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This report is based on the deliberations of experts who gathered for two days to discuss a series of questions about the microbiology of the built water distribution infrastructure. The report has been reviewed by all participants, and every effort has been made to ensure that the information is accurate and complete. The contents reflect the views of the participants and are not intended to reflect official positions of the American Academy of Microbiology, the American Society for Microbiology or the colloquium sponsor.

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INTRODUCTION

The construction of sanitary water delivery systems that ended the scourges of typhoid, cholera and other diseases was one of the public health triumphs of the last century. So successful was the investment in clean water distribution systems that in the developed world, we take for granted the safety of our water. Certainly, we do not look to this long-established public sector as the setting for major scientific or technological advances. But despite the vast improvements in water safety in the last century, drinking water is still occasionally contaminated with pathogens and waterborne illnesses continue to occur, albeit at much lower levels than in the past. Ensuring that the water distribution system delivers safe water at a reasonable cost requires ongoing vigilance and investment, and water utility companies are continually looking for ways to make our water even safer.

In the last decade, revolutionary advances in the field of microbiology are providing new ways to think about how water distribution systems are designed and managed. For the first time, the true diversity of microbes that live in our drinking water distribution system is something we can observe and study. The scientific and technological advances necessary to characterize distribution system ecosystems come at a time when the need to make informed decisions about replacing and upgrading existing water distribution systems has never been greater. The American Association of Civil Engineers in 2009 gave the U.S. drinking water infrastructure a grade of D- and estimated that in the next 30 years, 30% of the water distribution system infrastructure in the United States will need to be replaced at an estimated annual cost of 11 billion dollars. These numbers do not include additions to the systems that will be necessary to accommodate increased demand for drinking water over the next 30 years. At the same time new green technologies and water conservation and reuse strategies are becoming more popular and new water treatment strategies are being developed. All of these have the potential to significantly alter the microbial ecology of water distribution systems in ways that we cannot now predict.

Many non-microbiologists probably assume that the goal has always been, and should remain, the elimination of all microbes from our drinking water. But the water we drink has never been sterile; perfectly safe water contains millions of non-pathogenic microbes in every glassful — and this is just as true, by the way, of bottled water as it is of tap water. Like every other human built environment, the entire water distribution system — every reservoir, every well, every pipe, and every faucet — are home to hundreds or thousands of species of bacteria, algae, invertebrates, and viruses, most of which are completely harmless to humans. This has always been the case and in a way it is especially remarkable that our drinking water can contain so many microbes, but nevertheless be virtually pathogen-free. Indeed, it is possible that the non-pathogenic residents actually suppress pathogens.
A new opportunity has emerged whereby the people whose job it is to provide us with safe water, and the scientists who are working to understand the vast microbial world that surrounds us, can work together to their mutual advantage. Water utilities have the potential to apply deeper understanding of the microbial ecology of water distribution systems to develop and apply more effective microbiological monitoring, pathogen detection, and treatment approaches. Overall, the microbial ecology of the water distribution system is exceptionally diverse because there are so many different microhabitats. However, defined portions of the system (for example the biofilm on one stretch of pipe) is likely simpler than many natural habitats. As a result, water distribution systems could provide access for microbiologists to microbial communities that are less complicated than those found in many natural environments but are nevertheless genuine ecosystems that develop and change over time. In many ways, the water delivery infrastructure could serve as a ‘model system’ for the study of microbial communities, and it is likely that discoveries made in that environment will be highly transferable to our understanding of microbial communities in more complex natural settings. New partnerships between academic microbiologists and water utilities could take advantage of this historic opportunity to the benefit of both groups, and to society at large.

To consider these opportunities, the American Academy of Microbiology held a colloquium in April of 2012 focused on identifying specific challenges and gaps in our understanding of the microbial ecosystems of water distribution systems, and developing a research plan to address them. The colloquium brought together microbiologists with experts from many other science and engineering communities, including public health, infectious disease, epidemiology, risk assessment, materials science, civil and environmental engineering, water quality monitoring, and water policy, each of whom specialize in some aspect of the interaction between microbes and the water distribution infrastructure.

The participants addressed three areas of interest:

- What is the composition and activity of the microbial communities living in the water distribution system?

- What are realistic goals for understanding and influencing these communities? What would we like to achieve?

- What do we need to do to advance the study of these communities? What collaborations need to be fostered? What advances in science and technology are needed?

The group considered how an interdisciplinary, integrated approach to understanding the microbial ecology of water distribution infrastructure could leverage each discipline’s expertise and generate an understanding that is greater than the sum of the current parts. This report, the output of the colloquium, outlines the challenges that must be tackled to enhance our understanding of the ecology of water distribution systems, and a plan to address these challenges in the most effective way possible, as part of a greater effort to continue to ensure healthful drinking water within a sustainable infrastructure. Thus this report should be of use not only to microbiologists, but also utilities, government agencies, public health professionals, hospitals, and even industries that rely on safe water for the manufacture of products.
PART 1: UNDERSTANDING THE WATER DISTRIBUTION SYSTEM — WHY IT MATTERS

The outbreak of cholera in Haiti that began after the 2010 earthquake is a vivid reminder that the threats against which our modern water distribution systems protect us are not imaginary. But while major waterborne outbreaks are now uncommon in the United States, the true health burden associated with waterborne disease is not currently known, but based on hospitalization costs alone exceeds one billion dollars annually.

In the United States, the Centers for Disease Control and Prevention (CDC), Environmental Protection Agency (EPA), and Council of State and Territorial Epidemiologists (CSTE) manage the Waterborne Disease Outbreak Surveillance System (WBDOSS), a voluntary reporting service to monitor waterborne disease outbreaks. For example, in the U.S. in 2007-2008, 164 waterborne disease outbreaks from drinking water or treated recreational water, affecting thousands of people, were reported to WBDOSS. The reports gathered by WBDOSS are currently the most comprehensive resource available, but they undoubtedly capture only a fraction of actual waterborne disease cases. For an outbreak to be recorded, affected people must report their illness, it must be properly diagnosed and reported to local health authorities, and epidemiological investigations must convincingly link the illness to a specific water exposure. Less severe or misdiagnosed illnesses, most outbreaks where only a few people are affected, and sporadic cases, do not appear in the statistics at all. Additionally, some outbreaks may be attributed to contaminated food (and therefore classified as foodborne disease outbreaks) when they actually originated with contaminated water used to cook or prepare the food. Finally, reported outbreaks of waterborne disease always focus on people who are obviously sick with an acute disease. Chronic diseases known to be associated with municipal water systems remain undetected.

In addition to the human costs, waterborne illnesses also impose an economic burden. Because WBDOSS does not capture all cases, it is not possible to estimate the total economic costs of waterborne disease. However, there have been a few, well-documented waterborne disease outbreaks that suggest the cost may be large. In 1993, an outbreak of *Cryptosporidium* in Wisconsin sickened 403,000 residents and caused 725,000 productive work days to be lost at an estimated cost of over 50 million dollars. In 2000, contamination of water with *E. coli* O157:H7 in Walkerton, Ontario resulted in estimated economic damages of over 150 million dollars.

Efforts to quantify the impacts of waterborne diseases more accurately are ongoing at the CDC. A system similar to Foodnet — a recently established program that monitors foodborne disease outbreaks and trends, and tracks the source of outbreaks — might be an effective method of establishing a more accurate estimate of the health and economic burden of waterborne diseases. On a relatively small budget, Foodnet has been successful in quanti-
fying the health burden of foodborne disease in the United States. A companion program, Waternet, might be able to accomplish the same for waterborne diseases.

Lost productivity and health care expenses are not the only economic costs associated with the microbial inhabitants of water distribution systems. There is also the cost of microbially mediated degradation of the water infrastructure. Corrosion of pipes and other water system components degrades the system and necessitates repairs. It is estimated that US industries spend 276 billion dollars per year repairing damage from corrosion. Biocorrosion, corrosion influenced by microorganisms, is believed to account for approximately 50% of this cost. However, just as most of the microbes in our drinking water pose no health threat, it is likely that the vast majority of microbes also do no physical harm. Indeed, some of the microbial communities might protect pipes from chemical and physical stresses. Understanding the role of the various microbial communities, and their responses to disturbances of various kinds, could lead to more well-informed choices about which materials are used in different environments, more nuanced approaches to treatments designed to reduce microbial populations, and even preventative or restorative microbially based treatments. Such considerations are particularly important now, as so much of our water distribution system is due for replacement. The water system infrastructure could be an important model system for understanding how microbial communities affect the physical components of the system and better ways to monitor system integrity.

There is another important benefit to investing in the capability to quantify the disease burden and economic cost associated with current distribution system ecosystems. Accurate metrics are a critical ingredient in accountability — without an overall system of evaluation, the impact of new treatments, procedures or materials cannot be judged and lessons learned in one water system will be difficult to apply to others. Having a baseline for comparison of the state of the system before and after any intervention will help guide utilities to the most effective investments and convince policy-makers and taxpayers that the investments are worthwhile. With over 53,000 separate water utilities in the United States, common metrics could have an especially large benefit.
For the purpose of this report, water distribution systems are defined as the networks of pipes managed by water utilities that carry water from treatment plants to service lines at individual properties. Distribution systems are bracketed in the water system by treatment plants upstream and by premise plumbing — pipes that carry water from the property line to individual points of use in buildings — downstream.
PART II: WHAT DO WE KNOW ABOUT THE MICROBES IN OUR WATER DISTRIBUTION SYSTEMS NOW?

Conventionally, water utilities have relied on an indirect approach to monitoring the microbial safety of their distribution systems. Most disease-causing organisms are thought to be transient members of the microbial communities that inhabit water distribution systems; they are only present when the water has been contaminated somehow by animal or human waste. Thus, positive tests for particular microbes, mainly *Escherichia coli* (*E. coli*), alert utilities that waste, and thereby potentially pathogenic microorganisms, may have entered distribution systems.

Positive tests trigger responses that range from raising disinfectant levels all the way to alerting consumers of the need to boil water, and, of course, include investigations to determine the source of contamination. Coliform markers are extremely useful to indicate contamination of the distribution system but they have some important drawbacks. First, the coliform-based tests are not specific for pathogens. *E. coli* is a common organism, indeed, it is a normal resident of the human gut, and only a few strains of *E. coli* are pathogenic. Thus, a positive test may trigger a treatment response that is actually unnecessary from a public health perspective. Secondly, these tests do not detect other potential pathogens and we know that the distribution system can contain microbes that are capable of causing illness other than those associated with fecal contamination. At present, though, we simply do not know the full list of microorganisms, including pathogens, that can and do inhabit our water distribution systems.

Only a tiny fraction of the microbes that inhabit these systems are potentially pathogenic, and historically, little attention has been given to the non-coliform microbial inhabitants of the water distribution systems. However, some microbes that are considered opportunistic pathogens — that is, they generally cause illness only under certain conditions — can be long-term inhabitants of water distribution systems. One characteristic of microbial communities in water distribution systems that is particularly important is their propensity to form biofilms. Many microbes have two distinct lifestyles; sometimes they are *planktonic*, that is, floating freely in the water, and at other times, they attach themselves to surfaces by secreting sticky polymers made up of DNA, proteins and complex carbohydrates. The resultant biofilms can house a great variety of microbes, including opportunistic pathogens, and can shield their inhabitants from environmental stresses including disinfectants. Study of the ecology of distribution system biofilms is at an early stage, but their importance is already clear.
**BOX 1: OPPORTUNISTIC PATHOGENS IN THE WATER DISTRIBUTION SYSTEM**

Opportunistic pathogens are microbes that normally live innocuously in the environment, but that under some circumstances can infect susceptible humans. At special risk are infants and individuals whose immune systems are compromised in various ways. Diseases caused by these organisms generally are not transmitted directly between humans and so must be acquired from the environment. Some opportunistic pathogens are found particularly in water distribution systems.

- A collection of about 20 closely related bacteria known as “non-tuberculous mycobacteria” (NTM), such as *Mycobacterium avium*, *M. intracellulare*, *M. kansasii* and *M. fortuitum*, are the most important opportunistic pathogens in terms of numbers of clinical cases (estimated at least 20,000 cases in the US in 2010). These organisms can cause serious pulmonary and lymphatic disease, skin ulcerations and other syndromes.

- *Legionella pneumophila* is the causative agent of Legionnaires’ disease (pneumonia) and the milder Pontiac fever, and results in thousands of hospitalizations annually (8,000-18,000 est. in 2008 by CDC). *L. pneumophila* is the only opportunistic bacterial pathogen that is reportable to the CDC.

- *Pseudomonas aeruginosa* commonly occurs in low nutrient environments such as water supplies and can infect eyes, ears, skin and in some individuals can cause pulmonary disease. Infections probably are much more common than reported (a few hundred cases per year, mainly pneumonia). It is one of the most common gram negative bacteria isolated from hospital infections. The role of drinking water in the transmission of *Pseudomonas aeruginosa* has not always been clear. However, Trautmann et al. (2008) found a significant difference in *P. aeruginosa* nosocomial infections (monthly episodes reduced by 85%) in a hospital pre and post installation of point of use treatment devices thus implicating the water as the source of the infections.

- Protozoan opportunistic pathogens such as *Acanthamoeba* (keratitis, encephalitis) and *Naegleria* (encephalitis) are endemic to distribution systems, probably particularly in reservoirs, and not to be confused with *Cryptosporidium* (intestinal disease) whose resting stage (oocysts) may be introduced via fecal contamination. The main manifestation is *Acanthamoeba* keratitis, with an estimated 700 cases between 2005 and 2007, mainly from contact lenses washed or stored in tap water, with only a few cases of *Naegleria* encephalitis per year via nasal washes.
As habitats for microbes, biofilms can contain many times more bacterial cells than the water flowing through the pipes, and can act as reservoirs within which both bacterial and viral pathogens can persist. Bacteria can also find safe harbor within amoebae and other free-living protozoa (e.g. ciliates) that can live in the distribution system. In aquatic ecosystems, amoebae are predators that eat bacteria. Some bacteria, however, including pathogens like *Legionella pneumophila* and *Mycobacterium avium*, can resist digestion and actually grow and multiply within various biofilm amoebae. Even though most of the microbial biofilms in distribution systems probably do not pose health threats, they can still cause physical damage such as corroding pipes and blocking intake valves. In addition to forming biofilms, non-pathogenic microorganisms can also break down chemicals used to minimize microbial growth or inhibit biofilm formation, and still others may release nutrients into the distribution system that support downstream growth of opportunistic pathogens. The extent to which these processes occur in water distribution systems is largely unknown, because the makeup of the ecosystem of these systems has not been characterized.

Uncertainty as to the makeup of the microbial communities living within distribution systems complicates treatment efforts to control undesirable microorganisms. Utilities are forced to rely on treatment strategies designed and evaluated based on their success in eliminating a few known indicators of pathogens, or breaking down biofilms in general, without regard to what microbes they might contain. The same treatment strategy that will eliminate one pathogen could lead to conditions that favor the proliferation of another. For instance, use of chlorination to kill fecal pathogens may lead to selection of opportunistic pathogens such as *Mycobacterium avium* due to their relative chlorine resistance. Any change in water treatment strategies likely will influence the microorganisms that live in water distribution systems. Changes may encourage the development of an ecosystem favoring a sustainable infrastructure or encourage the proliferation of microorganisms capable of degrading the infrastructure. The recent change from chlorine to chloramine offers an excellent example of unanticipated consequences (Box 2).

**BOX 2: UNANTICIPATED EFFECTS OF CHANGES IN TREATMENTS**

Classically, chlorine (also known as bleach) has been added to water as it leaves treatment plants to inhibit the growth of many microorganisms. Some utilities have recently begun switching disinfection treatments from chlorine to chloramine, as chloramine residuals remain in the water longer and produce fewer harmful regulated disinfection by-products. But chloramine can also encourage the growth of certain nitrifying bacteria within biofilms that remove the disinfectant residual. Many systems have also found it necessary to add phosphate nutrients to water along with the ammonia to form chloramine, to control the corrosivity of the water to metals such as lead bearing plumbing. Is this a problem? Which treatment is better? Which will result in more healthy water and a more sustainable infrastructure? Without an understanding of the communities of microbes in the system it is difficult to predict the effects of any change in water treatment strategy. Without a detailed inventory of the microorganisms growing within distribution systems, water utilities are forced, in a sense, to “fly blind” when making treatment decisions.
At the same time that utilities are struggling with these uncertainties, the tools and expertise needed to characterize complex microbial communities, like those present in water distribution systems, are rapidly evolving. It is now economically feasible to sequence the pooled DNA from microbial communities and determine which species and functional genes are present. Pooling and sequencing messenger RNA provides an indication of what metabolic processes are being carried out. Through these techniques, known respectively as metagenomics and metatranscriptomics, microbial ecologists are able to probe microbial ecosystems in many environments. For example, scientists study the microbial communities in marine and soil environments, where microbes can have significant effects on biogeochemical cycles. The Human Microbiome Project, a five year effort undertaken by the NIH, has begun to characterize the microbial assemblages living within humans and unravel how microbes affect the immune system, nutrient absorption, various disease processes and other bodily processes. Studies of the microbial ecosystems in extreme environments are uncovering novel processes and enzymes that can be put to use in industrial settings. Study of the microbial ecology of water distribution systems has up to now received little attention from microbial ecologists, but there is reason to think that such study could be extremely valuable, both for its potential public health impact and because water distribution systems could prove to be excellent model environments.
PART III: WHAT AFFECTS THE MICROBIAL COMPOSITION OF THE WATER DISTRIBUTION SYSTEM?

There are approximately 53,000 distribution systems in the US, and no two are identical. The number and diversity of distribution systems may seem daunting, but while each system will be different, it is likely that their differences and similarities will be instructive and that the ecosystems of these distribution systems will share common themes.

As a result, water distribution systems represent an attractive model system for the study of microbial ecosystems. The lessons learned from studying water distribution systems not only may help enhance understanding of the ecosystems in the water infrastructure but microbial ecosystems in other environments as well.

In contrast to more complex environments, scientists can identify, monitor, and sometimes modify many of the factors that may influence the microbial ecosystems in distribution systems. These factors can be broken into three categories:

**Infrastructure characteristics:**

**Pipe material:**
Pipe material varies between distribution systems and within individual systems. New pipes are often constructed from PVC or lined ductile iron, but older systems were constructed with different materials including pipes with and without linings (asphaltic, cement, epoxy), and unlined cast iron. There are even some wooden pipes still in use in systems in the oldest areas of the United States. The composition of the pipes controls what chemicals may be released into the system and the types of bacteria that colonize the surface. Some pipe materials release bio-available iron, hydrogen, and phosphate compounds that support the growth, and proliferation of different types of bacteria and related microbial communities. Other materials such as copper can have bacteriostatic properties.

**Pipe age:**
Biofilms coating the interior surfaces of water distribution pipes develop slowly and probably do not reach maturity until years after new pipes are inserted into distribution systems. Composition of biofilms can change rapidly before they reach maturity, and it is not even clear whether mature biofilms are stable. There is little known about how existing biofilms react to the insertion of new pipe in distribution systems. Old iron pipes can become heavily tuberculated, with depths of scale or rust exceeding 10 centimeters, leading to even greater surface area for biofilm growth and protection of organisms from disinfectants. Attempts to extract the oldest pipes from every system are not always needed (it is not pipe age that is always
the main determinant for failure) nor always achievable (as the pipe replacement rate may be
every 100 years for larger systems). Chemicals used to coat old pipes to control corrosion
may have different effects on biofilms; for example, phosphates can stimulate bacterial biofilm
growth, while silicates can inhibit it.

**Physical integrity of the system:**
Over 7 billion gallons of water are leaked from distribution systems in the US each day.
Breaks or leaks in pipes can lead to low water pressure events and when repaired may draw
soil microbes into the distribution system. Some of these invaders may be able to colonize
the distribution system, while others will only be transient visitors and flow out of the system.
Sediment that enters distribution system pipes or forms from pipe corrosion may settle within
pipes and serve as a nutrient source as well as a carrier for microbes. New pipes and appur-
tenances are usually disinfected before use but are not sterile.

**Number and types of storage facilities in the system:**
Different types of storage facilities such as tanks in the distribution system can affect some of
the hydraulic properties of the system such as pressure and water age. Water storage tanks
represent a potential habitat for microbes and their addition typically increases water age,
which can lead to decreased disinfectant concentrations and, increased sediment accumula-
tion and therefore influence microbial growth.

**Water chemistry:**

**Source water:**
The micro and macro-nutrients in the water influence the microbial ecology in ways that
we do not yet understand. Microbes feed on a myriad of different carbon compounds.
Measurements such as total organic carbon (TOC), assimilable organic carbon (AOC), and
biodegradable organic carbon (BDOC) vary between distribution systems due to differences
in the source water feeding into a specific system. The ability to measure which specific
carbon compounds are present is less well-developed in the water industry, but no doubt this
variable has a considerable influence on which microbes will be able to thrive.

**Chemical Stability:**
Water changes as soon as it is treated and enters the distribution system in ways that can
favor microbiological growth. For example, the disinfectant residual decreases at rates
depending on water temperature and disinfectant demand. Ammonia is released from
the chloramine residual as it decays. Haloacetic acids and other disinfectant by-products
decrease in areas with longer detention times as influenced by microbiological activity.

**Treatment:**
Chemical additives from the source water, treatment plant or from the distribution system can
play a major role in distribution system ecosystems. One example is the use of phosphates
for corrosion control, which can stimulate bacterial growth. Another example is the free
ammonia residual from formation of chloramine and its degradation by bacteria.

**Temperature:**
Microbial processes are heavily influenced by temperature changes. Different microorganisms
have different optimal growth temperatures and different responses to changing tempera-
tures. Episodic and seasonal temperature changes may thus influence the balance and
diversity of microorganisms in an ecosystem.
Hydraulic and physical properties of the distribution system:

Water pressure, turbulence, flow rates, and flush regimes:
Changes in water pressure, turbulence, flow rates, and flushing regimes can all exert shear forces on the biofilms in the distribution system. Strong shear forces enhance mass transport of disinfectants, and can cause areas of biofilm to slough off, resulting in an alteration of the microbes found in the bulk water component of the water distribution system. System design will have a significant impact on biofilm deposition.

Water age:
Different architectures and sizes of distribution systems can affect how long water is present in the system. Heavily looped systems can have some areas where the water has been in the distribution system for considerably longer periods than others, and systems with dead ends also have long water ages. Larger systems will have “older” water as well, especially at the ends of lines. Chlorine residuals in water decay over time, thus the chemistry of “older” water may support different microorganisms than water that has just entered the distribution system.

We do not yet know what constitutes the range of variation of microbial ecosystems in distribution systems. Early indications, though, are that the microbial ecosystems of distribution systems are diverse. Many different types of microorganisms live in the water or are associated with the biofilms that coat the pipe walls. It is likely that each different distribution system and pipe material will have its own unique microbial ecosystem. However, there are likely to be common functions and preliminary studies have revealed a core set of shared genera. A systematic survey of the microbial ecosystems of water distribution systems could be extremely useful. Correlating the microbial composition of distribution systems to all of the physical characteristics described above would begin to tease out which physical variables have the strongest impact on microbial ecosystems, and how changes in those characteristics affect the magnitude and composition of the microbial populations.

It is important to consider that what grows in the distribution system can seed downstream building plumbing systems, both cold and hot water and HVAC systems. Opportunistic pathogens can then grow to health concern levels if these downstream systems, such as in hospitals and nursing homes, are operated improperly.

Finally, another characteristic shared by all distribution systems is that their microbial ecosystems are dynamic. Microbial ecosystems in distribution systems are likely to change over time, both in terms of species composition and distribution between biofilm-associated and planktonic organisms. Seasonal temperature changes can shift the balance of the ecosystems in biofilms and bulk water. Planktonic microorganisms can become trapped in the biofilm, while disruptions in flow rate and pressure of the pipes can dislodge portions of the biofilm, transiently changing the composition of the microorganisms in the flowing water. New microorganisms from outside the system can be introduced through breaks or leaks in the pipes and via poorly sealed water storage facilities. Again, comparisons across systems are likely to reveal patterns in how these communities adapt and change over time.
PART IV: WHERE DO WE GO FROM HERE?

Reaching a truly integrated understanding of the microbial ecology of water distribution systems and their impact on human health and the physical infrastructure will be no small task. However, what became clear at the colloquium is that there are at least three stakeholder groups who have a strong interest in understanding the microbial ecology of distribution systems and that if their efforts can be coordinated, progress could be rapid and mutually beneficial.

- Public health authorities need to know how much disease is being caused by pathogens that reach people through the water distribution system, and how best to prevent it.

- Water utilities are obliged to deliver safe water, but are operating in a climate of constrained resources and facing urgent decisions about the best way to replace or repair aging infrastructure.

- The field of microbial ecology is at a stage where it is possible and necessary to move beyond cataloguing which broad groups are present in a given environment, to developing model systems upon which experimentation and modeling of community establishment, succession, stability and function can be carried out. The results of those studies could feed back into the disease statistics gathered by public health authorities, and into water utilities treatment and infrastructure engineering decisions.

First, we need to better characterize a healthy distribution system’s microbiota. Then we need to be able to measure and track changes, both localized and systemic. This requires tools for monitoring. Perhaps water utilities can screen for changes and track indicators, thus collecting and sharing considerable amounts of needed data. We also need to understand how we can design and operate distribution systems to favor healthy or even protective microbiota. Indeed is there a ‘probiotic’ biofilm microbiota that limits unwanted guests? In all of this, the regulatory agencies and public stakeholders must not impose disincentives to collecting the needed data.

Together, these three groups have a unique opportunity to work together to advance understanding on several fronts. Indeed, together they could accomplish goals none could reach alone. The participants at the colloquium developed a vision for advancing our understanding the microbiology of the water distribution system through a collaborative and modular research program. Particularly appealing about the plan is that it can be implemented on several scales, ranging from a short-term partnership between a local water utility and an academic microbiology or environmental engineering department, to a national network integrating several utilities, universities and public health agencies. Even if implemented in a piece-meal fashion, such collaborations would bring benefits of greater understanding and more functional links between the public and academic sectors. But with a modest infusion
of forethought and planning, collaborations of various scales and types could reinforce each other and multiply the value of all results. The recommendations thus call for a group effort to develop a research strategy. Such an effort could begin with a planning meeting, to be followed by opportunities for stakeholder input. The goal of the process would be to:

- Engage stakeholders;
- Jointly determine what data will be needed to establish a comparable baseline across systems, and identify major variables affecting microbial composition from system to system;
- Develop a standardized minimal sampling protocol — determine whether adequate tools already exist, or define specifications for the necessary kits or samplers;
- Develop data standards to ensure comparability across systems and develop a plan for data sharing, including planning for the capacity to incorporate the data generated in current and future monitoring of waterborne disease outbreaks;
- Develop connections to funding agencies with responsibilities in public health and infrastructure development.

Each of the three major stakeholders — utilities, microbial ecologists, and public health authorities — would be responsible for gathering and sharing data: utilities about the physical system, microbiologists about the biological components, and public health authorities about disease outcomes. With a common database and standard sampling protocols, the program would be modular and accessible to individual researchers and utilities.
Step 1: Engage stakeholders:

**Water utilities:**
Advancing our understanding of the microbial ecology of the distribution system hinges upon the involvement of water utilities. Utilities control access to the distribution systems and will need to authorize sampling programs. It is usually difficult to access biofilms in pipes unless the pipes are being removed for maintenance or replacement. This may mean that utilities will need to implement ways to provide access to biofilms. As mentioned before, water utilities have collected vast amounts of metadata on distribution systems. Utilities’ chemical and physical distribution system data can be invaluable to researchers if it can be properly paired with ecological data. By pairing physical and chemical metadata with ecological data, researchers can parse out how changes in conditions in the distribution system affect the ecology. Access to utility metadata will be important to predicting how ecosystems will change when challenged by new environmental pressures. Water utilities have knowledge and expertise that researchers will need. Utility experts understand distribution systems and will be invaluable in helping researchers develop model or sentinel systems in their laboratories.

Two issues must be addressed during the development of partnerships with water utilities. First, water utilities will need to be convinced of the benefits of entering into collaboration with academic researchers on sensitive health issues. Initial research on distribution system ecosystems will be fundamental in nature and it may take some time before results of the collaboration can affect the bottom line for the water utility. It will be necessary to highlight the potential benefits of collaboration to utilities ahead of time. Secondly, utilities may fear that any investigation into distribution system ecosystems will open a “can of worms”. Although water utilities and researchers understand that distribution systems are not sterile, the public, regulators, and press may react fearfully. Characterizing the ecosystem associated with any specific distribution system is likely to reveal the presence of potentially undesirable microorganisms. Public outreach, communication and community involvement must be built in to the research program from the outset to minimize the risk of misunderstanding.

**Microbial ecologists:**
Microbial ecologists have the expertise to characterize complex microbial ecosystems like those present in distribution systems. But currently there are very few microbial ecologists working on these systems despite some features that make them an attractive model. Engaging this community will depend on informing researchers that distribution systems represent fairly controlled environments and many of the non-microbial aspects of the systems have already been well studied by water utilities. Utilities have large amounts of data describing water chemistry and the physical properties of the system. Perturbations to the system have been tracked for long periods of time and are well documented.

There are additional tangible benefits to working on these ecosystems. Understanding distribution system ecosystems has the potential to make an immediate impact on public health. Parsing out how interactions in the ecosystem affect biocorrosion and biofouling offer enormous potential economic benefits. Water utilities will need help developing testing, monitoring, and detection technologies, and may be willing to cooperate with research efforts if they can be convinced of the economic benefits of such research. Because investigation into these systems is so new, there is still much to be learned, and there is likely a significant amount of “low-hanging fruit” for microbial ecologists. Research done into these systems need not exist in a vacuum; insights into the interactions between members of the microbial communities in distribution systems will likely be applicable to more complex environmental systems.
Public health authorities:
Public health authorities are a vital party in any effort to determine what constitutes a healthy water distribution system. While it may be some time before clear correlations emerge between microbial community composition and health impacts, the ultimate goal of being able to monitor and prevent pathogen spread and other associated potential concerns (such as antibiotic-resistance genes) through the water system is sufficiently important that it is critical that the public health community be engaged from the outset. Today, public health authorities are concerned about what comes from the distribution system and grows in plumbing systems — such as Legionella and Mycobacterium. These opportunistic pathogens are known to have a significant impact on public health, and like antibiotic-resistance, there will always be new concerns in the future.
Step 2: Agree on what data to collect

Water utilities are proficient at monitoring the physical and chemical properties of water in distribution systems, but have focused less on the biological properties of the water. A suite of physical characteristics like flow rate, turbidity, and water pressure are routinely tracked. Chemical characteristics like pH and disinfectant residuals also are monitored. With this information, utilities are able to construct hydraulic models of distribution systems that can help predict qualities like the water age in different parts of the system. Other approaches have been designed to detect potential intrusion events. Biological measurements include testing water samples for the presence of total coliforms, E. coli and heterotrophic plate count (HPC) bacteria to estimate the presence or number of culturable bacteria in the water. These types of characterizations have proven useful for detecting intrusion events and tracking changes in distribution system environments, but do not address the other microorganisms present in the water and may poorly address those associated with biofilms.

The participants stressed the need for a robust set of guidelines for sampling of bulk water and biofilms that is longitudinal in nature. Samples should be collected at regular intervals from defined points in the distribution systems to help build an understanding of how microbial ecosystems change over time and place. Samples should also be collected following extreme events like fires, heavy rainfall, disinfection changes, chlorine burns, or line flushes. These extreme events may be transient but may also represent key times of change in the ecosystem. Supplemental sampling during and immediately after extreme events could give insights into how resilient the ecosystems are to change, or reveal periods of transient risk in water healthfulness. Smaller perturbations to distribution systems like those detected by changes in pressure, turbidity, or particle counts could also prompt sampling. Whenever possible, chemical sampling of the water should occur simultaneously with biological sampling, so that physical, chemical, and biological samples can all be correlated with one another.

One benefit from the development of standards for sampling distribution systems is the potential to “crowd-source” sampling efforts. With a uniform, simple set of standard techniques university laboratories could partner with local water utilities to collect large numbers of samples from distribution systems. Some participants even noted the potential for the crowd sourcing to expand beyond universities. Community groups, water associations, and even schools could be leveraged as a kind of citizen science project to help characterize their community's distribution system. The participants drew parallels to the “Adopt a Highway” system where community groups take responsibility for the cleanliness of a section of highway. Crowd sourcing sample collection could represent a rapid, inexpensive way of collecting samples for microbial ecologists to analyze.

Coming to an agreement about what biological measurements should be sought across all systems will be an important part of early discussions. Full characterization of the microbial community in even one system, much less 53,000, is clearly out of reach. But characterizing distribution system ecosystems will require that biological sampling be expanded beyond monitoring for microorganisms that indicate potential contamination. Both bulk water and biofilm-associated microorganisms will need to be sampled. Utilities already collect bulk water samples as part of their sampling regime; this sampling could be expanded to allow for characterization of other microorganisms present in bulk water. Sampling biofilm-associated microorganisms could be accomplished by the insertion of standardized sample gathering devices (known as coupons) into systems, or by using a sidestream with coupons, similar to techniques used to sample wastewater biofilms. Special procedures may have to be developed for older tuberculated pipes where extensive biofilm buildup might complicate...
Ideally, it will be important to move beyond determination of the specific microbes that occupy distribution networks — although that still represents a major gap in knowledge — to beginning to characterize how all the different inhabitants interact. Thus decisions on what to sample should develop a consensus on two areas:

- **Census of microbial members:** Water utilities have more or less detailed data on the abundance of culturable coliforms in distribution systems, but almost all of the other inhabitants and unculturable states of the ecosystem members are uncharacterized. In addition to bacteria, the ecosystems host fungi, protozoa, viruses, and archaea, and even less is known about these groups. No two distribution systems are identical — is the same true of the microbial ecosystems in them? Are there commonalities between ecosystems? Or can different characteristics of the distribution system sort ecosystems into different groups?

As public health authorities proceed with plans to expand monitoring efforts for waterborne diseases, it would be helpful to include them in discussions of sampling programs being put in place by microbial ecologists and water utilities. It will not be possible to predict exactly which measurements will eventually correlate with disease outcomes, but if there are factors that are suspected to be important in disease spread or pathogen survival, it would be better to begin collecting early rather than waiting until more detailed epidemiological information becomes available.

- **Interactions between organisms:** The behavior of an ecosystem can be thought of as the sum of its members and their interactions with one another. These interactions between members can have tremendous effects on the behavior of the ecosystem as a whole. For example, in marine environments, bacterial viruses are responsible for the turnover of about 50% of marine bacteria every day. Such viruses also are present in distribution systems, but their interactions with the other members are uncharacterized. There is a growing list of pathogenic bacteria, viruses, and even fungi that can live and grow inside free-living amoeba species and there is evidence that these associations protect the pathogens from the effects of disinfectants like chlorine. For example, Hartmanella, one type of free-living amoeba, is a common inhabitant of drinking water, raising the possibility that interactions between these amoeba and pathogens may play a significant role in the safety of the water. Interactions between members of the different species may also play an important role in the sustainability of the water infrastructure. Microbially induced corrosion, or biocorrosion, of pipes can be caused by single species of microorganisms, but the most aggressive rates of corrosion have been associated with the actions of diverse biofilms.

As discussed above, efforts are underway at the Centers for Disease Control and Prevention to expand its monitoring and surveillance capacity for foodborne illnesses, possibly through the expansion of a program similar to the successful Foodnet program. Correlation of foodborne illness data with the data collected by utilities and microbiologists could provide the basis for understanding the characteristics of “healthy” microbial ecosystems within the water infrastructure. In addition to improvements in national data gathering, it is possible that an intensive disease monitoring exercise in a limited area where a water utility and microbiologist are already collecting physical and microbiological data series could serve as an instructive case study and help to identify critical variables.
Step 3: Develop minimal standard sampling preparation and analysis protocols, including tools and/or kits if necessary

New metagenomic and molecular technologies are available to help characterize samples. Analysis of DNA and RNA from bulk water and biofilm samples could be used to characterize abundant microorganisms in distribution systems. To detect less abundant species it will likely be necessary to use qPCR (quantitative polymerase chain reaction) or other more sensitive techniques. Traditional nucleic acid analysis will not inform scientists of the viability of these organisms but renewed efforts in plate count and culturing techniques and other improvements to molecular methods might yield new approaches to determining organism viability. New metatranscriptomic techniques may be able to reveal what the inhabitants of the distribution systems are doing metabolically.

Efforts to standardize techniques in the field should not end with sample collection. It is important to set specific standards for techniques used to process and analyze the samples. Unified standards for these techniques will allow groups to compare results across studies and leverage discoveries. Specific sample processing techniques can introduce bias into data, so the use of differing techniques can make resulting data difficult to compare. For example, even mundane tasks like harvesting DNA from water samples can be biased by using different isolation techniques. Having a defined set of techniques for sample processing and analysis will help remove biases that could hinder comparisons between groups. Colloquium participants suggested convening a meeting of biofilm researchers and microbial ecologists with expertise in molecular tools and culture based methods of characterizing microbial ecosystems. The attendees at the meeting would share techniques and technologies that are used in the field so that the meeting organizers could develop a portfolio of tools that would be appropriate for the characterization of distribution system ecosystems.

With this information in hand, it would be possible to agree on a standard set of techniques for use by researchers working on characterizing the ecosystems in distribution systems. These techniques would cover sample collection, sample processing and analysis, and data collection and annotation. The panel of experts would invite engineers with experience in distribution system design to ensure that the sampling methods are compatible with the water utilities distribution systems. Ultimately, the sampling strategy should allow studying the ecosystem in the short term, focused on real time analysis of ecosystems, as well as integrating longer term sampling to identify changes in ecosystems after perturbations.
Step 4: Develop data standards and data sharing procedures

Standardization of sampling, processing, and analysis techniques can generate data that can be readily compared between groups, but to be useful in this regard the data should be collected and annotated in a uniform fashion. Metadata should be annotated in a standard fashion to enable transfer and analysis by other groups. Furthermore, standardized data is not useful if researchers cannot access it. The participants stressed the need to develop a central database for the storage of data pertaining to distribution system ecosystems similar to the database available from the human microbiome studies. Furthermore, it is important for participants to discuss which data will be fully shared and how quickly those data will be made available. The human genome and human microbiome project may offer useful models for data release practices.

Eventually, the goal of the process would be an improved ability to understand, monitor and maintain what characterizes a healthy microbial community in the distribution system. The pervasiveness of microbial infiltration into almost all environments dictates that distribution systems will never be sterile — there will always be an ecosystem inside water distribution systems. But how do we know if the ecosystem present in the pipes is the one we want? In other words, what are we aiming for in terms of the composition of the microbial assemblages living in distribution systems? A microbiologically ideal distribution system would deliver safe water, with good aesthetics, in a sustainable infrastructure. Correlating ecosystem data with health reports and surveys of infrastructure over time will allow us to address this question. As a starting point, participants in the colloquium identified several qualities of an ideal distribution ecosystem would exhibit:

Pathogen levels within safe limits:
A healthy ecosystem should not harbor pathogens at concentrations that represent an unacceptable risk of illness. A current suggested guideline is below $10^{-4}$ annual probability of infection due to tap water. It is likely that *Legionella* exceeds this goal. Quantitative microbial risk assessment will be needed for distribution systems. While dose-response models are available (QMRAwiki) there is a significant need to address exposure routes and understand the concentrations of these pathogens particularly for sensitive populations. Many fecal pathogens that can be transmitted in water are already known, but other, particularly opportunistic and endemic, pathogens may still await identification. Due to clearer health effects, pathogens that cause acute illness are better characterized than pathogens that cause chronic illness. Likewise, a microorganism that poses no threat to one person may be a potential pathogen for another. Elderly, very young, and certain immune compromised individuals are often more susceptible to pathogens than the rest of the population, and these sensitive members are increasing in number. This greatly complicates risk analysis and may necessitate educational outreach to vulnerable groups if outright elimination of pathogens turns out to be an unobtainable goal. Another complicating factor in determining acceptable pathogen levels in the distribution system is that while the water provider’s responsibility and control end when the water enters a building, the capacity of pathogens to grow and persist does not. It is possible that home and commercial building owners will have to work together with local and state officials and water utilities to ensure that the water that finally comes out of the taps is safe.
No microorganisms that introduce aesthetic concerns:
While not harmful, aesthetic concerns, or water nuisances, are undesirable characteristics that can be introduced into water by microorganisms in the distribution system. These can affect the color, odor, or taste of the water. For example, chlorophenols released by certain materials can be biotransformed to odorous chloroanisoles by fungi growing in the system. Thus, a microbial ecosystem in the water distribution system that favors these organisms would not be considered ideal.

Little corrosion and other infrastructure concerns:
Microbial biofilms have been implicated in some of the most aggressive, biologically induced corrosion events. In an ideal ecosystem microbially induced corrosion would be minimized, extending the life span of the water infrastructure, reducing aesthetic issues and enhancing maintenance of disinfectant residuals.

Stable:
Ideally, the microbial ecosystem would be robust so that periodic changes in the environment would not generate long-term undesirable changes in the microbial community. The ecosystem should be resilient, so that changes that affect microbial ecosystems would be transient. An ecosystem that can quickly recover from transient changes — like those that could occur from sporadic events (pressure changes, temperature spikes or intrusions) — would require less oversight and management. Stable ecosystems can competitively exclude pathogens from taking up residence. These types of systems require less disinfectant to kill transient intruders, limiting additional aesthetic concerns from the addition of chemicals, and decreasing the costs associated with water treatment.

Compromised ecosystems would have essentially the opposite features of those associated with healthy systems. Such ecosystems would harbor microorganisms that pose immediate health threats, introduce aesthetic concerns to drinking water, or contribute to microbially induced corrosion of the water infrastructure.

Water utilities already use indicators to flag ecosystems that may be compromised. The presence of *E. coli*, for instance, indicates that human or animal waste may have entered the system. Records of pressure drops in the distribution system can alert utilities to the possibility that an intrusion event has occurred. Increases in turbidity or decreased chlorine residuals in the system can signal that conditions in the system have become more favorable to the growth of microorganisms.

The ecosystem of any distribution system can be thought of as being graded on a spectrum of which one end is a healthy ecosystem, and the other is a compromised ecosystem. Understanding the characteristics of sick systems may help detect potential issues in healthy systems before they progress to “sick” states. If researchers can characterize what sick and healthy ecosystems “look” like, and what conditions are present in distribution ecosystems in real time, then they may be able to detect gradual changes leading to unfavorable ecosystems.
A shared data platform has another important benefit; all three sets of stakeholders will be able to use the data to design experiments, test hypotheses, evaluate new treatments, and build models. In turn, the results of those exercises will feed back into the shared data, making it increasingly more powerful. This will allow researchers to explore many new questions. For example, if the water temperature transiently changes in a distribution system, are the changes in the microbial ecosystem transient as well? Are intrusion events or the microorganisms that colonize upstream sand and carbon water treatment filters able to alter the downstream microbial ecosystem permanently? In other words, can water filter or soil microbes invade a niche in the distribution system and become permanent residents? Or do they simply flow through the system? Can some colonize and others not? If so which ones? Are pathogens harbored in certain kinds of biofilms and do particular events lead to their release? Understanding how baseline physical parameters and changes to them affect the composition of the ecosystem is necessary to understand and predict how changes in treatment regimes, pipe replacements, or new water use technologies could affect the microorganisms in distribution systems.

Answering complex and multi-faceted questions like these will require an iterative process that includes data gathering, experimentation, and modeling and analysis (see figure 2), but the impact could be substantial. The results of these efforts could become the basis for evidence-based regulations and treatment practices, and lead to new understanding of microbial community dynamics, resilience and vulnerability that could apply to microbial ecosystems in many other environments.
Public health agencies, water utilities, and microbial ecologists can pool their data and use the shared data to build models and design experiments. There will be benefits for each group.
CONCLUSION:

Characterizing the microbial ecology of water distribution systems and how it affects drinking water and the water infrastructure is not a small task. It will require the cooperative efforts of a diverse group of stakeholders. Many of the tools and technologies needed to undertake the challenge are available now, as is the expertise.

The present challenge is to harness and motivate the different stakeholders to work with one another. Microbial ecologists must be convinced that distribution systems offer a tractable model system to understand complex microbial interactions, likewise water utilities must be convinced of the practical impacts of studying these systems. Harnessing these groups’ potential will require a culture change in the way we think about water and the water infrastructure that provides it. The distribution systems are host to a diverse range of bacteria, archaea, protozoa, and fungi, all of which have viruses, and all potentially interact with one another. The members of these communities change, and alterations in their environments can radically change the face of these communities. Decisions made when replacing, upgrading, or treating the distribution system can greatly alter the microbial ecosystem, for better or for worse. Understanding how the microbial ecology of distribution systems affects drinking water may allow utilities to produce healthier water, or provide the same quality water more efficiently and may give utilities the opportunity to manipulate the microorganisms in distribution systems to decrease microbially mediated degradation. The extent of the benefits of studying the microbial ecology of water distribution systems may not be realized until the task is well underway, but looking into past efforts should prompt optimism. Investments in clean water technologies in the past have yielded as much as a 23-fold return on investment. While the benefits may not yet be clear, one thing is obvious — water distribution systems host complex, diverse ecosystems and understanding them may be critical to making effective decisions for water systems.