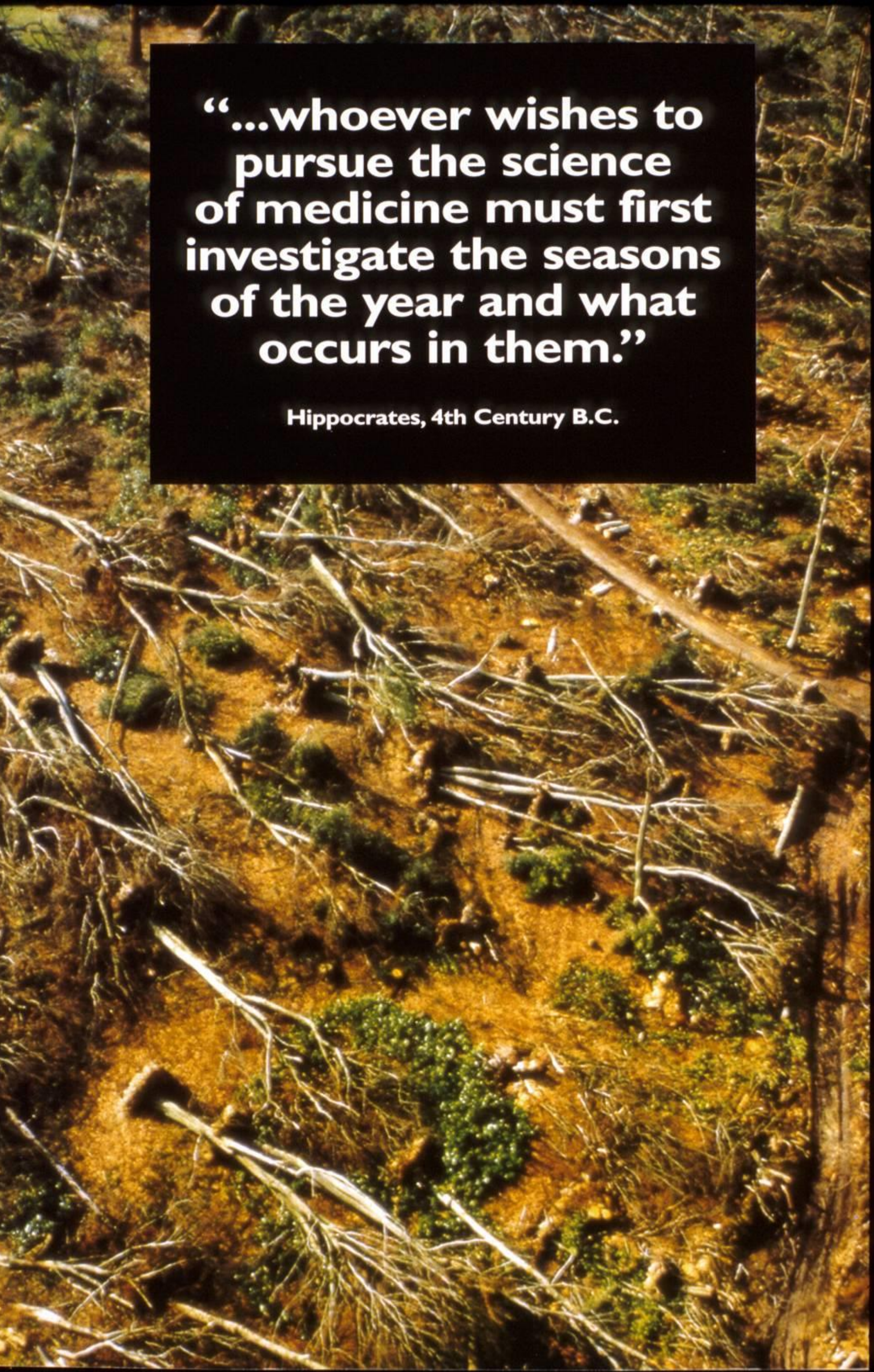


> “...whoever wishes to pursue the science of medicine must first investigate the seasons of the year and what occurs in them.”
>
> Hippocrates, 4th Century B.C.

Impact of climate change on water quality and health: a focus on cholera

3rd International One Health Congress
Amsterdam

March 17, 2015

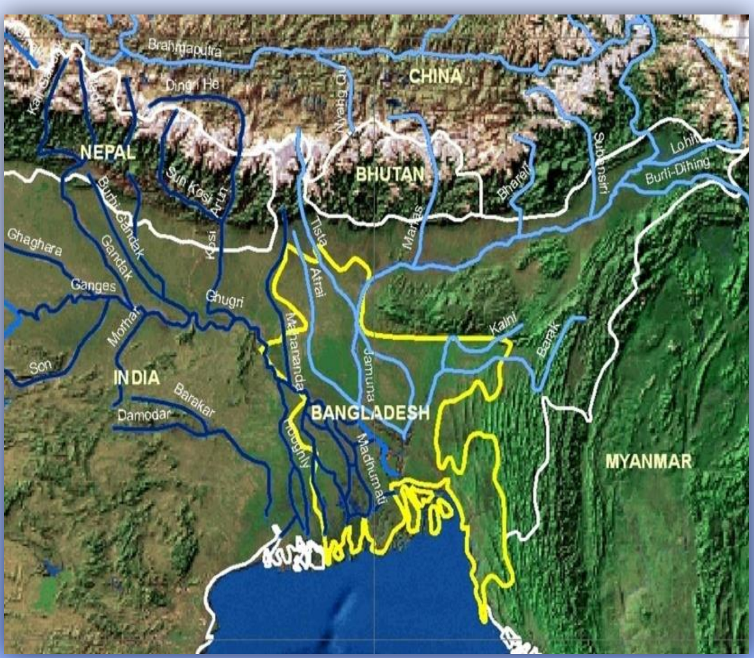
Rita R. Colwell, Ph.D., D.Sc.
Distinguished University Professor
University of Maryland College Park
and
Johns Hopkins University Bloomberg School of Public Health

Water-related diseases

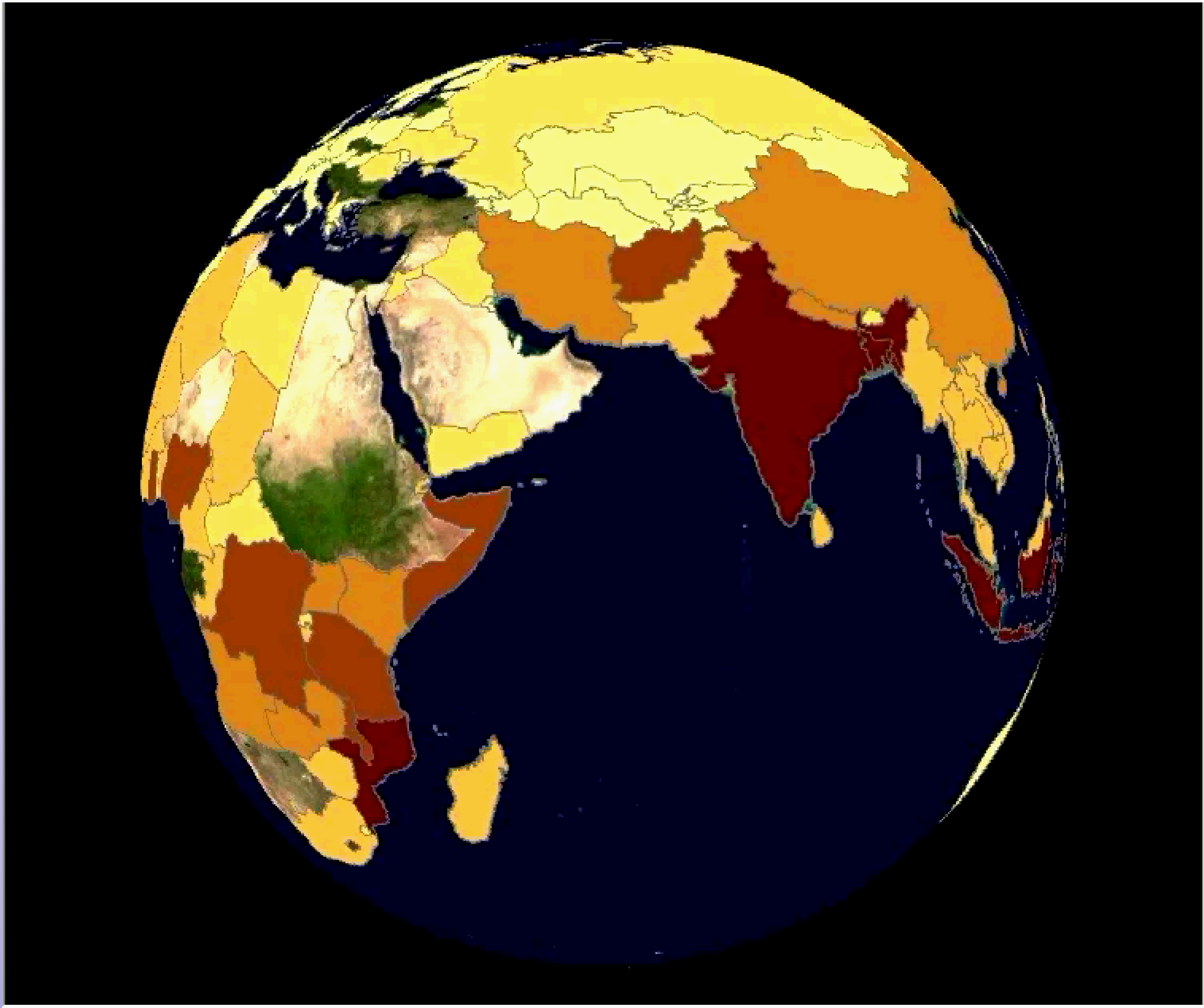
	Cases per year	Deaths per year
Amoebiasis	48,000,000	110,000
Arsenic	28-35m exposed to drinking water with elevated levels	
Diarrhoeal disease, Including cholera	1.5 billion	1,800,000
Dracunuliasis (guinea worm)	> 5000	-
Fluorosis	26 million (China)	-
Giardiasis	500,000	Low
Hepatitis A	1,500,00	-
Intestinal helminths	133,000,000	9400
Malaria	396,000,000	1,300,000
Schistosomiasis	160,000,000	> 10,000
Trachoma	500,000,000	-
Typhoid	500,000	25,000

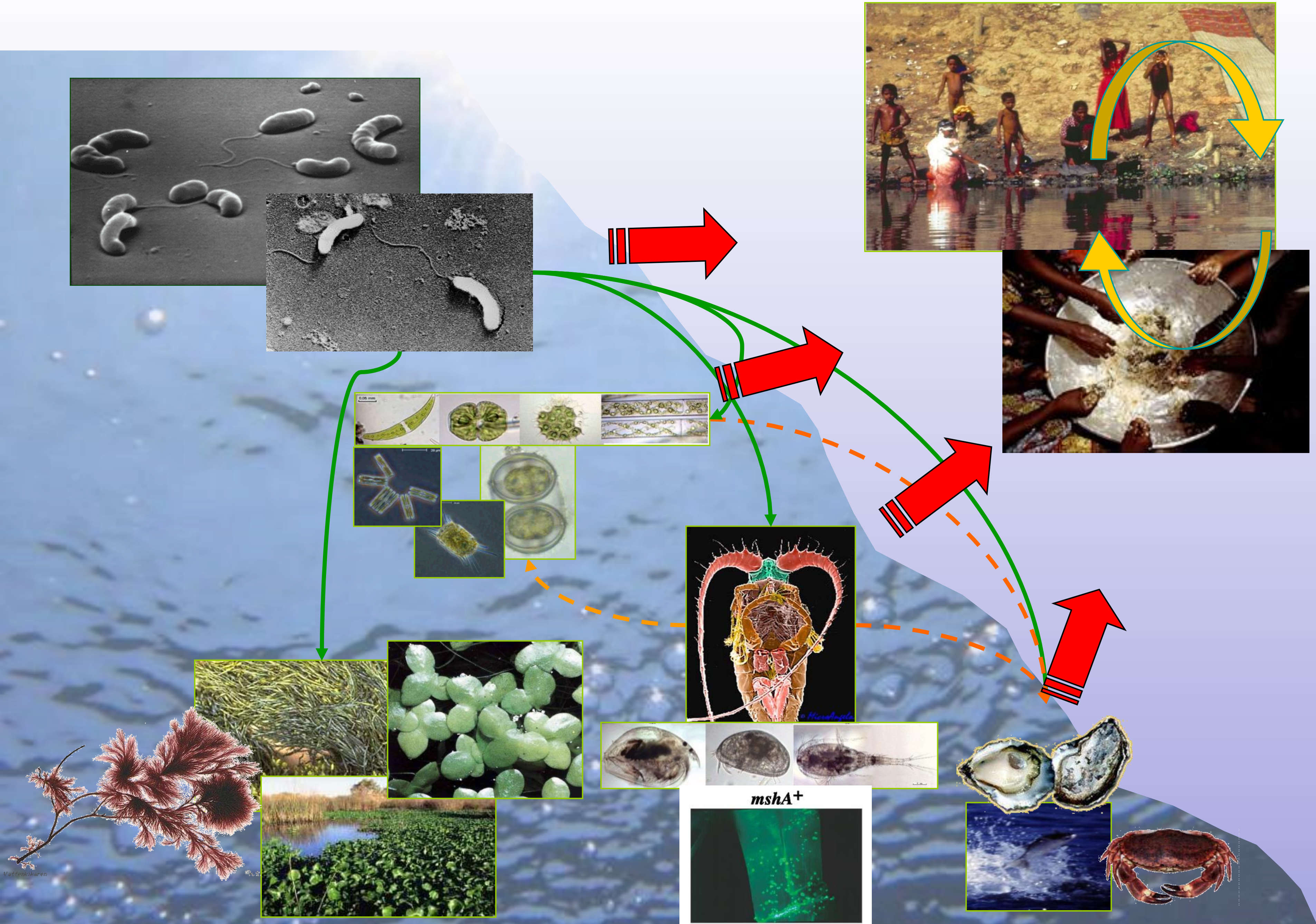
Cholera: A Global Disease

- **Acute water-related diarrheal disease**
- **Seventh pandemic started in 1960s**
- **Occurs in more than 50 countries affecting approximately 7 million people**
- **Bengal Delta is known as "native homeland" of cholera outbreaks**
- **Since cholera bacteria**
  - **exist naturally in aquatic habitats**
  - **evidence of new biotypes emerging, *it is highly unlikely that cholera will be eradicated but clearly can be controlled by provision of safe drinking water.***




CHOLERA.
SCIENCE
NEW YORK CITY
KENDRICK



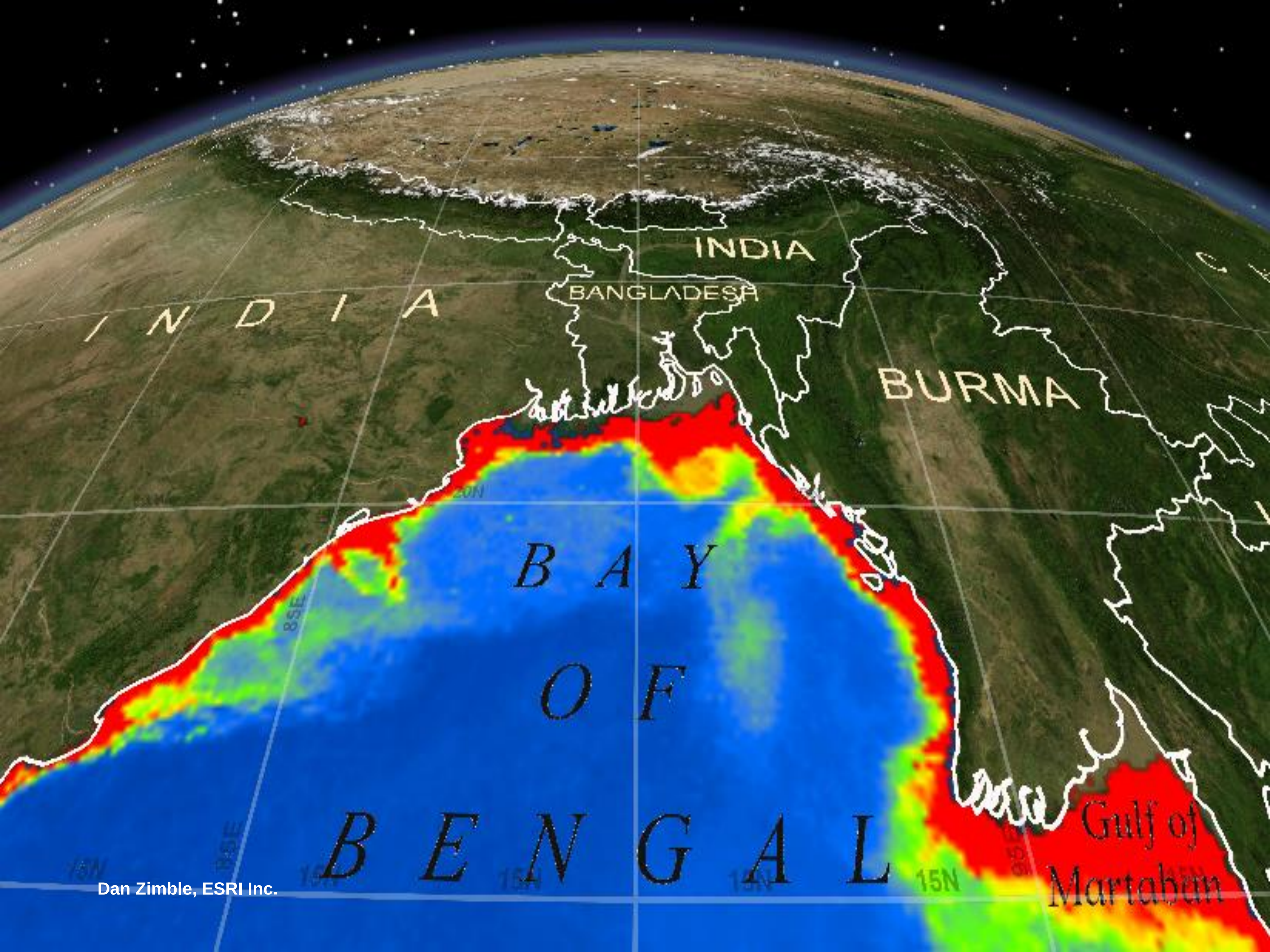


G. Constantin de Magny

What is reported about cholera and macro-scale processes?

Cholera outbreaks have been linked to environmental and climate variables

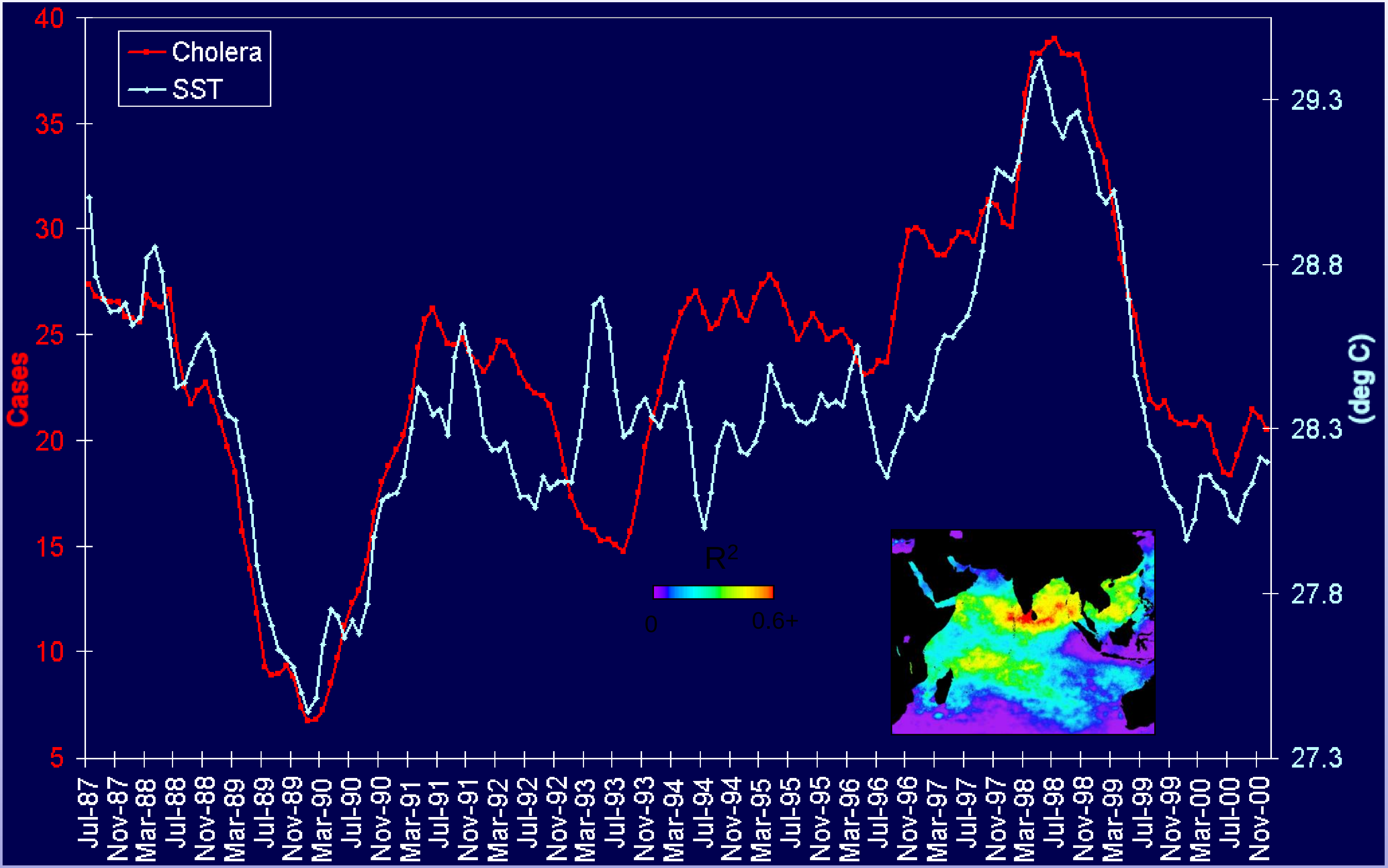
- precipitation (*Hashizume et al. 2008*)
- floods (*Koelle et al., 2005*)
- river level (*Emch et al., 2008*)
- sea surface temperature (*Colwell*, 1996; *Lobitz et al., 2000*)
- coastal salinity (*Miller et al., 1982*)
- dissolved organic material (*Worden et al., 2005*)
- fecal contamination (*Islam et al., 2006)*
- chlorophyll (*Lobitz et al., 2000, Magny et al., 2008)*



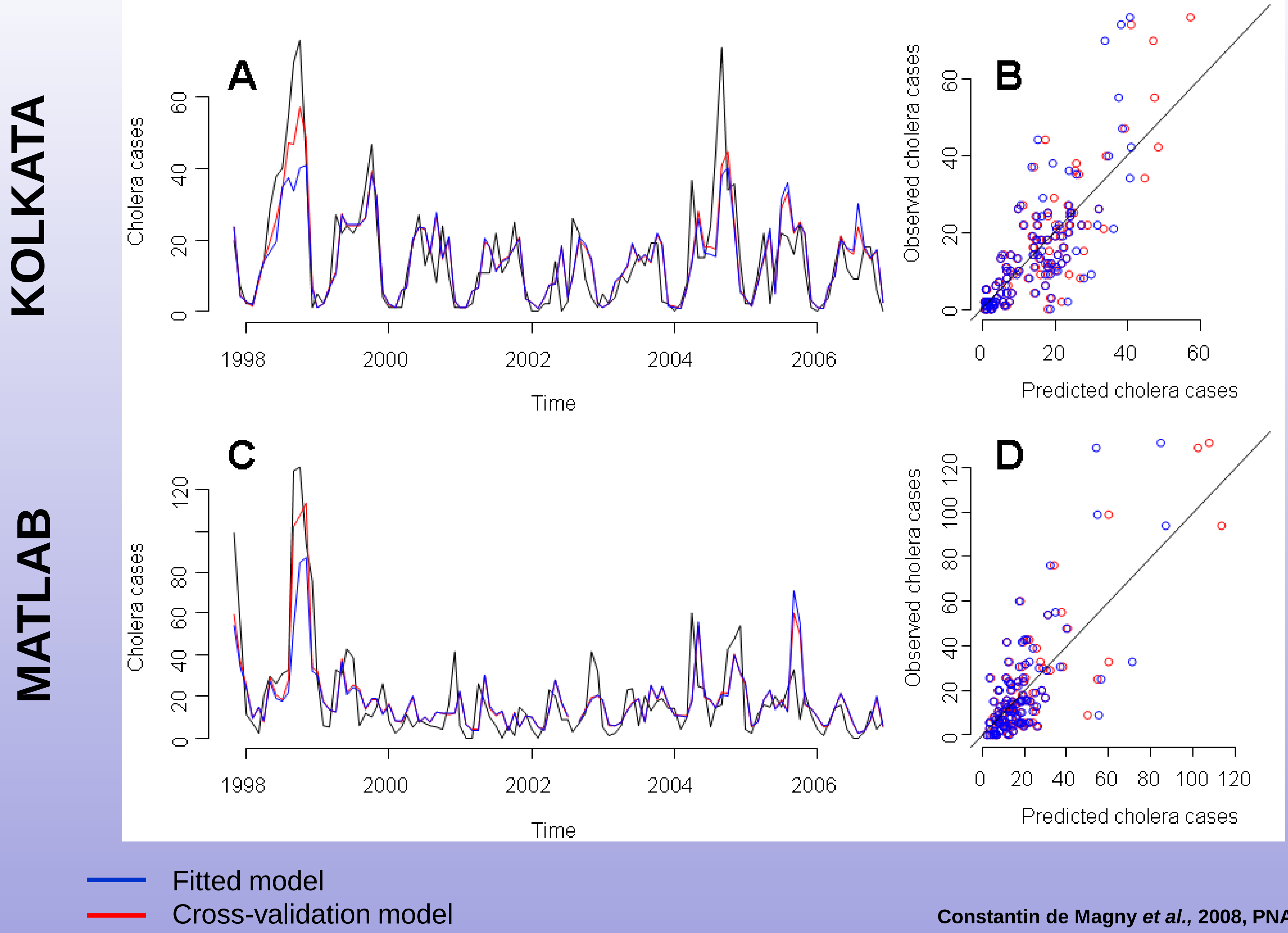
Dan Zimble, ESRI Inc.

Cholera and SST in the Indian Ocean

Six-month SST lead: $R^2 = 0.72$



Environmental Signatures Related To Cholera Epidemics



Constantin de Magny *et al.*, 2008, PNAS

Results

Kolkata: Significant and positive relationship between cholera and CHL(t) and Rain(t).

Matlab: Significant and positive relationship between cholera and Chl(t-1).

KOLKATA

+1 $mg.m^{-3}$ in CHL(t) => **+32.5%** in number of cholera cases (95% CI 8.3%-62.0%)

+1 $mm.day^{-1}$ in Rain(t) => **+6.5%** in number of cholera cases (95% CI 1.6%-11.7%)

MATLAB

+1 $mg.m^{-3}$ in CHL(t-1) => **+31.4%** in number of cholera cases (95% CI 13.0%-52.7%)

Constantin de Magny *et al.*, 2008, PNAS

Epidemic Cholera

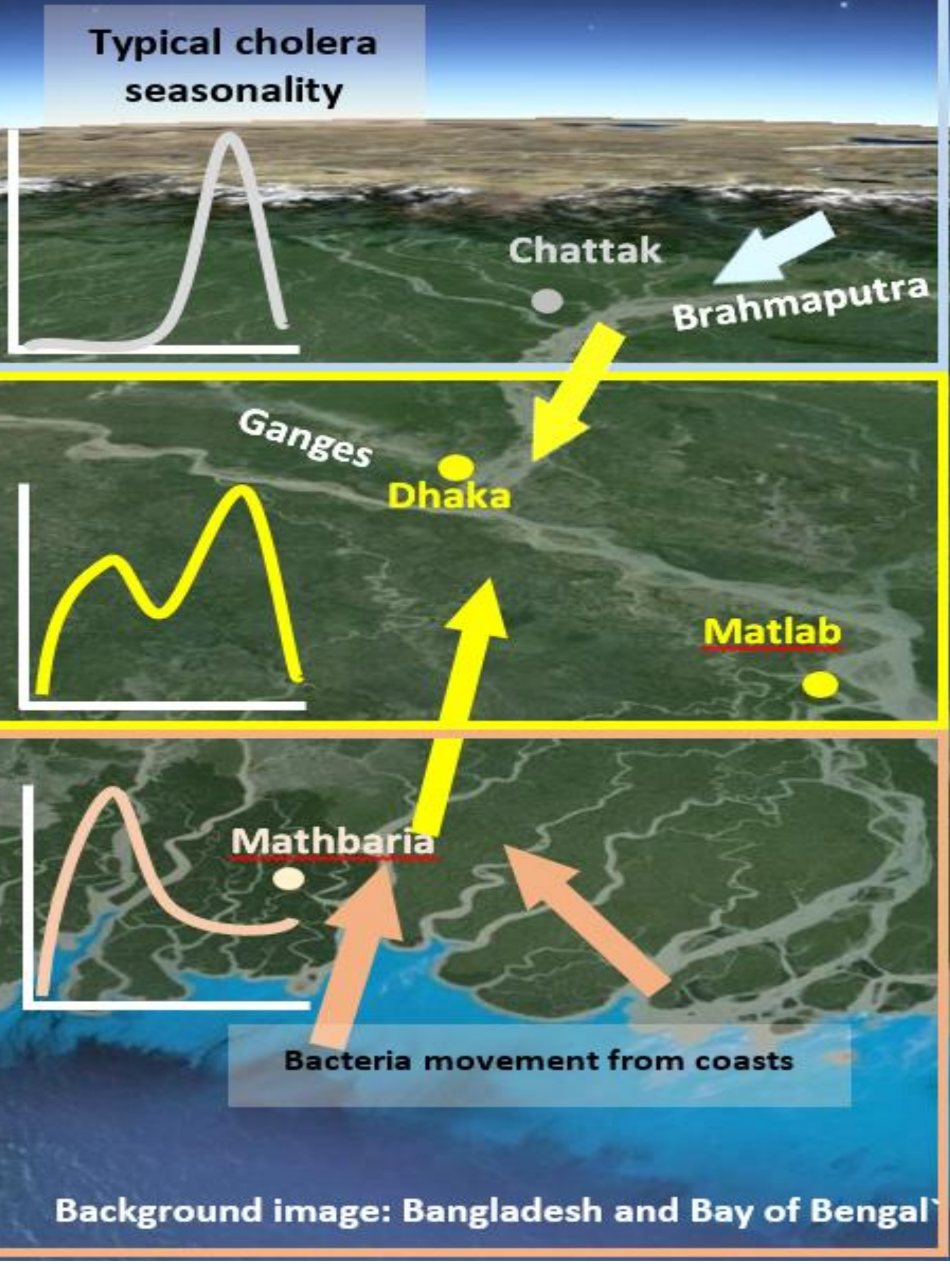
- Sporadic outbreak
- Usually occurs following floods or inundation of large landscapes
- Warm temperatures may increase growth of bacteria in aquatic bodies.

Mixed-mode Cholera

- Usually two seasonal peaks
- One peak related to seawater intrusion; Second peak associated with widespread inundation
- Specific to Bengal Delta region

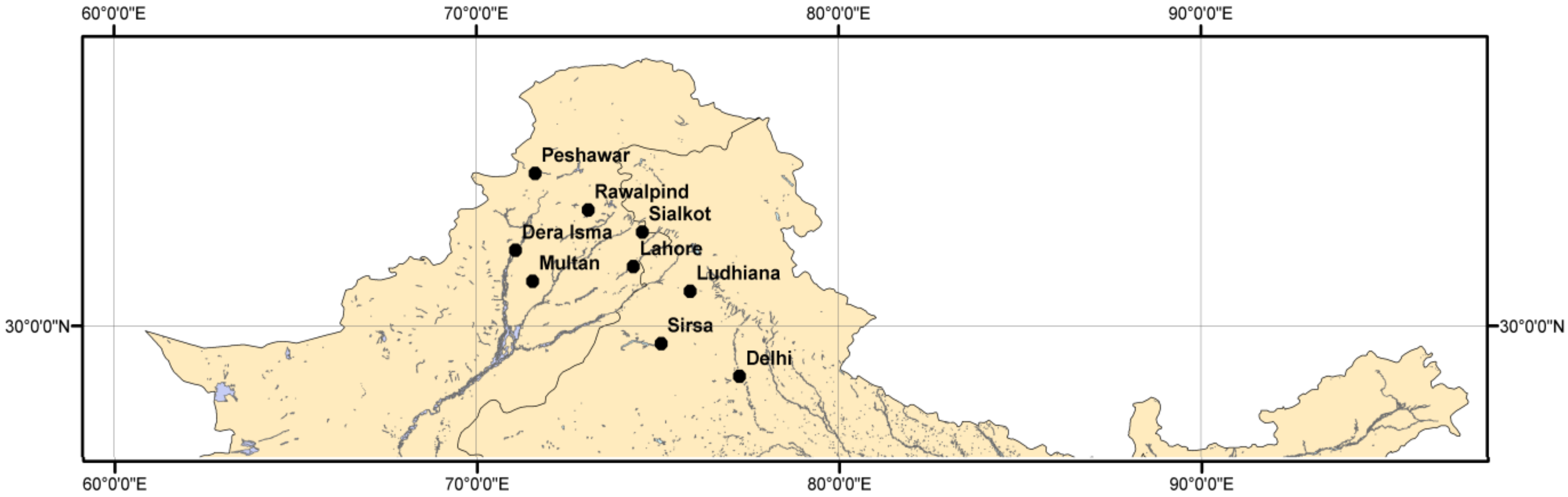
Endemic Cholera

- Cholera persists throughout year in coastal regions
- Seawater Intrusion from coasts to inland
- Cholera outbreaks occur during low river flow season

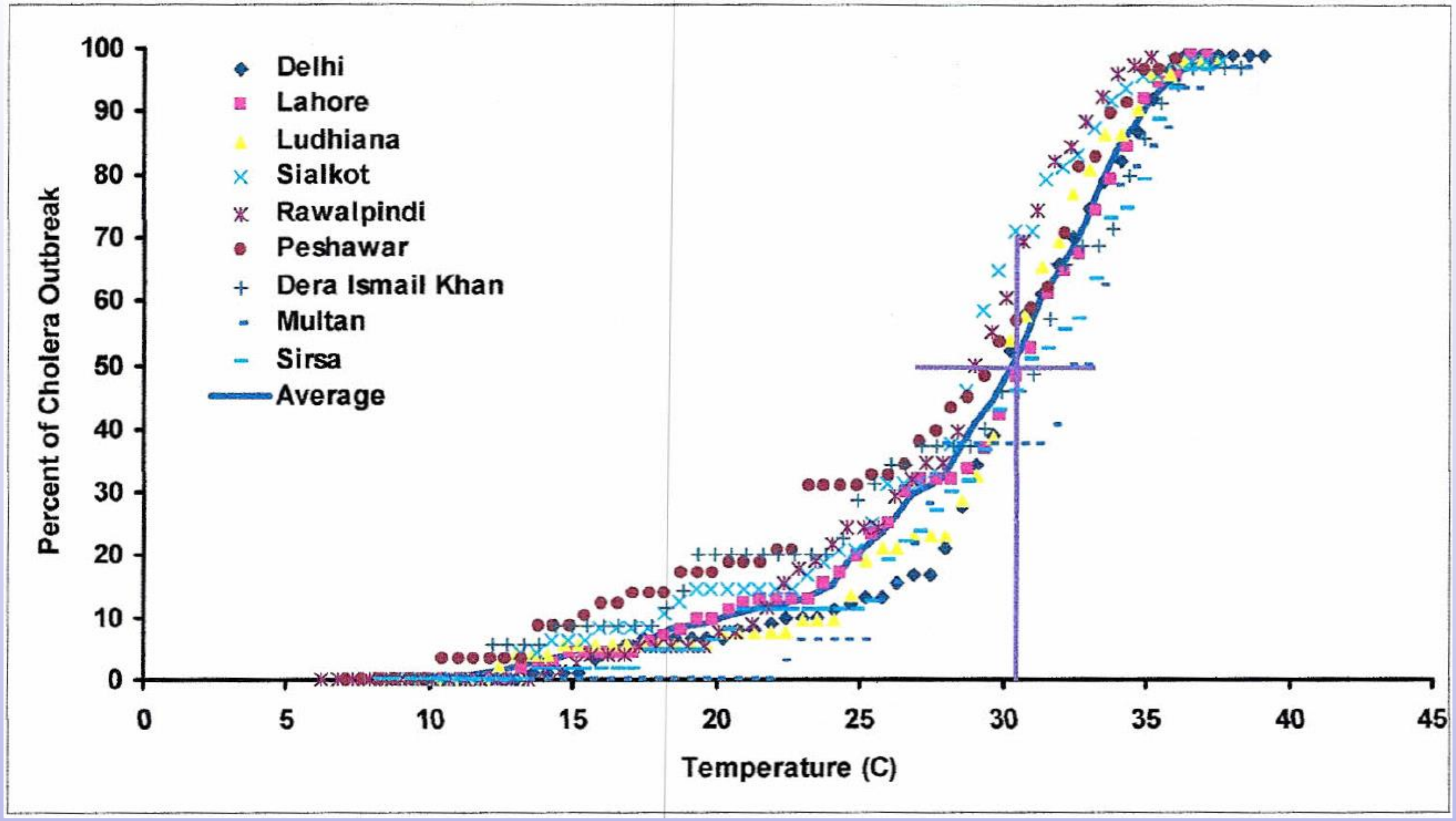


Background image: Bangladesh and Bay of Bengal


Afghanistan
Kabul
Islamabad
Srinagar
Borders of Kashmir are in dispute; India, Pakistan and China have differing claims.
China
Tongtran R. (Yangtze)
Mekong R.
Indus R.
Xiangquan R.
0 400 km
0 300 mi
Jammu
Amritsar
Pakistan
Chandigarh
Simla
30°
Yamuna R.
Ganges R.
Yarlung R.
(Brahmaputra) R.
Indus R.
Nepal
Kathmandu
New Delhi
Bhutan
Ledo
Itanagar
Jaipur
Agra
Lucknow
Siliguri
Kanpur
Ganges R.
Sillong
Kohima
Allahabad
Varanasi
Bangladesh
Imphal
Agartala
Gandhinagar
Bhopal
Aizawl
Kandla
Ahmadabad
Jabalpur
Calcutta
Dhaka
Jamshedpur
Myanmar (Burma)
Narmada R.
Mahanadi R.
Mouths of the Ganges
India
Veraval
Raipur
Cuttack
Bhubaneshwar
20°
Silvassa
Puri
Godavari R.
Mumbai
N
Pune
Irrawaddy R.
Vishakhapatnam
Arabian Sea
Hyderabad
Yangon (Rangoon)
Krishna R.
Mouths of the Irrawaddy
Panaji
Bay of Bengal
Marmagao
Guntakal
Andaman Islands (India)
Bangalore
Chennai
Mangalore
Cauvery R.
Laccadive Islands
Pondicherry
Port Blair
Calicut (Khozikode)
Cuddalore
Kavaratti
10°
Palk Strait
Andaman Sea
Madurai
Nicobar Islands (India)
90°
Sri Lanka
Tiruvanantapuram
70°
Laccadive Sea
Gulf of Mannar
©1997 MAGELLAN Geographix℠
(805) 685-3100 www.maps.com

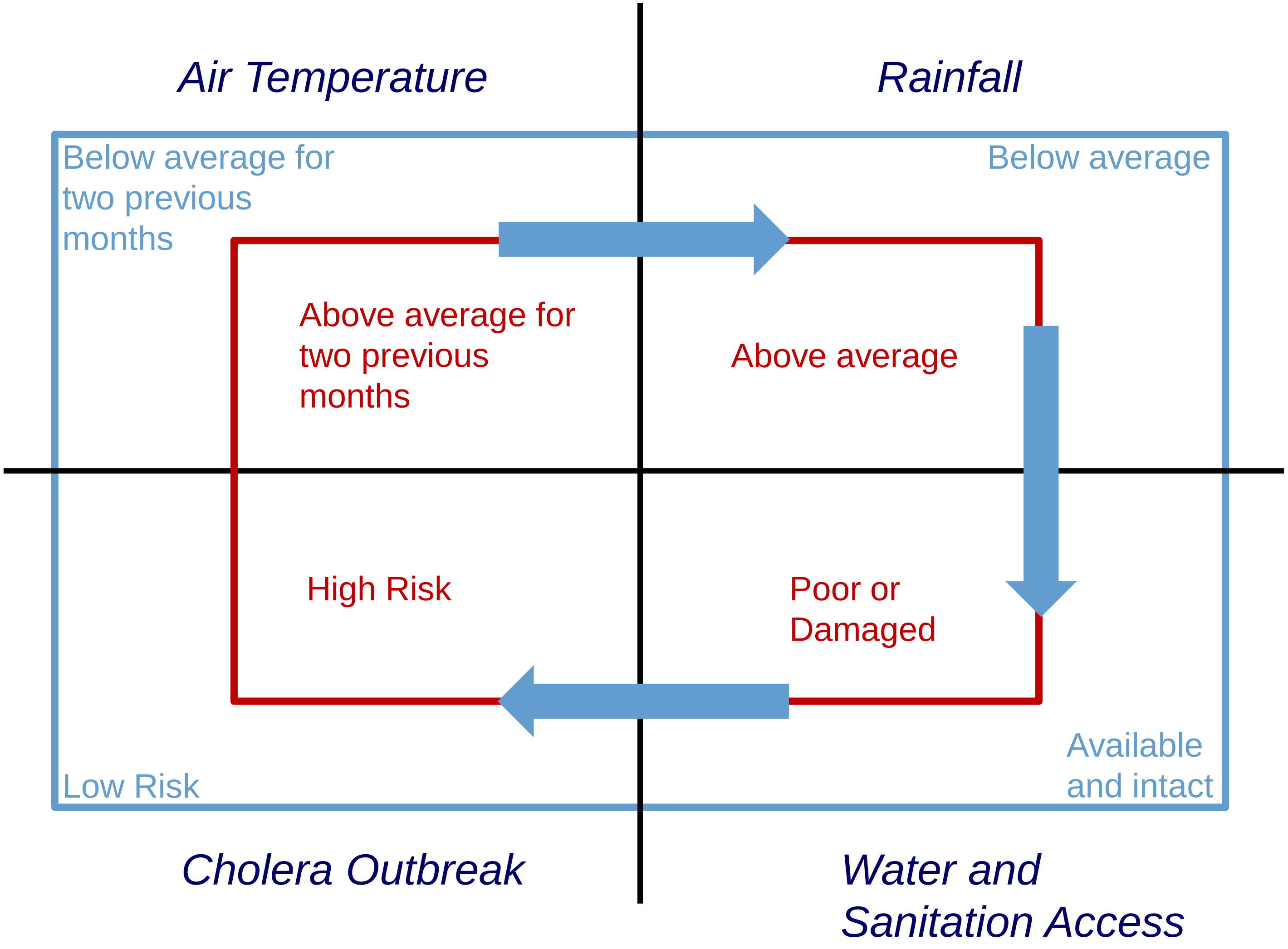


Location of areas in the Indus River Basin where cholera outbreaks were reported from 1875-1900.



Relationship between cholera outbreaks and air temperatures

Theoretical framework for predicting cholera outbreaks in epidemic regions

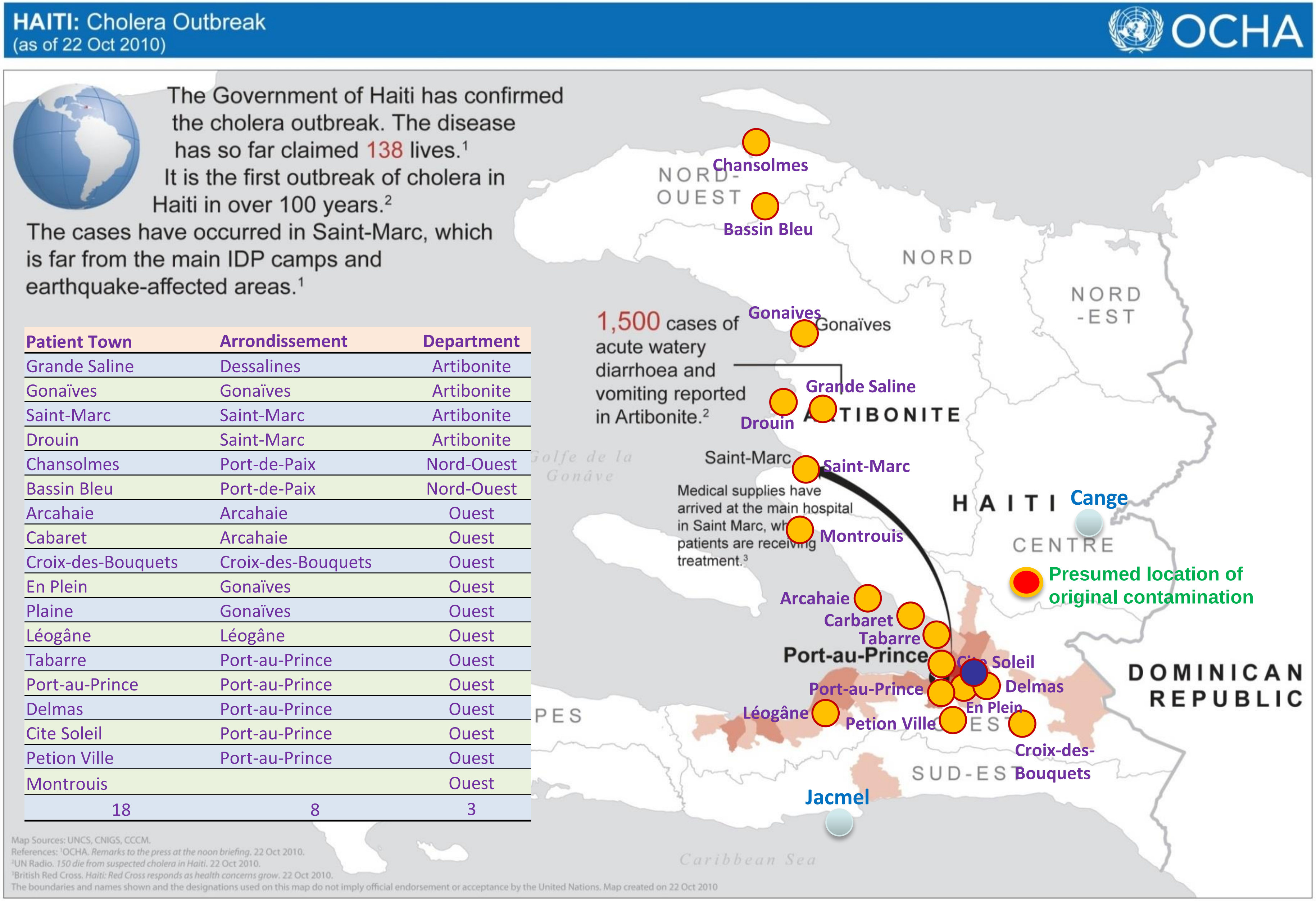


Could we have predicted the Haiti Cholera outbreak?

- **Recent cholera outbreak in Haiti indicated the disease remains a global threat.**
- **Framework for developing cholera prediction models in cholera endemic (ER) and non-endemic regions (NER)**
- **The sharp contrast in mortality rates between ER and NER exists not because we do not know how to treat cholera patients, but because of a persistent "knowledge barrier" between ER and NER.**
- **We propose a pragmatic and adaptive framework which hypothesizes that convergence of three enabling situations - Inception, Environmental Conditions, and Transmission - are necessary for a cholera outbreak to become an epidemic.**

Antarpreet Jutla, Elizabeth Whitcombe, Nur Hasan, Bradd Haley, Ali Akanda, Anwar Huq, Munir Alam, R. Bradley Sack and Rita Colwell. 2013. Environmental factors influencing epidemic cholera. Amer J Trop Med Hyg 89(3) 597-607

Source and Distribution of isolates collected from Haitian outbreak



HAITI: Cholera Outbreak
(as of 22 Oct 2010)

OCHA

The Government of Haiti has confirmed the cholera outbreak. The disease has so far claimed 138 lives.[1] It is the first outbreak of cholera in Haiti in over 100 years.[2]

The cases have occurred in Saint-Marc, which is far from the main IDP camps and earthquake-affected areas.[1]

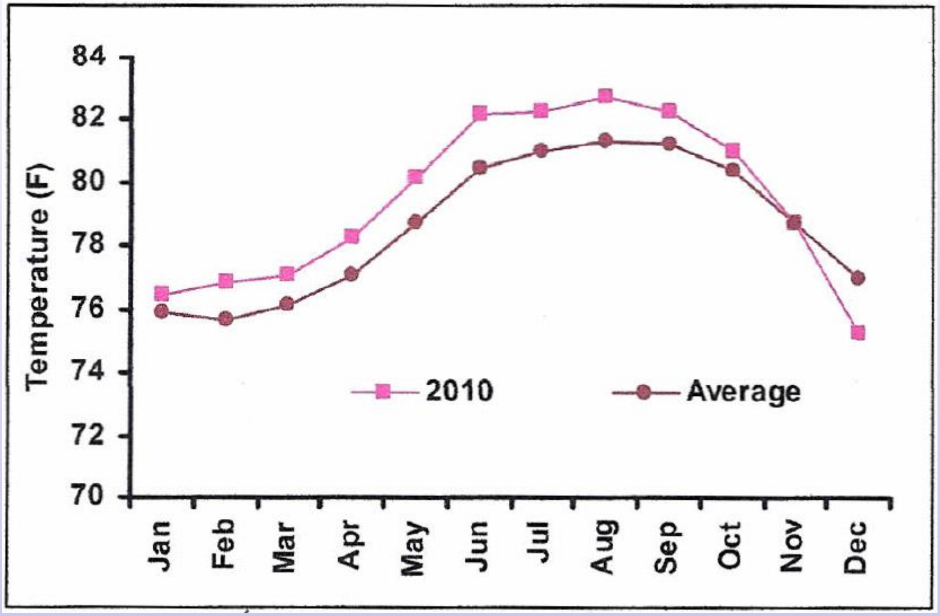
1,500 cases of acute watery diarrhoea and vomiting reported in Artibonite.[2]

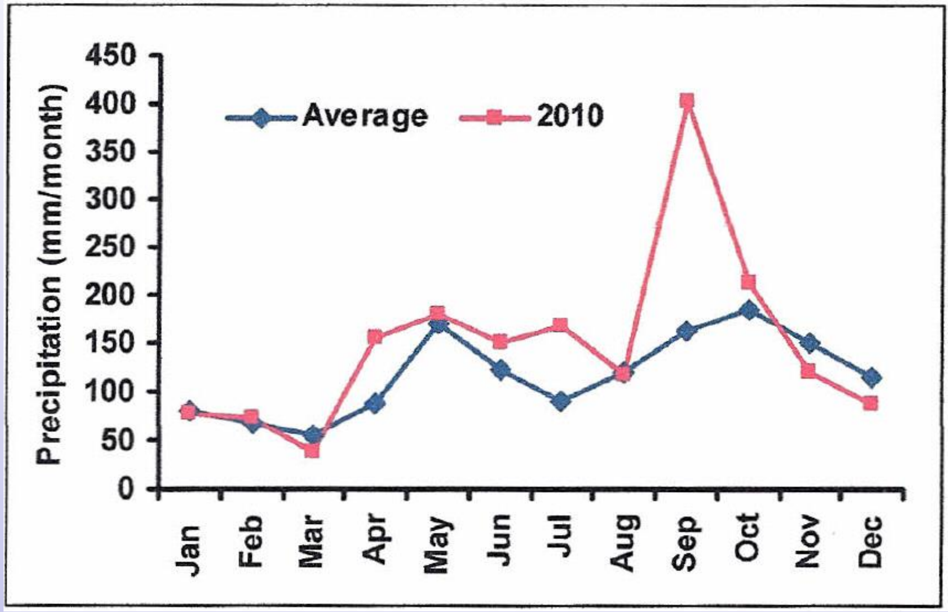
Medical supplies have arrived at the main hospital in Saint Marc, wh patients are receiving treatment.[3]

Patient Town	Arrondissement	Department
Grande Saline	Dessalines	Artibonite
Gonaïves	Gonaïves	Artibonite
Saint-Marc	Saint-Marc	Artibonite
Drouin	Saint-Marc	Artibonite
Chansolmes	Port-de-Paix	Nord-Ouest
Bassin Bleu	Port-de-Paix	Nord-Ouest
Arcahaie	Arcahaie	Ouest
Cabaret	Arcahaie	Ouest
Croix-des-Bouquets	Croix-des-Bouquets	Ouest
En Plein	Gonaïves	Ouest
Plaine	Gonaïves	Ouest
Léogâne	Léogâne	Ouest
Tabarre	Port-au-Prince	Ouest
Port-au-Prince	Port-au-Prince	Ouest
Delmas	Port-au-Prince	Ouest
Cite Soleil	Port-au-Prince	Ouest
Petion Ville	Port-au-Prince	Ouest
Montrouis		Ouest
18	8	3

Map Sources: UNCS, CNIGS, CCCM.
References: [1]OCHA. *Remarks to the press at the noon briefing.* 22 Oct 2010.
[2]UN Radio. *150 die from suspected cholera in Haiti.* 22 Oct 2010.
[3]British Red Cross. *Haiti: Red Cross responds as health concerns grow.* 22 Oct 2010.
The boundaries and names shown and the designations used on this map do not imply official endorsement or acceptance by the United Nations. Map created on 22 Oct 2010

www.reliefweb.int/haiti http://ochaonline.un.org http://oneresponse.info/disasters/haiti/



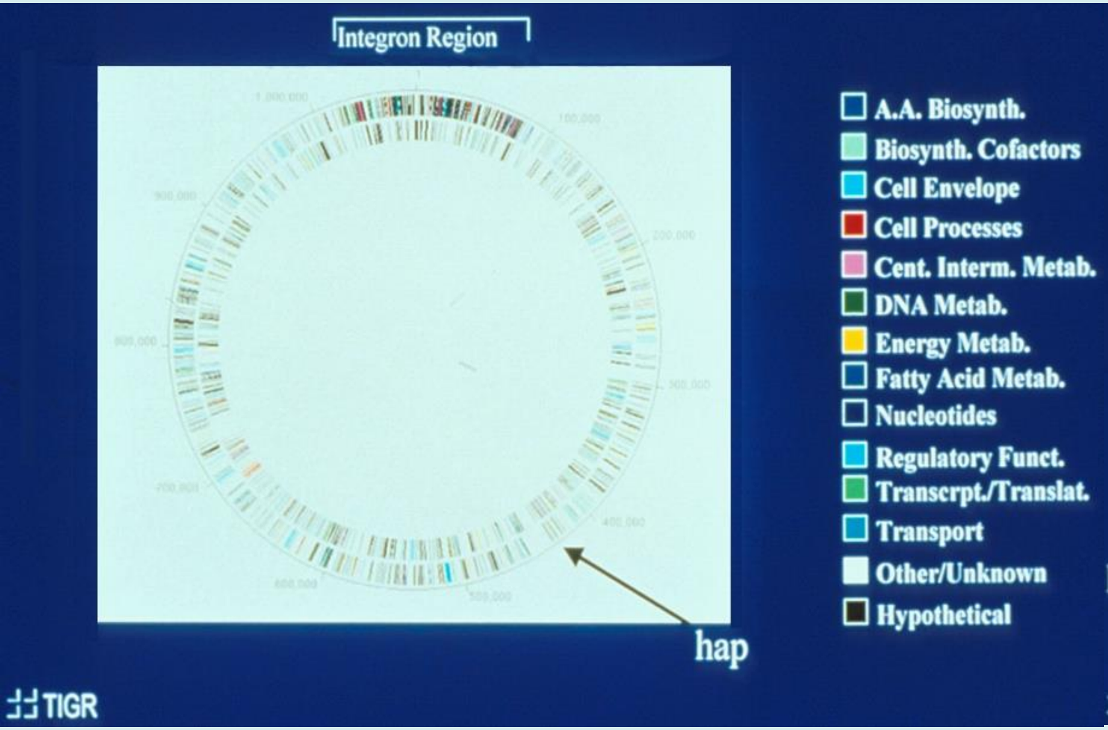


Air temperature in Haiti in 2010 compared with historical air temperature data

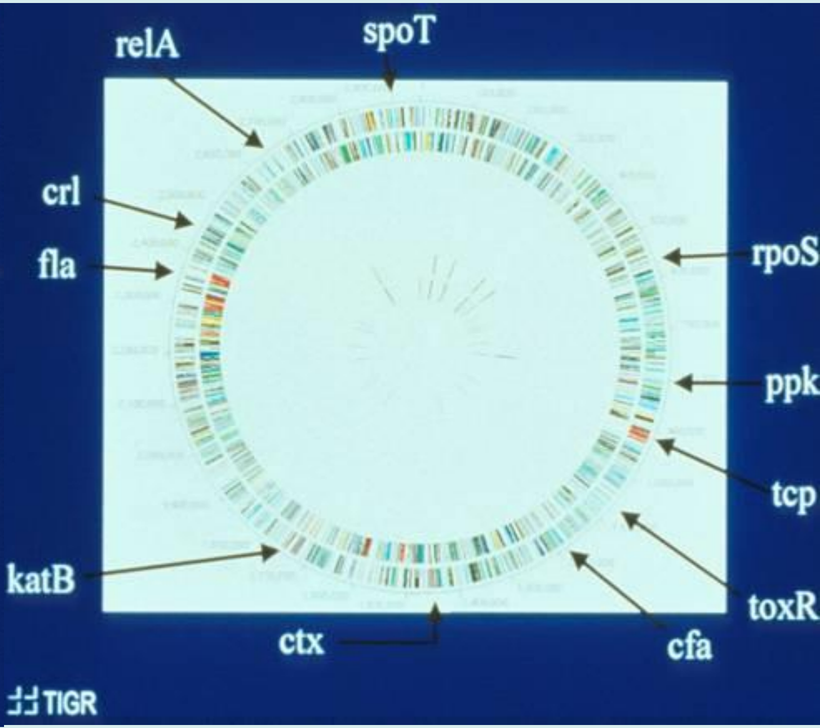
Monthly rainfall in Haiti in 2010 compared with historical rainfall data

Vibrio cholerae

Small Chromosome



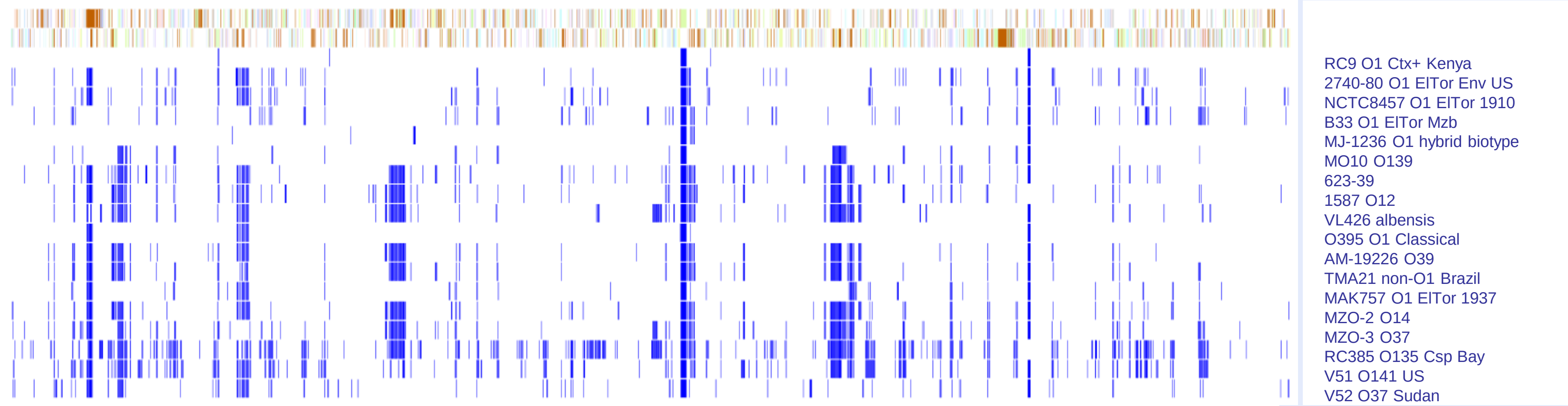
Large Chromosome



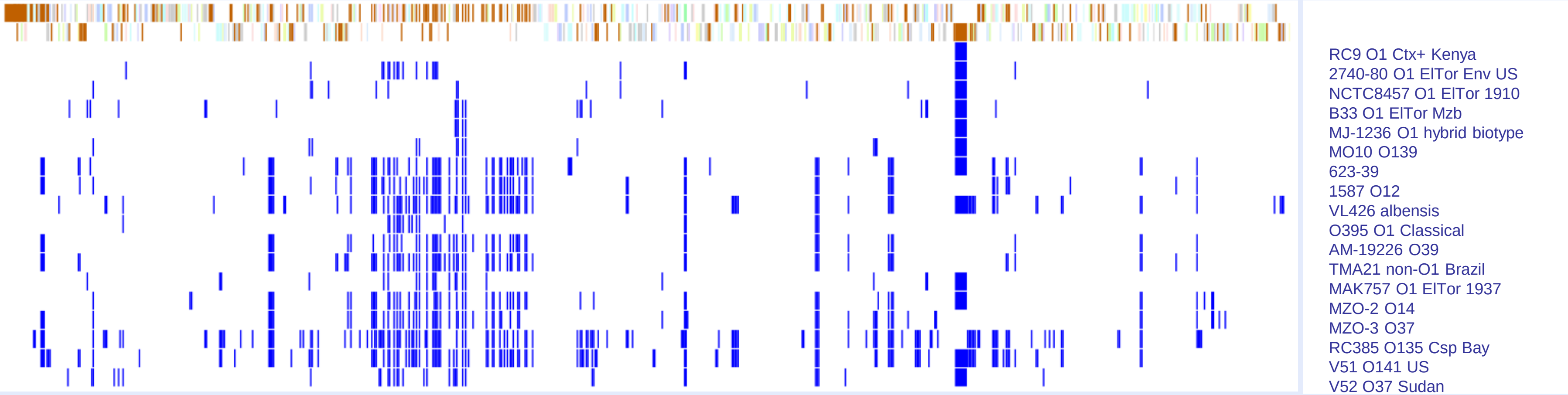
Source: The Institute for Genomic Research

Mosaic genomic structure of *V. cholerae* revealed by comparative genomics

Chromosome I (2,961,149 bp, 2,742 ORFs)



Chromosome II (1,072,315 bp, 1,093 ORFs)



🟦 Missing ORFs in *V. cholerae* strains (Reference: N16961; cutoff = 70% DNA similarity)

Clinical Sample:

- 81 stool samples

V. cholerae **O1**	**41 samples**
V. cholerae **Non-O1/O139**	**21 samples**

- Both *V. cholerae* O1 and Non-O1/O139 have been isolated from 6 stool samples

Environmental Sample:

A. **From Cange in the Central Plateau:** ca. 199 meters above sea level, is located near the Artibonite River.

- From the hospital: tap water, greywater , and a latrine sample
- From the school a latrine sample was also collected.
- *V. cholerae* non-O1/O139 were isolated from all samples, all are *ctxA* negative (by PCR).

B. From Jacmel in the Sud-Est Department in southern Haiti:

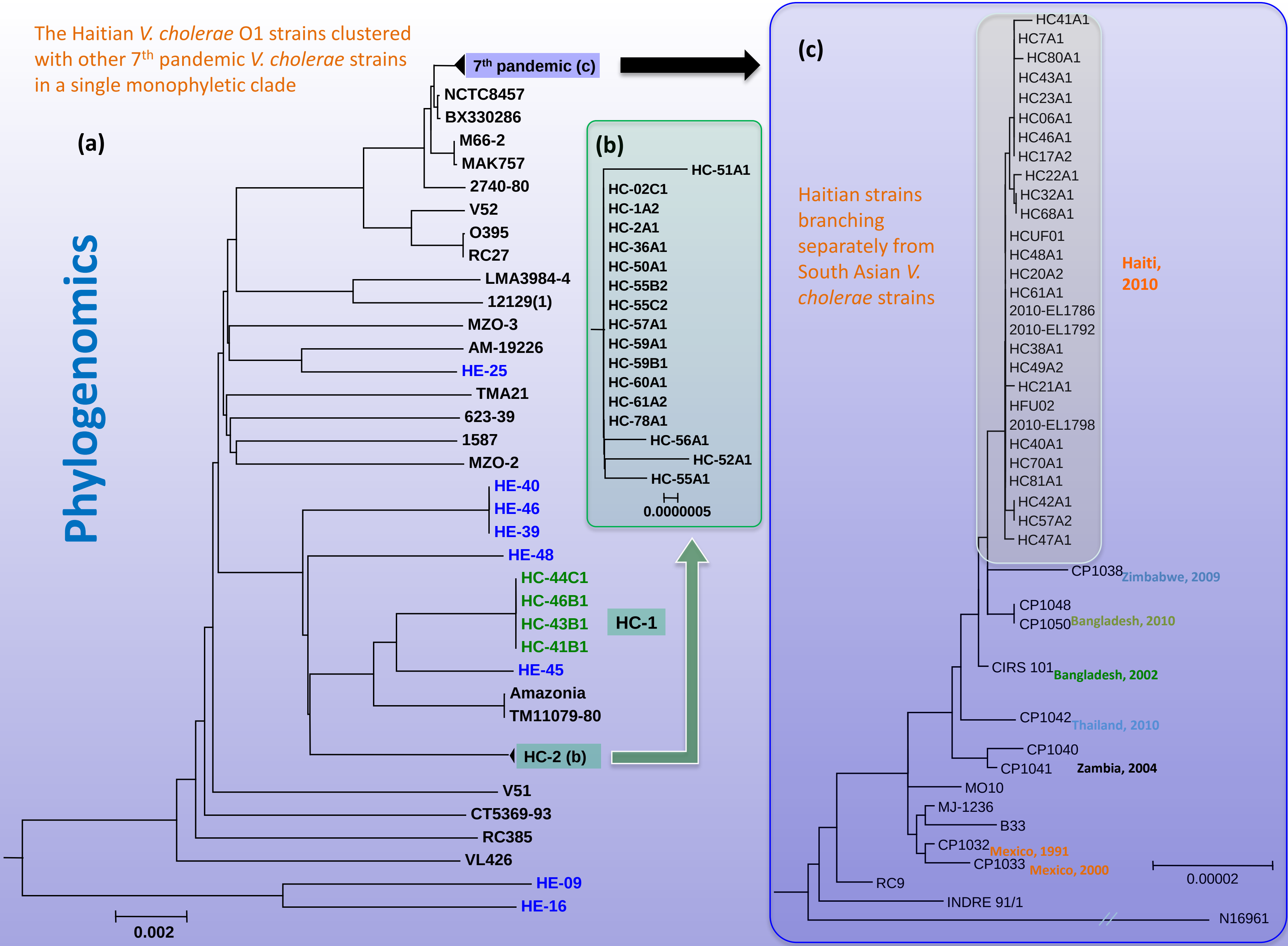
- Water samples were collected from community tube well, river, and ocean
- All samples were negative for *V. cholerae* by toxR PCR,
- Some presumptive *V. alginolyticus* were isolated from these samples which showed positive bands of different size for *ctxA* and the O1 *rfb* by Hoshino PCR.

C. Surface water samples from the south of Haiti, Grand'Anse, Nippes, and Sud.

- *V. cholerae* non-O1/O139 have been isolated and are currently being investigated.

Phylogenomics

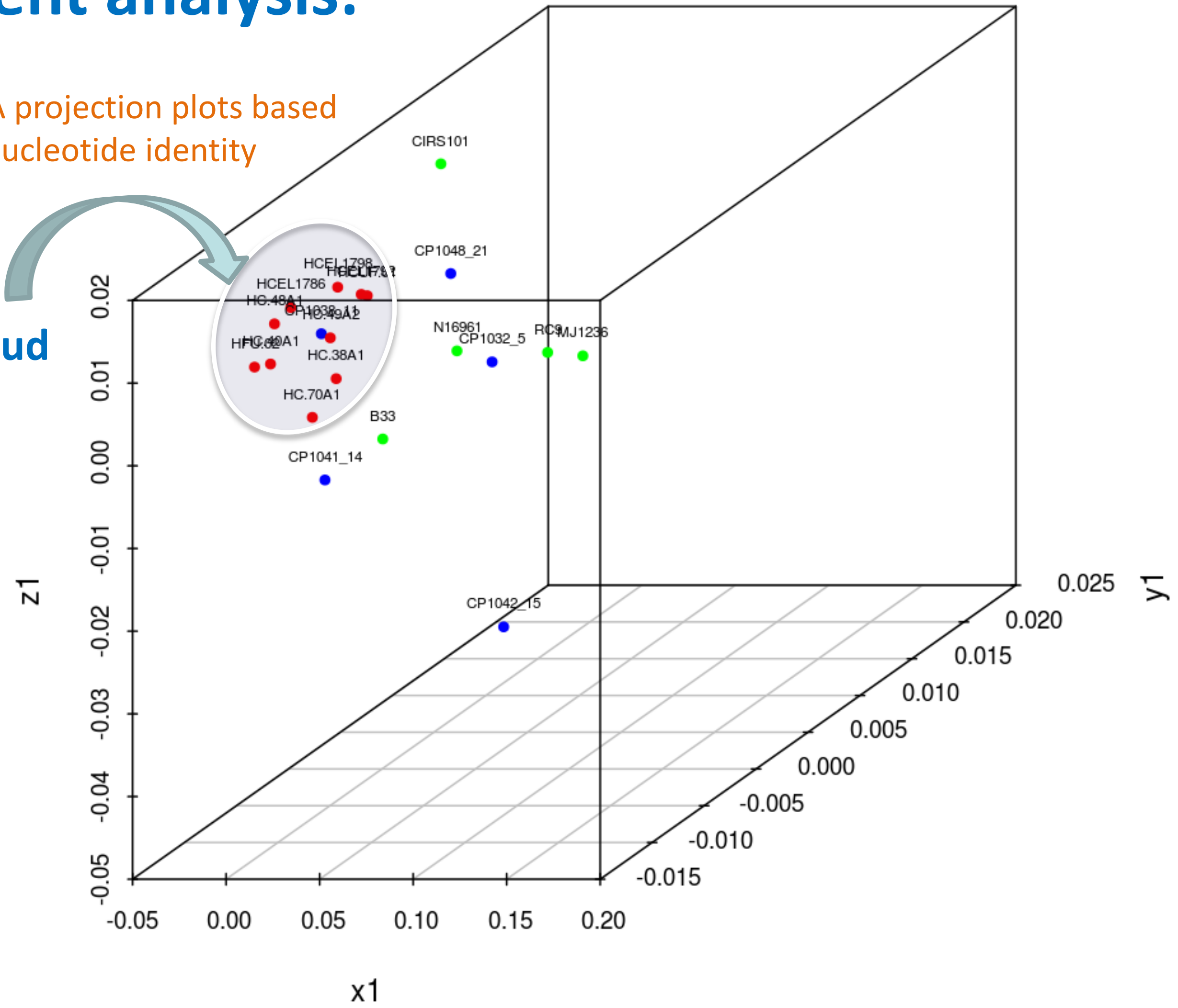
The Haitian *V. cholerae* O1 strains clustered with other 7th pandemic *V. cholerae* strains in a single monophyletic clade



Haitian strains branching separately from South Asian *V. cholerae* strains

Principal component analysis:

The three-dimensional PCA projection plots based on divergence of average nucleotide identity



Haitian Cluster Cloud

- ❑ **10 Haitian strains (red) form a cluster cloud, distinct and yet, distant, from CP genomes (concurrent epidemic isolates form different parts of the world) (blue) and others (green).**
- ❑ **Interestingly, one reference strain CP 1038 (from Zambia) genome falls into the Haitian cluster.**

Conclusion:

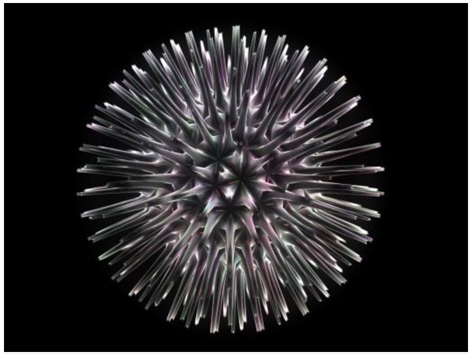
- ☐ **Genomic analysis provided evidence that two distinct *Vibrio* populations, *V. cholerae* O1 and *V. cholerae* non-O1/O139, contributed to the cholera epidemic in Haiti.**

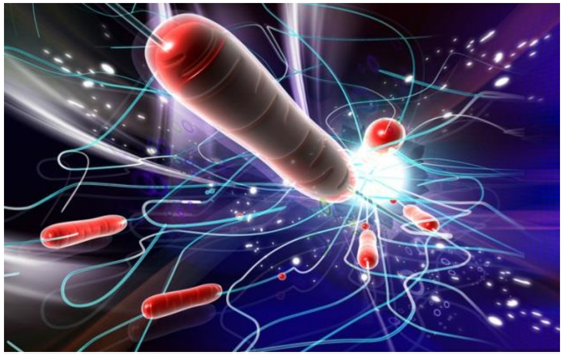
- ☐ **Comprehensive genomic analysis showed:**
  - ✓ ***V. cholerae* O1 populations were clonal, resembling concurrent epidemic isolates from South Asia and Africa.**
  - ✓ ***V. cholerae* non-O1/O139 populations were not clonal but most probably serve as a reservoir for genomic and pathogenicity islands.**

- ☐ ***V. cholerae* non-O1/O139 populations in Haiti harbor a genomic backbone similar to that of toxigenic *V. cholerae* O1 circulating in the Western hemisphere.**

- ☐ **Genomic analysis of Haitian *V. cholerae* O1 strains has provided evidence of:**
  - ✓ a distinct VNTR genotype,
  - ✓ genetic polymorphisms of *rst*B and *ctx*B,
  - ✓ nucleotide (GTA) deletions in *rst*B,
  - ✓ an increased number (*five*) of ToxR binding repeats,
  - ✓ mutations in *gyr*A and *par*C gene, and
  - ✓ a genetically similar set of MGE's shared with isolated elsewhere

- ☐ **Core gene and SNP-derived phylogenies suggest, and PCA findings reinforce, that quite quickly, i.e., within a three week period early in the cholera epidemic, significant genomic diversity accumulated in the circulating population.**





NEXT GEN SEQUENCING AND METAGENOMICS IDENTIFICATION

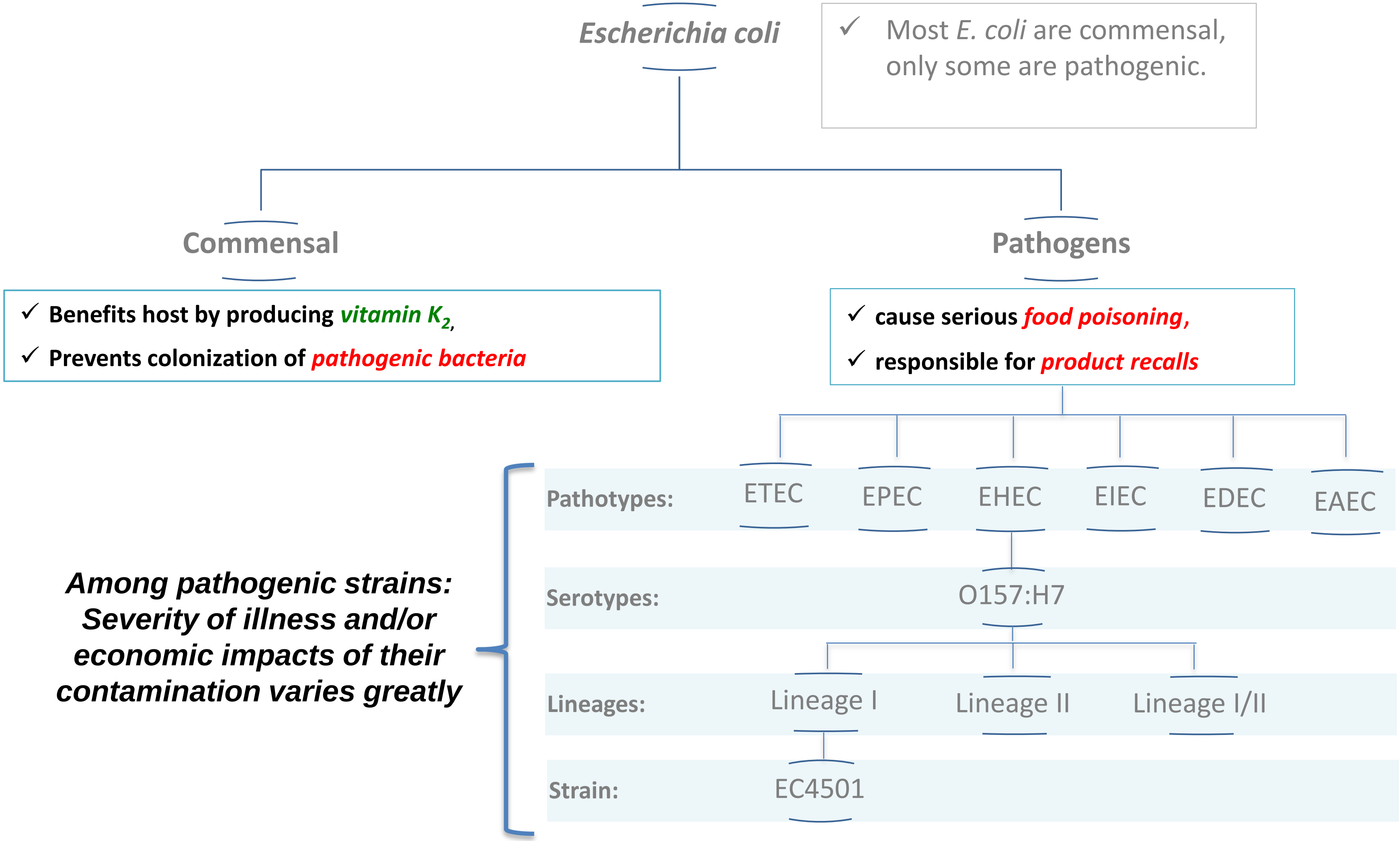
GENIUS Solution

- Software suite for analyzing WGS metagenomic sequence data
  - Runs on a Linux server
  - Remote client tool to conduct analysis, retrieve and visualize results (runs on desktop Linux, Windows, & OS X)
- Expertly curated genome databases available for
  - Bacteria
  - Viruses (DNA viruses and bacteriophages)
  - Fungi
  - Antibiotic Resistance
  - Virulence Factors
  - *S. aureus* (MRSA, MSSA) etc.
- Database is organized as a phylogenetic tree
- Types of markers
  - Unique to the organism
  - Shared with other neighboring organisms in the tree
- Provide identification at or near sub-species and strain level (*natural concept*)

Uses
entire genome
(tens of thousands of biomarkers)

WHY IS STRAIN ID IMPORTANT?

- **Typically different strains of a species elicit different impacts, critical to human health or business.**



Escherichia coli

✓ Most *E. coli* are commensal, only some are pathogenic.

Commensal

✓ **Benefits host by producing *vitamin K_2*,**

✓ **Prevents colonization of *pathogenic bacteria***

Pathogens

✓ **cause serious *food poisoning*,**

✓ **responsible for *product recalls***

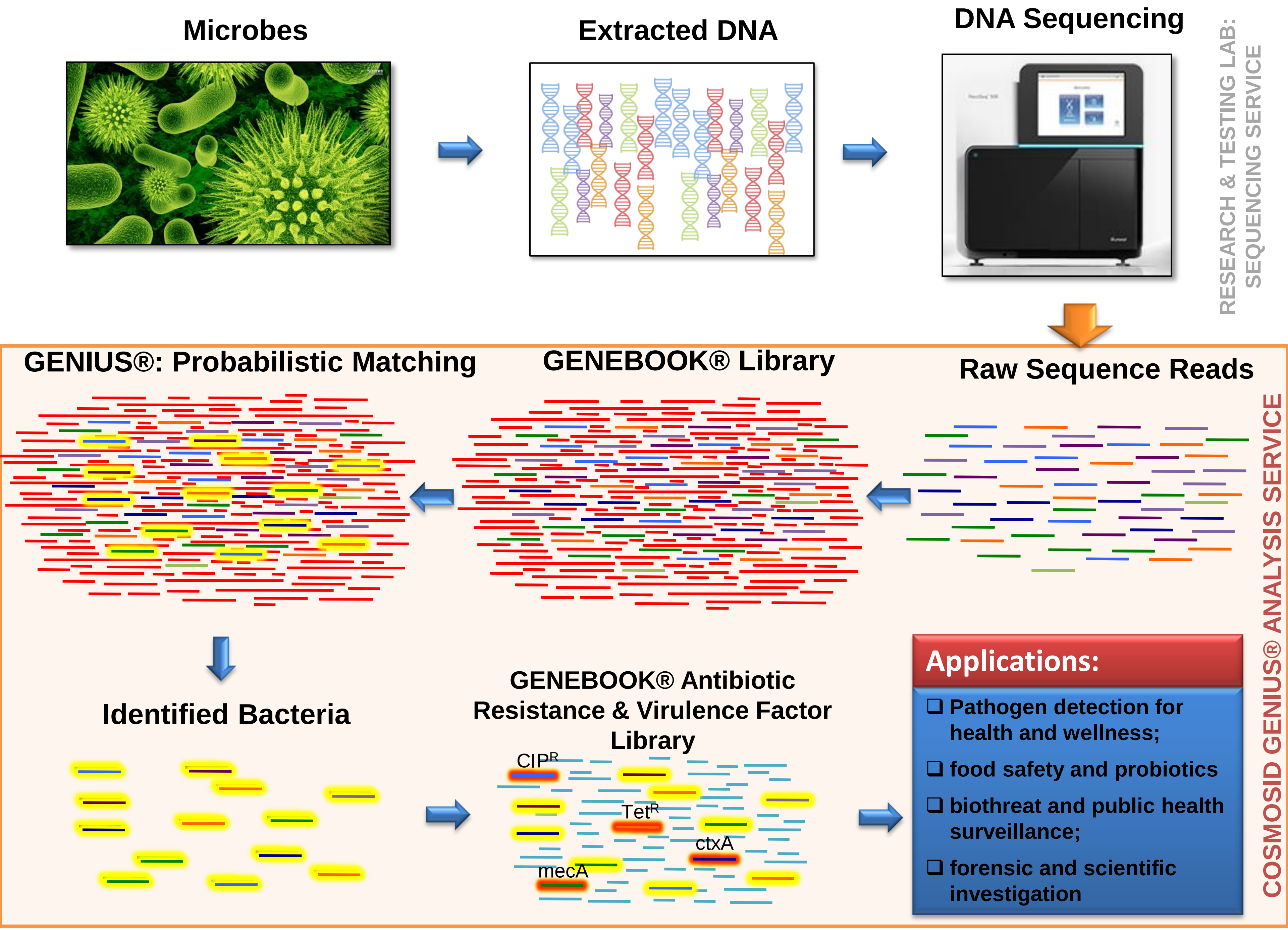
Among pathogenic strains: Severity of illness and/or economic impacts of their contamination varies greatly

Pathotypes: ETEC EPEC EHEC EIEC EDEC EAEC

Serotypes: O157:H7

Lineages: Lineage I Lineage II Lineage I/II

Strain: EC4501



Microbes

Extracted DNA

DNA Sequencing

RESEARCH & TESTING LAB:
SEQUENCING SERVICE

GENIUS®: Probabilistic Matching

GENEBOOK® Library

Raw Sequence Reads

Identified Bacteria

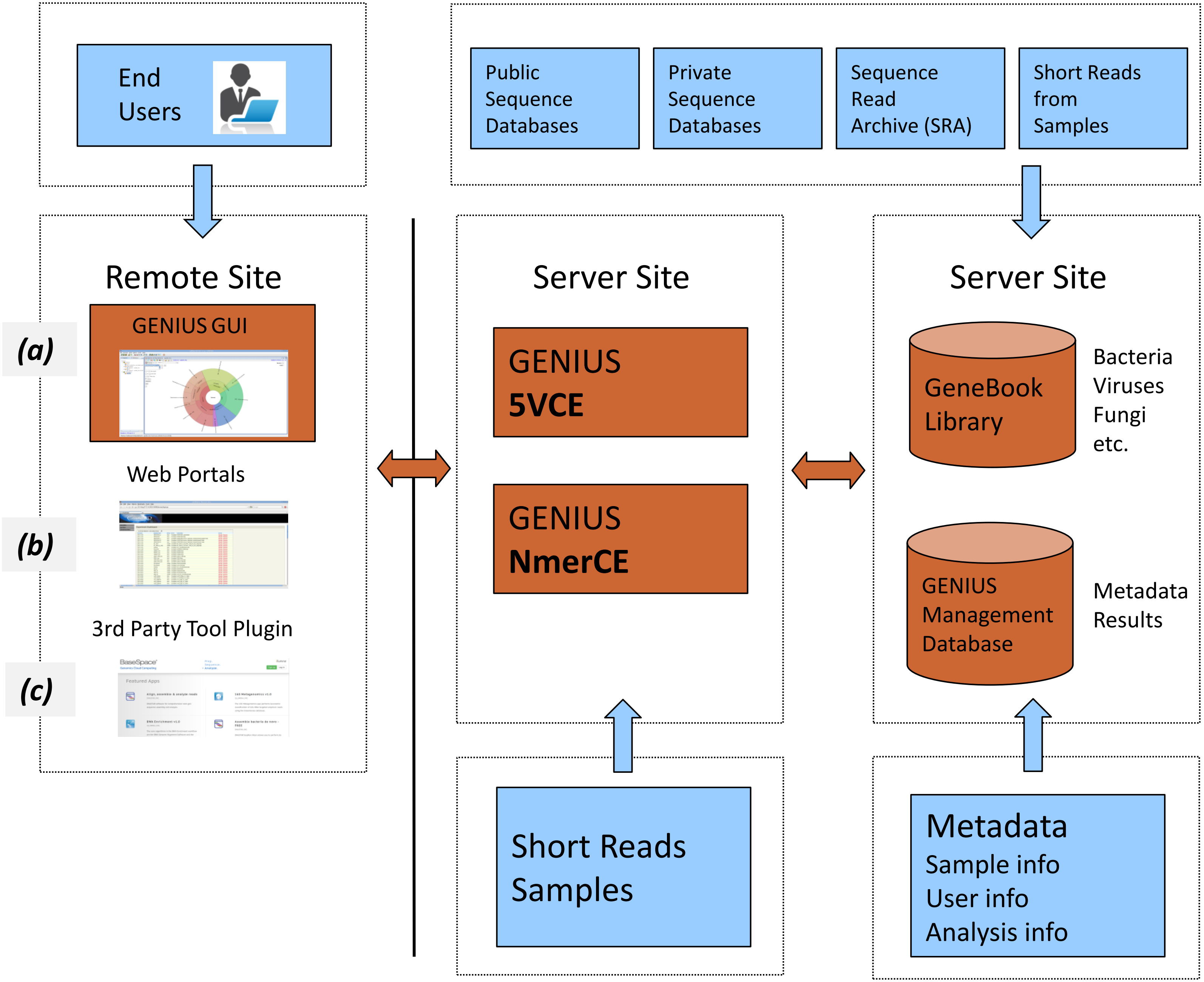
GENEBOOK® Antibiotic Resistance & Virulence Factor Library

Applications:

- ☐ Pathogen detection for health and wellness;
- ☐ food safety and probiotics
- ☐ biothreat and public health surveillance;
- ☐ forensic and scientific investigation

COSMOSID GENIUS® ANALYSIS SERVICE

GENIUS® SYSTEM OVERVIEW



End Users

Public Sequence Databases

Private Sequence Databases

Sequence Read Archive (SRA)

Short Reads from Samples

Remote Site

(a) GENIUS GUI

(b) Web Portals

(c) 3rd Party Tool Plugin

Server Site

GENIUS **5VCE**

GENIUS **NmerCE**

Short Reads Samples

Server Site

GeneBook Library

Bacteria
Viruses
Fungi
etc.

GENIUS Management Database

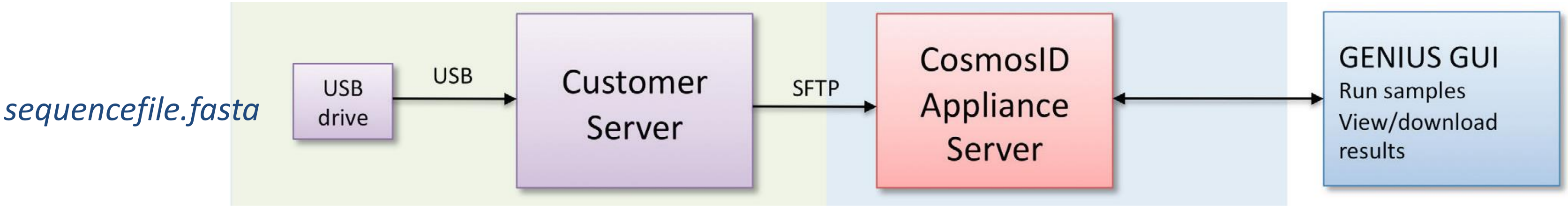
Metadata
Results

Metadata
Sample info
User info
Analysis info

Post-sequencing Bioinformatics Analysis of Clinical Specimens

Process:

1. Transfer sample sequence data to the CosmosID GENIUS appliance server.



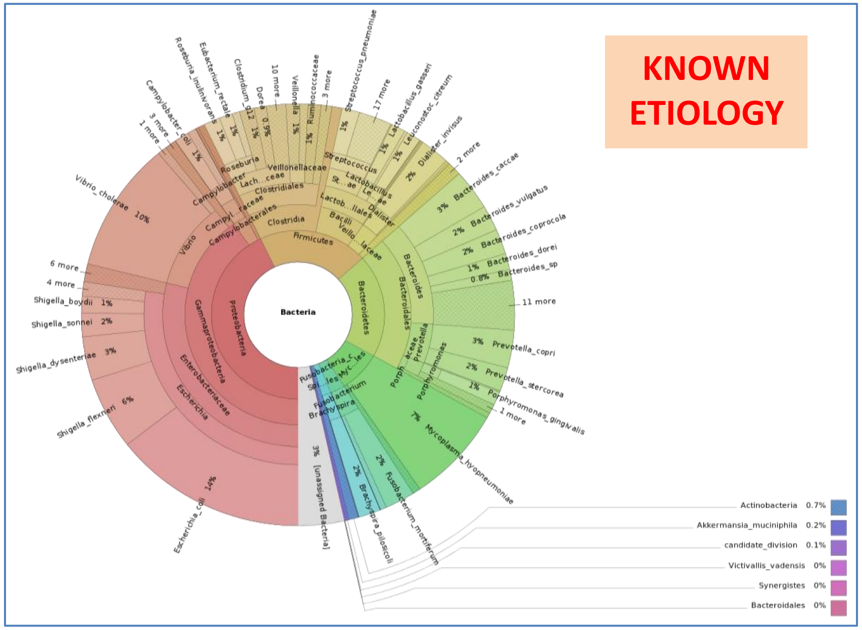
2. Open GENIUS GUI for the analysis of sample sequence data:
   a) Load samples,
   b) Select sample(s),
   c) Run analysis

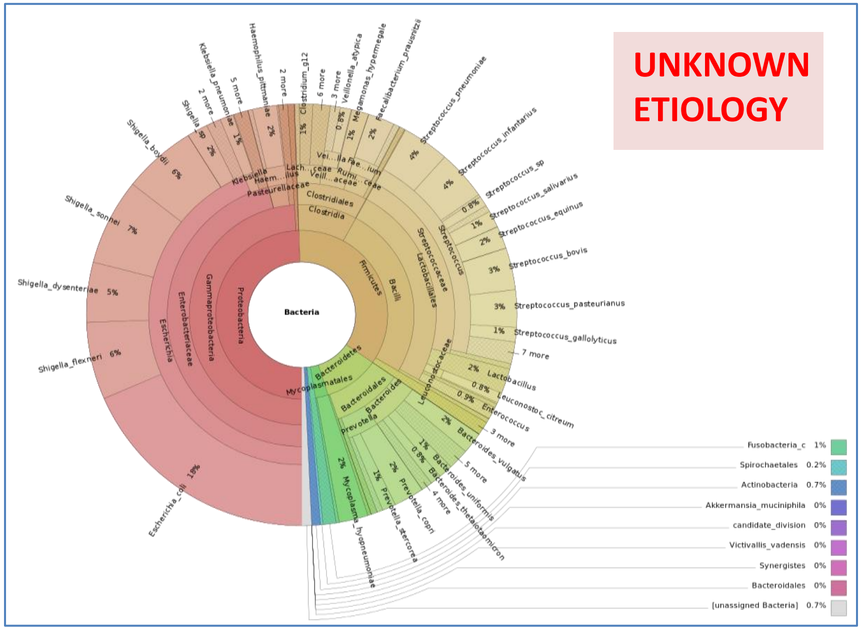
3. At the end of the analysis, a report will be generated, which includes information on bacterial strains identified and their respective relative abundances

Microbiome Analysis of Acute Diarrheal Patients Compared with Healthy Individuals

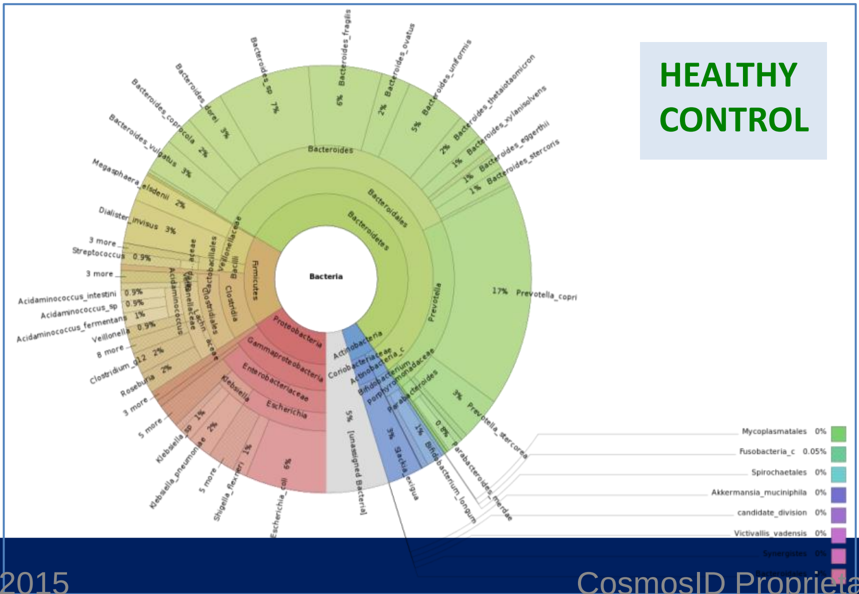
A SUBPOPULATION OF THE MICROBIOME IS OVER- REPRESENTED IN THE DIARRHEAL PATIENTS COMPARED TO HEALTHY INDIVIDUALS

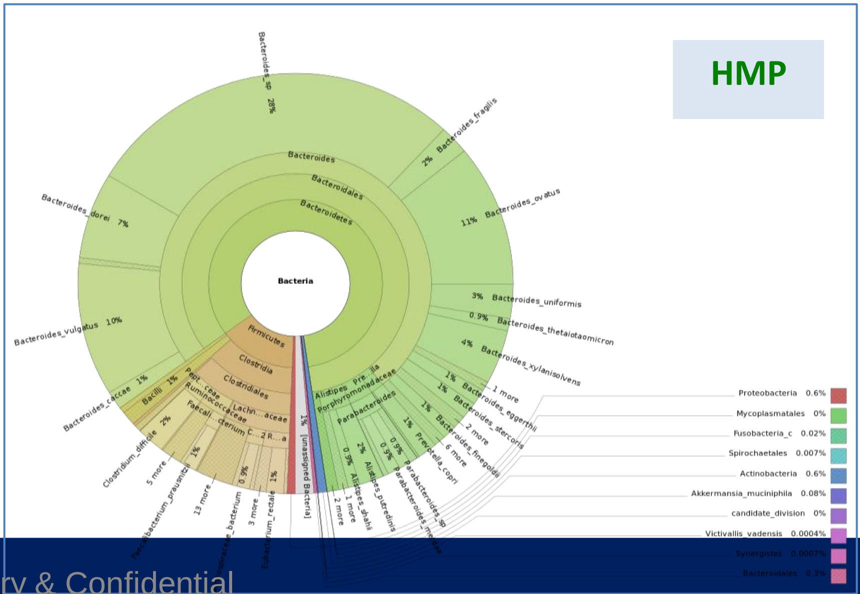
DIARRHEAL PATIENTS


KNOWN ETIOLOGY

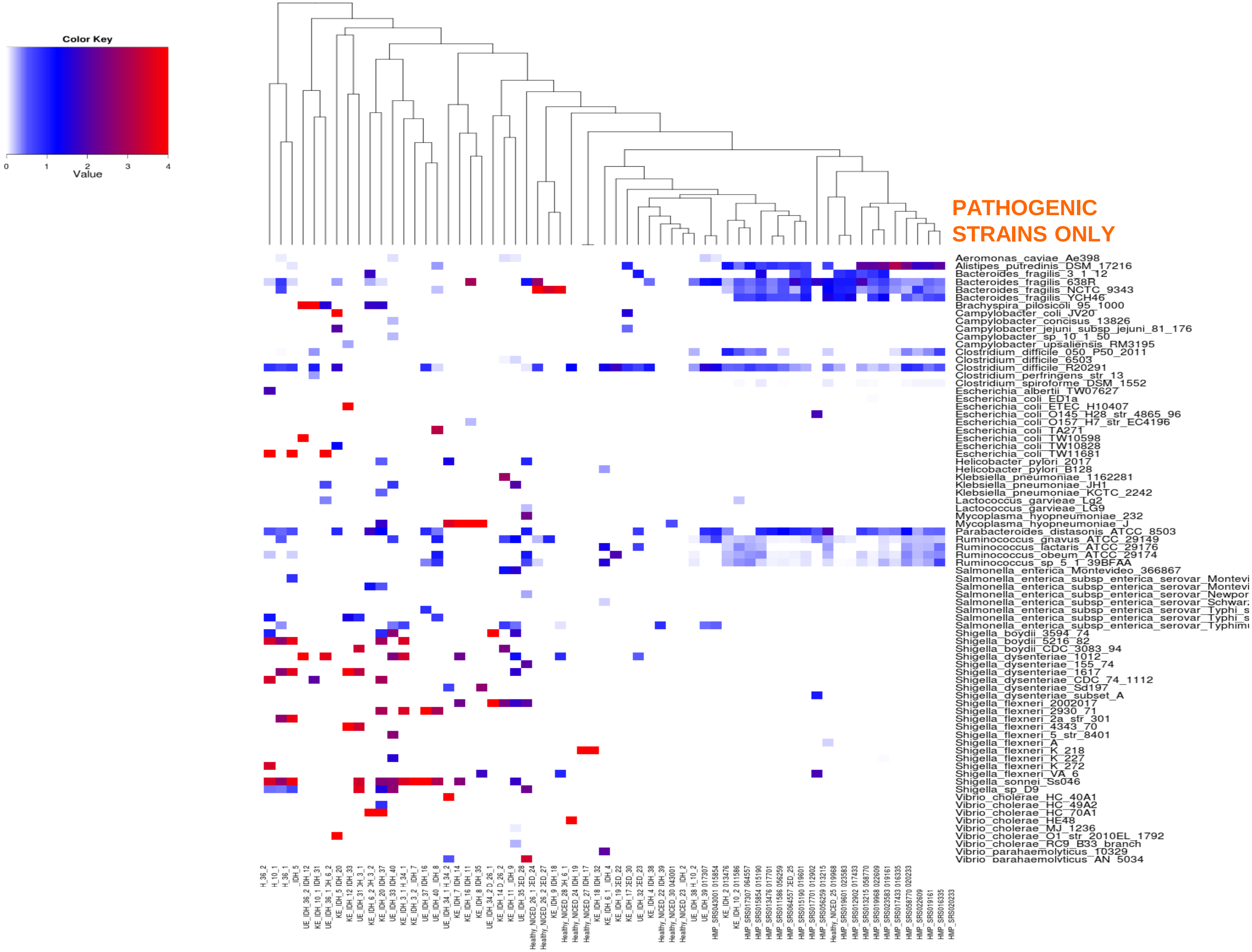

UNKNOWN ETIOLOGY

HEALTHY INDIVIDUALS


HEALTHY CONTROL


HMP

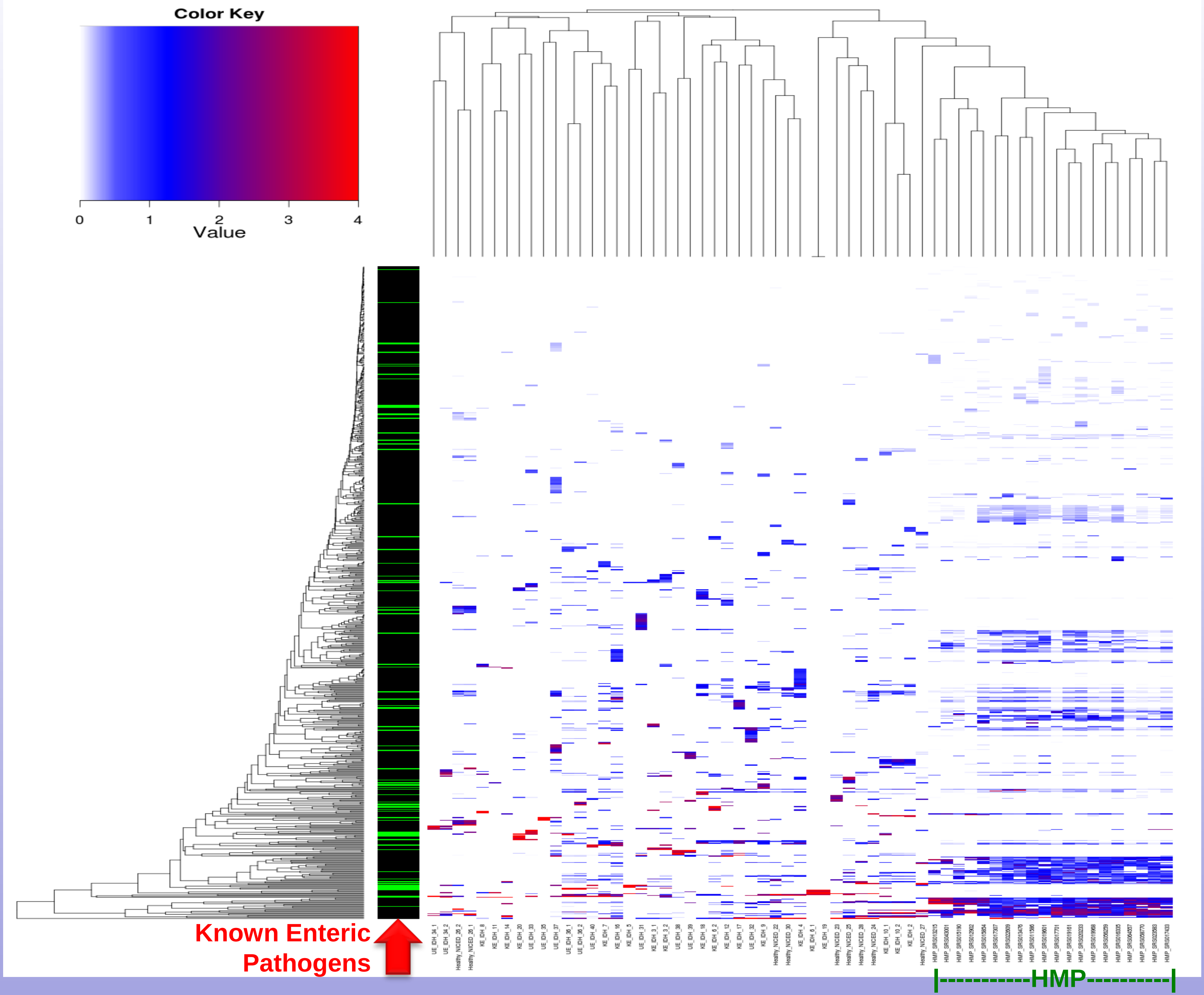
MULTIPLE PATHOGENS CAN READILY BE IDENTIFIED FROM DISEASE PATIENTS



PATHOGENIC STRAINS ONLY

THE POPULATION OF KOLKATA, INDIA MAY TOLERATE A SMALL NUMBER OF PATHOGENIC MICROORGANISMS THAT WOULD COMPRISE A DISEASE STATE IN WESTERN EUROPEANS

IDENTIFIED BACTERIAL COMMUNITIES AT STRAIN LEVEL



FDA/UVA/CosmosID Hospital Biofilm Study

- **Data – NGS reads**
  - Biofilm samples collected from sink traps
  - Single isolate samples cultured from patients

- **Analysis**
  - Assembled isolate strains & incorporated into our database
  - Ran GENIUS on all samples using both bacterial and antibiotic resistance factor (AR/V) databases
  - NCBI BLAST search to check if AR/V factors are from plasmid(s)

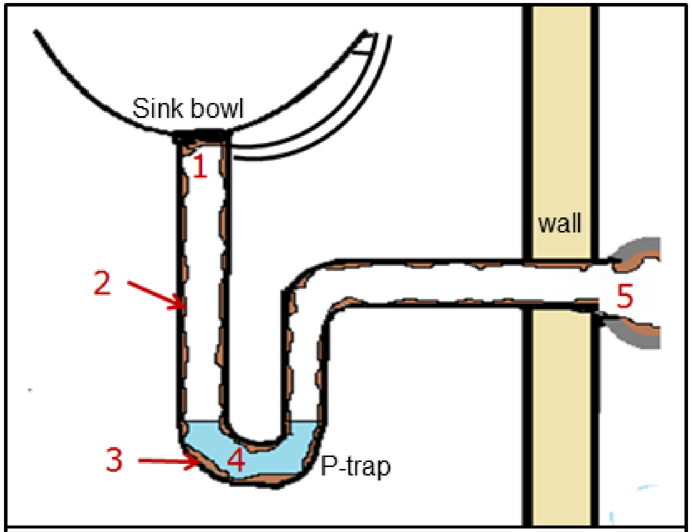
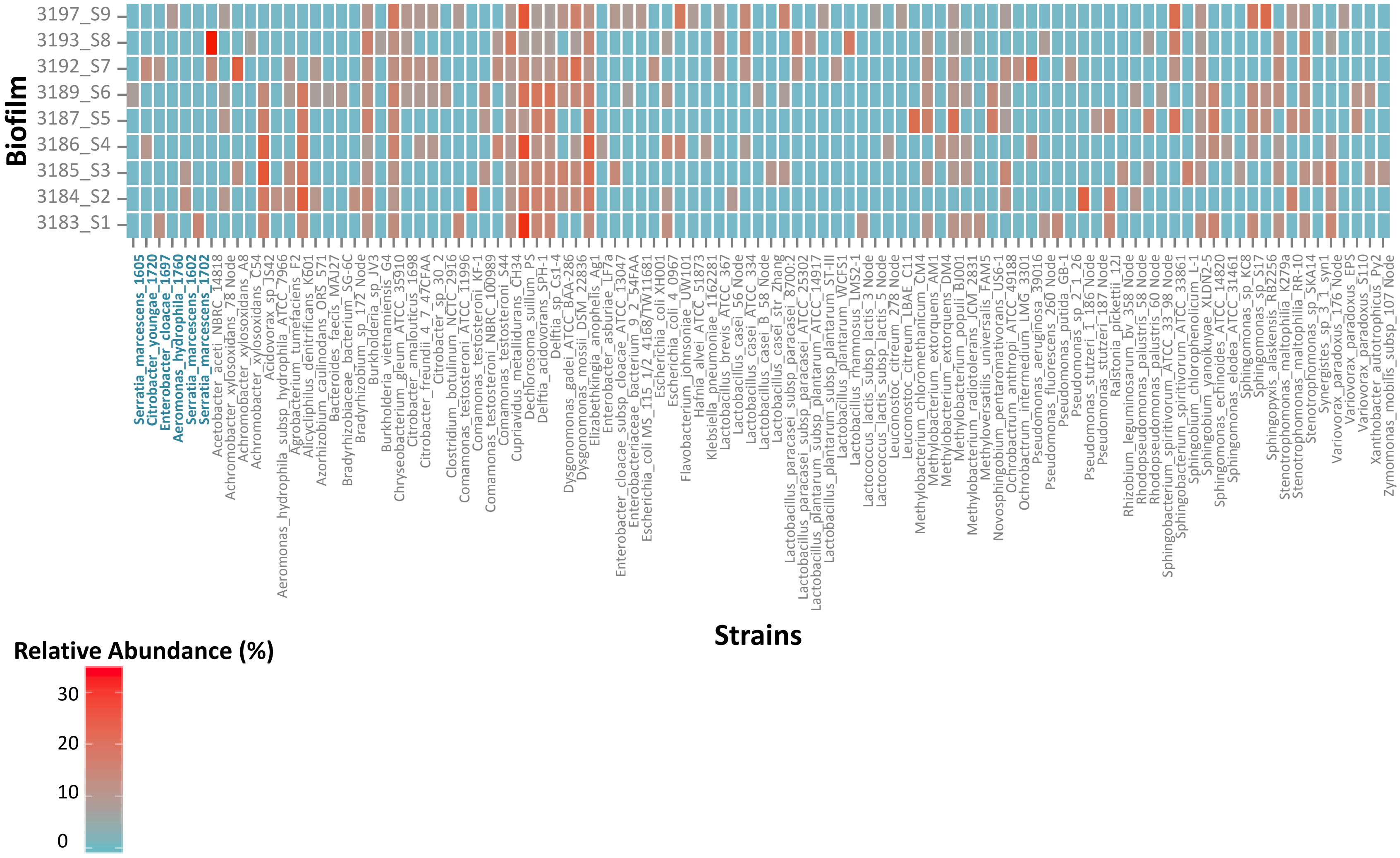
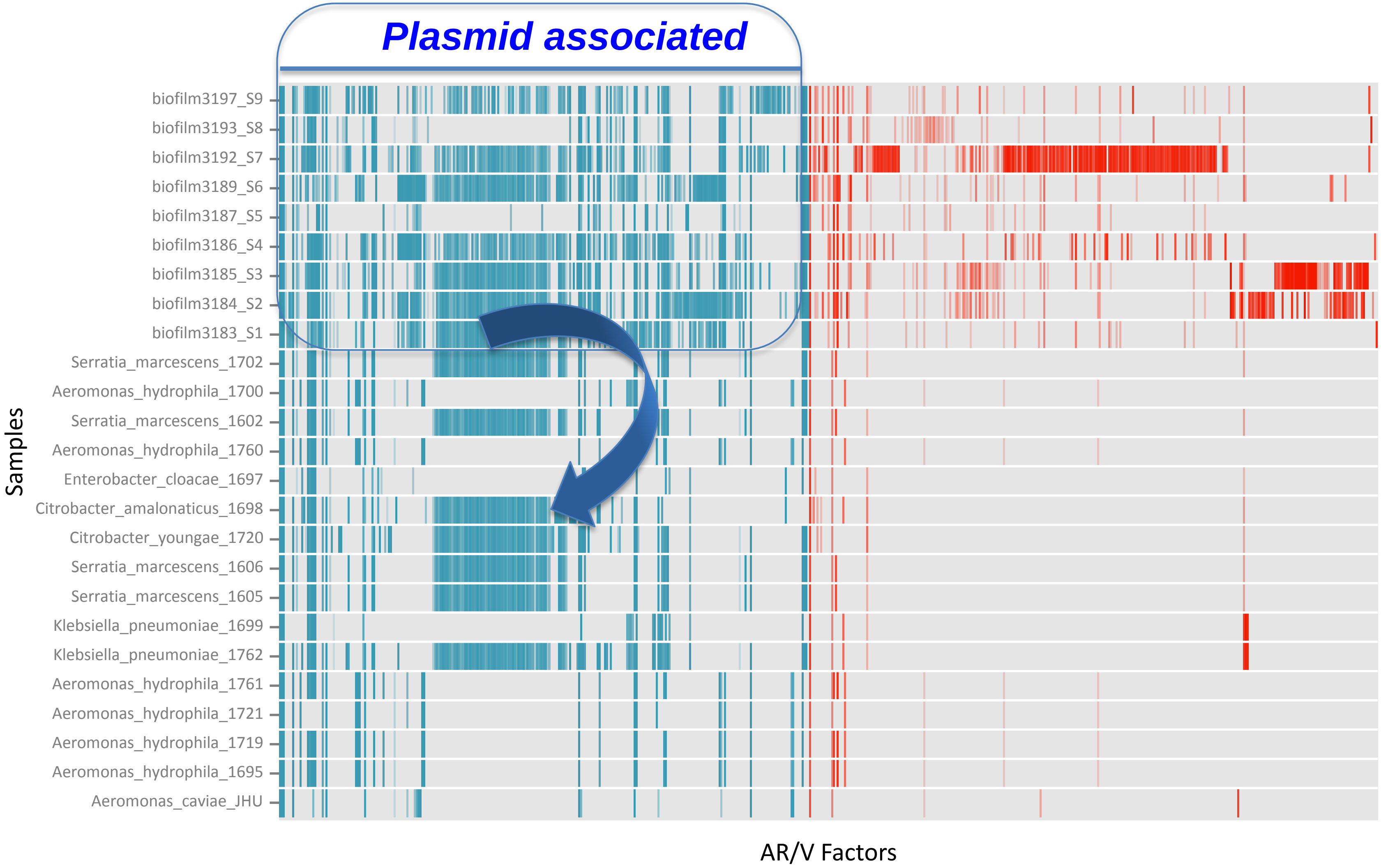


Figure 3. Colony count and diversity of across a single sink drain. Site, density of carbapenem resistant (*bla*$_{KPC}$ positive) Gammaproteobacteria (cfu/mL), Species: **1**, 1.0 X10^5, *Serratia marcescens & Citrobacter freundii;* **2**, 1.0X10^2 *S. marcescens*; **3**, 1.0X10^4 *S. marcescens*; **4**, 5.0X10^3, *S. marcescens & Aeromonas spp.;* **5**, 4.0X10^7 *Enterobacter absuriae, C. freundii & Aeromonas spp.*

Identification Resistant Bacterial Strains from Biofilms



Biofilms AR/V Factor Results – Biofilms & Isolates



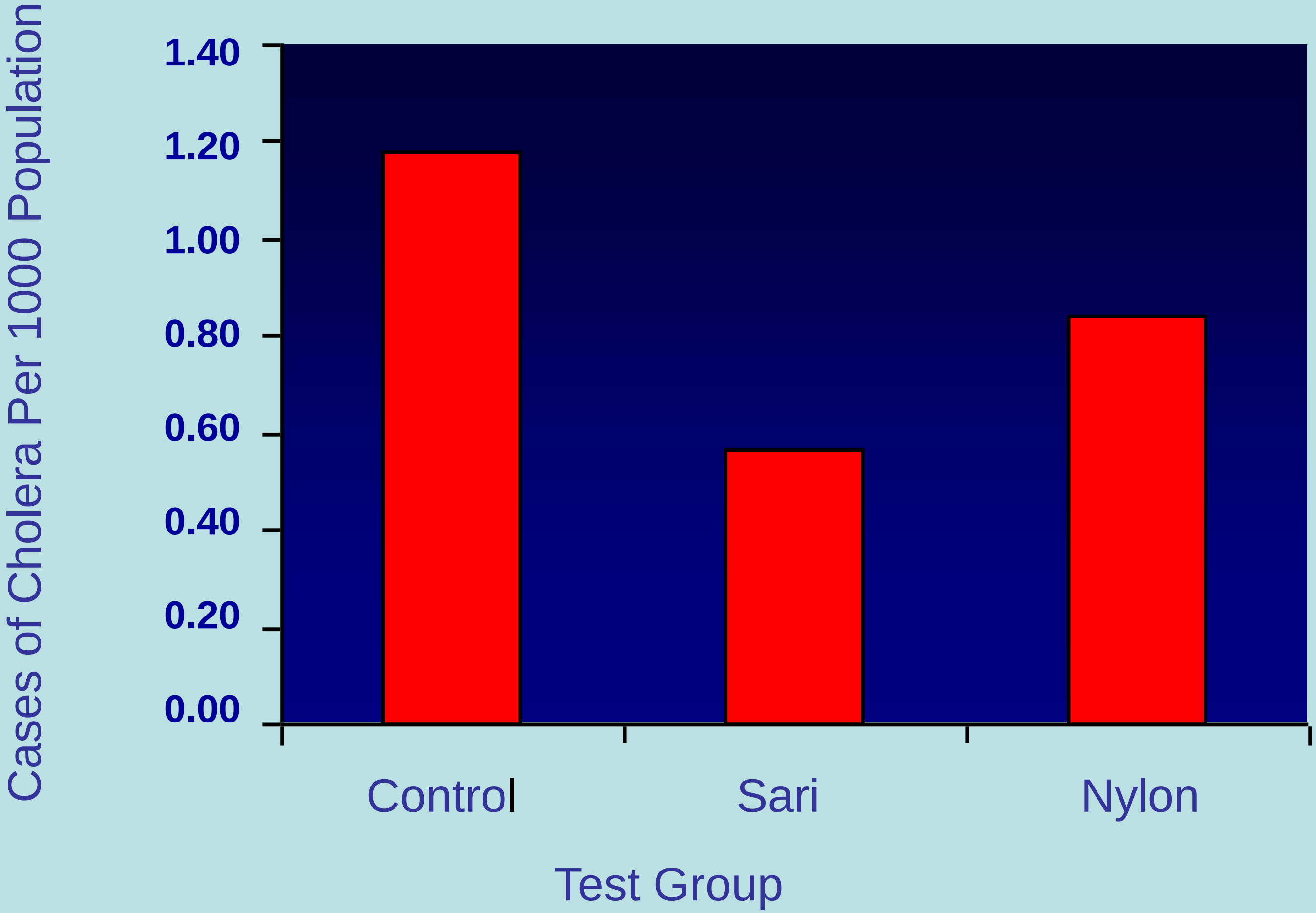
Milestone Technologies

Year	Algorithm	Approach	Algorithm	Relative Speed
1981	Smith-Waterman	Global Sequence Alignment	Exact matches and complete alignments. Hashes the query.	1.0
1988	FASTA	Local Sequence Alignment	Focuses on common sub-sequences (words) shared between query and database sequences. Hashes the query.	50x SWA
1990	BLAST	Local Sequence Alignment	Focuses on high-scoring sub-sequences (words) shared between query and database sequences. Hashes the query.	50x SWA
2002	BLAT	Local Sequence Alignment	Hashes the database.	50x WUBLAST
2006	ScalaBLAST	Local Sequence Alignment	Utilizes parallel processing	4x BLAST using 50 processors
2013	**GENIUS® 5VCE**	**Probabilistic Matching**	**Hashes the database.**	10,000X BLAST

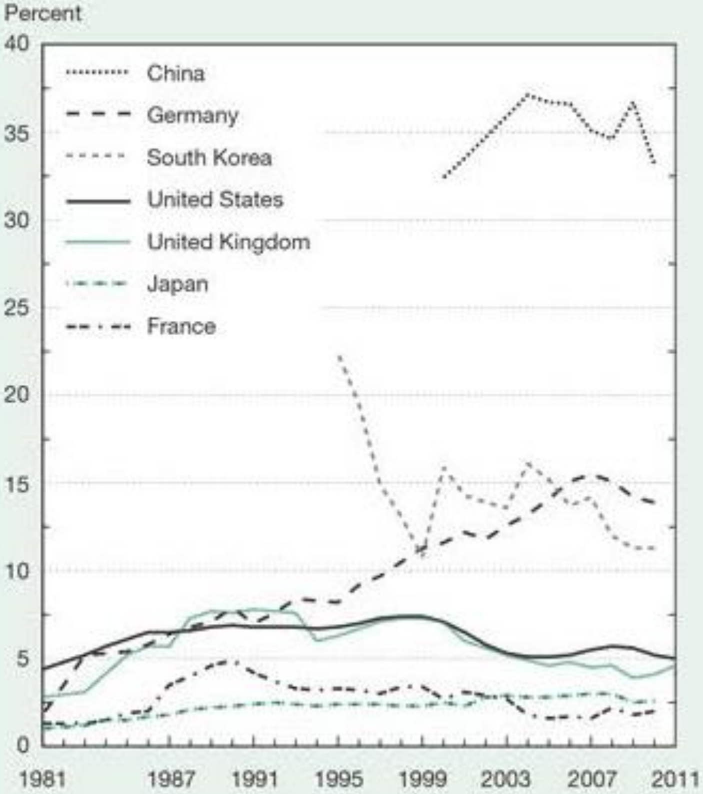
A Simple, Sustainable Method for Reducing Cholera

Vibrio cholerae

Full Study


Cases of Cholera Per 1000 Population
1.40
1.20
1.00
0.80
0.60
0.40
0.20
0.00
Control
Sari
Nylon
Test Group

Academic R&D financed by business for selected countries: 1981–2011



NOTES: Data are from the top seven R&D performing countries. Data are not available for all countries for all years. Data for Japan for 1996 onward may not be consistent with earlier data due to changes in methodology. Data for China for 2001 and 2002 are estimated by the National Science Foundation. Data for the United States are collected as part of *National Patterns of R&D Resources* and differ from Higher Education Research and Development expenditures data; pass-through funds are removed.

SOURCE: Organisation for Economic Co-operation and Development, *Main Science and Technology Indicators* (2012/2).

Science and Engineering Indicators 2014

The Gulf of Mexico Research Initiative

From Response to Coordinated Research
Established 2011

www.gulfresearchinitiative.org

www.gomri.org

GULF OF MEXICO RESEARCH INITIATIVE

Deepwater Horizon

Credit: US Chemical Safety Board

Deepwater Horizon

Credit: AP Photo/Charlie Riedel

GoMRI is not part of National Resource Damage Assessment or Clean Water Act

Credit: SAUL LOEB/AFP/Getty Images

GoMRI is not part of the National Academy of Science or National Fish and Wildlife Foundation

Deepwater Horizon

Credit: AP Photo/Charlie Riedel

Credit: SAUL LOEB/AFP/Getty Images

GoMRI is not part of
National Resource Damage Assessment or
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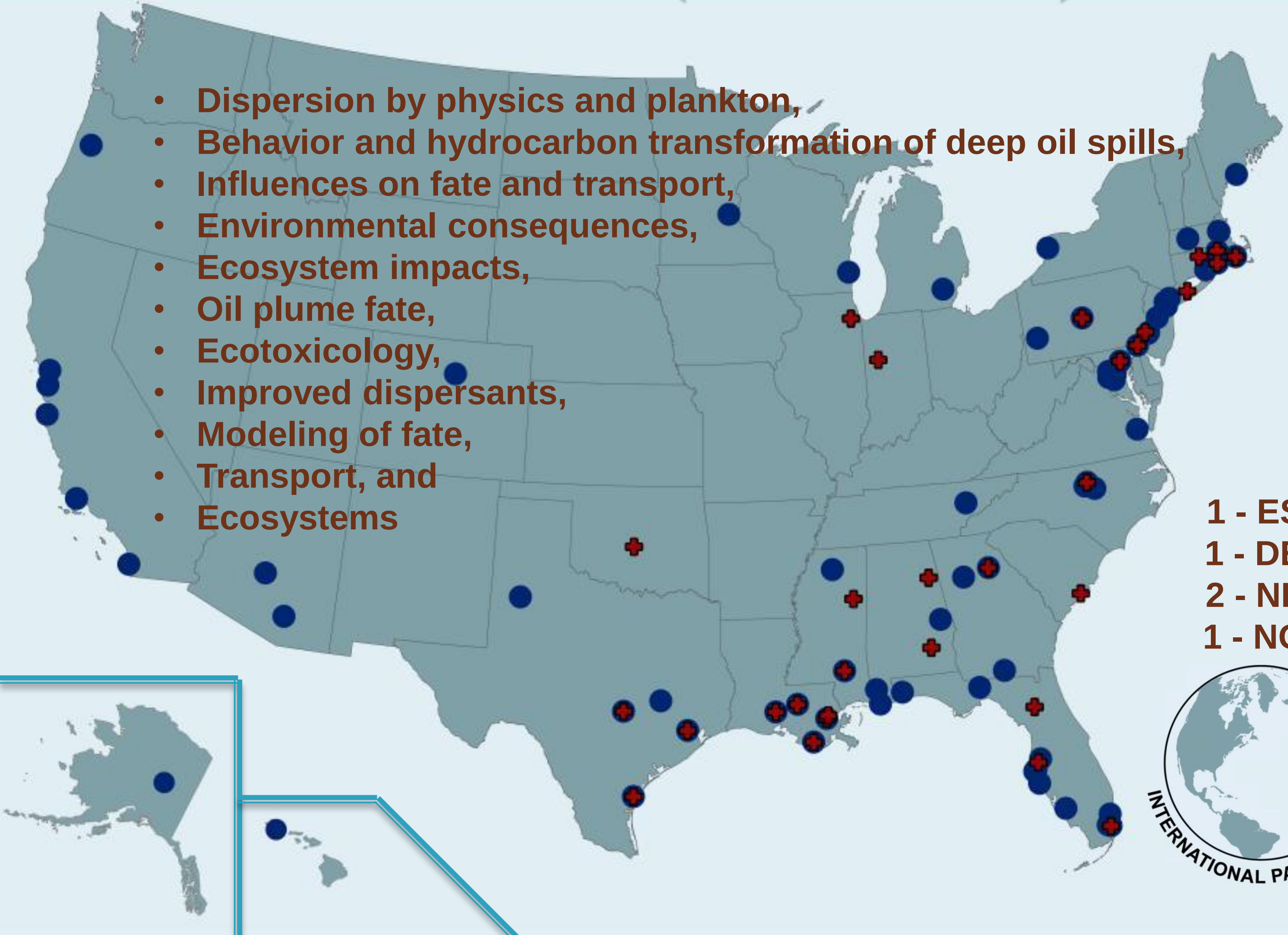
National Fish and Wildlife Foundation

GoMRI Grants (RFP I & II)



- Dispersion by physics and plankton,
- Behavior and hydrocarbon transformation of deep oil spills,
- Influences on fate and transport,
- Environmental consequences,
- Ecosystem impacts,
- Oil plume fate,
- Ecotoxicology,
- Improved dispersants,
- Modeling of fate,
- Transport, and
- Ecosystems

1 - ESP
1 - DEU
2 - NLD
1 - NOR

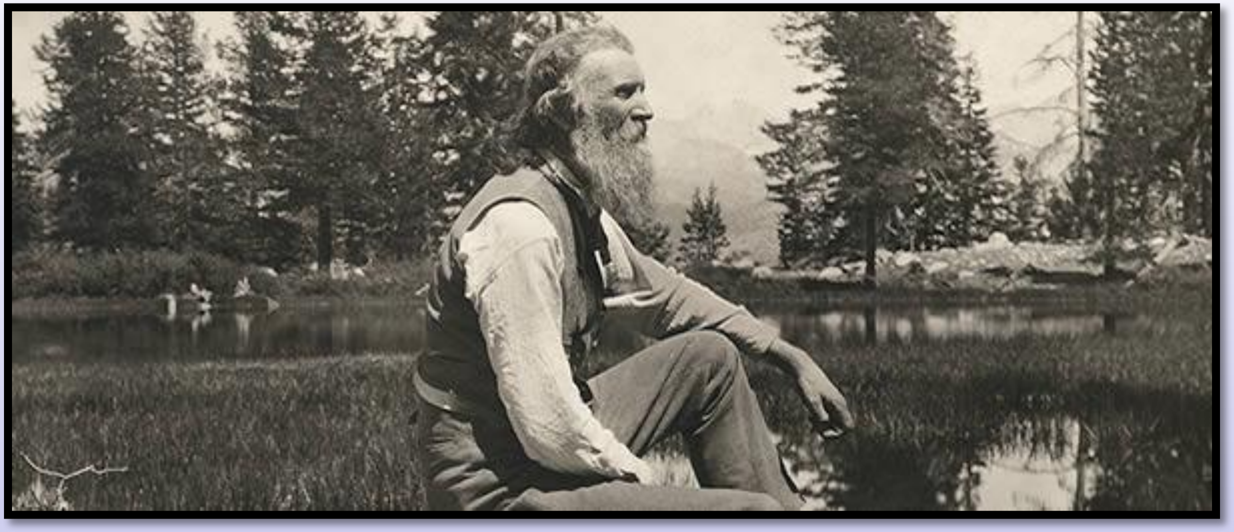
INTERNATIONAL PARTICIPANTS

All Funded Research to Date

- **Year One Block Grants - $45M, 149 Projects (completed)**
- **Summer 2011 Bridge Grants (RFP III) - $1.5M, 17 Projects (completed)**
- **2012 - 15 Eight Consortia Grants (RFP I) - $110 M, 8 RC (NCEs)**
- **2013 -16 Investigator Grants (RFP II) - $18.6 M, 19 projects**
- **2015 – 17 Consortia Grants (RPFIV) @ $140 M, 12 projects**
- **2016-18 Individual Grants (RFP V) release- Nov 14 @ $30 M**
- **2018 – 2020 last RFP(s) and wrap up.**
- **Metrics as of Jan 31, 2015**
  - About 480 scientific peer-reviewed publications/book chapters
  - Over 1930 presentations and poster sessions at conferences/scientific meetings
  - Over 688 graduate students

States and Countries who have received GOMRI funding

- 41 states
- 240 academic institutions
- 16 countries



“When one tugs at a single thing in nature, he finds it hitched to the rest of the universe.”

John Muir
(1838-1914)

Collaborators and Colleagues

ICDDR,B

- Dr. Munir Alam
- Dr. David Sack
- Dr. M. A. Salam
- Dr. A.S.G. Faruque
- Dr. Peter Kim Streatfield
- Dr. Carel van Mels
- Mr. Sarker M. Nazmul Sohel
- Dr. Mohammad Yunus
- A.K. Ashraful Aziz
- Dr. M. Imadadul Huq
- Dr. Sirajul M. Islam
- Huda Khan
- Rezaur Rahman

NICED, Kolkata, India

- Dr. Balakrish Nair
- Dr. T. Ramamurthy

University of Maryland

- Sittipan Chayanan
- Nipa Choopun
- Jafrul Hasan
- Anwarul Huq
- Christopher Grim
- Shameem Huq
- Guillaume Constantin de Magny
- Chenyang Jiang
- James Kaper
- Erin Lipp
- Valerie Louis
- David Maneval
- Tonya Rawlings
- Janie Robinson
- Estelle Russek-Cohen
- Paul West
- Young Gun Zo
- Norma Brinkley
- Jennifer Papp Newlin
- Victoria Lord

Collaborators and Colleagues

- Richard Atwell, England
- Brad Lobitz, NASA Ames
- Louisa Beck, NASA Ames
- Byron Wood, NASA Ames
- Phyllis Brayton, NIAID, NIH
- Jongsik Chun, Seoul, Korea
- Ana Gil, Lima, Peru
- Jay Grimes, Univ. of Southern Mississippi
- Sunny Jiang, Univ. of California
- Tatsuo Kaneko, San Diego
- L. Lizaragga-Partida, Mexico
- Huai-shu Xu, China
- Norma Binsztein, Argentina
- Crystal Johnson, Louisiana State University
- Carla Pruzzo, University of Genoa, Italy
- Tom Brettin, Los Alamos National Laboratory
- Nell Roberts, Louisiana State University
- Betty Lovelace, NCI/NIH
- Minnie Sochard, Catholic University, Washington, DC, USA
- Irma Rivera, Univ. of Sao Paolo, Brazil
- R. Bradley Sack, Johns Hopkins University, School of Public Health
- Fred Singleton, Gainesville, Florida
- Miguel Talledo, Lima, Peru
- Jack Dangermond, ESRI, Redlands, CA
- William Davenport, ESRI, Redlands, CA
- John Calkins, ESRI,, Redlands, CA
- Glenn Morris, Florida State University
- Ron Taylor, Darmouth, College, NH
- Matt Luck, ISciences, Burlington, VT
- Thomas Parris, ISciences, Burlington, VT
- Ric Ciccone, ISciences, Burlington, VT
- Fred Zimmerman, ISciences, Burlington, VT
- Linda Zall, Office of the Chief Scientist

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- Dr. Thomas A. Cebula, CSO, CosmosID Inc.
- Dr. Huai Li, Director, Product Development, CosmosID Inc.
- Dr. Seon Young Choi, Bioinformatic Scientist, CosmosID Inc.
- Dr. Poorani Subramanian, Bioinfromatician, CosmosID Inc.

Collaborators and Colleagues

Anwar Huq,
Professor
University of Maryland,
College Park, MD

Antarpreet Jutla,
Assistant
Professor,
West Virginia
University
Morgantown, WV

Nur Hasan
Vice-President,
Research and
Development
CosmosID, Inc.
College Park, MD

Dr. Seon Young
Choi,
Bioinformatic
Scientist,
CosmosID Inc.
College Park, MD

Courtesy of GB Nair, NICED, Kolkata, India