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

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University of Maryland College Park
and
Johns Hopkins University Bloomberg School of Public Health

“...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.
## Water-related diseases

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

*Note: The number of people exposed to drinking water with elevated levels of arsenic is 28-35 million.*
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.*
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*)
Environmental Signatures Related To Cholera Epidemics

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

A fitted model cross-validation model

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
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

- **Air Temperature**
  - Below average for two previous months
  - Above average for two previous months
- **Rainfall**
  - Below average
  - Above average

- **Cholera Outbreak**
  - Low Risk
  - High Risk

- **Water and Sanitation Access**
  - Available and intact
  - Poor or Damaged
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.

HAITI: Cholera Outbreak
(as of 22 Oct 2010)

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

Table:

<table>
<thead>
<tr>
<th>Patient Town</th>
<th>Arrondissement</th>
<th>Department</th>
</tr>
</thead>
<tbody>
<tr>
<td>Grande Saline</td>
<td>Dessalines</td>
<td>Artibonite</td>
</tr>
<tr>
<td>Gonaïves</td>
<td>Gonaïves</td>
<td>Artibonite</td>
</tr>
<tr>
<td>Saint-Marc</td>
<td>Saint-Marc</td>
<td>Artibonite</td>
</tr>
<tr>
<td>Drouin</td>
<td>Saint-Marc</td>
<td>Artibonite</td>
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<tr>
<td>Chansolmes</td>
<td>Port-de-Paix</td>
<td>Nord-Ouest</td>
</tr>
<tr>
<td>Bassin Bleu</td>
<td>Port-de-Paix</td>
<td>Nord-Ouest</td>
</tr>
<tr>
<td>Arcahaie</td>
<td>Arcahaie</td>
<td>Ouest</td>
</tr>
<tr>
<td>Cabaret</td>
<td>Arcahaie</td>
<td>Ouest</td>
</tr>
<tr>
<td>Croix-des-Bouquets</td>
<td>Croix-des-Bouquets</td>
<td>Ouest</td>
</tr>
<tr>
<td>En Plein</td>
<td>Gonaïves</td>
<td>Ouest</td>
</tr>
<tr>
<td>Plaine</td>
<td>Gonaïves</td>
<td>Ouest</td>
</tr>
<tr>
<td>Léogâne</td>
<td>Léogâne</td>
<td>Ouest</td>
</tr>
<tr>
<td>Tabarre</td>
<td>Port-au-Prince</td>
<td>Ouest</td>
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<tr>
<td>Port-au-Prince</td>
<td>Port-au-Prince</td>
<td>Ouest</td>
</tr>
<tr>
<td>Delmas</td>
<td>Port-au-Prince</td>
<td>Ouest</td>
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<tr>
<td>Cite Soleil</td>
<td>Port-au-Prince</td>
<td>Ouest</td>
</tr>
<tr>
<td>Petion Ville</td>
<td>Port-au-Prince</td>
<td>Ouest</td>
</tr>
<tr>
<td>Montrouis</td>
<td>Ouest</td>
<td></td>
</tr>
</tbody>
</table>

Map: Source and Distribution of isolates collected from Haitian outbreak

18 8 3

References:
1. UN Radio, 150 die from suspected cholera in Haiti, 22 Oct 2010.
2. British Red Cross, Haiti: Red Cross responds as health concerns grow, 22 Oct 2010.

The boundaries and names shown on this map do not imply endorsement or acceptance by the United Nations. Map created on 22 Oct 2010

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

<table>
<thead>
<tr>
<th>V. cholerae O1</th>
<th>41 samples</th>
</tr>
</thead>
<tbody>
<tr>
<td>V. cholerae Non-O1/O139</td>
<td>21 samples</td>
</tr>
</tbody>
</table>

- 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.
The Haitian *V. cholerae* O1 strains clustered with other 7th pandemic *V. cholerae* strains in a single monophyletic clade.
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 *rstB* and *ctxB*,
  - nucleotide (GTA) deletions in *rstB*,
  - an increased number (five) of ToxR binding repeats,
  - mutations in *gyrA* and *parC* 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*)
Typically different strains of a species elicit different impacts, critical to human health or business.

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

**Benefits host by producing vitamin K$_2$,**
**Prevents colonization of pathogenic bacteria**

**Pathogens**
- cause serious *food poisoning*,
- responsible for *product recalls*

**Pathotypes:**
- ETEC
- EPEC
- EHEC
- EIEC
- EDEC
- EAEC

**Serotypes:**
- O157:H7

**Lineages:**
- Lineage I
- Lineage II
- Lineage I/II

**Strain:**
- EC4501

**Among pathogenic strains:**
*Severity of illness and/or economic impacts of their contamination varies greatly*
COSMOSID GENIUS®
ANALYSIS SERVICE

Microbes

Extracted DNA

DNA Sequencing

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
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.
MUTIPLE PATHOGENS CAN READILY BE IDENTIFIED FROM DISEASE PATIENTS
THE POPULATION OF KOLKATA, INDIA MAY TOLERATE A SMALL NUMBER OF PATHOGENIC MICROORGANISMS THAT WOULD COMPRIS A DISEASE STATE IN WESTERN EUROPEANS

Identified Bacterial Communities at Strain Level

Known Enteric Pathogens
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 (blaKPC 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

Plasmid associated

Samples

biofilm3197_S9
biofilm3193_S8
biofilm3192_S7
biofilm3189_S6
biofilm3187_S5
biofilm3186_S4
biofilm3185_S3
biofilm3184_S2
biofilm3183_S1
Serratia_marcescens_1702
Aeromonas_hydrophila_1700
Serratia_marcescens_1602
Aeromonas_hydrophila_1760
Enterobacter_cloacae_1697
Citrobacter_amalonaticus_1698
Citrobacter_youngae_1720
Serratia_marcescens_1606
Serratia_marcescens_1605
Klebsiella_pneumoniae_1699
Klebsiella_pneumoniae_1762
Aeromonas_hydrophila_1761
Aeromonas_hydrophila_1721
Aeromonas_hydrophila_1719
Aeromonas_hydrophila_1695
Aeromonas_caviae_JHU

AR/V Factors

4/7/2015
CosmosID Proprietary & Confidential
# Milestone Technologies

<table>
<thead>
<tr>
<th>Year</th>
<th>Algorithm</th>
<th>Approach</th>
<th>Algorithm</th>
<th>Relative Speed</th>
</tr>
</thead>
<tbody>
<tr>
<td>1981</td>
<td>Smith-Waterman</td>
<td>Global Sequence Alignment</td>
<td>Exact matches and complete alignments. Hashes the query.</td>
<td>1.0</td>
</tr>
<tr>
<td>1988</td>
<td>FASTA</td>
<td>Local Sequence Alignment</td>
<td>Focuses on common sub-sequences (words) shared between query and database sequences. Hashes the query.</td>
<td>50x SWA</td>
</tr>
<tr>
<td>1990</td>
<td>BLAST</td>
<td>Local Sequence Alignment</td>
<td>Focuses on high-scoring sub-sequences (words) shared between query and database sequences. Hashes the query.</td>
<td>50x SWA</td>
</tr>
<tr>
<td>2002</td>
<td>BLAT</td>
<td>Local Sequence Alignment</td>
<td>Hashes the database.</td>
<td>50x WUBLAST</td>
</tr>
<tr>
<td>2006</td>
<td>ScalaBLAST</td>
<td>Local Sequence Alignment</td>
<td>Utilizes parallel processing</td>
<td>4x BLAST using 50 processors</td>
</tr>
<tr>
<td>2013</td>
<td>GENIUS® 5VCE</td>
<td><strong>Probabilistic Matching</strong></td>
<td>Hashes the database.</td>
<td>10,000X BLAST</td>
</tr>
</tbody>
</table>
A Simple, Sustainable Method for Reducing Cholera
Cases of Cholera Per 1000 Population

Test Group

Control Sari Nylon

Full Study
Academic R&D financed by business for selected countries: 1981-2011

Percent

- China
- Germany
- South Korea
- United States
- United Kingdom
- Japan
- France


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
Deepwater Horizon

Credit: US Chemical Safety Board
Deepwater Horizon

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

Credit: AP Photo/Charlie Riedel

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

Credit: SAUL LOEB/AFP/Getty Images

gulfresearchinitiative.org
Deepwater Horizon

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

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

Credit: AP Photo/Charlie Riedel

Credit: SAUL LOEB/AFP/Getty Images

gulfresearchinitiative.org
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
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

<table>
<thead>
<tr>
<th>ICDDR,B</th>
<th>University of Maryland</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dr. Munir Alam</td>
<td>Sittipan Chayanan</td>
</tr>
<tr>
<td>Dr. David Sack</td>
<td>Nipa Choopun</td>
</tr>
<tr>
<td>Dr. M. A. Salam</td>
<td>Jafrul Hasan</td>
</tr>
<tr>
<td>Dr. A.S.G. Faruque</td>
<td>Anwarul Huq</td>
</tr>
<tr>
<td>Dr. Peter Kim Streatfield</td>
<td>Christopher Grim</td>
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<tr>
<td>Dr. Carel van Mels</td>
<td>Shameem Huq</td>
</tr>
<tr>
<td>Mr. Sarker M. Nazmul Sohel</td>
<td>Guillaume Constantin de Magny</td>
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<tr>
<td>Dr. Mohammad Yunus</td>
<td>Chenyang Jiang</td>
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<tr>
<td>A.K. Ashraful Aziz</td>
<td>James Kaper</td>
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<tr>
<td>Dr. M. Imadadul Huq</td>
<td>Erin Lipp</td>
</tr>
<tr>
<td>Dr. Sirajul M. Islam</td>
<td>Valerie Louis</td>
</tr>
<tr>
<td>Huda Khan</td>
<td>David Maneval</td>
</tr>
<tr>
<td>Rezaur Rahman</td>
<td>Tonya Rawlings</td>
</tr>
<tr>
<td>NICED, Kolkata, India</td>
<td>Janie Robinson</td>
</tr>
<tr>
<td>Dr. Balakrish Nair</td>
<td>Estelle Russek-Cohen</td>
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<tr>
<td>Dr. T. Ramamurthy</td>
<td>Paul West</td>
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<td></td>
<td>Young Gun Zo</td>
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<tr>
<td></td>
<td>Norma Brinkley</td>
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<tr>
<td></td>
<td>Jennifer Papp Newlin</td>
</tr>
<tr>
<td></td>
<td>Victoria Lord</td>
</tr>
</tbody>
</table>
Collaborators and Colleagues

- Richard Atwell, England
- Brad Lobitz, NASA Ames
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- Byron Wood, NASA Ames
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