



Molecular Epidemiology and Global Transmission of Cholera

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

Intended Learning Objectives

Specific objectives of this session:

1. Understand the epidemiological background of cholera, including its global burden and historical context
2. Describe the molecular characteristics of *Vibrio cholerae* and its relevance in public health
3. Explain the role of whole genome sequencing (WGS) in outbreak investigations
4. Analyze genomic data to trace transmission pathways, using the 2010 Haitian cholera outbreak as a case study
5. Evaluate the importance of international collaboration and rapid response in managing infectious disease outbreaks

Outline

This session consists of the following elements:

1. Introduction to the epidemiological background of cholera
2. Genomic studies and outbreak investigations using WGS framed around the 2010 Haitian cholera outbreak
3. Explanation of genomic finding and conclusions

Introduction of cholera

- The Italian, Filippo Pacini first discovered *V. cholerae* in 1854, though it was originally believed to be Robert Koch who discovered it thirty years later in Berlin in 1884
- Gram negative motile curved rods, one flagella
- Belongs to Vibrionaceae and the genus consist of more than 30 species
- Generally halophilic, thrives at alkaline conditions, prefer pH > 7
- Grow at a temperature range of 18-37°C (optimal: 30°C)
- Most *Vibrio* spp. are harmless marine organisms but a few are truly pathogenic
- Bacteriologist Waldemar Haffkine: first cholera vaccine around 1900

Introduction of cholera

Species	Disease	Ecology
V. cholerae	Cholera	Water, food
V. parahaemolyticus	Gastroenteritis, wound infections	Shellfish, seafood, seawater
V. vulnificus	Gastroenteritis, wound infections, bacteremia	Shellfish, seafood, seawater
V. alginolyticus	Wound and ear infections	Seawater, seafood

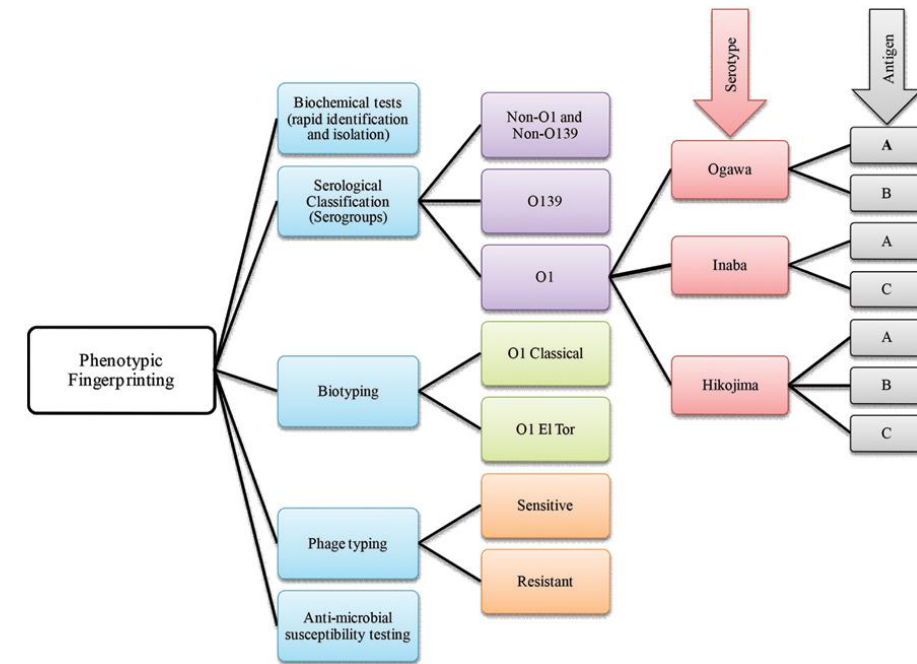
Introduction of cholera

- John Snow (15 March 1813 – 16 June 1858)
- English physician and a leader in the adoption of anesthesia and medical hygiene
- He is considered one of the fathers of modern epidemiology
- He published his theory in 1849, "On the Mode of Communication of Cholera" - in 1866, he map a cholera outbreak in London
- Skeptic of the theory that diseases such as cholera and bubonic plague were caused by pollution or a noxious form of "bad air"
- Lead to the germ theory of disease (1850 and 1910)
- Link between cholera and contaminated drinking water in the Soho epidemic of 1854

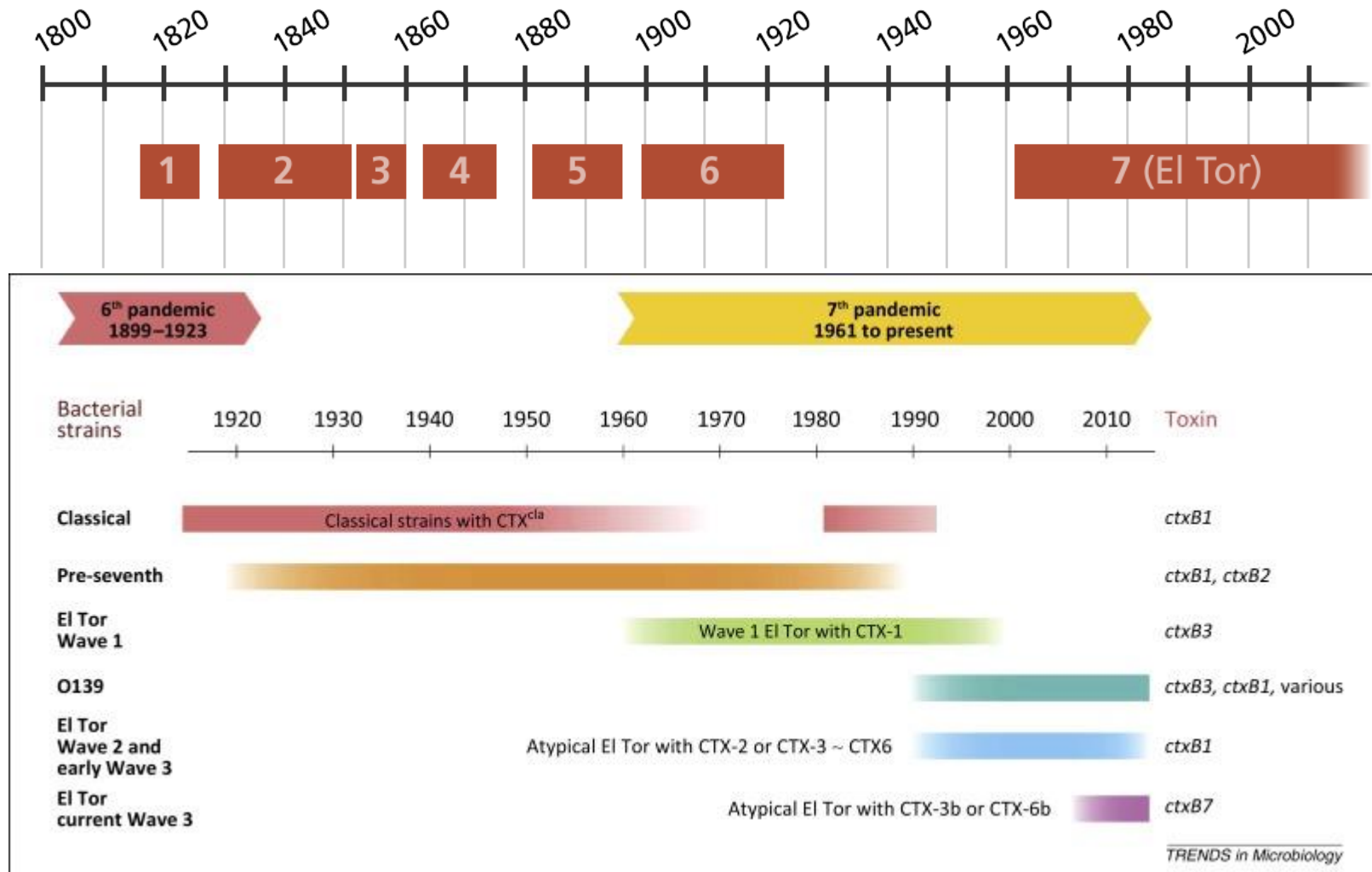


Introduction of cholera

- O1 serotype predominant, O139 emerging (evolved from O1)
- O1: based on phenotypes subdivided into:
 - biotypes Classical and El Tor The latter is able to express a haemolysin and resistance to polymyxin B susceptibility to specific phages
- O1: based on the O antigens subdivided into:
 - subtypes Inaba, Ogawa and Hikojima
 - Antigen: A, B, C



Introduction of cholera



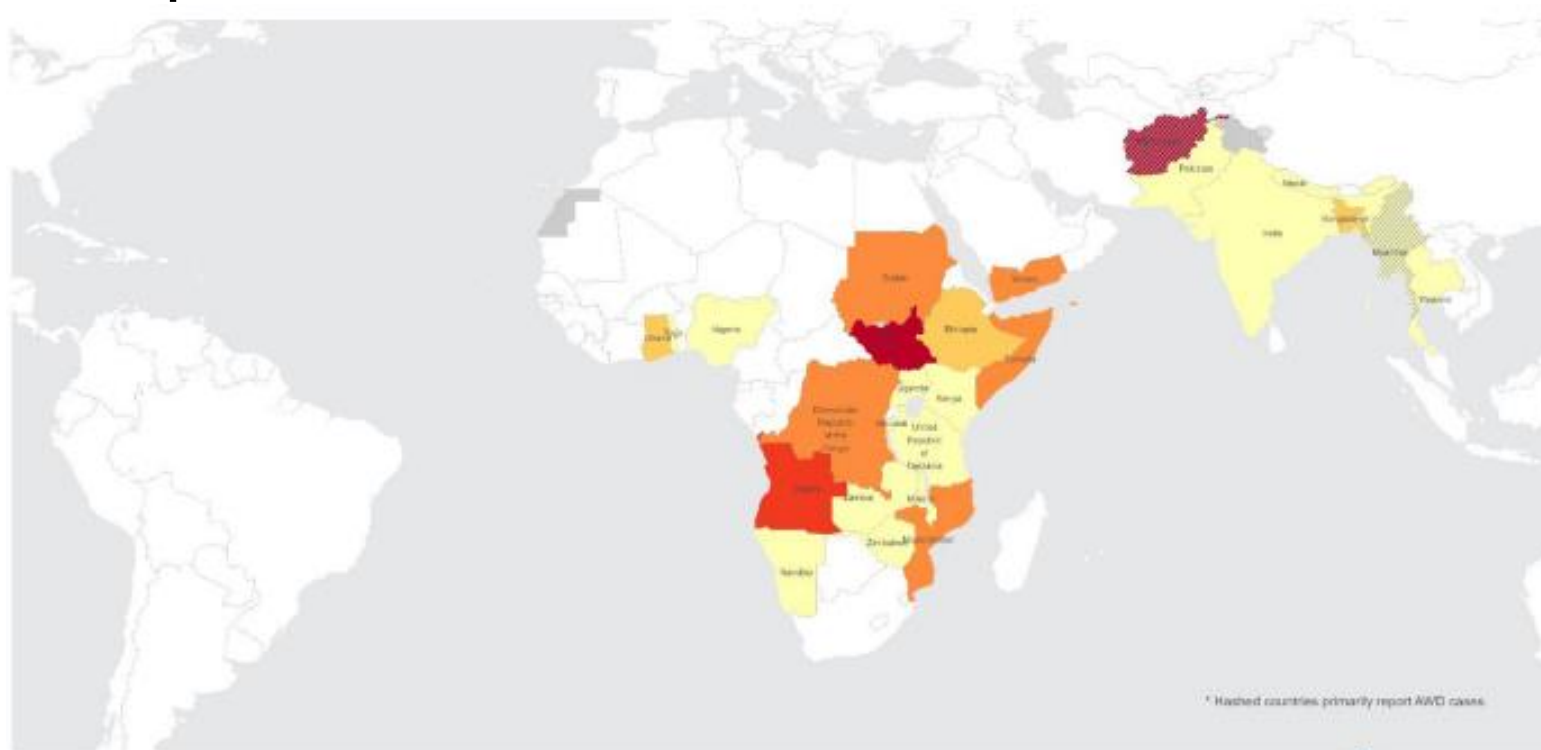
Introduction of cholera



- Massive investment in clean water supply and well-separated sewage treatment infrastructures between the mid-1850s and the 1900s
- Eliminated the threat of cholera epidemics from the major developed cities in the world
- Still a huge problem in low-and-middle-income countries
- Transmitted by food or water that is contaminated - often disaster areas
- In 2022, 38 countries reported a total of 132,121 cases
(<https://www.who.int/data/gho/publications/world-health-statistics/2022>)
- An estimated burden of 1.3 to 4.0 million cases, and 21 000 to 143 000 deaths per year worldwide (<https://www.who.int/en/news-room/fact-sheets/detail/cholera>)

Introduction of cholera

Cholera and acute watery diarrhoea (AWD) cases per 100 000, 1 January to 27 April 2025



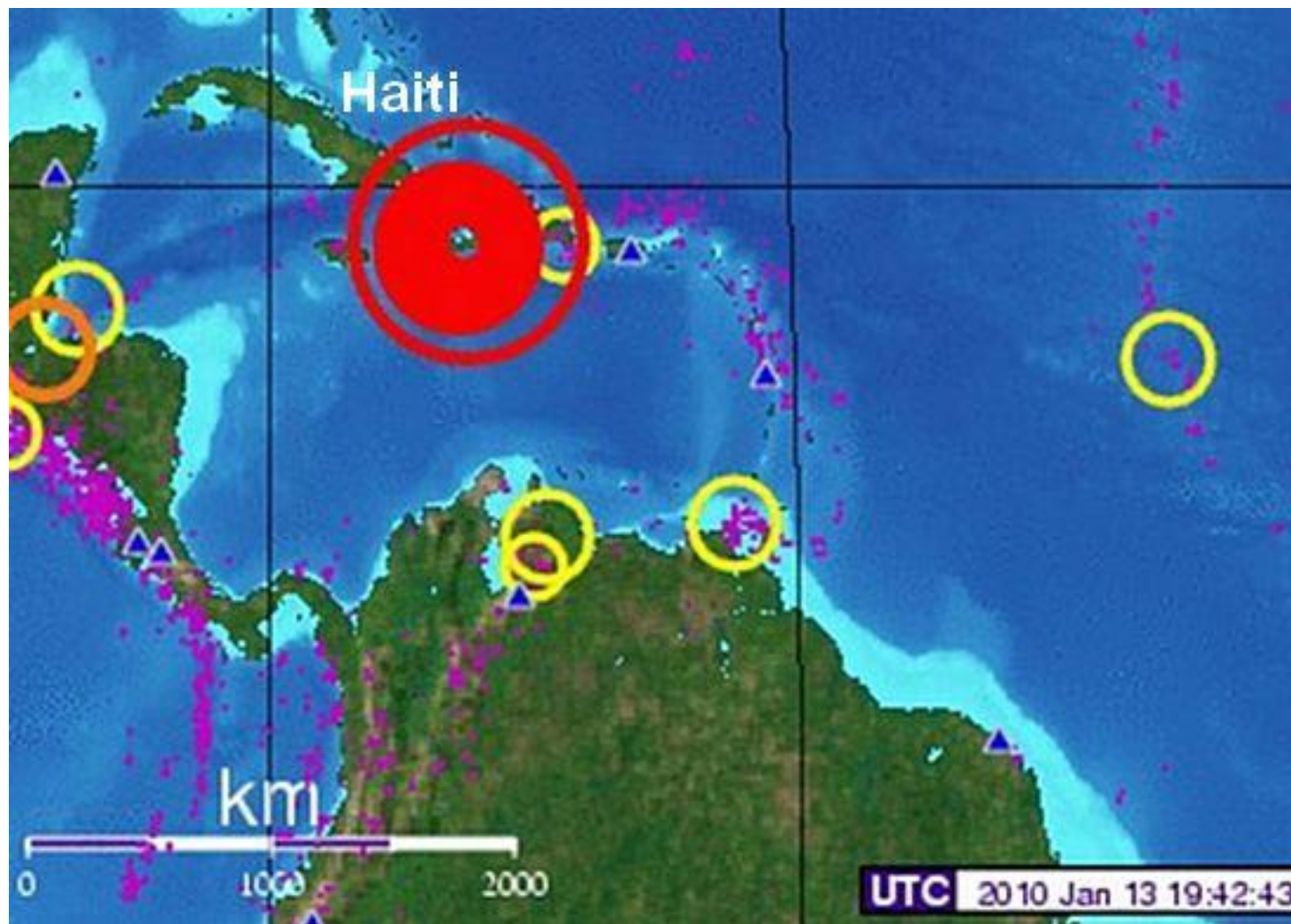
Cases per 100k 0.01 - <5 5 - <10 10 - <50 50 - <100 100+ Not applicable No data



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Data Source: World Health Organization
Map Production: WHO Health Emergencies Programme
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Setting the scene in Haiti - 2010



Setting the scene in Haiti

The earthquake caused massive destruction in the city of Port-au-Prince including the sanitary system

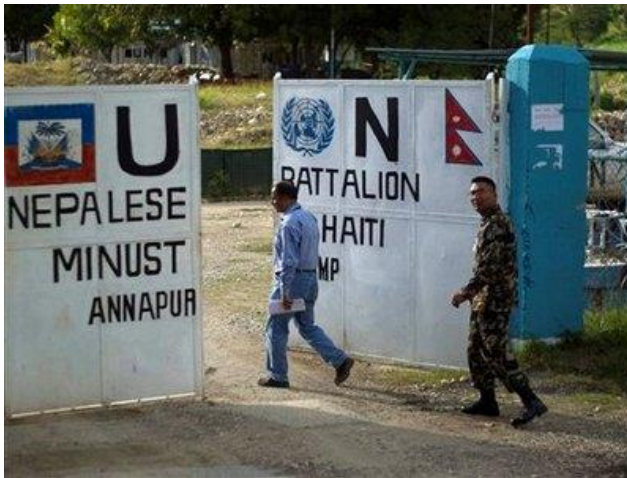


Reports of a cholera outbreak in Nepal

- 6 months later - A cholera outbreak were reported at the 28th of July 2010 in Nepal
- The outbreak was controlled by the 14th of August and reached endemic levels
- In total 1,400 cholera cases were reported
(<https://www.thenewhumanitarian.org/news/2010/08/20/cholera-outbreak-nepal-under-control-says-who>)
- Since 21st August 2010, incessant monsoon rains have resulted in flash floods, inundation and landslides in 43 districts across all of Nepal
(<https://www.unocha.org/publications/report/nepal/nepal-floods-and-landslides-ocha-situation-report-no-1-3>)
- Drinking water was contaminated in most of Nepal
(<https://kathmandupost.com/health/2025/08/25/birgunj-cholera-outbreak-largest-since-jajarkot-in-2009>)

The United Nations Stabilization Mission in Haiti

- In the early days of the disaster in Haiti - the global communities sent aid relief worth of 3.3 bill. USD
(https://en.wikipedia.org/wiki/Humanitarian_response_to_the_2010_Haiti_earthquake)
- Among others, a battalion of Nepalese soldiers were dispatched as part of an UN contribution (https://en.wikipedia.org/wiki/2010s_Haiti_cholera_outbreak)



The first cases of cholera in Haiti

- On the 12th of October 2010, the first case of cholera occurred in Haiti (Ivers et al.,2012)
- A 28 years old man with a history of untreated psychiatric disorder died after 24 hours after having drinking water from the Latem River
- On the 17th of October 2010, two undertakers preparing the body of the 28 years old man developed watery diarrhea and were hospitalized in Mirebalais
- On 21st of October 2010, the Haitian PH authorities confirmed a cholera outbreak (*V. cholerae* O1 biotype El Tor) with 3,020 cases and 129 reported deaths
- The 30th November 2010, 515,699 cases and 6.942 deaths were reported (Haiti Health Cluster Bulletin) (<https://www.paho.org/sites/default/files/HealthCluster-Bulletin30-2011-eng.pdf>)

“They came to the island with the best of intentions – only to sow disease and death”

- The Nepalese peacekeeping forces were accused of introducing the disease to Haiti (Science news)
- 1000's of liters of contaminated feces were disposed into the Artibonite River and in open pit on hilltops
- Artibonite River is widely used as drinking water and for washing, bathing and playing



EPIDEMIOLOGY

Despite Sensitivities, Scientists Seek To Solve Haiti's Cholera Riddle



HAITI'S CHOLERA OUTBREAK

Cholera Linked to U.N. Forces, But Questions Remain



The hunt for the source!

“A matter of scientific curiosity for the future”

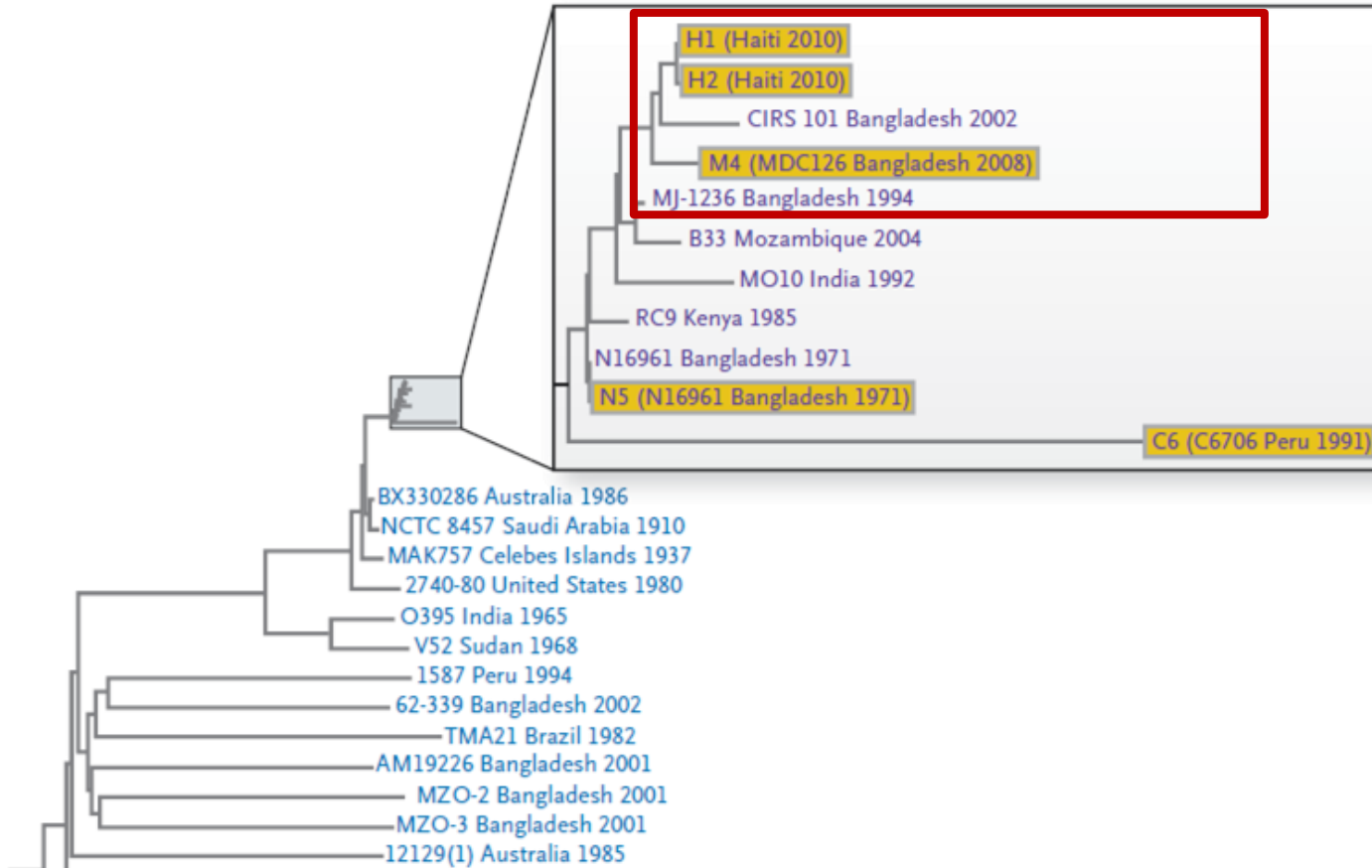


- Declared by the Lancet Infectious Diseases in a recent editorial
- Wrong-headed attitude! Proclaimed by Matthew Waldor (Harvard Medical School)
- Matthew Waldor stated that it is important to know the origin of the source and how the outbreak started for future prevention
- Later Waldor states, that “he would like to see a comparison of whole genome sequence of Haitian strains with one from the cholera outbreak in Nepalese capital Katmandu”
- This kick-started a bunch of scientific articles using WGST to pinpoint the origin of the outbreak strains

The search for “the smoking gun”!

- Chin et al. whole genome sequenced 5 strains using the PacBio RS system and compared those with 23 that had previously been sequenced
- The 5 strains were: 2 from the Haitian outbreak (2010), 1 strain from Latin America (1991), and 2 isolates from South Asia (2002, 2008)
- The 23 genomes were of an older global collection (1910-2002)
- Chin et al. performed a SNP analysis and investigated the structure of the hyper-variable elements

The search for “the smoking gun”!





The search for “the smoking gun”!

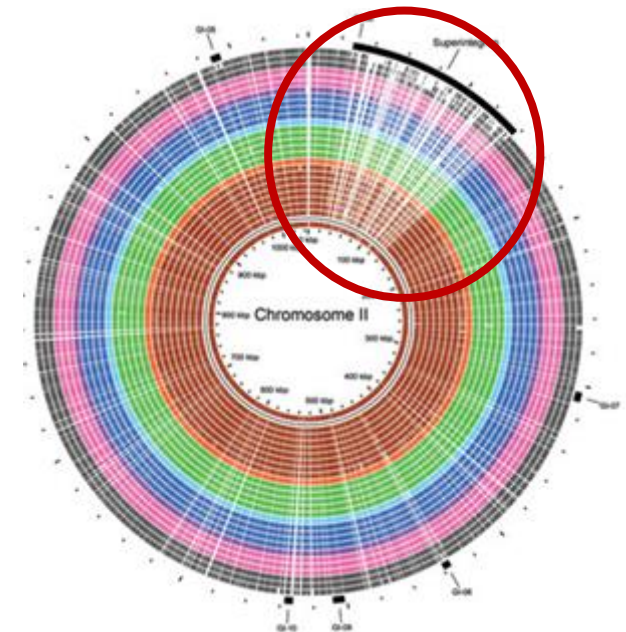
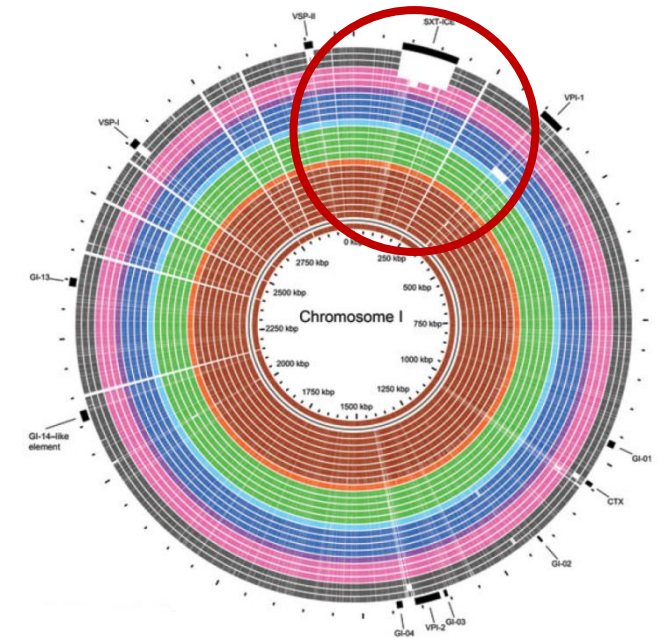
- Ceccarelli et al. analyzed whole genomes of 3 clonal isolates from the Haitian outbreak recently sequenced by US-CDC
- Ceccarelli et al. found
 - An integrative conjugative element of the SXT-ICE family
 - An antimicrobial spreading vector
 - Resistance genes for cotrimoxazole, sulfamethoxazole, streptomycin
 - Genotype 7 ctxB gene encoding toxin subunit B
- The Genotype 7 ctxB has only been associated with an altered El Tor variant from the epidemic in Orissa, India 2007
- Alignment and comparative analysis with isolates from also Central America and Indian subcontinent were conducted
- Alignment confirmed the outbreak isolates phylogenetically related with the reference genome Cirs101 from Bangladesh

The search for “the smoking gun”!

- Reimer et al. analyzed 23 whole genomes
 - 9 isolates from the Haitian outbreak
 - 12 PFGE matched isolates linked to Asia / Africa
 - 2 PFGE none-matched isolates
- Sequencing
 - One Haitian isolate were fully closed using Sanger sequencing
 - Remaining isolates were sequenced using Illumina sequencer; GAIIE
- All genomes were aligned and core genome phylogeny were performed including 5 available reference genomes

The search for “the smoking gun”!

- Low overall diversity on chromosome 1
- No macroscopic differences were observed in SXT-ICE among Haiti / PFGE matched isolates
- Some variability in the hyper-variable superintegron in chromosome 2
- No major deletions in the superintegron

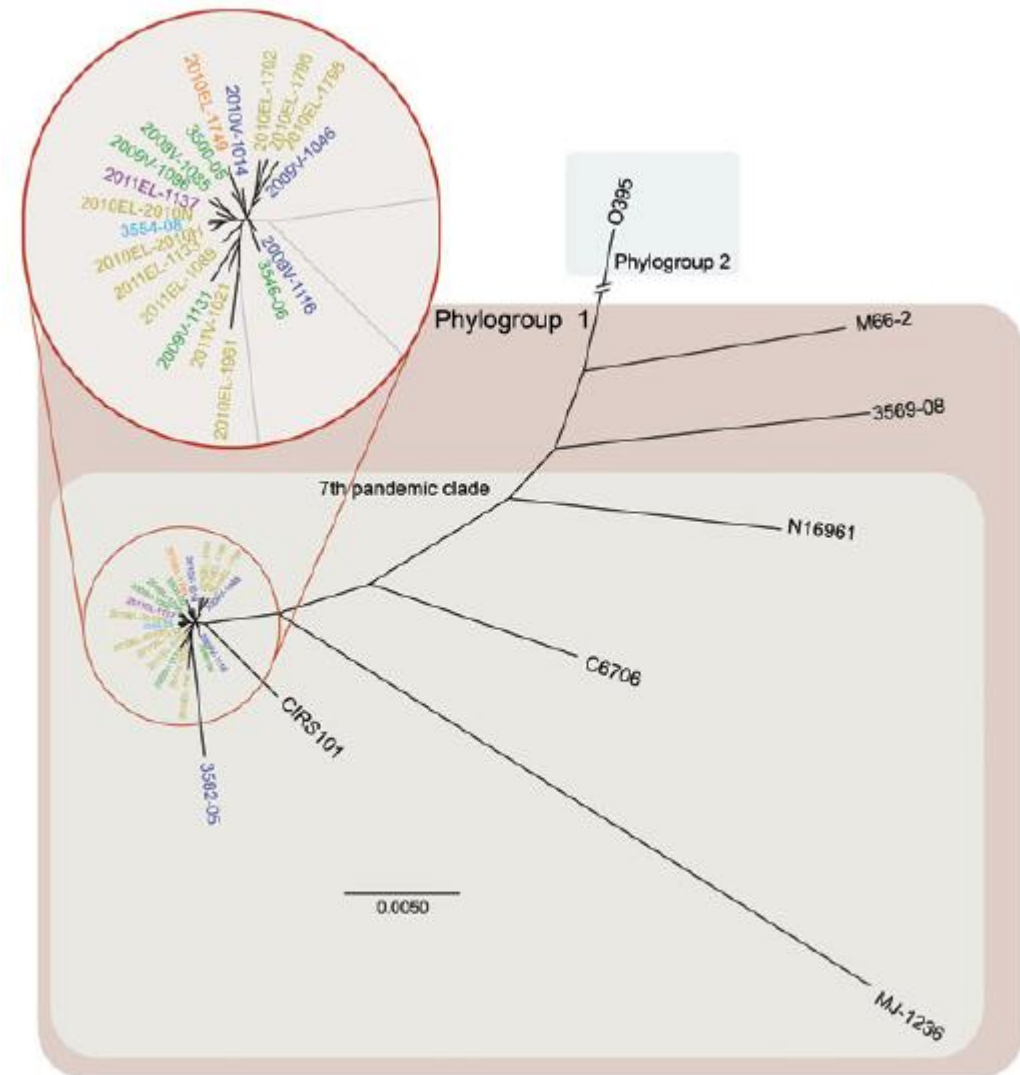


Origin of infection

- Haiti/Dominican Republic
- Cameroon
- India
- Nepal
- Pakistan
- South Africa
- Bangladesh
- Other

The search for “the smoking gun”!

- 0-2 SNPs were observed among the 9 Haitian outbreak isolates
- Haitian isolates differed with 4-25 SNPs to the 12 PFGE matching isolates clustered
 - Closest match was 4-7 SNPs (India / Cameroon)
- Closest ancestor of historical ref. genomes was CIRS101 (Bangladesh)
- No identical match why the genetic origin remains to be identified



The search for “the smoking gun”!

- Based on the earlier comment by Waldor – I contacted colleagues in Nepal
- The 2010 outbreak strains from Nepal were shared due to trustful friendship developed during laboratory training as part of WHO Global Foodborne Infections Network (GFN) former Global Salm Surv (GSS)



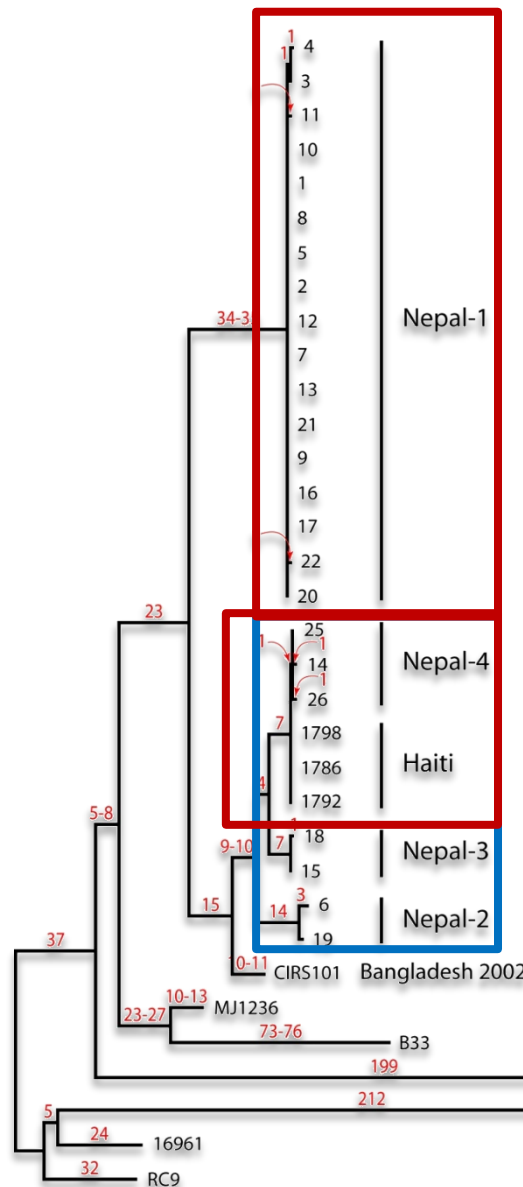
The search for “the smoking gun”!

- 24 contemporary *V. cholerae* isolates from Nepal were received at National Food Institute, Denmark
 - Originated from 30th July to 1st November 2010
 - Five districts in Nepal
 - 15 isolates were of the Nepalgunj outbreak
- 10 previously sequenced isolates were included the analysis
 - incl. 3 from the Haitian outbreak sequenced by the US-CDC
- The Nepalese isolates were characterized by
 - MIC determination
 - PFGE
 - MLVA (not published)
 - WGST

The search for “the smoking gun”!

- Pooled paired-end libraries were sequenced using Illumina GAIIx
 - Sequence data were deposit in the Short Read Archive at NCBI
- Data were aligned with V.cholerae O1 biovar El Tor N16961
 - BWA and BWA-SW alignment tool was utilized
 - Reads containing insertions / deletions or regions mapping to multiple locations were not included the alignment
- ID of single-nucleotide polymorphism
 - Alignments were analyzed for SNP's using SolSNP and customized scripts
- Phylogenetic analysis was performed using parsimony criteria

The search for “the smoking gun”!



Group V

24 *V. cholera* genomes

2 major Nepalese clades

Haiti nested within Nepal

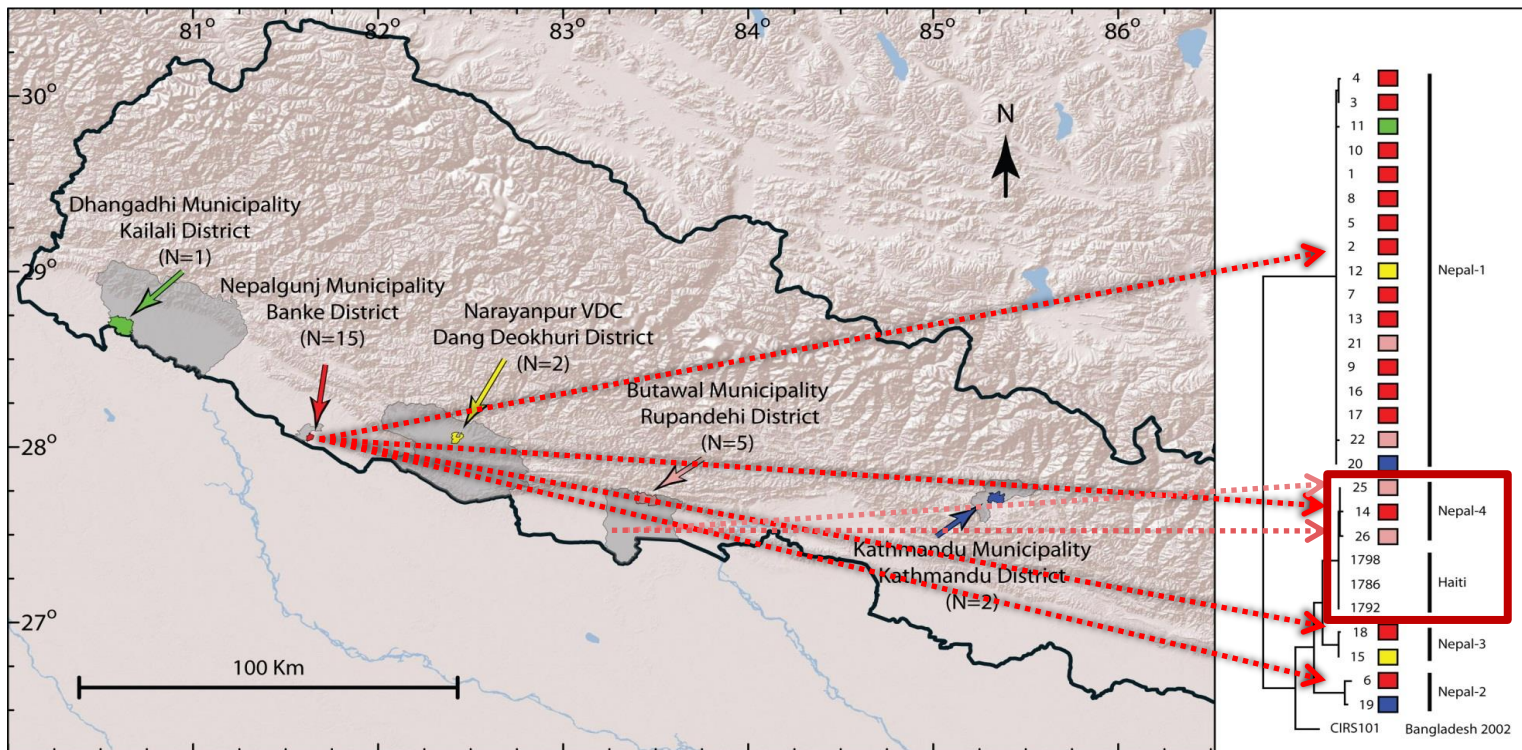
Near Match: Haiti – Nepal-4

1 to 2 SNPs difference between Nepal-4 and Haitian genomes)

Nucleotide or amino acid in:			Haitian isolate			Nepalese isolate		
Chromosome	Position	Reference strain	1786	1792	1798	14	25	26
I	2787016	C	C	C	C	T	T	T
		Gly	Gly	Gly	Gly	Arg	Arg	Arg
I	1090536	T	T	T	T	T	T	G
		Ile	Ile	Ile	Ile	Ile	Ile	Ser
II	962762	C	C	C	C	T	C	C
		Ala	Ala	Ala	Ala	Ala	Ala	Ala

The search for “the smoking gun”!

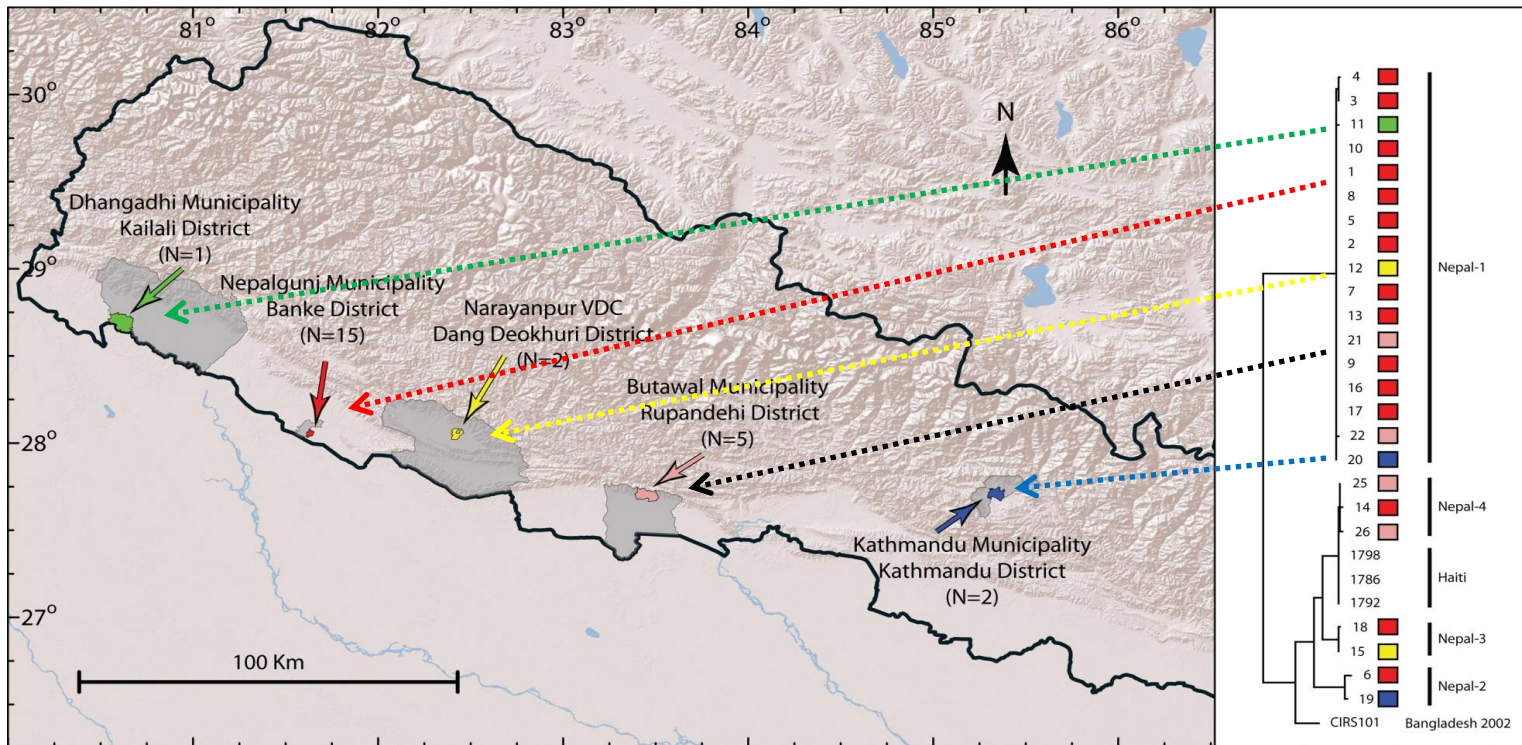
- Outbreak isolates from Nepalgunj were observed in all four clusters
- Isolates from Nepal 4 cluster was present in 2 locations separated by only 100km



Classified as ECDC NORMAL

The search for “the smoking gun”!

- Nepal 1 cluster has a dramatically low diversity and presented in all 5 locations separated by app. 200km



Classified as ECDC NORMAL

Did we find the smoking gun?

- Cholera seems to have been in Nepal for years due to the distribution of the SNP clones
- Very strong indications that cholera was transmitted from Nepal to Haiti
- Strains from the Nepal 4 cluster representing a common ancestor with the Haitian isolates
- The Haitian strains differed with only one SNPs from the Nepalese – “the closest to a molecular proof so far”
- It is possible that a closer related isolate will emerge from a neighboring country than Nepal – so far it hasn’t!
 - Still confirms a single introduction of Vc to Haiti

In summary

- Whole genome sequencing (WGS) is a powerful tool for outbreak investigation
- SNP analysis enables high-resolution tracking of pathogen transmission
- Genomic data can reveal evolutionary relationships and outbreak origins
- Cholera remains a major public health threat in low- and middle-income countries
- Historical and modern outbreaks highlight the importance of sanitation and clean water
- International travel and humanitarian missions can inadvertently spread infectious diseases
- Genomic epidemiology provides actionable insights for public health responses
- Integration of molecular tools with traditional epidemiology enhances outbreak preparedness
- Global collaboration and data sharing are essential for managing transboundary health threats – also in Europe!

References

Genomic Epidemiology and Cholera:

- Chin et al., 2011. The Origin of the Haitian Cholera Outbreak. New England Journal of Medicine
- Ceccarelli et al., 2011. Genomic Analysis of Haitian Cholera Strains. Lancet Infectious Diseases
- Reimer et al., 2011. Whole Genome Sequencing of Haitian Cholera Isolates. Emerging Infectious Diseases
- Hendriksen et al., 2011. Population Genetics of Vibrio cholerae from Nepal. mBio.

Global Health and Cholera Surveillance:

- WHO Fact Sheet on Cholera: <https://www.who.int/news-room/fact-sheets/detail/cholera>
- PAHO Health Cluster Bulletin on Haiti: <https://www.paho.org>
- The New Humanitarian: Cholera Outbreak in Nepal Under Control (2010)

Historical and Epidemiological Context:

- Ivers et al., 2012. Cholera in Haiti: One Year Later. American Journal of Tropical Medicine and Hygiene
- Piarroux et al., 2011. The Spread of Cholera in Haiti. Emerging Infectious Diseases

Acknowledgements

The creation of this training material was commissioned by ECDC to Institut Pasteur with the direct involvement of Rene Hendriksen

Slido 1



Have you ever worked with cholera

☐ Yes

☐ No

Slido 2

What was your role working with Cholera?

- ☐ Clinical microbiologist
- ☐ Epidemiology
- ☐ Bioinformatician
- ☐ Public health coordination
- ☐ Other: _____

Slido 3



How well prepared is your institution for a cholera outbreak?

- ☐ Very prepared
- ☐ Somewhat prepared
- ☐ Not prepared
- ☐ Don't know