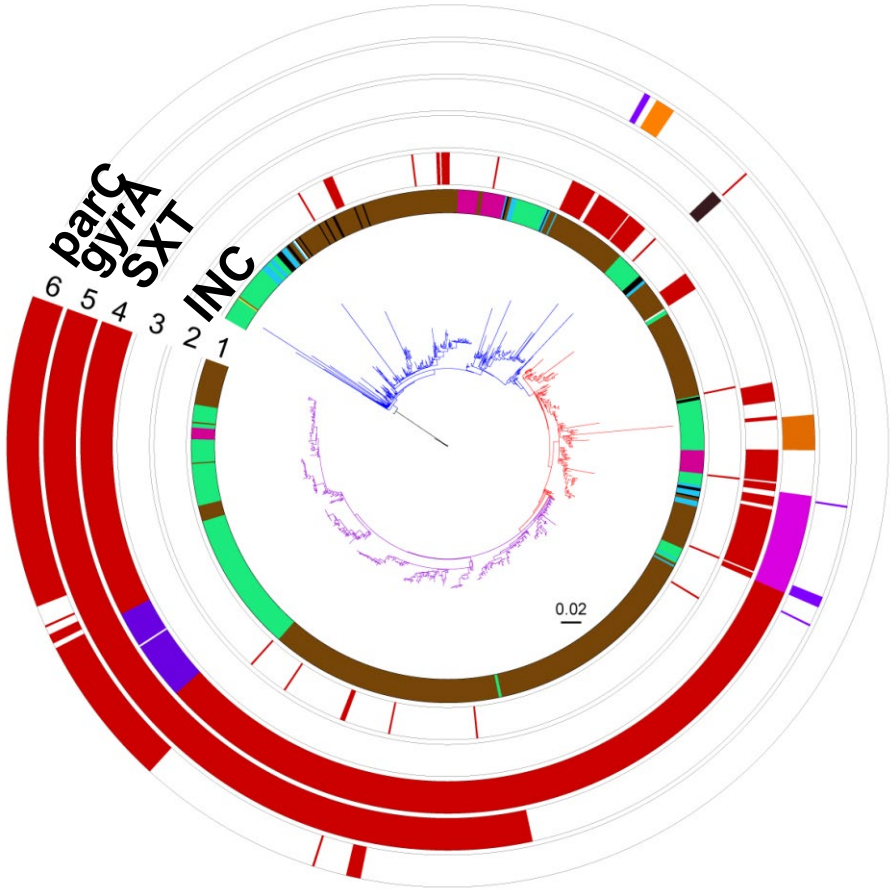
1970-1984

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

ARG, antibiotic resistance gene

2000-2014

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



Keys

1. Geographic area
   - Africa
   - Asia
   - Americas
   - Europe
   - Middle East
   - Oceania
   - Unknown
2. IncA/C (AMP STR KAN SUL COT TET)
   - Presence
   - Absence
3. GI-15 (STR SUL)
   - Presence
   - Absence
4. SXT/R391 (STR SUL COT CHL TET)
   - ICE*Vch*Ind5/ICE*Vch*Ban5
   - ICE*Vch*Ban9/ICE*Vch*Moz10
   - ICE*Vch*Ind6
   - ICE*Vch*Ban11
   - SXT/ICE*Vch*Ind4
   - Absence
5. *gyrA* (NAL)
   - S83I
   - S83R
   - D87Y
   - Wild-type
6. *parC* (CIP^{DS})
   - S85L
   - Wild-type

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

gyrA and ***parC*** mutations

Acquired in South Asia

Yemen, 2016

~27 000 000 inhabitants (2015)
528 000 km^2
Human development index 154/187 (2014)

Civil war (March 2015)

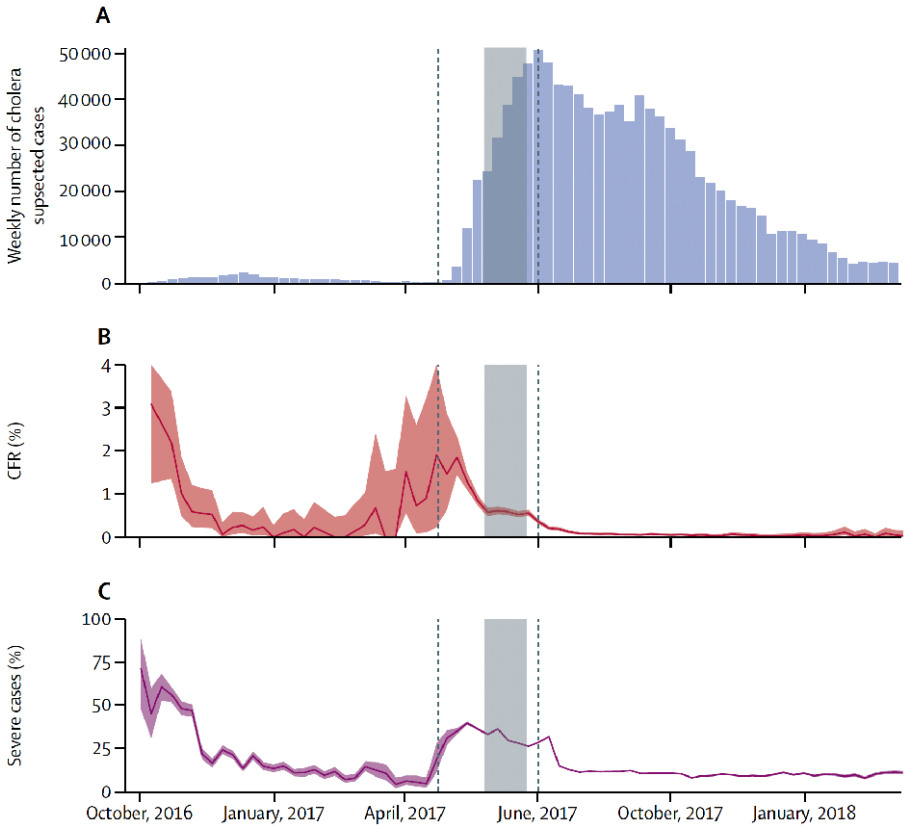
Fatalities: 8757

Injuries: 50 000

3 000 000 displaced people

Massive disruption of basic infrastructures and health system

October 6th 2016: Report of the first cholera cases



Two epidemic waves:

-Sept 28, 2016 to April 23, 2017

25 839 suspected cases
120 deaths (0.46%)

-April 24, 2017 to now

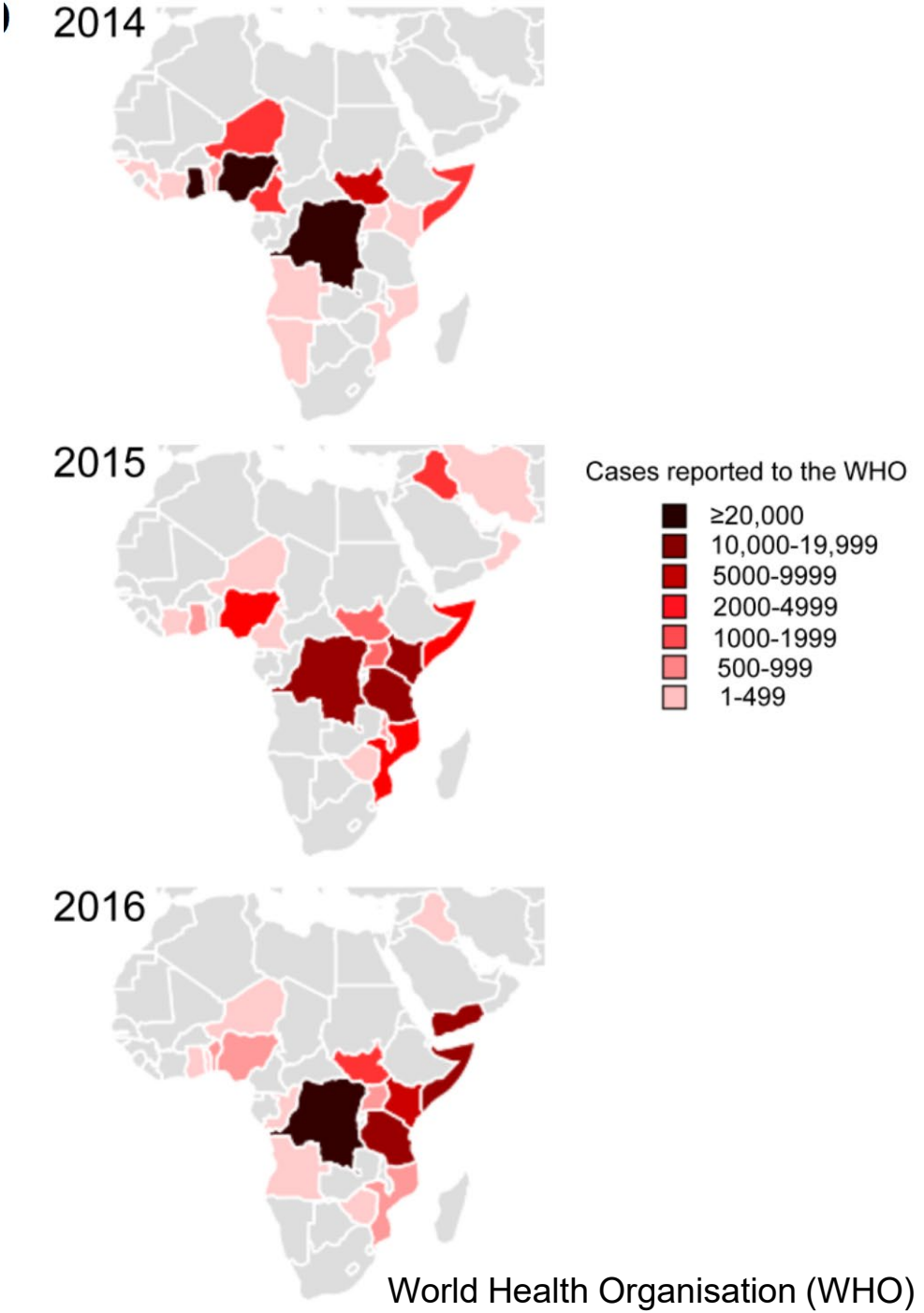
>1 000 000 cases
2265 deaths (0.2%)

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 ?



World Health Organisation (WHO)

LETTER

https://doi.org/10.1038/s41586-018-0818-3

Genomic insights into the 2016–2017 cholera epidemic in Yemen

François-Xavier Weill[1,21]*, Daryl Domman[2,3,21], Elisabeth Njamkepo[1], Abdullrahman A. Almesbahi[4], Mona Naji[4], Samar Saeed Nasher[4], Ankur Rakesh[5], Abdullah M. Assiri[6], Naresh Chand Sharma[7], Samuel Kariuki[8], Mohammad Reza Pourshafie[9], Jean Rauzier[1], Abdinasir Abubakar[10], Jane Y. Carter[11], Joseph F. Wamala[12], Caroline Seguin[13], Christiane Bouchier[14], Thérèse Malliavin[15], Bita Bakhshi[16], Hayder H. N. Abulmaali[17], Dhirendra Kumar[7,18], Samuel M. Njoroge[8], Mamunur Rahman Malik[10], John Kiiru[8], Francisco J. Luquero[5], Andrew S. Azman[19], Thandavarayan Ramamurthy[18], Nicholas R. Thomson[2,20,22] & Marie-Laure Quilici[1,22]

10 JANUARY 2019 | VOL 565 | NATURE | 231

Objectives

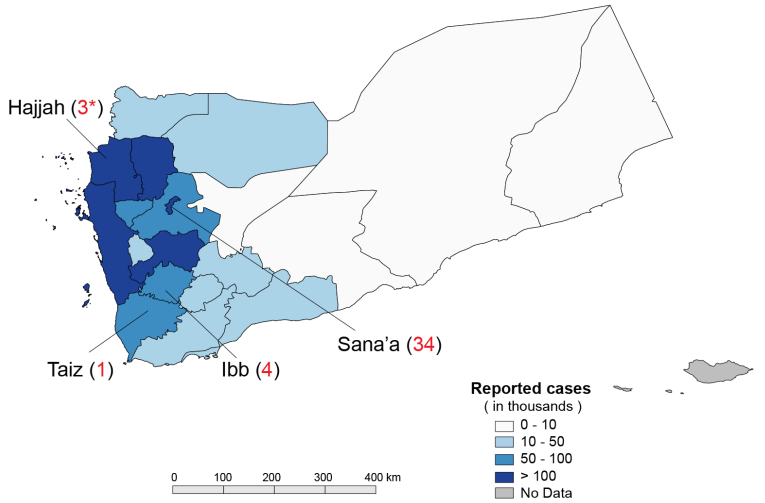
Confirmation of the pathogenic agent

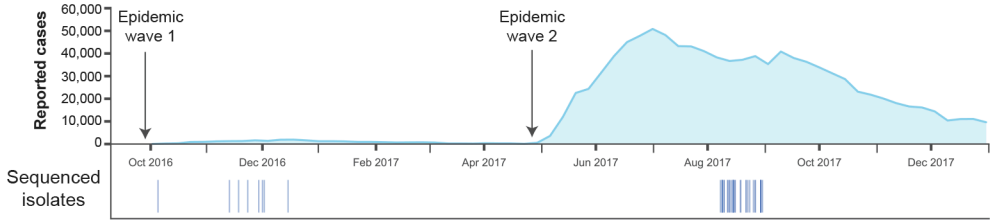
Description of the genomic features (including AMR)

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

Normal AMR phenotype of Wave 3 7PET isolates:

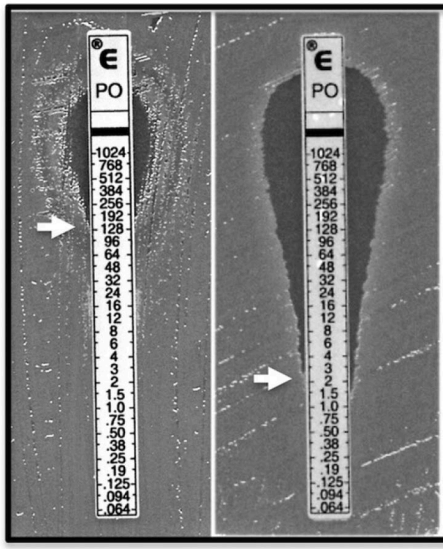
STR^R SUL^R TMP^R SXT^R TET^R CHL^R NAL^R FT^R POL^R

TMP^R NAL^R FT^R

TMP, trimethoprim
NAL, nalidixic acid
FT, nitrofurantoin

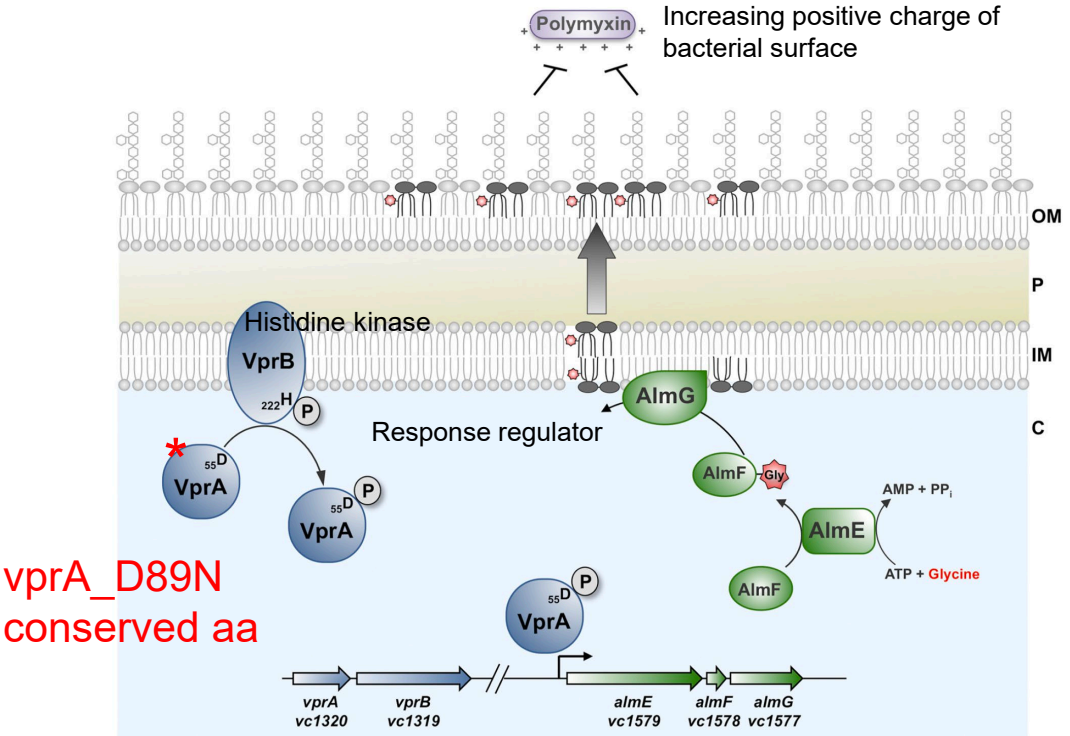
POLYMYXIN B

7PET El Tor Yemen



MIC (µg/ml) 128 2

VprA-VprB two component system

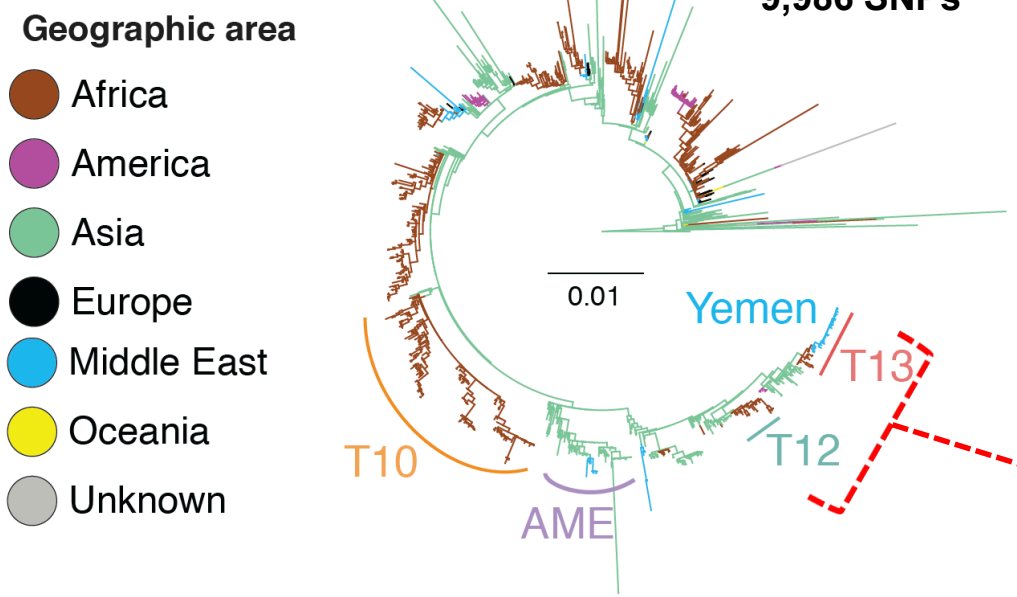


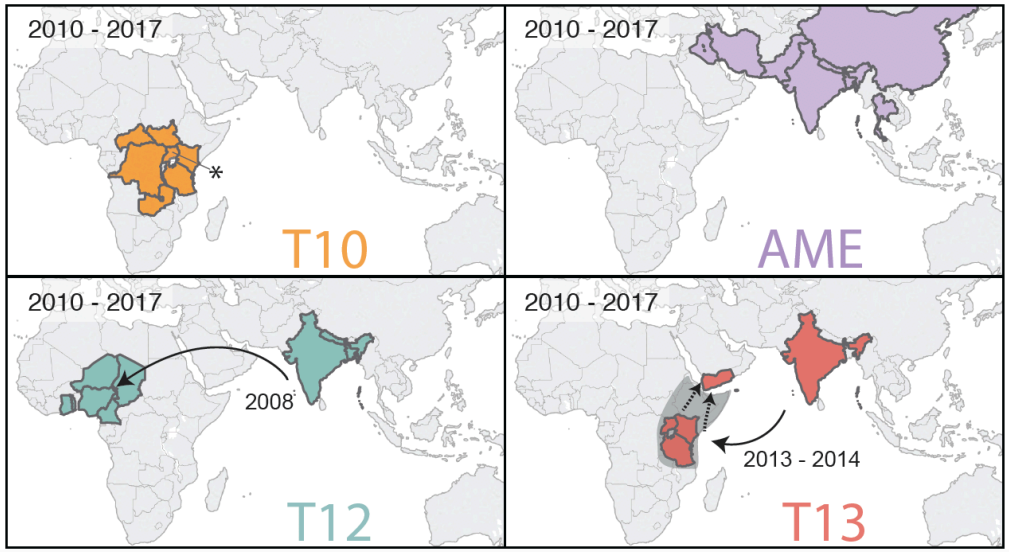
vprA_D89N conserved aa

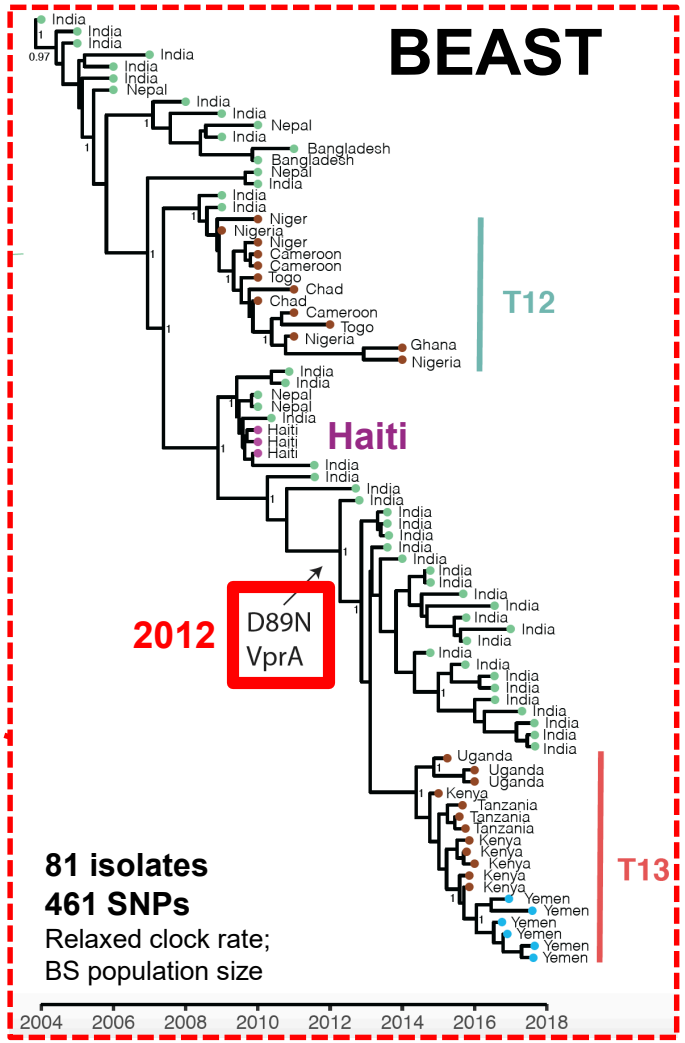
Herrera et al, Mbio 2014

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

Maximum likelihood







Recent sublineage (*ctxB7*)
Not linked with Middle Eastern isolates but with Eastern African isolates from a new introduction (T13)

Conclusions

- ✓ 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)


NEWS | IN DEPTH

EPIDEMIOLOGY

Genomes rewrite cholera's global story

sciencemag.org SCIENCE

Collaborators

wellcome trust sanger institute

D Domman
G Dougan
J Parkhill
N Thomson

National Institute of Cholera and Enteric Diseases

A Mukhopadhyay
T Ramamurthy

CDC

C Tarr

K Keddy
F Luquero
AL Page
A Rakesh

epicentre

MEDECINS SANS FRONTIERES

L Bonte
M Henkens
C seguin

APHM Assistance Publique Hôpitaux de Marseille

S Moore
R Piarroux

UNIVERSITÀ DEGLI STUDI DI BARI ALDO MORO

C Pazzani

ROBERT KOCH INSTITUT

R Grunow

Service de santé des armées

JL Koeck

Institutul Cantacuzino

M Damian

Université Libanaise

M Hamze

amp AGENCE DE MÉDECINE PRÉVENTIVE

B Lafourcade
M Mengel

E Monakhova

Institut Pasteur

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

More…

World Health Organization

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

Johns Hopkins University

A Azman

Iran B Bakhshi (TMU)

Iraq H Abulmaali (CPHL)

Saudi Arabia A Assiri (MoH)

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

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)

Thank you for your attention !