Waterborne Bacterial Infections in Georgia:
Pathogens of Concern, Environmental Factors and Potential Biocontrol Strategies

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Waterborne diseases

- Caused by pathogenic microorganisms (protozoa, viruses, bacteria), transmitted through contaminated water
  - Direct contact or ingestion of water
    • Drinking water
    • Water used in preparation of food (= foodborne disease)
    • Irrigation water (= foodborne disease)
    • Recreational waters
  - Bathing water (fresh, brackish or marine)
Waterborne infections

Waterborne infections may occur in the areas/countries experiencing:
- Freshwater deficiency (arid lands, deserts)
- Excess of water (due to flooding)
- Natural catastrophes (earthquakes etc)
- Wars/ethnoconflicts (crowded refugee camps)

Global climate change - triggering factor

Vital Water Graphics 2, 2009
Waterborne diseases

- Major reason of water borne diseases – Unsafe water supply, poor sanitation and hygiene
- World-wide around
  - 1.1 billion people lack access to safe water sources
  - 2.4 billion have no basic sanitation.

- Diarrheal disease
  ~ 4.1% of global burden of disease
  - cause of 1.8 million deaths / year (4% of all deaths)
- In Southeast Asia and Africa
  - up to 8.5% and 7.7% of all deaths respectively
  - children under 5 year most affected

http://www.who.int/water_sanitation_health/diseases/diseasefact
Waterborne bacterial infections

- Dysentery (bacillary) – *Shigella* spp., *Sh. disenteriae*
- Campylobacteriosis – *C. jujuni*, *C. coli*
- *Cholera* – *V. cholerae* O1, O139
- *E. coli infections* – *E. coli* species - ETEC, EHEC, EIEC,
- Typhoid fever - *S. typhi*
- Salmonellosis - *Salmonella* spp.
- *Vibrio illness* – *V. parahaemolyticus*, *V. vulnificus*, *V. algynolyticus*
- *Aeromonas illness* – *A. hydrophyla*, *A. caviae*, *A. sobria* (Toxicoinfection)
- Leptospirosis - *Leptospira* spp
- Legionellosis – *Legionella* spp. (Legionar’s and Pontiac’s disease)-
- *Helicobacter disease* – *Helicobacter pylori* (indirect evidence of water transmission)
- *F. tularensis* (possibility for Y. pestis) – Class A bacterial pathogens - high risk of water transmission
Water borne infections in Georgia

Water resources in Georgia

- **Unequal** natural availability of freshwater resources in the South Caucasus region
- **Georgia** is the richest country in water resources
- **Azerbaijan** suffers from water shortages the most.

- **Total renewable water resources (TRWR) per inhabitant- 11 637 m3/yr**
  
  *(the international water poverty line of 1,000m3 /yr)*

Caucasus Environment Outlook (CEO) 2002
Water borne infections in Georgia

Issues related to safe water supply

- Potable water supply problems
  Centralized water supply systems in rapidly growing cities do not meet the requirements (outdated, low technical capabilities)
  - 13.3-17.3% and 15.2-16.0% of samples from centralized water supply systems did not meet existing drinking water quality standards for toxicity and bacteriological quality (*WHO/MoH of Georgia, report 2001*).
  - *Tbilisi water Supply system has been significantly improved during last decade* and water quality is high
Water borne infections in Georgia

Issues related to safe water supply

Rural areas
- Many sites do not have central water supply systems
- The rural population uses the water from artesian wells and rivers that might be contaminated.
- >36% of well water samples didn’t meet existing drinking water quality standards (WHO/MoH of Georgia, report 2001).
- 56% rural drinking water samples exceeded drinking water quality standards (Eliava Lab data, 2006-2010)
Water borne infections in Georgia

Issues related to safe water supply

Recent ethnic conflicts
- Destroyed sanitation infrastructure, aggravated sanitary-hygienic conditions
- Internally displaced population (IDP) is at risk of acquiring water borne infections due to cramped, crowded and unhygienic living conditions
  - Diarrhea is prevalent among children of IDPs.

Intensified international traffic

Europe-Caucasus-Asia transport corridor
- East- West, South- North
  - Ground transportation (trucks)
  - International workers
  - Migration routes

Possible source of non-endemic infections
Water borne infections in Georgia

Morbidity data of main waterbone infectious diseases (2007)

- Cholera
- Typhoid fever
- Salmonella Infections
- Shigellosis
- E.coli infections
- Bacterial intestinal infections
- Amebiasis
- Infectious Diarrhoea and Gastroenteritis
- Leptosirosis
- Tularemia

Indication of Morbidity for 100000 population

- High morbidity rate for presumed infectious diahhrea and gastroenteritis (etiologic agents not identified)

* Prepared based on NCDC, Annual report 2007-2008
Water borne infections in Georgia

Etiologic structure of Intestinal infections in children Hospitalized patients, 2004-2008
Children's Infectious Disease Hospital, Tbilisi

I. Shalamberidze et al, 2009
Water borne infections in Georgia

Etiologic structure of Intestinal infections
(Data of the Eliava Analytical-Diagnostic Center, Tbilisi)

Intestinal bacterial pathogens or opportunistic bacteria are isolated ~ 25% of stool samples from patients with intestinal disorders

- Pathogenic E.coli: 30%
- Proteus spp.: 24%
- S.aureus: 7%
- Enterococcus spp.: 11%
- Other bacteria: 17%
- K. pneumoniae: 7%
- S. enteritidis: 1%
- P. aeruginosa: 3%
- Other bacteria: 17%

n=226, Sept.- Dec., 2008

N. Topuria, 2009
Water borne infections in Georgia: Outbreaks of Amebiasis and tularemia

- In July -September 1998, 177 cases of suspected amebiasis were identified.
  - 52 persons had diagnosed liver abscesses
- Drinking water was the source of infection
- inadequate treatment or contamination of municipal water distribution system


- In November 2006, an outbreak of waterborne tularemia occurred in an eastern region of Georgia.
  - 26 cases:
    - 21 oropharyngeal cases
    - 5 glandular tularemia cases.
- Transmission – through contaminated spring water

Water Borne Infections in Georgia

Recreational waters: Black Sea Coastal Zone of Georgia

- Highly populated cities, recreational/tourist places
- Ports, factories, farms
- Possible health threat: typhoid, cholera, dysentery, hepatitis A, other... exotic diseases
- Favourable conditions: low salinity, warm climate, nutrient rich environment in estuaries etc

Insufficiently purified urban municipal, domestic and industrial effluents, surface runoffs

Introduction of non-indigenous, potentially harmful species into marine environment

Balast water and waste from ships

Spreading of bacterial, viral or protozoan infections.
Water Borne Infections in Georgia

Vibrio-related infections

- Warm, subtropical climate in Georgia provide favorable conditions for emergence and spread of Vibrio-related infections

- In the early 1970's the outbreak of cholera was reported in Georgia (Batumi and Tbilisi), caused by *V. cholerae O1*, biotype El-Tor
  - Several Reports on NAG-Vibrio isolation in Coastal waters in 70’-80’s
  - Two small outbreaks of cholera in 90’s (imported cases)

- Periodic outbreaks & sporadic cases in neighboring countries

- No published reports were available before 2006 on detection of toxigenic *V. cholerae* in water reservoirs
  - Waterborne diarrheal diseases of unknown etiology in the warm season - possible vibriosis?
Water borne infections in Georgia: Pathogenic vibrios in Georgian water environment

Project GG-13

Main Goal
Development of strategies for monitoring, prevention, and control of cholera and other Vibrio-related infections

- Ecology and biodiversity of pathogenic vibrios, especially *V. cholerae*, in different geographical regions
- Occurrence and spread of epidemic strains
- Ecology and biodiversity of *Vibrio* – specific bacteriophages

*Monitoring in the Black Sea Georgian Coastal Zone and freshwater lakes in Tbilisi area*
Pathogenic vibrios in Georgian water environment

Sampling in different water environment (2006-2008; 2009)

Black Sea coast of Georgia
- Ports
- Oil terminals
- Sea resorts
- Agriculture
- Fishery

4 Coastal sites
- Chorokhi,
- Batumi Boulevard
- Green Cape,
- Supsa

- Tbilisi Sea (used for drinking, recreation)
- Lisi Lake (recreation, fishing)
- Kumisi Lake (recreation, fishing, irrigation)
Pathogenic vibrios in Georgian water environment

*Isolation and identification of environmental Vibrio spp*

- 11 pathogenic *Vibrio* spp. cultured from marine environment and 8 from freshwater lakes (phenotypical identification)

- Prevalence of non-halophylic vibrios in freshwater environment

- In total, >2000 primary isolates of *Vibrio* spp. have been studied by phenotypic properties
  - 800 *Vibrio* spp. isolates identified by ITS-PSR

- *V. cholerae* – 58,9%
- *V. parahaemolyticus* - 10,4%
- *V. vulnificus* - 5,1%
- *V. algynolyticus* – 3,8%
- *V. metschnikovii* - 3,1 %,
- *V. mimicus* - 0,8% etc
Pathogenic vibrios in Georgian water environment

Direct detection of Vibrio spp. in water

- Novel pathogen detection system using PCR ionization electro spray mass spectrometry (PCR/ESI-MS)
  - Determination of the full spectrum of vibrios in water samples (Total DNA’s)

- 440 DNA samples from marine and lake water analyzed by PCR/ESI-MS
  - 67% of water samples positive for Vibrio spp.

- 9 pathogenic Vibrio species detected
  - V. cholerae, V. parahaemolyticus, V. vulnificus were prevalent the Black Sea samples
  - The lake waters were dominated by V. cholerae
Pathogenic vibrios in Georgian water environment

Direct detection of epidemic serotypes of Vibrio cholerae in the environment

- Direct Fluorescent Antibody (DFA) Assay
  - *V. cholerae* O1 and O139 DFA kit (NHD, MD)
  - Epifluoroscent microscope ZEISS Axioscop 40

- Up to 450 Samples examined
  - 85 Samples positive for *V. cholerae* 01
    - 29 - Freshwater samples (mainly Lisi and Kumisi Lakes)
    - 59 – Marine samples (all sites)

- Presumptively positive signals for *V. cholerae* 0139 in
  - 7 marine samples
  - 1 sample from Kumisi Lake

*Percentage DFA-positive samples increased during 2007 - 2009*
Pathogenic vibrios in Georgian water environment

Study of genetic relatedness of V. cholerae and V. parahaemolyticus isolates

- 210 V. cholerae isolates studied by PFGE
- 500 V. cholerae isolates by ERIC-PCR

V. parahaemolyticus isolates
PFGE (160), ERIC-PCR (70)

High Genetic diversity of Environmental isolates
Pathogenic vibrios in Georgian water environment

What we have found

- Data obtained (DFA, PCR/ESI-MS, and culture) provide evidence for environmental reservoirs for toxigenic *V. cholerae* O1 in recreational waters and drinking water sources in Georgia, in freshwater lakes near Tbilisi, and in the Georgian coastal zone of the Black Sea.

- Direct detection methods (DFA, PCR/ESI-MS) serve as effective and precise tools for detection and enumeration of selected *Vibrio spp.* in the aquatic environment. It is important that bacteria in VBNC state can be detected.
Pathogenic vibrios in Georgian water environment

What we have found

- Temperature dependence of pathogenic vibrios might be a triggering factor for their massive propagation in the water environment (even in the considerably clean zones) in the conditions of continuing global warming.

- High density of *Vibrio* populations may facilitate genetic exchange between pathogenic and non-pathogenic vibrios and support evolving of potentially pathogenic variants/ serotypes of *Vibrio* species in the aquatic environment.
Waterborne infections

Current and Future challenge

- Worsening of environmental conditions (elevated temperature, drought, heavy rainfalls etc), also enforced anthropogenic impact may trigger further decline in water microbial quality and safety.

- In the era of natural catastrophes the probability of emergence and spread of waterborne infections increases, including the areas non-endemic for a particular pathogen (Haiti lesson).

- Increasing antibiotic resistance of Intestinal pathogens is in many areas of the world.

- Waterborne infections may pose a risk, the impact of which reaches beyond local and national borders.

Haiti: by February 9, 2011, there were 231,070 cases and 4,549 deaths due to cholera.
**Waterborne infections**

*Future strategy (Monitoring and preparedness)*

- Regular monitoring in recreation and drinking water reservoirs for:
  - Early detection of human pathogenic species, including pathogenic *Vibrio spp.* and other potentially harmful autochthonous microflora.

- Research targeting the predictive models and biological indicators;
  - Disease surveillance, collecting clinical data;

- Necessity for joint collaborative efforts/projects on regional level in environmental monitoring and disease surveillance.
Elaboration of effective biological control measures to prevent and treat emerging waterborne infections.

- Biological measures /preparations – flexible, genetic plasticity, overcome of bacterial resistance
  - Bacteriophages - alternative ecologically safe biocontrol means (especially for antibiotic resistant water borne pathogens).
Phages and Water borne infections

- Detection/diagnostics
- Infections source tracking
- Subtyping (for Epidemiological studies)
- Prevention
- Therapy
Phages as indicators

• Somatic and male – specific Coliphages - indicators of water microbial pollution

• Specific phages as indicators of presence of particular *pathogenic* bacteria

• Phage amplification assay -- indication of a pathogen in VBN state

• Correlation between Etiologic structure of water borne infections and phage profile of water samples

• Phages as predictive or retrospective indicators of disease outbreaks
Seasonal monitoring of microbial quality

South - East coastal zone of the Black Sea
Batumi city area (2002-2004)

• Seasonal variability of microbial quality indices
• Highest level of microbial contamination of sea water in summer season
• Abundance of indicator somatic phages in sea water - proportional to total bacterial count (TBC)
• Two distinct (clean and polluted) zones in Batumi aquatoria: Gonio-Kwariati and Bartskhana
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<th>Depth(m)</th>
<th>Sample type</th>
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<th>Total coliforms cell/L</th>
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<th>ENT-Index cell/L</th>
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Table 1. Indices of Microbial pollution of the Black sea water and sediments in Adjara coastal zone (Winter, 2003)
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<td>18,0-20,0</td>
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<td>3x10^2</td>
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<td>620</td>
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<td>9</td>
<td>Kvariati 3</td>
<td>20,0-21,0</td>
<td>water</td>
<td>-</td>
<td>-</td>
<td>-</td>
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<td>sediments</td>
<td>3,6x10^6</td>
<td>2,0x10^4</td>
<td>210</td>
<td>60</td>
<td>-</td>
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<tr>
<td>10</td>
<td>Kvariati 4</td>
<td>15,0-16,5</td>
<td>water</td>
<td>5x10^2</td>
<td>1x10^2</td>
<td>700</td>
<td>210</td>
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<td></td>
<td>sediments</td>
<td>1,8x10^6</td>
<td>6,8x10^3</td>
<td>60</td>
<td>46</td>
<td>-</td>
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</table>
Phages as indicators
Vibriophages in Georgian water environment (2006-2009)

• Vibriophages were isolated from freshwater and marine sampling sites
• **Vibrio** species vary by their phage potential
• Majority of *V. cholerae* phages - freshwater isolates (72%)
• *V. parahaemolyticus* -specific phages prevailed at the Black Sea sites

- Peak of phage isolation summer – autumn period
- Correlation with isolation dynamics of host bacteria

Correlations between average monthly vibriophage counts, and TVC

\[ r = 0.28 \]
Water borne infection Source tracking

**Phage tracing**

- Properties of the tracer phages resemble those of infectious agents (pathogenic bacteria and viruses).
- Phage is only harmful to a specific bacterial host(s)
- No limitation in the concentration of the initial inoculum
- The water course can be traced over long distances (>20 km)
- The low detection limit for plating - comparable to that for chemicals
Water borne infection Source tracking
Phage tracing in Black Sea costal Zone

• Testing of water treatment plant
• Impact of ballast waters from ships
• Swimming associated infections

I. Chkonia et al, 1988;1997
Control Water borne infections: Phage therapy and prevention

• **Prevention**
  Prophylactic use of polyvalent phage preparations (e.g. Intesti phage) in the population under the risk of infection spread

• **Therapy**
  Treatment of sick (infected) people with polyvalent or specific phage preparations
  • Phages alone
  • Phages in combination with antibiotics
Bacteriophage preparations for treatment and prophylactics of intestinal disorders / enteric infections

**Salmonella phage preparation**
- 11 components, active against
  - *S. typhimurium*
  - *S. cholera suis*
  - *S. dublin, S. enteritidis*
  - *S. paratyphi A, B*
  - *S. newport etc*

**INTESTY-bacteriophage**
- 17 components, active against
  - *Shigella (3 comp.)*
  - *Salmonella (6 comp.)*
  - *E.coli (5 comp.)*
  - *Enterococcus (2 comp)*
  - *Staphylococcus (2 comp)*
  - *Proteus (2 comp)*
  - *Pseudomonas aeruginosa*

**Phages to be used for waterborne enteric infections**
Study of the efficacy of INTESTY Phage and Antibiotics

![Graph showing the comparison of % of recovered patients over days after starting the treatment for INTESTY Phage, Antibiotic, and INTESTY Phage+Antibiotic treatments.](image)

- **INTESTY Phage**: Blue line with diamonds.
- **Antibiotic**: Red line with circles.
- **INTESTY Phage+Antibiotic**: Yellow line with triangles.

Days after starting the treatment:
- 2--3
- 4--5
- 6--7
- 8--9
- 10--11
- 12--13
- 14--15
- 16--17
- 20--22
- 26--28

% of recovered Patients:
- INTESTY Phage: 32.1
- Antibiotic: 21.4
- INTESTY Phage+Antibiotic: 7.14
Prophylactic use Polyvalent Salmonella Bacteriophage

- SALPhage in children of 0-2 year of age
- 1283 children treated with Sal phage
- 1238 children - control group

- 5 days 3-4 times /day
- Children 5-10-15 ml _per os or per rectum_
- _Adults_ -30-50ml
  or 1-2 tablets 3Xday

Positive prophylactic effect: decrease of disease manifestation 5.5 times

( E. Tsereteli et al., 1983 )
Phages against other water borne pathogens available at the Eliava institute

- *Vibrio choleare*
  - *V.parahaemolyticus*
  - *V.vulnificus*
  - *V.mimicus*
  - *V. algynolyticus*
  - *V. fluvialis*

- *Aeromonas hydrophyla*
  - *A. caviae*
  - *A. sobria*
  - *A. salmonicida*
Phenotypic and genetic diversity of Vibriophages

Phage Virion Morphology
- All three types of **Tailed phages**
- Majority belong to **Myoviridae** family

Restriction analyses of V. cholerae phages
EcoRI and Not I digest

V. cholerae phages
THANK you