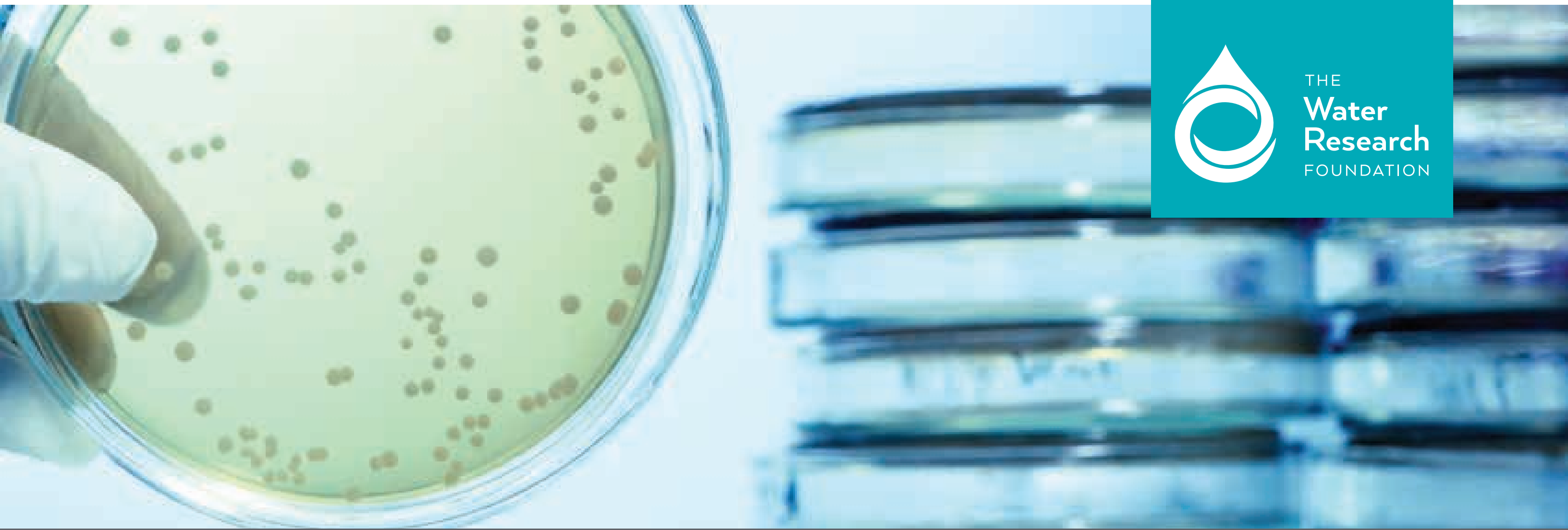


Microbes & Pathogens

THE CHALLENGE

Control of microbes in water systems is critical to protecting water quality and public health. While most microbes are not human pathogens, certain microbes can pose health risks or contribute to undesirable tastes and odors in drinking water. Since the early 20th century, drinking water treatment has made great advancements in the detection, removal, and inactivation of bacteria, viruses, and protozoa—especially fecal-derived pathogens. More recently, however, new challenges have arisen in the form of opportunistic pathogens in premise plumbing systems.

Wastewater and stormwater utilities play an essential role in reducing the pathogen load to receiving waters used for recreation. Additionally, advancements in direct potable reuse demand more understanding of pathogen detection, removal, and inactivation in wastewater.

THE RESEARCH

WRF has published over 200 projects on microbes and pathogens. Topics include detection, inactivation, and removal strategies; customer messaging; and risk assessment for microbes and pathogens at drinking water, wastewater, stormwater, and reuse utilities.

WRF has partnered on this research with the U.S. Environmental Protection Agency (EPA), UK Water Industry Research (UKWIR), the Electric Power Research Institute (EPRI), the California Energy Commission (CEC), the U.S. Bureau of Reclamation, and more. WRF's pioneering research on microbes and pathogens has been included in *Standard Methods for the Examination of Water and Wastewater* (Standard Methods) and informed various EPA guidelines.

Drinking Water

WRF research is leading the way in detection of microbes and pathogens. A notable example is *The Colilert System for Total Coliforms and* Escherichia coli (205), which developed the Colilert® method for detection of total coliforms and *E. coli* in drinking water and wastewater. Based on this research, the Colilert® method was approved by EPA and is included in Standard Methods. Colilert® is the most widely used coliform testing method in the world.

WRF has also published several impactful studies on *Cryptosporidium*, a microscopic parasite that can pose human health risks. With at least 20 different recognized species and approximately 50 genotypes, better genotyping techniques can more accurately detect those species that impact human health. Cryptosporidium *Genotyping Method for Regulatory Microscope Slides* (4099) and Cryptosporidium *Genotyping Workshop and Round Robin* (4284) help to advance this science and make an important connection between technology and regulations.

Analysis of the microbial community in particular environments, the microbiome, has advanced rapidly in the last five years. Nevertheless, the use of advanced meta-omic techniques to study the drinking water microbiome is in its infancy. Meta-omic tools may help utilities better understand drinking water microbiomes, allowing them to better manage water quality and ultimately protect public health. Through a partnership with the National Science Foundation and the University of Illinois at Urbana-Champaign, WRF published a literature review and hosted a workshop on *Advancing Understanding of Microbiomes in Drinking Water Distribution Systems and Premise Plumbing Using Meta-omics Techniques* (4700 and 4733) to enhance understanding of the feasibility and applicability of metagenomic and microbiome approaches to analyze engineered water systems.

SOLUTIONS IN THE FIELD

The City of Milwaukee

On April 7, 1993, the City of Milwaukee issued a boil-water advisory after *Cryptosporidium* passed through their drinking water treatment system and into the finished water. The advisory was lifted after 7 days, and the city embarked on an immediate $89M facilities renovation and upgrade to strengthen barriers related to source water protection, disinfection, and filtration.

To help find a solution, Milwaukee Water Works turned to WRF for help. With more than 100 research projects and over 150 related resources on ozone, which can be used to break down and destroy *Cryptosporidium*, WRF offered a road map to a solution. Based on this body of research, Milwaukee decided to replace chlorine with ozone as the primary disinfectant. This highly reactive gas destroys illness-causing microorganisms and harmful compounds (including *Cryptosporidium*), removes taste and odor compounds, and reduces the formation of disinfection byproducts. To expedite the project, the Department of Public Works used a design-build contract. At the time, the $51M project was the largest ozone retrofit in the world.

Opportunistic Pathogens (OPs)

In 2008, the U.S. Centers for Disease Control and Prevention (CDC) acknowledged higher waterborne disease occurrence from premise plumbing than from pathogens passing through treatment plants into the distribution system. Given increasing populations of elderly and immunocompromised individuals, coupled with advances in detection, the public health impacts of opportunistic pathogens (OPs) are increasing. Control of OPs is complex and requires a multifaceted approach. To advance understanding and improve monitoring and control of OPs, WRF initiated the Waterborne Pathogens in Distribution and Plumbing Systems Research Priority Area in 2015.

Customer Messaging on Opportunistic Pathogens in Plumbing Systems (4664) developed and validated a series of messages for OPs, with a focus on *Legionella*, and offered targeted strategies for educating the various customer groups on the steps they need to take to protect themselves and their tenants from waterborne pathogens.

In 2018, WRF contributed to *Management of* Legionella *and Other Pathogenic Microorganisms in Building Water Systems* (4726), which assembled experts and hosted three webcasts on various topics related to *Legionella*: analytical and monitoring methods, technologies for mitigation, and management and guidelines.

Most early *Legionella* research focused on its incidence in potable water systems, but there are crucial differences between potable and reclaimed water systems, namely, the relatively higher level of nutrients in reclaimed water compared to drinking water. In 2018, WRF published the project *Development of a Risk Management Strategy for* Legionella *in Reclaimed Water Systems* (Reuse-12-05/4756) to provide answers. Through monitoring at six diverse reclaimed water utilities, the study reinforces that *Legionella* occurrence is influenced by complex ecological interactions, such as protozoan hosts and water quality parameters. It also finds that indicator bacteria (heterotrophic bacteria) do not always correlate with the presence of *Legionella*, suggesting that *Legionella* needs to be directly monitored to accurately evaluate the efficacy of mitigation efforts. The findings underscore the importance of adapting detection methods to enable successful monitoring, maintaining disinfectant residual in distribution systems, and managing nutrients in upstream treatment processes to minimize regrowth in the system. Additionally, utilities should clean and flush systems to manage *Legionella* risk.

Wastewater and Stormwater

Wastewater and stormwater utilities play an essential role in reducing the pathogen load to receiving waters used for recreation. WRF research has advanced water quality monitoring, sampling, and analysis at these facilities to protect public health. Water quality criteria for pathogens help regulatory agencies establish baselines to support designated uses, and drive requirements for wastewater and stormwater management programs. Accurate, quantitative information to support the development of appropriate criteria is critically important.

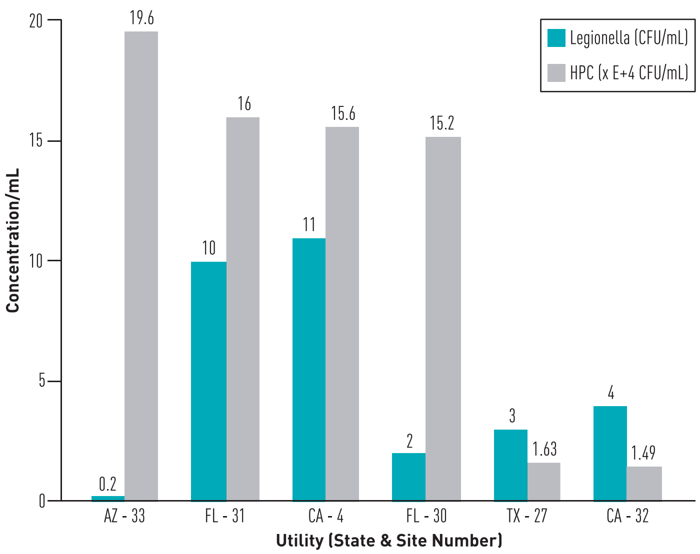
In 2009, WRF initiated the Waterborne Pathogens and Human Health Research Program to evaluate potential human health risk from waterborne microbes. The results inform the implementation requirements for EPA's 2012 Recreational Water Quality Criteria (RWQC). Research topics include rapid methods for monitoring pathogen indicators, risk assessment tools, source tracking microorganisms, and indicators in inland, tropical, and subtropical waters.

EPA is considering RWQC updates that include male-specific coliphages and/or somatic coliphages as fecal indicator organisms in primary contact recreational waters. Use of these coliphages is motivated by research that suggests viral pathogens in recreational waters can cause gastroenteric diseases. Furthermore, wastewater treatment design and regulations have traditionally focused on the use of bacterial indicators, and not explicitly on the reduction of viral indicators or viral pathogens. Although certain wastewater processes provide better viral reduction, it can be limited compared to bacterial reduction. *Evaluating the Fate of Coliphages in WRRFs and Potential Costs to Reduce Coliphages in WRRF Effluents* (U3R15/4880) evaluates the fate and treatability of bacterial indicators, viral indicators, and enteric viruses in wastewater, in relation to potential updates to EPA's RWQC.

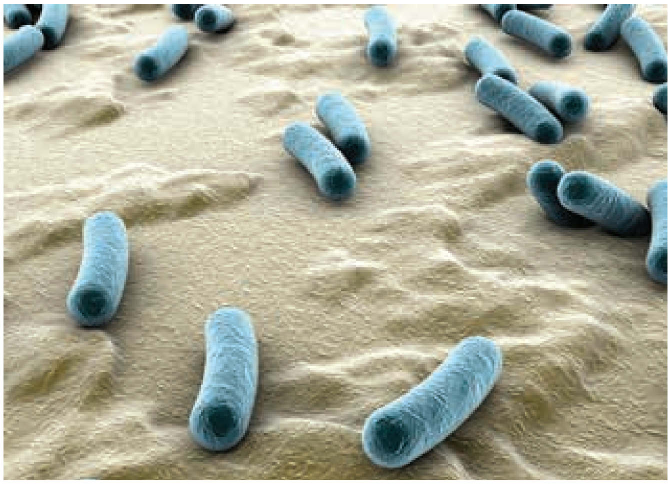
The fate of antibiotic resistance genes (ARGs) and antibiotic resistant bacteria (ARB) in wastewater treatment is not well understood. *Occurrence, Proliferation, and Persistence of Antibiotics and Antibiotic Resistance during Wastewater Treatment* (WERF1C15/4887) quantified public and environmental health risks related to antibiotic resistance. On the one hand, enrichment of bacteria that can degrade specific compounds under certain conditions is the most important aspect of successful wastewater treatment and may efficiently treat ARGs and ARB. However, biological secondary wastewater treatment processes may also proliferate ARGs and ARB owing to horizontal gene transfer (HGT).

The Ebola virus epidemic of 2014–2016 revealed shortcomings in the wastewater sector's response to infectious disease outbreaks. During the outbreak, the World Health Organization and CDC recommended direct disposal of liquid waste from Ebola patients into sanitary sewers or latrines, despite the unknown persistence of Ebola in

Concentration of *Legionella* vs. Indicator Bacteria



Source: WRF Project Reuse-12-05/4756



In a recent study, indicator bacteria did not always correlate with the presence of *Legionella*.

wastewater. This recommendation was met with uncertainty by Ebola Treatment Units in the United States. *Environmental Persistence and Disinfection of Lassa Virus to Protect Worker and Public Safety* (5029) will help prepare the wastewater industry for the next highly infectious virus outbreak.

Early during the COVID-19 pandemic, scientific studies demonstrated that the genetic material of the Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2)—the virus that causes the disease COVID-19—can be detected in the feces of infected individuals. This suggested that the genetic signal of SARS-CoV-2 could be detected in wastewater.

To further this science, WRF initiated *Interlaboratory and Methods Assessment of the SARS-CoV-2 Genetic Signal in Wastewater* (5089). This project assessed the reliability and repeatability of laboratory methods being used to test for the genetic signal of SARS-CoV-2 in untreated wastewater. Wastewater sampling was conducted at two WRRFs, and the samples were analyzed by 32 U.S.-based laboratories. The study evaluated 36 methods (standard operating procedures) for reproducibility, sensitivity, and other impacts.

Understanding the Factors That Affect the Detection and Variability of SARS-CoV-2 in Wastewater (5093) will increase understanding of sample design factors affecting the detection, variability, and dynamic range of SARS-CoV-2 genes in wastewater, which is crucial for the interpretation of results from wastewater surveillance. The results will include a framework with specific recommendations for sample design to enable implementation of wastewater surveillance at large urban sewersheds, medium-sized regional sewersheds with small bore sewers, and small regional systems.

Reuse

Potable reuse requires various treatment processes to remove pathogens and pollutants and protect public health. For direct potable reuse (DPR) systems, where there is no environmental barrier between wastewater treatment and introduction into drinking water systems, the risk posed by even short-duration, reduced-performance events or failures must be understood. In 2018, WRF published *Pathogen Risk Evaluation of Treatment and Monitoring System Performance for Potable Reuse* (Reuse-14-16/4767), which provides a detailed approach to pathogen risk analysis using quantitative microbial risk assessment. This risk analysis includes an evaluation of the failure mechanisms and frequencies of treatment processes for potable reuse and the resulting impact on pathogen risk.

Another 2018 project, *Review of Non-Culture Based Methods for Pathogen Monitoring in Potable Reuse* (Reuse-14-17/4768), identifies three major areas of potable reuse that require significant research on pathogen presence: pathogen concentrations in raw and treated wastewater, challenge tests for pathogen removal process validation, and real-time measurements to ensure pathogen removal. The researchers reviewed pathogen and microorganism detection methods that can aid in addressing these knowledge gaps, with a focus on new developments. This work is being continued in the ongoing project, *Molecular Methods for Measuring Pathogen Viability/ Infectivity* (Reuse-15-07/4774).

WHAT'S NEXT

Because monitoring of pathogens and indicator organisms is critical for potable reuse, specifically in determining whether treatment processes can sufficiently achieve stringent public health criteria, WRF will continue its work in this area. *Indicator Viruses for Advanced Physical Treatment Process Performance Confirmation* (4955) will identify and evaluate potential viral indicator(s) for assessing the performance of physical treatment processes during advanced water treatment for potable reuse.

Recently, there has been a great interest in NGS technologies because it provides the holistic insight of microbial communities and their functional capacities in water and wastewater systems. *The Use of Next Generation Sequencing (NGS) Technologies and Metagenomics Approaches to Evaluate Water and Wastewater Quality Monitoring and Treatment Technologies* (4961) will assess the key barriers to the application of NGS and provide a road-map for advancing practical application of NGS for the water industry.

Another ongoing project, *Fate of Antibiotic Resistance Genes (ARGs) and Antibiotic Resistant Pathogens in Full-Scale Activated Sludge Processes and the Optimization of Activated Sludge Processes for Reduction of ARGs* (5028) will analyze the fate of a suite of ARGs through the secondary treatment processes for six municipal wastewater treatment plants.

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