

# Microbiology of Drinking Water Systems: Contemporary Perspectives on Pathogen Detection, Antimicrobial Resistance, and Public Health Challenges

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## Abstract

The microbiological safety of drinking water remains a critical public health priority worldwide. Coliforms in drinking water pose public health challenges in developing countries. This comprehensive review examines the complex microbial ecology of drinking water systems, emerging pathogenic threats, detection methodologies, and current challenges in water quality management. Recent advances in molecular techniques have revolutionized our understanding of water microbiology, revealing diverse microbial communities and novel pathogens that conventional methods fail to detect. This article synthesizes current knowledge on bacterial, viral, and parasitic contaminants, antimicrobial resistance in aquatic environments, and the impact of treatment processes on microbial populations.

**Keywords:** Drinking Water; Coliforms; Multiple Tube Test

## Introduction

Safe drinking water is fundamental to human health, yet waterborne diseases continue to cause significant morbidity and mortality globally. The World Health Organization estimates that contaminated drinking water causes over 500,000 diarrheal deaths annually [1]. The microbiology of drinking water encompasses a complex interplay between indigenous microbiota, pathogenic organisms, environmental conditions, and treatment interventions [2]. Understanding this microbial ecosystem is essential for developing effective water safety strategies and protecting public health.

Traditional microbiological assessment of drinking water has relied on indicator organisms, particularly total coliforms and fecal coliforms (*Escherichia coli*) [3]. However, these indicators have limitations in predicting the presence of certain pathogens, especially viruses and protozoa. Modern molecular approaches, including next-generation sequencing and quantitative PCR, have transformed our ability to characterize water microbiomes and detect emerging threats.

## Microbial ecology of drinking water systems

### Indigenous microbiota

Drinking water systems harbor complex microbial communities that establish biofilms on pipe surfaces and exist as planktonic populations in bulk water. The core microbiome of drinking water typically includes members of *Proteobacteria*, *Actinobacteria*, *Bacteroidetes*, and *Firmicutes* [4]. These communities play crucial roles in nutrient cycling, biofilm formation, and may influence the survival and proliferation of pathogenic organisms.

Recent metagenomic studies have revealed that treated drinking water may contain  $10^3$ - $10^4$  bacterial cells per milliliter, with significant spatial and temporal variation. The composition of these communities is influenced by source water characteristics, treatment processes, distribution system materials, water age, and residual disinfectant concentrations.

Biofilm formation and significance

Biofilms represent the predominant microbial lifestyle in drinking water distribution systems, with biomass estimates reaching  $10^5$ - $10^7$  cells per square centimeter of pipe surface [5]. These structured communities provide protection against disinfectants, facilitate horizontal gene transfer, and serve as reservoirs for opportunistic pathogens including *Legionella pneumophila*, *Mycobacterium avium* complex, and *Pseudomonas aeruginosa*.

The architecture and composition of drinking water biofilms vary depending on pipe material, flow dynamics, and nutrient availability. Copper and copper alloys demonstrate superior antimicrobial properties compared to plastic materials such as polyvinyl chloride (PVC) and polyethylene, though trade-offs exist regarding corrosion and leaching concerns.

Waterborne pathogens of public health significance

Bacterial pathogens

| Pathogen                           | Disease                              | Infective Dose        | Environmental Persistence     | Detection Method          |
|------------------------------------|--------------------------------------|-----------------------|-------------------------------|---------------------------|
| <i>Escherichia coli</i> O157:H7    | Hemorrhagic colitis, HUS             | 10-100 cells          | Moderate (weeks)              | Culture, PCR, immunoassay |
| <i>Salmonella</i> spp.             | Typhoid, gastroenteritis             | $10^2$ - $10^6$ cells | High (months)                 | Culture, PCR              |
| <i>Shigella</i> spp.               | Bacillary dysentery                  | 10-200 cells          | Moderate (weeks)              | Culture, PCR              |
| <i>Campylobacter jejuni</i>        | Gastroenteritis                      | 500-800 cells         | Low (days-weeks)              | Culture, PCR              |
| <i>Vibrio cholerae</i>             | Cholera                              | $10^3$ - $10^6$ cells | High (months)                 | Culture, PCR              |
| <i>Legionella pneumophila</i>      | Legionnaires' disease, Pontiac fever | Unknown (inhalation)  | Very high (years in biofilms) | Culture, PCR, immunoassay |
| <i>Mycobacterium avium</i> complex | Pulmonary disease, lymphadenitis     | Variable              | Very high (years)             | Culture, PCR              |
| <i>Pseudomonas aeruginosa</i>      | Opportunistic infections             | Variable              | Very high                     | Culture, PCR              |

Table 1: Major bacterial pathogens in drinking water.

Classic enteric bacterial pathogens including *Salmonella*, *Shigella*, and enteropathogenic *E. coli* remain significant concerns in developing nations where water treatment infrastructure is inadequate [6]. However, in developed countries, opportunistic premise plumbing pathogens have emerged as the predominant bacterial threat. An example is *Pseudomonas aeruginosa* present in drinking water, that is now the most important cause of *H. pylori*-negative gastritis in man.

*Legionella pneumophila*, the causative agent of Legionnaires' disease, has shown increasing incidence in recent years, with reported cases quadrupling in the United States between 2000 and 2022 [7]. This pathogen thrives in warm water environments (25-42°C), particularly in building plumbing systems, cooling towers, and hot tubs. Amoebae serve as important environmental reservoirs, providing intracellular protection and facilitating *Legionella* replication.

Nontuberculous mycobacteria (NTM), particularly the *Mycobacterium avium* complex, colonize drinking water distribution systems and premise plumbing. These organisms demonstrate exceptional disinfectant resistance due to their hydrophobic, lipid-rich cell walls and biofilm-associated growth. NTM infections have increased significantly, especially among immunocompromised populations and individuals with underlying lung disease.

Viral pathogens

| Virus             | Disease                     | Genome Type | Environmental Stability | Detection Method        |
|-------------------|-----------------------------|-------------|-------------------------|-------------------------|
| Norovirus         | Gastroenteritis             | ssRNA       | High                    | RT-qPCR, ELISA          |
| Rotavirus         | Gastroenteritis             | dsRNA       | High                    | RT-qPCR, ELISA, culture |
| Hepatitis A virus | Hepatitis                   | ssRNA       | Very high               | RT-qPCR, culture        |
| Hepatitis E virus | Hepatitis                   | ssRNA       | High                    | RT-qPCR                 |
| Adenovirus        | Respiratory/GI illness      | dsDNA       | Very high               | qPCR, culture           |
| Enterovirus       | Various (polio, meningitis) | ssRNA       | High                    | RT-qPCR, culture        |
| Astrovirus        | Gastroenteritis             | ssRNA       | Moderate                | RT-qPCR                 |
| SARS-CoV-2        | COVID-19                    | ssRNA       | Low-moderate            | RT-qPCR                 |

Table 2: Viral pathogens in drinking water.

Enteric viruses represent a major waterborne disease burden globally, with an estimated 1.5 million cases of viral gastroenteritis attributable to drinking water annually in developed countries alone. Human noroviruses are the leading cause of waterborne viral gastroenteritis, with extremely low infective doses (10-100 viral particles) and high environmental persistence [11].

Hepatitis A and E viruses cause acute liver inflammation with potential for severe outcomes, particularly hepatitis E in pregnant women and immunocompromised individuals. Waterborne hepatitis outbreaks continue to occur sporadically, often associated with groundwater contamination and inadequate disinfection (Martinez-Hernandez., et al. 2024).

The COVID-19 pandemic highlighted the utility of wastewater surveillance for viral pathogens, though SARS-CoV-2 transmission through drinking water has not been documented due to the virus’s susceptibility to conventional treatment and disinfection processes (Chen., et al. 2023). Nonetheless, this experience has catalyzed development of viral monitoring programs for drinking water sources.

Parasitic pathogens

| Parasite                       | Disease                             | Infective Stage  | Size (µm) | Disinfectant Resistance | Detection Method             |
|--------------------------------|-------------------------------------|------------------|-----------|-------------------------|------------------------------|
| <i>Cryptosporidium</i> spp.    | Cryptosporidiosis                   | Oocyst           | 4-6       | Very high               | Microscopy, PCR, immunoassay |
| <i>Giardia lamblia</i>         | Giardiasis                          | Cyst             | 8-12      | High                    | Microscopy, PCR, immunoassay |
| <i>Entamoeba histolytica</i>   | Amoebiasis                          | Cyst             | 10-20     | High                    | Microscopy, PCR              |
| <i>Cyclospora cayetanensis</i> | Cyclosporiasis                      | Oocyst           | 8-10      | High                    | Microscopy, PCR              |
| <i>Toxoplasma gondii</i>       | Toxoplasmosis                       | Oocyst           | 10-12     | High                    | PCR, serology                |
| <i>Acanthamoeba</i> spp.       | Keratitis, encephalitis             | Cyst             | 10-25     | Very high               | Microscopy, culture, PCR     |
| <i>Naegleria fowleri</i>       | Primary amebic meningo-encephalitis | Cyst/trophozoite | 10-35     | Moderate                | Microscopy, culture, PCR     |

Table 3: Protozoan parasites in drinking water.

*Cryptosporidium* and *Giardia* are the most significant protozoan pathogens in drinking water. *Cryptosporidium* is particularly problematic due to its exceptional chlorine resistance, with oocysts capable of surviving conventional disinfection concentrations. The Milwaukee cryptosporidiosis outbreak of 1993, affecting over 400,000 individuals, remains the largest documented waterborne disease outbreak in United States history.

*Cryptosporidium hominis* and *C. parvum* are the primary species affecting humans, with low infective doses (10-100 oocysts) and potential for severe, life-threatening disease in immunocompromised individuals. Physical removal through filtration (particularly membrane filtration) and ultraviolet disinfection are the most effective control measures (Rodriguez-Martinez, *et al.* 2023).

Free-living amoebae, including *Acanthamoeba* spp. and *Naegleria fowleri*, represent emerging concerns. While infections are rare, *N. fowleri* causes primary amebic meningoencephalitis with a case fatality rate exceeding 97%. These organisms thrive in warm freshwater environments and premise plumbing systems.

Indicator organisms and microbial water quality assessment

Traditional indicator organisms

| Indicator                       | Significance                   | Advantages                                | Limitations  |
|---------------------------------|--------------------------------|---|--|
| Total coliforms                 | General water quality          | Easy detection, established methods       | May grow in distribution systems, poor viral/protozoan predictor |
| <i>E. coli</i>                  | Recent fecal contamination     | Specific for fecal pollution              | May not predict all pathogens, die-off in environment            |
| <i>Enterococci</i>              | Fecal contamination            | More persistent than coliforms            | Variable correlation with pathogens                              |
| <i>C. perfringens</i> spores    | Historical fecal contamination | Highly persistent, disinfectant resistant | Not specific to recent contamination                             |
| Coliphages                      | Viral contamination indicator  | Similar behavior to enteric viruses       | Methodology complexity, no standardized method                   |
| HPC (Heterotrophic Plate Count) | General bacterial load         | Indicates treatment efficacy, regrowth    | No health significance, variable methodology                     |

Table 4: Microbiological indicators for water quality.

The concept of indicator organisms is predicated on identifying surrogates that are present in greater numbers, easier to detect, and demonstrate similar or greater persistence than pathogens of concern. Total coliforms have been used since the early 20<sup>th</sup> century, with *E. coli* serving as the definitive indicator of recent fecal contamination (Thompson, *et al.* 2023).

However, significant limitations exist with traditional indicators. Neither total coliforms nor *E. coli* reliably predict the presence of viruses or protozoa, particularly in treated water where differential inactivation occurs. *Cryptosporidium* oocysts, for instance, may persist despite complete elimination of bacterial indicators through chlorination.

Alternative and supplementary indicators

Bacteriophages, particularly somatic coliphages and F-specific RNA coliphages, have gained attention as potential viral indicators due to their structural and behavioral similarities to enteric viruses. *Clostridium perfringens* spores serve as indicators of remote fecal contamination and treatment efficacy against chlorine-resistant pathogens, given their exceptional environmental persistence.

Recent research has explored molecular indicators including human-specific genetic markers (e.g. human polyomaviruses, crAssphage) that may better indicate human fecal contamination and associated health risks (Martinez-Hernandez, *et al.* 2024). However, standardization and regulatory acceptance remain challenges for these novel approaches.

### Detection and quantification methods

#### Culture-based methods

Traditional culture techniques remain the gold standard for many regulatory applications, particularly for bacterial indicators. Membrane filtration followed by culture on selective media allows for enumeration and confirmation of coliforms, *E. coli*, and enterococci. Despite advances in molecular methods, culture provides information on organism viability and phenotypic characteristics essential for public health assessment.



**Figure:** Figure showing excessive coliforms in water, collected from a site having intermittent water supply by Multiple tube test (image: authors).

Cultivation of certain pathogens, notably *Legionella* spp. and *Mycobacteria*, requires specialized media and extended incubation periods. ISO 11731 describes standard methods for *Legionella* isolation using buffered charcoal yeast extract (BCYE) agar with selective supplements [7]. Mycobacterial culture may require 4-8 weeks for slow-growing species, representing a significant diagnostic delay. For detecting remote contamination, Bile aesculin azide broth is able to detect *Enterococci* well. Reinforced Clostridial medium is useful to detect *Clostridium* spp. It should be remembered that total and fecal coliforms indicate recent compromise or contamination of drinking water, while presence of *Enterococci* and *Clostridia* show remote contamination.

Multiple tube test is used to detect total coliforms in drinking water while Eijkman's test (ability of *E. coli* to grow, and produce Indole and gas at 44.5°C) is useful to detect fecal coliforms.

#### Immunological methods

Immunoassay techniques, including enzyme-linked immunosorbent assay (ELISA) and immunofluorescence assay (IFA), provide rapid detection of specific pathogens and antigens. The USEPA Method 1623 employs immunomagnetic separation (IMS) coupled with immunofluorescence for *Cryptosporidium* and *Giardia* detection. While these methods offer improved sensitivity compared to direct microscopy, they cannot definitively determine organism viability.

Molecular methods

| Method                          | Principle                                 | Advantages                        | Limitations                                    | Applications                                   |
|---------------------------------|---|-----------------------------------|--|--|
| Conventional PCR                | DNA amplification                         | High specificity                  | Not quantitative, requires post-PCR analysis   | Pathogen identification, genotyping            |
| qPCR/RT-qPCR                    | Real-time DNA/RNA amplification           | Quantitative, rapid, sensitive    | Detects dead cells, matrix inhibition          | Pathogen enumeration, gene detection           |
| ddPCR                           | Droplet-based absolute quantification     | No standard curve needed, precise | Expensive, limited multiplexing                | Absolute quantification, rare target detection |
| Next-generation sequencing      | Massively parallel sequencing             | Comprehensive community analysis  | Expensive, complex analysis, no viability info | Microbiome characterization, metagenomics      |
| Isothermal amplification (LAMP) | DNA amplification at constant temperature | Simple equipment, rapid           | Less established, optimization needed          | Field testing, point-of-care                   |
| Flow cytometry                  | Cell counting and characterization        | Rapid, total cell counts          | Requires specialized equipment                 | Intact vs. damaged cells, total cell counts    |

Table 5: Molecular detection methods for waterborne pathogens.

Quantitative PCR (qPCR) has revolutionized waterborne pathogen detection, offering sensitivity, specificity, and rapid results compared to culture methods (Chen., *et al.* 2023). Multiplex qPCR assays can simultaneously detect multiple pathogens in a single reaction, improving efficiency and reducing costs. However, molecular methods detect DNA from both viable and non-viable organisms, potentially overestimating public health risk.

Viability PCR, employing propidium monoazide (PMA) or ethidium monoazide (EMA) pre-treatment, selectively amplifies DNA from intact cells with compromised membranes, providing better correlation with infectious organisms (Rodriguez-Martinez., *et al.* 2023). This approach has shown promise for assessing disinfection efficacy and actual pathogen threats.

Next-generation sequencing (NGS) technologies, including 16S rRNA gene amplicon sequencing and shotgun metagenomics, enable comprehensive characterization of microbial communities without cultivation bias (Kumar., *et al.* 2024). These approaches have revealed extensive microbial diversity in drinking water systems and identified previously unrecognized organisms. However, challenges include high costs, complex bioinformatic analyses, and limited functional information regarding metabolic activity and pathogenicity.

Antimicrobial resistance in drinking water

The aquatic environment serves as a reservoir and transmission route for antimicrobial resistance (AMR) genes and resistant bacteria. Municipal wastewater discharge, agricultural runoff, and pharmaceutical manufacturing effluents introduce antibiotics, resistant bacteria, and mobile genetic elements into water sources [8]. Drinking water treatment provides a barrier, yet studies consistently detect AMR genes and resistant bacteria in treated drinking water and distribution systems.

Metagenomic studies reveal that drinking water distribution systems harbor diverse antibiotic resistance genes (ARGs), with sulfonamide, tetracycline, and beta-lactam resistance genes most frequently detected. While concentrations are generally lower than in wastewater, the potential for horizontal gene transfer through mobile genetic elements (plasmids, transposons, integrons) raises concerns about resistance dissemination.

| Resistance Type | Common Genes                                     | Associated Bacteria                                       | Public Health Concern              |
|-----------------|--|---|------------------------------------|
| Beta-lactam     | <i>bla</i> TEM, <i>bla</i> CTX-M, <i>bla</i> KPC | <i>E. coli</i> , <i>Klebsiella</i> , <i>Pseudomonas</i>   | High - common resistance           |
| Quinolone       | <i>qnr</i> , <i>aac</i> (6')-Ib-cr               | <i>E. coli</i> , <i>Salmonella</i> , <i>Campylobacter</i> | High - widespread use              |
| Tetracycline    | <i>tet</i> (A), <i>tet</i> (M), <i>tet</i> (W)   | Diverse bacteria  | Moderate - agricultural impact     |
| Sulfonamide     | <i>sul</i> 1, <i>sul</i> 2                       | <i>E. coli</i> , <i>Aeromonas</i>                         | Moderate - common in environment   |
| Aminoglycoside  | <i>aac</i> , <i>aph</i> , <i>ant</i>             | <i>Pseudomonas</i> , <i>Acinetobacter</i>                 | Moderate - clinical importance     |
| Carbapenem      | <i>bla</i> NDM, <i>bla</i> KPC, <i>bla</i> OXA   | <i>Klebsiella</i> , <i>E. coli</i> , <i>Acinetobacter</i> | Critical - last-resort antibiotics |
| Colistin        | <i>mcr</i> -1, <i>mcr</i> -2                     | <i>E. coli</i> , <i>Salmonella</i>                        | Critical - last-resort antibiotic  |

Table 6: Antimicrobial resistance in water environments.

Opportunistic premise plumbing pathogens, including *Pseudomonas aeruginosa* and mycobacteria, frequently exhibit intrinsic and acquired antimicrobial resistance. Biofilm-associated growth further enhances resistance through reduced antibiotic penetration and metabolic heterogeneity [9]. The clinical implications of drinking water-associated AMR transmission require further investigation, though epidemiological evidence suggests this route contributes to the overall burden of resistant infections.

Water treatment and microbial control

Conventional treatment processes

| Process                              | Bacteria (log removal) | Viruses (log removal) | Protozoa (log removal)  | Mechanism               |
|--------------------------------------|------------------------|-----------------------|-------------------------|-------------------------|
| Coagulation/Flocculation             | 1-2                    | 1-2                   | 1-2                     | Particle aggregation    |
| Sedimentation                        | 0.5-1                  | 0.5-1                 | 1-2                     | Gravity separation      |
| Rapid sand filtration                | 1-2                    | 0-1                   | 1-2                     | Physical straining      |
| Slow sand filtration                 | 2-4                    | 2-3                   | 2-3                     | Biological + physical   |
| Chlorination (free Cl <sub>2</sub> ) | 3-6                    | 2-4                   | <1 ( <i>Crypto</i> : 0) | Oxidation               |
| Chloramination                       | 2-4                    | 1-3                   | <1                      | Oxidation (slower)      |
| Ozonation                            | 4-6                    | 3-4                   | 2-3                     | Oxidation               |
| UV irradiation                       | 3-6                    | 3-5                   | 3-4                     | DNA damage              |
| Membrane filtration (MF/UF)          | 4-6                    | 2-4                   | >4                      | Size exclusion          |
| Reverse osmosis                      | >6                     | >5                    | >6                      | Size exclusion + charge |

Table 7: Efficacy of treatment processes against waterborne pathogens.

The multi-barrier approach to drinking water treatment employs sequential processes to achieve cumulative pathogen reduction (Thompson., *et al.* 2023). Conventional treatment combining coagulation, flocculation, sedimentation, filtration, and disinfection typically achieves 4-6 log removal of bacteria, 3-5 log removal of viruses, and 3-4 log removal of protozoa when properly optimized.



Chlorination remains the most widely employed disinfection method globally, providing both microbial inactivation and residual protection in distribution systems. Free chlorine demonstrates excellent efficacy against vegetative bacteria and enveloped viruses at concentrations of 0.5-2.0 mg/L with contact times of 30 minutes [10]. However, chlorine exhibits limited activity against *Cryptosporidium* oocysts, bacterial spores, and mycobacteria, necessitating supplementary treatment barriers.

Chloramines (combined chlorine) are increasingly employed as secondary disinfectants in distribution systems due to greater stability and reduced disinfection by-product (DBP) formation compared to free chlorine. While chloramines provide superior residual maintenance, they demonstrate slower microbial inactivation kinetics and reduced efficacy against biofilms [11].

### **Advanced treatment technologies**

Ultraviolet (UV) disinfection has gained widespread adoption for inactivation of chlorine-resistant pathogens, particularly *Cryptosporidium* and *Giardia* (Martinez-Hernandez, *et al.* 2024). UV radiation at 254 nm causes thymine dimer formation in DNA, preventing replication. The USEPA LT2ESWTR regulations recognize UV treatment for achieving 3-log *Cryptosporidium* inactivation at doses of 12 mJ/cm<sup>2</sup>. UV systems provide rapid disinfection without chemical addition or DBP formation, though they lack residual protection in distribution systems.

Membrane filtration technologies, including microfiltration (MF), ultrafiltration (UF), nanofiltration (NF), and reverse osmosis (RO), provide absolute barriers to pathogens based on size exclusion (Rodriguez-Martinez, *et al.* 2023). Properly operated MF/UF systems achieve >4 log removal of protozoa and >6 log removal of bacteria. These technologies have particular application in small systems, point-of-use treatment, and challenging source waters.

Ozonation offers powerful oxidation potential, effectively inactivating bacteria, viruses, and protozoa while reducing taste, odor, and color. Ozone demonstrates superior efficacy compared to chlorine against resistant pathogens but requires sophisticated generation equipment and careful process control. Ozone rapidly decomposes, necessitating secondary disinfection for residual protection.

### **Disinfection by-products and health considerations**

Chemical disinfection, while essential for microbial control, generates potentially harmful disinfection by-products (DBPs) through reactions with natural organic matter. Trihalomethanes (THMs) and haloacetic acids (HAAs) are regulated DBPs associated with chlorination, while bromate and chlorite arise from ozonation and chlorine dioxide treatment, respectively.

Epidemiological studies have linked long-term exposure to chlorination DBPs with increased bladder cancer risk and adverse reproductive outcomes, though evidence remains mixed and health benefits of disinfection vastly outweigh potential DBP risks [8]. The challenge lies in optimizing disinfection to achieve adequate microbial control while minimizing DBP formation through source water protection, enhanced organic matter removal, and alternative disinfection strategies.

### **Distribution system challenges**

#### **Microbial regrowth**

Despite disinfection residuals, microbial regrowth occurs in drinking water distribution systems when biodegradable organic carbon, nutrients, and suitable temperatures exist. This regrowth can compromise water quality, lead to elevated heterotrophic plate counts, degrade disinfectant residuals, and promote biofilm development. Controlling regrowth requires minimizing assimilable organic carbon (AOC) and biodegradable dissolved organic carbon (BDOC) through enhanced treatment, maintaining disinfectant residuals, and reducing water age through proper system design and operation.



### **Premise plumbing contamination**

The final segment of drinking water systems-premise plumbing in buildings, presents unique microbiological challenges. Extended water stagnation in infrequently used fixtures, elevated temperatures in hot water systems, and complex plumbing configurations with increased surface area-to-volume ratios favour opportunistic pathogen growth. *Legionella*, *Mycobacteria*, and *Pseudomonas* thrive under these conditions, causing diseases primarily affecting vulnerable populations.

Water management programs for large buildings, as recommended by ASHRAE Standard 188 and CDC guidelines, employ strategies including temperature management (cold water <20°C, hot water >50-55°C at point-of-use), periodic flushing of low-use fixtures, and point-of-use filtration for high-risk areas [9]. Copper-silver ionization and chlorine dioxide supplementation show promise as supplementary control measures, though optimal implementation strategies remain under investigation.

### **Climate change and emerging challenges**

Climate change exacerbates water quality challenges through multiple mechanisms including increased temperatures, altered precipitation patterns, more frequent extreme weather events, and changing pathogen ecology. Warmer water temperatures accelerate microbial growth, reduce disinfection efficacy, and extend the environmental survival of certain pathogens.

Extreme precipitation events cause source water quality deterioration through increased turbidity, organic matter loading, and pathogen introduction from agricultural and urban runoff. The increasing frequency of floods overwhelms treatment infrastructure and damages distribution systems, leading to contamination events. Conversely, drought conditions concentrate contaminants, promote cyanobacterial blooms producing toxins, and challenge water availability.

Harmful algal blooms (HABs) producing cyanotoxins represent an emerging threat at the intersection of microbiology and toxicology. Microcystin, cylindrospermopsin, anatoxin-a, and saxitoxin pose health risks through drinking water exposure, with conventional treatment showing variable efficacy. Advanced treatment including ozone, activated carbon, and membrane filtration may be necessary for cyanotoxin removal.

### **Regulatory framework and water safety plans**

#### **International guidelines and standards**

The WHO Guidelines for Drinking-water Quality provide the international reference for water safety standards, employing a risk-based approach encompassing source protection, treatment optimization, and distribution system management. These guidelines establish guideline values for chemical and radiological parameters while recommending microbiological targets achievable through good practice rather than specific limits.

National regulations vary considerably, with the United States employing the Safe Drinking Water Act (SDWA) and associated regulations including the Total Coliform Rule, Revised Total Coliform Rule (RTCR), Long Term 2 Enhanced Surface Water Treatment Rule (LT2ESWTR), and Groundwater Rule. European Union member states follow the Drinking Water Directive (DWD) establishing quality standards and monitoring requirements.

#### **Water safety plans**

The Water Safety Plan (WSP) approach promotes comprehensive risk assessment and management throughout the water supply chain, from catchment to consumer. This preventive framework identifies hazards, assesses risks, implements control measures, and establishes monitoring systems to ensure consistent delivery of safe drinking water. WSPs have demonstrated effectiveness in improving water quality, particularly in resource-limited settings, though implementation challenges persist.

## Future Directions and Research Needs

Several critical research areas require attention to advance drinking water microbiology:

1. **Improved understanding of the “viable but non-culturable” (VBNC) state:** Many waterborne pathogens enter dormancy under stress conditions, evading detection by culture methods while potentially retaining pathogenicity. Developing methods to assess VBNC organisms and their public health significance remains crucial.
2. **Microbiome-based water quality assessment:** Moving beyond single indicators toward comprehensive microbial community analysis may provide superior water quality assessment and early warning of contamination events. Machine learning approaches analyzing metagenomic data show promise for predictive modeling.
3. **Antimicrobial resistance monitoring and intervention:** Establishing comprehensive surveillance programs for AMR in drinking water, understanding transmission dynamics, and developing targeted interventions represent critical needs.
4. **Novel pathogens and One Health integration:** Climate change, ecological disruption, and globalization facilitate pathogen emergence and geographic expansion. Integrating human, animal, and environmental health surveillance through the One Health framework will enhance outbreak detection and prevention.
5. **Point-of-use treatment technologies:** Developing affordable, effective point-of-use and point-of-entry treatment systems for vulnerable populations and challenging environments remains a priority for global water security.
6. **Advanced oxidation processes:** Research into novel disinfection technologies including photocatalysis, electrochemical oxidation, and combined treatment systems may provide enhanced pathogen inactivation with reduced DBP formation.

## Conclusion

The microbiology of drinking water encompasses a complex, dynamic ecosystem requiring sophisticated understanding and management to protect public health. While tremendous progress has been achieved in water treatment and pathogen control, emerging challenges including climate change, antimicrobial resistance, opportunistic premise plumbing pathogens, and novel organisms demand continued vigilance and innovation.

Modern molecular tools have revolutionized our ability to characterize microbial communities and detect pathogens, yet translating these capabilities into operational practice and regulatory frameworks remains challenging. The integration of advanced detection methods, comprehensive risk assessment through Water Safety Plans, infrastructure investment, and research addressing knowledge gaps will be essential for ensuring universal access to safe drinking water.

The COVID-19 pandemic demonstrated the critical importance of water, sanitation, and hygiene (WASH) for disease prevention while highlighting the vulnerability of water systems to disruption. Moving forward, resilient water infrastructure, trained workforce capacity, adequate funding, and international cooperation will be essential for achieving Sustainable Development Goal 6 and securing water security for future generations.

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