

Thursday, April 14
10:30 a.m.–12:00 p.m.

Session 6:
**Advances in Alternative Indicators
and Measurement**



Evaluation of Three Culture-Based Methods for Enumeration of Coliphage from Ambient Waters

Asja Korajkic, PhD

U.S. Environmental Protection Agency

Abstract

Despite a long history of use, many uncertainties exist about the ability of fecal indicator bacteria to predict the presence of enteric pathogens in ambient waters. Coliphage densities could provide a useful alternative approach. Here, we evaluate the ability of three methods to enumerate F+ and somatic coliphage from 1-liter samples from Lake Michigan and Trail Creek waters (n=37 each) collected in 2015. Methods tested include direct membrane filtration (MF) on 0.45-um pore size nitrocellulose filters, deadend hollow-fiber ultrafiltration combined with single agar overlay (D-HFUF-SAL) and U.S. Environmental Protection Agency method 1602 (1602). Overall, somatic coliphage levels ranged from nondetectable (ND) to 4.38 log₁₀ plaque forming units (PFU) per liter and were consistently higher compared to F+ (ND to 3.35 log₁₀ PFU per liter), irrespective of method or water type. Concentrations of both coliphage types were significantly higher ($P < 0.0001$) in creek samples than in lake waters. The MF method recovered significantly less somatic (lake and creek) and F+ coliphage (creek) ($P < 0.0001$) than either 1602 or D-HFUF-SAL. Coliphage levels in creek water detected by D-HFUF-SAL and 1602 were not significantly different ($p > 0.05$). In lake waters, D-HFUF-SAL recovered significantly more F+ coliphage than 1602 ($p > 0.0001$), but there was no significant difference in levels of somatic coliphage detected by either method ($p > 0.05$). Results suggest that D-HFUF-SAL and 1602 perform in a similar manner, consistently recovering greater levels of somatic and F+ coliphage than the MF method, regardless of water type.

Biosketch

Dr. Asja Korajkic is a microbiologist in the U.S. Environmental Protection Agency's Office of Research and Development, National Exposure Research Laboratory in Cincinnati, Ohio. She received her bachelor of science degree in microbiology and doctorate in environmental and ecological microbiology from the University of South Florida. Dr. Korajkic's research interests include characterizing fate and transport of bacterial and viral indicator organisms and microbial source tracking markers, as well as pathogens in aquatic habitats. More recently, she has been involved in the development and optimization of viral concentration/detection methods.



Coliphage Levels in the Effluents of Wastewater Treatment Plants Discharging Nearby Great Lakes Beaches

Marirosa Molina, PhD

U.S. Environmental Protection Agency

Abstract

Coliphages are being considered as potential surrogates for human viruses and thus an alternative indicator of fecal contamination in recreational waters. During the summer of 2015, the U.S. Environmental Protection Agency conducted a field study to determine the seasonal and temporal variability of coliphages in selected Great Lakes (GL) beaches. Complementary to this work, a study was conducted to determine coliphage levels in the effluents of wastewater treatment plants (WWTPs) in close proximity to the selected beaches, with the objective of obtaining point source information for future modeling exercises of coliphage densities. The study was conducted from June 9 through September 9, 2015. Effluents from four WWTPs serving the Great Lakes region were analyzed for somatic and male-specific (F+) coliphages on a weekly basis using both the double agar overlay (DAL) and the deadend hollow-fiber ultrafiltration with single agar overlay (HFUF-SAL) methods. Based on the variable concentrations across treatment plants, both analytical methods were necessary to properly detect the presence of phages in the various effluents. Concentrations ranged from 1 to 4.7 log PFU/L for F+ and from 1 to 4.67 for somatic phages in the effluents of three WWTPs, with both types of coliphages detected at similar concentrations per individual plant. No coliphages were detected in one of the plants throughout the course of the study. The high variability observed in coliphage densities between treated WWTP effluents highlights the importance of these data when explaining the dynamics of microbial contaminants in nearby

beaches. These results will be combined with fate and transport information and relationships to human pathogens and health data will be explored to better understand the potential advantages of coliphage as indicators of microbial water quality.

Biosketch

Dr. Marirosa Molina is a research microbiologist with the Exposure Methods and Measurement Division of the National Exposure Research Laboratory located in Athens, Georgia. Dr. Molina has a bachelor of science degree in industrial microbiology from the University of Puerto Rico-Mayagüez, and a master of science degree in microbiology and doctorate in ecology from the University of Georgia. Her research focuses on (1) assessing the fate and transport of microbial contaminants, microbial source tracking markers, and pathogens from point and nonpoint sources in watersheds through the application of field, laboratory, and modeling approaches; and (2) studying the response of microbial communities to land use and climate changes, including extreme events impacting agricultural and urban landscapes. Dr. Molina also has participated in the development and evaluation of Virtual Beach, a software tool designed to predict the concentration of fecal indicator bacteria in surface waters using meteorological, hydrological, and physicochemical parameters in an effort to provide nowcast capabilities to beach managers and stakeholders.



Coliphage Levels in the Effluents of Wastewater Treatment Plants Discharging Nearby Great Lakes Beaches

Marirosa Molina¹, Kelvin Wong², Mike Cyterski¹, Richard Zepp¹, and Gene Whelan¹
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The views expressed in this presentation are those of the authors and do not necessarily reflect the views or policies of the U.S. Environmental Protection Agency

Introduction

- Coliphages as alternative indicators
- Understanding sources of contamination
 - critical
- Objective
 - Waste Water Treatment Plant coliphage contributions
 - F & T modeling = source
 - F+ or somatic coliphages
 - Is there a difference?

2015 Beach Study Sites

MSS – Milwaukee South Shore
 SM – South Milwaukee Wastewater Treatment
 MC – Michigan City Sanitary District
 NFO – Northwestern Ohio Regional Sewer
 Edgewater
 Washington Park
 Grant Park

Michigan City Sanitary District, IN

Discharge: 12 MGD
 Disinfection: Chlorine gas diffusion of tertiary effluent

Northeastern, OH

Discharge: 26 MGD
 Disinfection by chlorination (Sodium hypochlorite)

South Milwaukee and Milwaukee South Shore, WI

Discharge: 6 MGD
 UV Disinfection System

Discharge: 90 MGD
 Disinfection by Chlorination of secondary effluent



EPA Sampling and Analysis

- Samples collected directly from WWTP effluents
- One sample /week
 - 14 weeks
 - June - Sept 2015
- Analysis
 - HFUF-SAL
 - DAL
- Targets
 - F+ coliphages
 - Somatic coliphages

EPA Results

- Both types of coliphages were detected at similar concentrations in the effluents of the WWTP.
- Northeastern OH had a significantly higher concentration relative to the other three plants
- No phages were detected at Michigan City (which is in close proximity to Washington Park) except for one instance during August 2015

EPA Results

- FIB in WWTP Effluents

EPA Results

Correlation between FIB and Coliphages Monitored in WWTP Effluents

WWTP Plant	FIB	Coliphage	R ²
Milwaukee South Shore	<i>E. coli</i> (MPN/100 ml)	F+	0.09
		Somatic	0.11
South Milwaukee	Fecal Coliforms	F+	0.42
		Somatic	0.31
Northeastern OH	<i>E. coli</i> (CFU/100 ml)	F+	0.09
		Somatic	0.01

* Michigan City did not have enough coliphage densities to develop a correlation

EPA WWTP Characteristics

	Northeastern Ohio	South Milwaukee	Milwaukee South Shore	Michigan City
Nearby Beach	Edgewater	Grant Park	Grant Park	Washington Park
Outfall	L. Erie	L. Michigan	L. Michigan	Trail Creek
Design Flow (MGD)	35	6	300	15
Average Flow (MGD)	26	6	90	12
Disinfection	chlorination (sodium hypochlorite)	UV	chlorination of secondary effluent	chlorination of tertiary effluent
FIB (method)	<i>F. coli</i> (Method 1603 modified m-Tec)	Fecal Coliform	<i>E. coli</i> (enzyme substrate, SM method 9223 B)	<i>F. coli</i> (Method 1603 modified m-Tec)
Correlation	Low	High	Low	N/A

EPA Results

Solar Irradiation Experiment

- Phage photoinactivation could be described by first order kinetic expressions.
- Delays of up to seven orders of magnitude were observed.
- Surrogate somatic phage exhibited the largest decay > F+RNA/Somatic phage Communities > Surrogate F+ RNA

Poster: Process Relationships for Evaluating the Role of Light-Induced Inactivation of Coliphages at Selected Beaches and Nearby Tributaries of the Great Lakes. Richard G. Zopp¹, Maritessa Molina¹, Mike Cytuski¹, Gene Whelan¹, Rajbir Panwar¹, Kelvin Wong², Brad Acree³, and Rama Georgakopoulos³



Summary of Results

- Both types of coliphages were detected at similar concentrations.
- No correlation was identified between coliphages and *E. coli* in any of the WWTP effluents tested. However, a significant correlation was observed between fecal coliforms and both types of coliphages in one of the treatment plants.
 - Disinfection treatment, treatment capacity??



Final Considerations

- The potential impact of the WWTP on adjacent beaches based on the phage effluent concentrations is as follows:
 - Edgewater > Grant Park > Washington
 - For FIB : Grant Park > Edgewater > Washington
 - Basically, no phages were detected in the effluent of Michigan City WWTP, therefore, any phages detected at Washington Park are likely coming from other sources in the watershed.



Next steps

- Factors affecting fate and transport
 - Effect of sunlight irradiation
 - Interaction with organic matter
- Predictive vs. process modeling approaches

Thanks!



Advances in Measurements and Indicators for Determining Shellfish Growing Area Classifications Adjacent to Wastewater Treatment Plant Outfalls

Yaping Ao

U.S. Food and Drug Administration

Abstract

Since 1987, the U.S. Food and Drug Administration (FDA) has recommended at training courses and other venues the use of a 1000:1 dilution as the minimum level of dilution needed around a wastewater treatment plant (WWTP) outfall to mitigate the impact of viruses. In 1995, this estimated level of necessary dilution was further calculated and explained by FDA using assumptions based on the most relevant scientific literature available at that time. Since then major advances in the detection and enumeration of norovirus in wastewater and shellfish have been made, and advances in fluorometer technologies have enabled more sophisticated and accurate hydrographic dye study methods. Using these advances, FDA has conducted hydrographic dye dilution studies within estuaries of various geographic locations and conditions. Shellfish sentinels placed at various dilutions were tested for enteric viruses and male-specific coliphage. This has afforded FDA, for the first time, with a means to directly determine the viral risk posed by WWTP effluent on shellfish resources. The results of these studies provided the scientific basis behind FDA's dilution guidance that was recently adopted into the National Shellfish Sanitation Program. The results also have proven valuable for a joint United States/Canada quantitative norovirus risk assessment for molluscan shellfish as well as calibration and validation of hydrodynamic models of WWTP discharges to growing areas currently being developed by FDA. The data collected might additionally support future forecasting models used to predict the sanitary impacts to

growing areas attributed to forecasted storm-related events.

Biosketch

Ms. Yaping Ao is a visiting associate with the U.S. Food and Drug Administration, Center for Food Safety and Applied Nutrition in College Park, Maryland. She serves as a lead modeler in the application of computer fate and transport models to assess pollution source impacts to shellfish growing areas. She assisted with the development of dilution models to support a joint U.S.-Canada norovirus risk assessment and has provided training on and led specialized field and hydrographic studies to identify and assess pollution sources in the environment. Ms. Ao's expertise includes supporting the development of guidance for irrigation water use for produce safety. She received her master of science degree in civil engineering from Marquette University in Wisconsin and her bachelor of science degree in environmental engineering from the Chengdu University of Technology, China.



FDA U.S. Food and Drug Administration
Protecting and Promoting Your Health
www.fda.gov/food

Advances in Measurements and Indicators for Determining Shellfish Growing Area Classifications Adjacent to Wastewater Treatment Plant Outfalls

Yaping Ao¹, Gregory Goblick¹, Steven Tidwell¹, Eric Tate¹, Julie Anbarchian¹, Jacqueline Woods², William Burkhardt, III², and Kevin R. Calc²

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Disclaimer: Some of the information contained in this presentation is unpublished. The data, the presentation slides, or portions of the presentation slides may not be further disseminated.

National Shellfish Sanitation Program – MO Section II

Chapter IV - Shellfish Growing Areas
@.03 - Growing Area Classification
E - Prohibited Classification
(5) - Wastewater Discharges

(a) An area classified as prohibited shall be established adjacent to each sewage treatment plant outfall or any other point source outfall of public health significance.

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Why are Enteric Viruses a Concern?

- Viruses have a longer survival time than the coliform bacteria group
- Viruses are more resistant to disinfection and may be found in treated WWTP effluent
- Build-up of virus levels can occur in poorly flushed estuaries
- Shellfish can bio-accumulate viruses >50 fold overlying waters
- Bacterial indicators do not adequately index viral risk posed by WWTP effluents – especially for conventional WWTP treatment

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Advances in Indicators

Male-Specific Coliphage:

- Virus which infect coliform bacteria
- Culturable using rapid, inexpensive techniques
- Chlorine resistant
- More resistant to environmental stresses than fecal coliforms

Good indicator of:

- Raw or treated municipal sewage
- exfiltration from a sewage collection system
- overflows or spills from a collection system
- viral impact on shellfish from the above sources

- 50 PFU/100 g (shellfish) - has been adopted by the NSSP – re-opening after sewage contamination events from WWTPs
- 100 PFU/100 g (shellfish) – level at which outbreaks in EU shellfish at market begin to occur

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Advances in Measurements/Tools

Submersible Fluorometers:

- High sensitivity
- Less expensive
- Small and light weight
- No calibrations – regressions for dye
- No false positives from air or carryover
- Can use in moored mode
- Can use in plume tracking mode
- Several parameters possible

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Advances in Analytical Tools

Hydrodynamic and Transport Models:

- Models were calibrated and validated against dye studies and were used to evaluate wet weather driven pollution events
- Models allow different conditions and factors to be assessed that were not captured during dye study
- Can be used for comparing factors to determine worst case and useful for management and classification of growing areas

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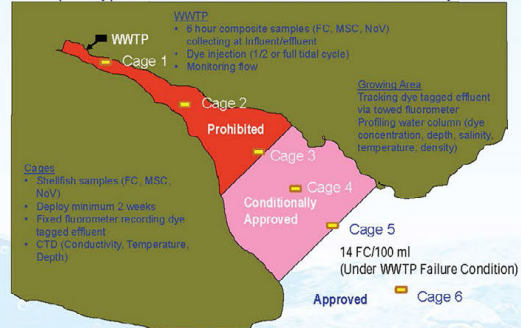
FDA studies

- In recent years FDA has conducted >50 hydrographic dye dilution studies
- Since 2007, FDA has conducted combined dilution/shellfish meat bio-accumulation studies:
 - Gulf Coast – AL*, MS*
 - Atlantic Coast - VA
 - West Coast – OR, WA, CA[^]
 - East Coast – ME*, NH**, MA**, RI**, CT
- 216 samples determined dilution and analyzed shellfish for Male-Specific Coliphage (MSC) and 161 of these for Human Norovirus (HuNoV)
 - Samples collected collaborative effort with States and Industry
 - Many additional samples provided by Spinney Creek Shellfish
 - On-going studies/samples with: CT, NH, WA
- Shellfish species: pacific and eastern oysters, hard and soft-shell clams, mussels
- WWTP Disinfection technologies: conventional chlorination, UV, Membrane

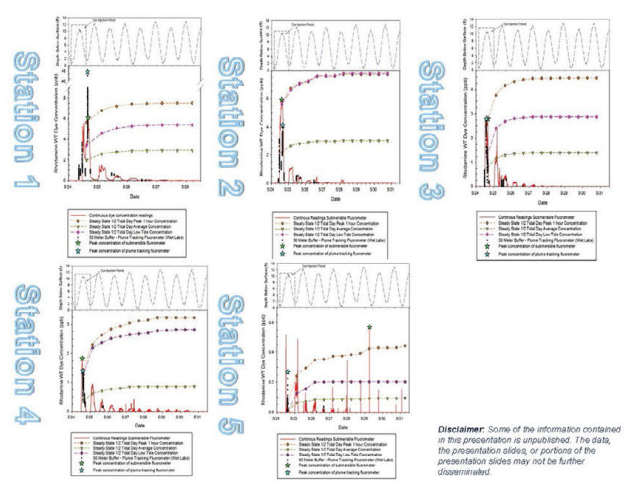
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FDA Field Studies

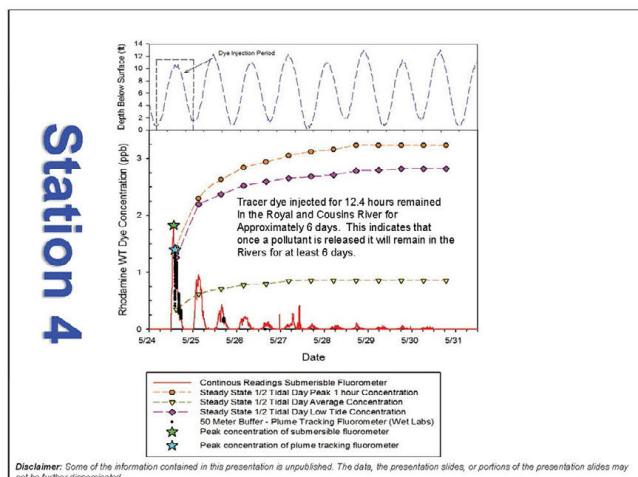
Example: Typical Technical Assistance & Research Study



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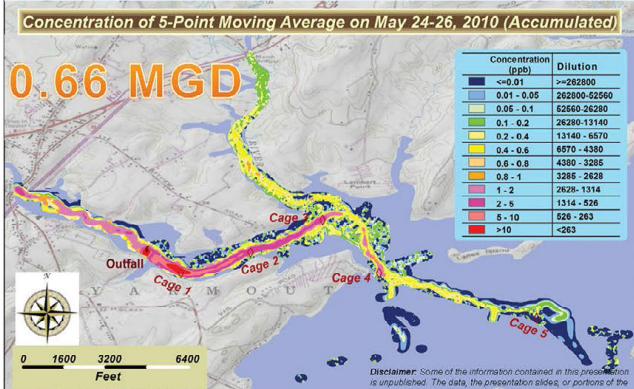


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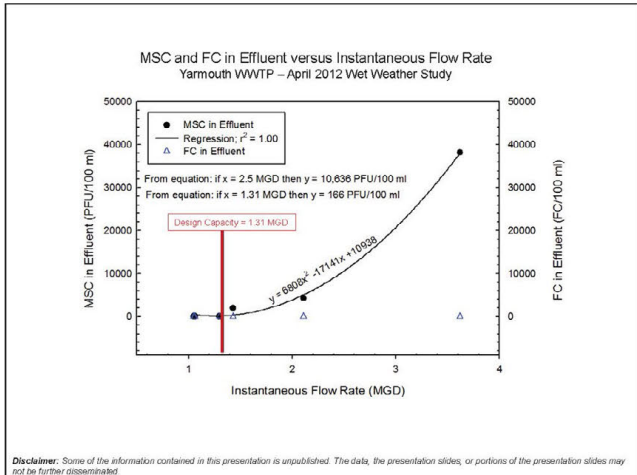
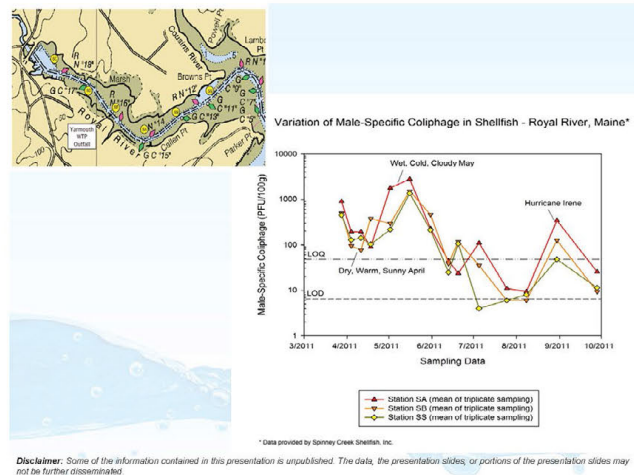
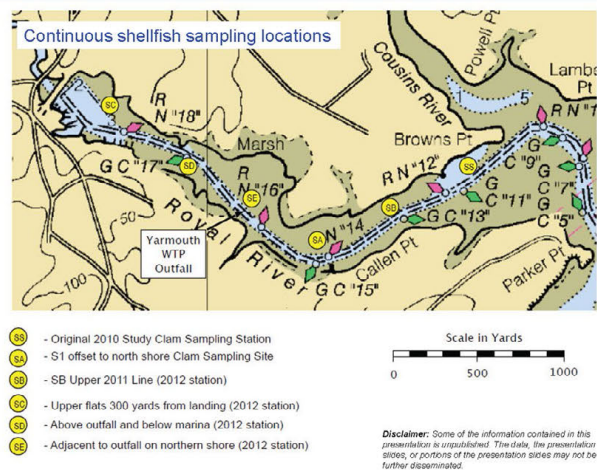
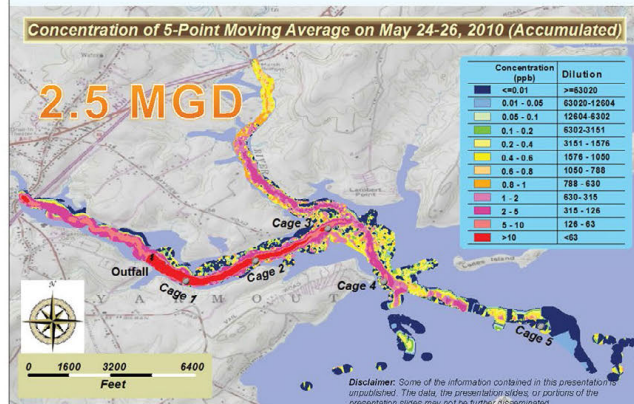
Yarmouth, ME – Dye Study Results



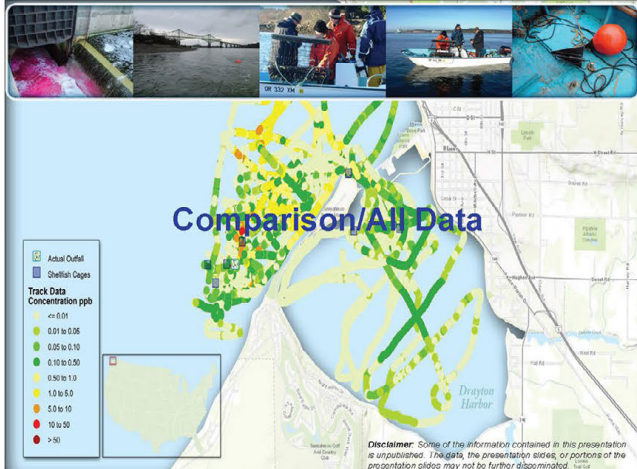
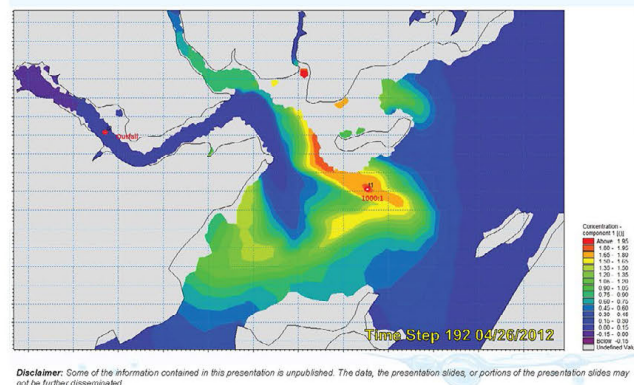
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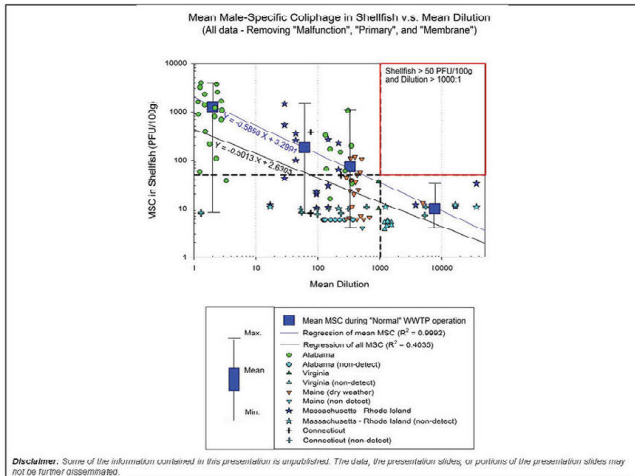
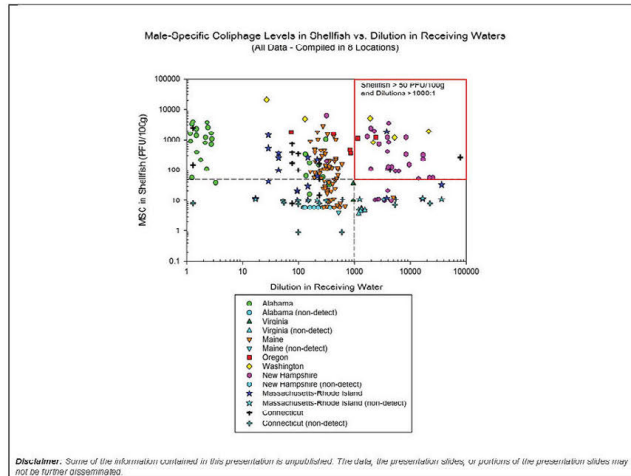


Yarmouth, ME – Empirical Model Results



Hydrodynamic Model Simulation – Determine 1000:1 Dilution under wet weather





Conclusion

- These new advances in tools has enabled FDA to develop **Dilution Guidance** on how to size a prohibited buffer zone protective of viral impacts
- When WWTPs operating within "normal" conditions results fall below MSC end-point within the 1000:1 recommended dilution
- 1000:1 **does not appear** to be applicable for:
 - When WWTPs "malfunction" frequently – such as bypass primary or secondary or change operations frequently causing degradation of effluent
 - Unconventional treatment technologies that have not been validated – e.g. some membrane technologies
 - WWTPs with only primary treatment

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Uses of MSC

- To characterize the performance of Wastewater Treatment Plants (WWTP).
- To characterize the shellfish growing area catchment, the pollution sources and strength.
- To classify shellfish harvest areas that are adjacent to WWTPs.
- To manage sewage spill events and to determine when shellfish can be safely harvested again after the event.
- To manage shellfish relaying operations where shellfish are moved to clean environmental waters for long term cleansing.
- To assess viral illness events associated with shellfish.

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Acknowledgements

Mikkel Anderson
DHI Group

Tim Bridges
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Yarmouth Wastewater Treatment Plant

Tom Howell
Spinnecreek Shellfish

Maine Department of Marine Resources

Joint Canada-U.S. Risk Assessment working group members

FDA employees that have assisted on field studies





Coliphages as Indicators of Gastrointestinal Illness in Recreational Waters: A Pooled Analysis of Six Prospective Marine Beach Cohorts

Jack Colford, Jr., MD, PhD

University of California—Berkeley

Abstract

Background: Coliphages have been proposed as potential indicators of fecal contamination of marine recreational waters because they might be able to predict the presence of viruses better than fecal indicator bacteria. We estimated the association between coliphages and gastrointestinal illness.

Methods: We pooled data from six prospective cohort studies conducted from 2003 to 2009 that enrolled beachgoers in the summer at coastal beaches in Alabama, California, and Rhode Island. Studies collected water samples and recorded incidents of gastrointestinal illness within 10 days of the beach visit. Samples were tested for male-specific and somatic coliphage using U.S. Environmental Protection Agency (EPA) methods 1601 and 1602. We estimated cumulative incidence ratios (CIRs) for the association between gastrointestinal illness and coliphage when human fecal pollution was suspected to be present (“high-risk conditions”) and not present (“low-risk conditions”).

Results: Under high-risk conditions, a 1-log₁₀ increase in male-specific coliphage levels was associated with a CIR of 1.30 (95% CI 0.94, 1.81) (EPA 1601; n=6 beaches) and 2.20 (95% CI 1.30, 3.71) (EPA 1602; n=2 beaches); under low-risk conditions the CIRs were 0.83 (95% CI 0.70, 1.00) (EPA 1601) and 0.71 (95% CI 0.19, 2.72) (EPA 1602). The CIRs for a 1-log₁₀ increase in somatic coliphage (EPA 1602) were 1.27 (95% CI 0.92, 1.76) under high-risk conditions and 0.98 (95% CI 0.82, 1.16) under low-risk conditions (n=2 beaches).

Conclusion: Coliphage was associated with increased gastrointestinal illness risk at beaches with suspected human fecal pollution.

Note: This abstract does not represent EPA policy.

Biosketch

Dr. Jack Colford is a professor of epidemiology at the University of California (UC), Berkeley School of Public Health. He trained at Johns Hopkins University (doctorate in medicine), Stanford University (chief medical resident), UC San Francisco (residency in internal medicine and fellowships in infectious diseases and HIV/AIDS), and UC Berkeley (doctorate in epidemiology). Dr. Colford has served as the principal investigator for numerous randomized controlled trials and observational studies evaluating the impact of water, sanitation, and hygiene interventions in India, Bolivia, Guatemala, Bangladesh, Kenya, Mexico, and the United States. His research has been supported by the National Institutes of Health, Centers for Disease Control and Prevention, U.S. Environmental Protection Agency, and Gates Foundation. He teaches courses each year at UC Berkeley on epidemiologic methods, the design of randomized controlled trials, and impact evaluation for health professionals.



EPA's Development of Recreational Water Quality Criteria for Coliphage: Updates and Experts Coliphage Workshop Overview

Sharon Nappier, PhD

U.S. Environmental Protection Agency

Abstract

Recreational Water Quality Criteria (RWQC) are recommendations intended to be used by states in adopting water quality standards (WQSs) to protect the designated use of primary contact recreation. WQSs are then used to develop point source permits, to identify impaired waters, and for beach notifications. Historically, RWQC recommendations have been based on fecal indicator bacteria *E. coli* and enterococci. The U.S. Environmental Protection Agency (EPA) is now evaluating coliphage, a viral indicator, to help prevent viral associated illnesses. EPA recently held an experts workshop to engage a group of internationally recognized experts on the state of the science of coliphage and their usefulness as a viral indicator for the protection of public health. Topics for discussion included the need for a viral indicator, coliphage as a predictor of gastrointestinal illnesses, coliphage as an indicator of wastewater treatment performance, male-specific vs. somatic coliphage, a systematic literature review of viral densities, and data gaps and future research. EPA will provide an overview of the recent Experts Coliphage Workshop and an update on the development of RWQC for coliphage.

Biosketch

Dr. Sharon Nappier specializes in environmental microbiology and quantitative microbial risk assessment and has more than 13 years of national and international experience working on foodborne and waterborne diseases; microbial method development and evaluation; program and contract management; national water policy development; and science communication. Dr. Nappier received her master's degree from the University of North Carolina at Chapel Hill in environmental sciences and engineering and her PhD from the Johns Hopkins Bloomberg School of Public Health in environmental health engineering. She has been working at the U.S. Environmental Protection Agency for the past 6 years. Her major projects include chairing the 2012 Recreational Water Quality Criteria workgroup; leading efforts to develop recreational water quality criteria for coliphage; and assessing the microbial risks associated with direct potable reuse. Since 2011, Dr. Nappier also has served as a professorial lecturer at The George Washington University's School of Public Health, teaching applied environmental health microbiology.



Recreational Water Quality Criteria for Coliphage: Updates and Experts Workshop Overview

Sharon P Nappier, MSPH, PhD
Office of Water, Office of Science and Technology
US Environmental Protection Agency
April 14, 2016

Outline

- Recreational Water Quality Criteria
- Experts Workshop
- Next Steps

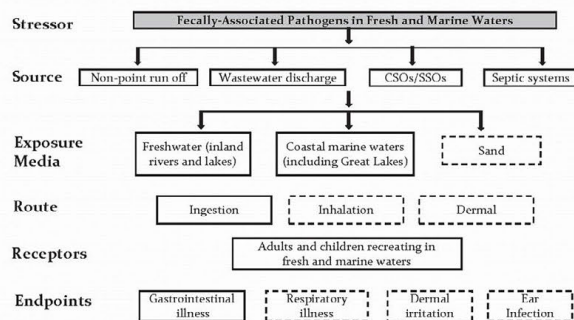
Clean Water Act (CWA)

- Goal: Restore and maintain oceans, watersheds, and their aquatic ecosystems to protect human health, support economic and recreational activities, and provide healthy habitat for fish, plants and wildlife.
- Establishes basic structure for state water quality standards, including regulation of pollutant discharge into the waters of the United States.

Recreational Water Quality Criteria (RWQC)

- Intended to be used by states adopting water quality standards to protect the designated use of primary contact recreation.
- BEACH ACT (CWA 304(a)(9)(B)) requires EPA to review coastal RWQC every five years (next review: 2017)
- RWQC recommendations:
 - **Prevent illness**
 - By preventing fecal contamination and/or pathogens from entering surface waters
 - Point source permits (NPDES permits)
 - **Identify impaired waters**
 - 303(d) Listing, Total Maximum Daily Loads (TMDLs)
 - **Identify potentially hazardous conditions**
 - Beach notifications

Conceptual Model



2012 Recreational Water Quality Criteria

The 2012 RWQC for primary contact recreation are associated with bacterial indicators of fecal contamination.

Highlights:

- **Indicators:**
 - Enterococci (marine and freshwater) and *E. coli* (freshwater)
- Specified magnitude, duration (30 day), and frequency
 - Two sets of recommended criteria, each corresponds to a different illness rate
- Includes supplemental tools
 - qPCR method for same-day notification
 - Beach action values for precautionary notification



Current Status

To prevent illness

- **Bacterial** pathogens targeted through bacterial indicators
 - Historically bacteria were thought to cause majority of illnesses
 - Wastewater treatment improvements and permits based on bacterial indicators effectively control bacterial pathogens
- QMRA, epidemiological, and microbial water quality studies indicate **viruses** cause majority of swimming-associated illnesses in human-impacted waters
 - Current treatments, indicators, and permits do not specifically target viruses
 - Thus, viruses enter surface waters from treated & untreated human sources

To identify impaired waters or potentially hazardous conditions

- Culturable **bacterial** indicators used
 - Effective at predicting bacterial impairments of water quality
 - Epi studies indicate they may not always be predictive of **viral** illnesses

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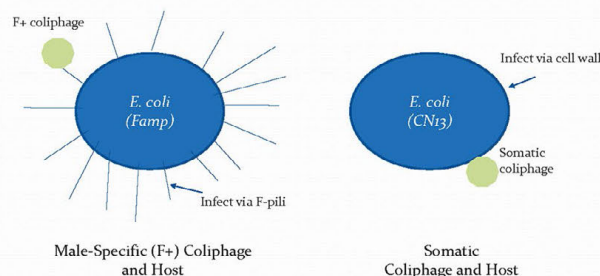
Coliphage – a viral indicator

In use since the 1970's:

- EPA: Ground Water Rule recommended coliphage to detect and/or quantify viral indicators in ground water
- ISSC/FDA: Evaluating the use of male-specific coliphage for shellfish bed closure decisions
- NWRI: Framework for Direct Potable Reuse recommends coliphage be used as a surrogate for evaluating virus removal in reuse configurations

8

Coliphage – a viral indicator



9

Recreational Water Quality Criteria - Coliphage

Coliphage advantages:

- Of fecal origin/highly concentrated in sewage
- Physically similar to enteric viruses of concern
- Similar persistence patterns to enteric viruses of concern
 - To treatment and to environmental insults
- No appreciable re-growth in ambient waters
- Non-pathogenic

Indicators rather than pathogenic viruses:

- Currently not feasible to assess all pathogenic viruses due to methodological and time constraints

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Recreational Water Quality Criteria - Coliphage

- **Prevent viral illness** ✓
 - Coliphage-based discharge permits can prevent viruses entering source waters, thus preventing viral illnesses
- **Identify impaired waters or potentially hazardous conditions** ✓
 - Epidemiological studies indicate coliphage may provide a tool to better protect from viruses

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Coliphage Experts Workshop: March 1-2, 2016

12



Coliphage Experts Workshop

Purpose: Have internationally recognized experts engage on the topic of how best to protect public health from viral contamination of water given currently available information.

Specific Goals:

- Obtain input on science questions from experts in fields of environmental microbiology, microbial risk assessment, and environmental epidemiology.
- Gather scientific insight to determine the best coliphage type (male-specific and/or somatic) for use in CWA 304(a) criteria.
 - Identify situations where these coliphage types may be most useful for preventing illnesses and identifying impaired waters
- Identify research needs that can be addressed by 2017.

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Coliphage Experts Workshop – Experts

Name	Affiliation
Nicholas Ashbolt	University of Alberta
William Burkhardt	U.S. Food and Drug Administration
Kevin Calci	U.S. Food and Drug Administration
Jack Colford	University of California, Berkeley
John Griffith	Southern California Coastal Water Research Project
Vincent Hill	Centers for Disease Control and Prevention
Juan Jofre	University of Barcelona, Spain
Naoko Munakata	Sanitation Districts of Los Angeles County
Rachel Noble	University of North Carolina, Chapel Hill
Joan Rose	Michigan State University
Mark Sobsey	University of North Carolina, Chapel Hill
Timothy Wade	U.S. Environmental Protection Agency

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Coliphage Experts Workshop - Scope

- Focused on recreational risks associated with fecal contamination
 - Other risks not considered: sunburns, shark attacks, etc.
- Focused on science aspects of criteria development
 - Minimized policy and implementation discussions

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Coliphage Experts Workshop – Topic Areas

1. Need for a Viral Indicator
2. Coliphage as a Predictor of Gastrointestinal Illness
3. Coliphage as an Indicator of WWTP Performance
4. Male-specific vs Somatic Coliphage
5. Systematic Literature Review of Viral Densities

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Coliphage Experts Workshop – Meeting Format

- Experts assigned a topic with associated charge questions
 - Experts provided written responses to charge questions to EPA prior to Workshop
 - Responses compiled and provided to all experts prior to Workshop
- Each expert gave 10-15 min presentation, based on their answers to charge questions
- Group collectively discussed charge questions
- Group captured main points in discussion summary

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Coliphage Experts Workshop – Highlights (1)

Topic 1: Need for a Viral Indicator

- Individual experts agreed that viruses are a source of illness in recreational water exposures.
- Viruses can enter surface waters via WWTP effluent.
 - Especially during wet weather and when WWTPs exceed design flows.
- Coliphages are more similar to human pathogenic viruses compared to the traditional fecal indicator bacteria (FIB).
 - Mimic human pathogenic viruses.
- Coliphages have demonstrated value added for managing risks and are used full-scale to address WWTP water quality and related applications.
 - Ex: NC reclaimed water, Ground Water Rule, and by FDA for reopening shellfish harvesting areas after catastrophic spills.
- Coliphage methods are available, inexpensive, and could be developed into easy-to-use commercial kits.
 - Faster methods (less than 8 hours) are available.

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Coliphage Experts Workshop – Highlights (2)

Topic 2: Predictor of GI Illness

- Future epidemiological studies should specifically include coliphages as measured indicators.

Topic 3: Indicator of WWTP performance

- Coliphages are consistently present in municipal sewage, and provide a baseline for looking at different WWTP processes under varied conditions.
 - Some experts indicated the literature suggests coliphage and human viruses have more similar log-reductions during wastewater treatment, compared to traditional FIB.

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Coliphage Experts Workshop – Highlights (3)

Topic 4: Male-specific vs Somatic Coliphages

- Opinions ranged on whether somatic, male-specific coliphage, or both would be better for various applications.
 - Evidence for both showing relationship to GI illness.
 - Male-specific coliphage behave more similarly to RNA viruses under some conditions and are currently used successfully by FDA/ISSC.
 - Somatic may persist longer than male-specific coliphage and may be present in greater concentrations in raw sewage.
 - Hosts are available that can detect both.

Topic 5: Review of Viral Densities

- Individual experts supported how the systematic analysis was structured and conducted.

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Coliphage Experts Workshop – Products

Presentations:

- 2016 UNC Water Microbiology Conference (May 2016)

Publications:

- Fact-sheet (summer 2016)
- Peer-reviewed Proceedings Report (winter 2017)

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Status and Timeline

date	milestone
04/17/2015	Review of Coliphages as Possible Viral Indicators of Fecal Contamination for Ambient Water Quality
10/15/2015	Stakeholder Webinar
03/01/2016	Coliphage Expert Workshop fact sheet (summer 2016) and proceedings (winter 2017)
2016	Listening Sessions/Webinars <ul style="list-style-type: none"> • Conferences (New Orleans and Chapel Hill) • States • Other stakeholders (industry/environmental groups)
summer 2016	Analytical method multi-lab validation
late 2017	Draft Criteria released for public review

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Questions?

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Question & Answer Session

Question 1

Mark Sobsey: I'm glad to see the interest in viruses. I have a comment to Asja [Korajkic]. The EPA study involved three methods. There are other methods, why weren't some of them included? MPN [most probable number] 1601 is easy to do and less expensive, but it takes longer because there are two steps. There is membrane filtration and an adsorption dilution method. If you took that method and stuck it in a dilution and waited, I think the dilution would be more successful. You could then do another assay. If you revisit, you should consider looking at this.

Answer 1

Asja Korajkic: Thanks for developing all those methods. When we looked between methods 1601 and 1602, we wanted to take the standard methods. So, it added 1 day for 1601, which is why we didn't use it. It was because of the logistics. All your points are great, and we have a system for alternative methods. This is to get the ball rolling. These aren't the end-all-be-all. I'd love to try to modify some other methods, especially to get results in a shorter time frame: 16 hours or so. All of these methods were initially developed for groundwater and we're trying to get them suitable for surface water. Dilution might improve it, but we opted to go for simplified versions of the methods first.

Comment 1

Mark Sobsey: I think there is a future for reducing the time for results. There is an opportunity for revisions of these methods.

Question 2

*Dan Shapely: Regarding wastewater treatment, in New York there has been some analysis and push for new standards. Our plants, with holding time, can't meet enterococcus standard but can meet *E. coli* standard. They require smaller holding tanks than other states require. Have there been any studies about holding times in wastewater treatment with coliphages or viral indicators?*

Answer 2

*Marirosa Molina: When we talked to WWTP [wastewater treatment plant] operators, they were very helpful and cooperative. It was interesting that Michigan City has a specific reservoir for exposure to chlorine; it goes above the minimum of 15 minutes. That has something to do with it. We also found no coliphage at the plant that never exceeded their *E. coli* standard.*

Answer 2 (follow-up)

Sharon Nappier: Free chlorine is different though, so it depends what they use for disinfection.

Question 3

Ali Boehm: For your low versus high risk, did you categorize sources? Why isn't there a relationship with low-risk beaches?

Answer 3

Jack Colford: Yes, we did parse based on times of day. I don't know about lack of relationship. Maybe there was not enough evidence yet.

Answer 3 (follow-up)

Steve Weisberg: Phages are not as abundant in the environment as fecal indicators. So, you see a lot more minimums in the study. That is one reason for low numbers in low-risk conditions, there are lots of nondetects.



Answer 3 (follow-up)

Sharon Nappier: All those samples were under 100 mL [milliliters]. Future studies for EPA will sample using 2 L [liters]. I recommend we include the larger volumes so there is a better chance of catching coliphage

Question 4

Phil Scanlan: I'm here to try to share what we did in New Jersey. Many states have told me they want to cut the amount of pollution at their beaches in half by 2020. In 2013, the average number of exceedances was 10 to 12 percent of beaches. If we change to coliphage, how does that change exceedances? What will the exceedance be then?

Answer 4

Steve Weisberg: That is a fair question but it is complicated. It depends on the threshold.

Question 5

Linda Pechacek: When you discussed the results of your study, you said there were two possible factors: disinfection time and flow. And, one plant was operating near capacity and one was operating far below its permitted flow. Could there be a third factor, like tertiary treatment in Michigan City?

Answer 5

Marirosa Molina: Yes, there could be. They do have tertiary treatment as well. There are a number of factors affecting things.