

Waterborne pathogen mitigation: Decoding techno-ecological synergies in multiscale transmission networks

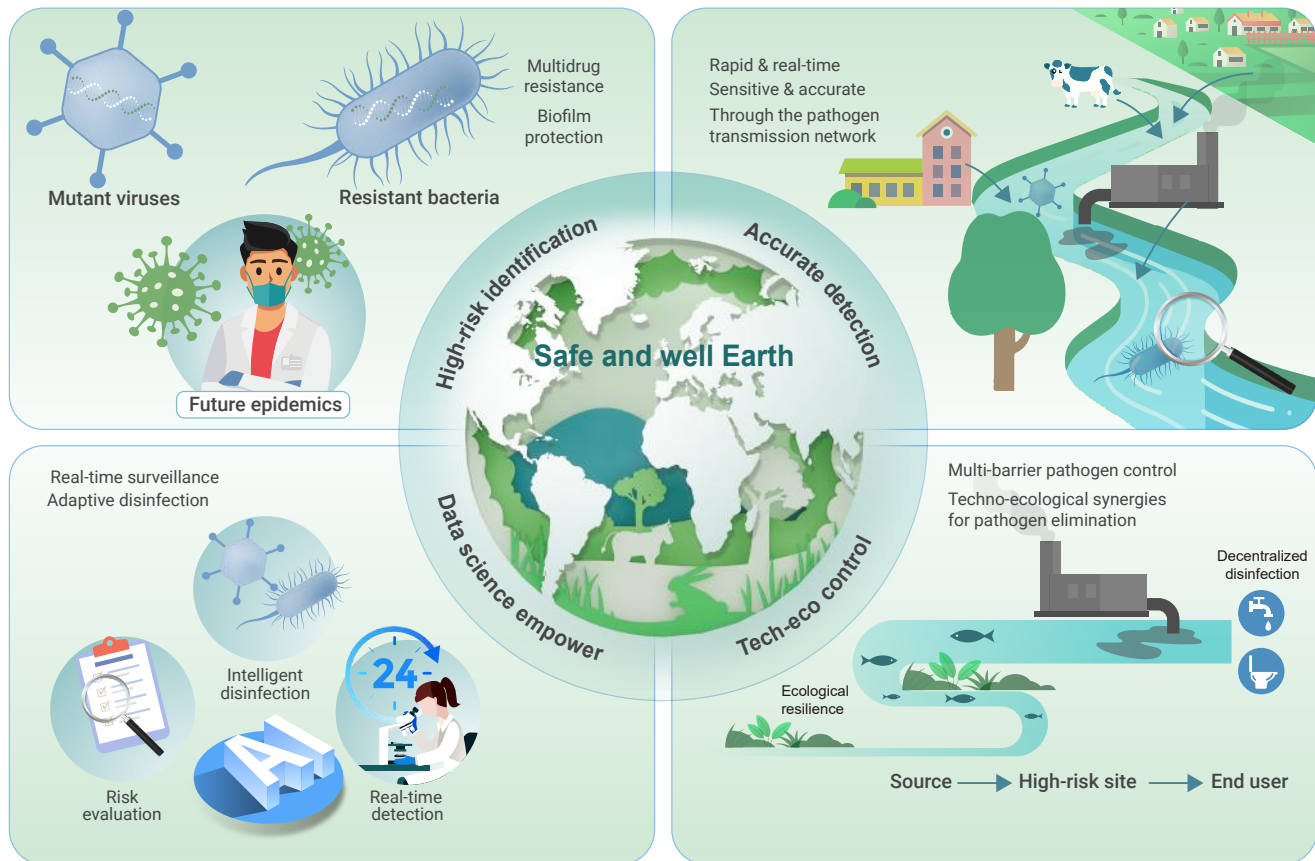
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GRAPHICAL ABSTRACT



PUBLIC SUMMARY

- Pathogenic mutant viruses and resistant bacteria may lead to future epidemics of primary concern.
- Rapid, sensitive on-site detection of low-level pathogens is essential for accurate risk assessment.
- Versatile, decentralized disinfection at high-risk sites offers a multi-barrier pathogen control system.
- Bridging ecological resilience and multi-barrier disinfection enables synergistic pathogen mitigation.
- Data science enables real-time surveillance and adaptive disinfection for intelligent pathogen control.

Waterborne pathogen mitigation: Decoding techno-ecological synergies in multiscale transmission networks

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Pathogen spread and infection represent paramount global challenges, their intricate transmission pathways fundamentally shaped by human behavior and anthropogenic influences. Here, we elucidate pathogen transmission networks in the environment and identify the increasing risks resulting from mutant viruses and resistant bacteria. We examine the advantages and limitations of techniques for pathogen detection and advocate the development of real-time, high-precision, point-of-need assays capable of detecting microorganisms in waterborne matrices, providing a new conceptual and technological approach to future detection methods. We also highlight the inadequate protection of existing centralized disinfection methods and propose the implementation of decentralized disinfection (i.e., chemical-free and energy-efficient point-of-use disinfection) as a form of multi-barrier protection throughout the different pathways of pathogen transmission. A robust and resilient ecosystem can prevent containment sources and inhibit the bioactivity of residual pathogens, and, when working in synergy with multi-barrier disinfection, can achieve a techno-ecological framework for pathogen mitigation. We further address the fact that data-driven technologies (e.g., artificial neural networks and machine learning methods) provide a route for intelligent detection-guided disinfection and the accurate selection of pathogen indicators that are directly relevant to human health. Finally, we highlight concerns regarding potential high-risk pathogens due to climate change.

INTRODUCTION

Pathogenic challenges have constituted an ongoing and persistent pressure throughout human history.^{1–4} Until the last few decades, pathogen infections have remained a notable challenge to public health and have become the second leading cause of death for humans, surpassed only by cardiovascular disease.^{5–7} The emergence and spread of high-risk pathogens, such as the coronavirus pandemic in 2019, and Ebola and Zika epidemics, pose a significant threat to public health, particularly in low-resource and disaster-stricken areas, thereby leading to more than 1.8 million deaths per year.^{1,7,8} In particular, the rapid urbanization and industrialization of our society, coupled with increasing environmental pollution and more frequent extreme weather events, have exacerbated the dynamics of pathogen mutation and transmission, leading to an increased risk of infection.^{4,9,10} As a result, a reimagined paradigm for a more scientific, rational framework for pathogen risk assessment and control throughout the whole transmission network has the potential to address the urgent need to curb pathogen infections.^{11–13}

The primary challenge in pathogen assessment and control is that pathogens have multiscale transmission networks in a range of environments.^{14–16} Pathogens, including viruses, bacteria, and protozoa, have a wide range of sizes (nm to mm) and can be transmitted within and throughout multiple media (i.e., air, water, and soil) by floating, swimming, and attaching to solid surfaces. Due to their self-protective structures, which include viral envelopes, bacterial biofilms, and protozoan cysts, pathogens are able to survive for prolonged periods with a high level of pathogenicity under a range of adverse environmental condi-

tions.^{17–19} The transmission of pathogens from primary sources (e.g., animals, farms, and hospitals) to the end user is closely linked to human activity, making pathogen infection ubiquitous in our daily life; as a result, it is challenging to achieve overall protection by conventional centralized sanitation facilities alone.^{13,20,21} Once stimulated by adverse external stimuli, which include antibiotics, organic pollutants, and pharmaceuticals, pathogens are also likely to evolve into new mutant species with significantly enhanced resistance to environmental stressors, thereby leading to highly infectious viruses and multidrug-resistant bacteria.^{22,23} However, current studies on pathogen control fail to ensure overall prevention throughout the complete transmission network and are particularly inadequate at decentralized locations for high-risk and resistant pathogens.^{24,25}

Current pathogen detection technologies, such as culturing and the polymerase chain reaction (PCR), are time-consuming and operator dependent, thereby undermining their suitability for real-time, accurate assessment of environmental pathogens.^{26,27} In particular, the process of culturing can only detect cultivatable bacteria, representing less than 10% of total microorganisms, and typically requires 24–48 h.²⁶ Although PCR facilitates the detection of non-cultivable pathogens, the prerequisite of concentrating trace-level organisms from complex matrices introduces delays that undermine its applicability for real-time surveillance.^{27,28} Conventional pathogen detection workflows mandate skilled operators and qualified laboratories, limiting on-site and automated implementation. As progressive technical developments continue, emerging molecular biology-based methods can provide sensitive and rapid identification of low-level pathogens within complex environments.^{29,30} For example, the gene editing method based on clustered regularly interspaced short palindromic repeats (CRISPR) approach is able to recognize targeted nucleic acids and trim them using endonuclease activity associated with the CRISPR-associated enzyme. These specific, programmable, and user-friendly CRISPR-based gene editing systems have made it feasible to detect environmental pathogens.³¹ Additionally, fluorescence and electrochemical biosensors, together with paper-based microfluidic detectors, hold promise for sensitive detection of low levels of pathogens without enrichment, making them readily available for non-experts to use in low-resource areas.^{32–35} Furthermore, multi-omics approaches provide a high-throughput and comprehensive evaluation of the pathogenic infectious capacity (i.e., levels of active genes and functional proteins), to uncover deeper insights into pathogen transmission and infection.^{36–38} However, these emerging methods still face challenges relating to reliance on operators (CRISPR), potential interruption by coexisting pollutants (biosensors), and the time-consuming and costly nature (multi-omics methods). Thus, the development of on-site and sensitive detection methods for precise, real-time risk evaluation is invaluable for dissecting multiscale pathogen transmission networks, and this should be a focus of future technological development.

Disinfection is essential for pathogen control; however, current disinfection methods are largely confined to centralized facilities, such as wastewater treatment plants. This centralized approach limits pathogen protection throughout the transmission network, in particular in decentralized high-risk locations associated with daily activities.^{13,39,40} Following centralized disinfection, the

subsequent regrowth of inactivated pathogens and the introduction of new pathogens during the distribution process can increase the health risks posed to end users.^{41,42} Additionally, chlorination, the most commonly used disinfection method, is compromised by the formation of carcinogenic chlorinated by-products.^{43,44} Alternative approaches, such as ultraviolet (UV) irradiation and ozonation, although effective, remain costly and reliant on an uninterrupted power supply.⁴⁵ This underscores the urgent need for decentralized, cost-efficient disinfection technologies that deliver layered protection throughout the pathogen transmission network, complementing centralized wastewater treatment. Alternative methods, such as the advanced oxidation process (AOP), use reactive radicals generated *in situ* to control pathogens. However, these methods typically require an external energy source (e.g., electricity or solar power) or a chemical additive (e.g., hydrogen peroxide or persulfate) to activate them. The need for immediate and near-complete pathogen inactivation further limits the available scenarios of AOP disinfection methods, as these methods typically require relatively continuous chemical dosages or high energy consumption (0.1–10 kWh/m).⁴⁶ Equally important is the reinforcement of ecosystem resilience through enhanced ecological functions and intrinsic self-purification capacity to suppress pathogen persistence and proliferation.^{47,48} Consequently, the integration of multi-barrier disinfection strategies with enhanced ecosystem resilience establishes a synergistic techno-ecological framework for robust prevention and control of environmental pathogens.

In this review, we explore novel strategies for addressing the challenges related to environmental pathogens through accurate, real-time detection methods and provide a techno-ecological perspective that bridges ecological resilience and multi-barrier disinfection systems throughout the pathogen transmission network. We first delineate the multiscale transmission network of pathogens and their self-protective structure, highlighting the increasing threat posed by mutant pathogenic viruses and biofilm-protected and multidrug-resistant bacteria. We then critically evaluate the time-consuming challenges of existing pathogen detection methods and envision the need for sensitive, real-time, on-site identification of low-level pathogens in complex environments. We also identify the gaps in current centralized disinfection throughout the pathogen transmission network, and accordingly provide an ideal solution by developing decentralized disinfection as a multi-barrier protection system and involving it with improved ecological resilience to reduce pathogen introduction. Finally, we illustrate the practical feasibility of this techno-ecological solution for environmental pathogen control using data-driven technologies and highlight potential high-risk pathogens as a result of climate change.

ENVIRONMENTAL PATHOGENS AND THEIR MULTISCALE TRANSMISSION NETWORK

Environmental pathogens, including viruses, bacteria, and protozoa, exhibit a wide range of sizes (nm to mm) and robust, self-protective structures that facilitate their transmission through multiscale environments and survival under adverse environmental conditions (Figure 1A).⁴⁹ In particular, viruses with dimensions from 20 to 500 nm can be readily transmitted through micro-sized aerosols (<1 μm) that are produced by infected patients by coughing and contaminated water from toilet flushing or wastewater treatment.^{15,49} Aerosols containing viruses can travel for kilometers when airborne.⁵⁰ Once attached to indoor surfaces (e.g., walls, chairs, and floors), some viruses remain infectious for weeks and are able to readily infect people located nearby through accidental inhalation or skin contact.⁵¹ As a result of their pervasive distribution and high virulence, pathogenic viruses, which are responsible for pneumonia, influenza, and the recent coronavirus disease, have posed a significant threat to public health. Notably, some pathogenic viruses, such as hepatitis B and poxvirus, have envelopes outside their protein capsids, which consist of phospholipids and proteins (i.e., derived from portions of the host cell membranes) and viral glycoproteins.^{52,53} The presence of a robust protective envelope enhances their viral resistance to environmental stressors while simultaneously augmenting high pathogenicity through easy binding to host cell receptors.^{54,55}

In contrast, bacteria with larger dimensions ranging from 0.2 to 5 μm are predominantly transmitted through water and food, or solid surface contact. As an example, *Pseudomonas aeruginosa*, *Enterococcus faecalis*, and *Acinetobacter baumannii* are likely to form biofilms under adverse environmental conditions.^{56–58} Once attached to sediments, water pipes, and indoor surfaces (e.g., in hospitals, schools, and public restrooms), these bacteria can produce

extracellular polymeric substances (EPS) that contain a variety of polymers such as polysaccharides, lipids, and DNA to form a protective layer.^{59,60} These biofilms encapsulate bacterial communities, shielding the cells from disinfectants and antimicrobial agents. They also act as reservoirs, periodically releasing viable bacteria and perpetuating persistent infections.^{59,61,62} Additionally, motile pathogens such as *Vibrio cholerae* and *Escherichia coli* exploit flagella to traverse water systems over long distances, thereby amplifying transmission risks.^{63,64} Furthermore, *Bacillus* and *Clostridium* are able to form a protective spore of reduced size (<1 μm), thicker walls, and a low water content structure that makes it highly resistant to environmental stress (e.g., low temperature, drought, and nutrient deprivation).^{65,66} The spores are also highly resistant to a range of physical and chemical sterilization methods used in water treatment and food processing. Upon encountering favorable conditions, surviving spores can rapidly germinate into bacterial nutrients, thereby resulting in water contamination and food spoilage.

Protozoa are typically larger in size, typically μm to mm, and are another critical environmental pathogen with complex structures than viruses and bacteria. *Cryptosporidium* and *Giardia* can swim and transport through water flow for potential infection.^{67,68} Their unique ability to form cysts or oocysts provides significant resistance to conventional disinfection methods, such as chlorination and UV irradiation, thereby making them hard to inactivate.⁶⁹ *Amoeba* is another type of protozoan that tends to attach itself to water pipes, sewage treatment plants, and soil surfaces.⁷⁰ The specific trophozoite structure enables *Amoeba* to climb above the attached surface for movement and predation, resulting in strong resistance and adaptability to adverse environmental conditions.^{18,71} Overall, the wide variety of genera and sizes facilitates the spread of pathogens through the environment via various pathways, resulting in ubiquitous and difficult-to-control pathogenic infections. For example, a commonly used water treatment method, membrane filtration, faces challenges in terms of notable pressure drop when it comes to removing all forms of pathogens (e.g., protozoa, bacteria, and viruses), since the filtration process is size dependent.

Persistent pathogenic challenges have shaped human history and civilization (Figure 1B). The “Justinian Plague” is the first recorded severe epidemic that has clear reasons for pathogenic infection. The plague was a result of *Yersinia pestis*, a pathogenic bacterium carried by fleas on rodents, which began in Constantinople in 541 and swept across the Mediterranean for more than 200 years, killing millions of people.⁷² *Yersinia pestis* also led to other severe pandemics, such as the Black Death, which devastated medieval Europe in the 14th century and killed approximately 30 million people, and the Third Plague, which began in East Asia in the 19th century and spread worldwide with 12 million deaths.⁷² In addition to pathogenic bacteria, viruses have also led to serious diseases throughout history, which include smallpox, the 1918 flu, and the Asian flu.

In light of the analysis of severe pandemics, it is clear that both bacterial and viral pathogens have posed a persistent threat to public health throughout history. However, the driving causes of global disease outbreaks have changed in recent decades. With remarkable improvements in public health and modern interventions, including the wide use of antibiotics, the rapid development of sanitation, and fully protective vaccination, previous pandemics, particularly those caused by bacterial infections, have been minimized considerably and even disappeared. Conversely, viral pathogens continue to pose an evolving challenge due to their high mutation rates, which facilitate immune evasion. Emerging mutant pathogenic viruses have therefore become the primary cause of worldwide epidemics in recent years, such as severe acute respiratory syndrome (SARS) in 2003, the Middle East respiratory syndrome in 2012, and the coronavirus disease in 2019 (Figure 1B).^{73–75}

Viruses are able to jump from animals to humans due to their widespread and highly pathogenic nature, significantly increasing the frequency of zoonotic virus spillover.⁷⁶ This issue leads to emerging mutated pathogenic viruses and can result in diseases that have never occurred, potentially resulting in severe global disease. Additionally, some viruses have RNA, a simple single-stranded structure, as their genome, which tends to show rapid mutation and adaptation when stimulated by environmental perturbations (e.g., high temperature, intensive solar irradiation, and antiviral treatment). In particular, SARS-CoV-2 is able to mutate rapidly to produce multiple variants, such as the Delta and Omicron variants. The Omicron mutants exhibit a notably increased infection ability with a high basic reproduction number (18.6) as compared with the initial

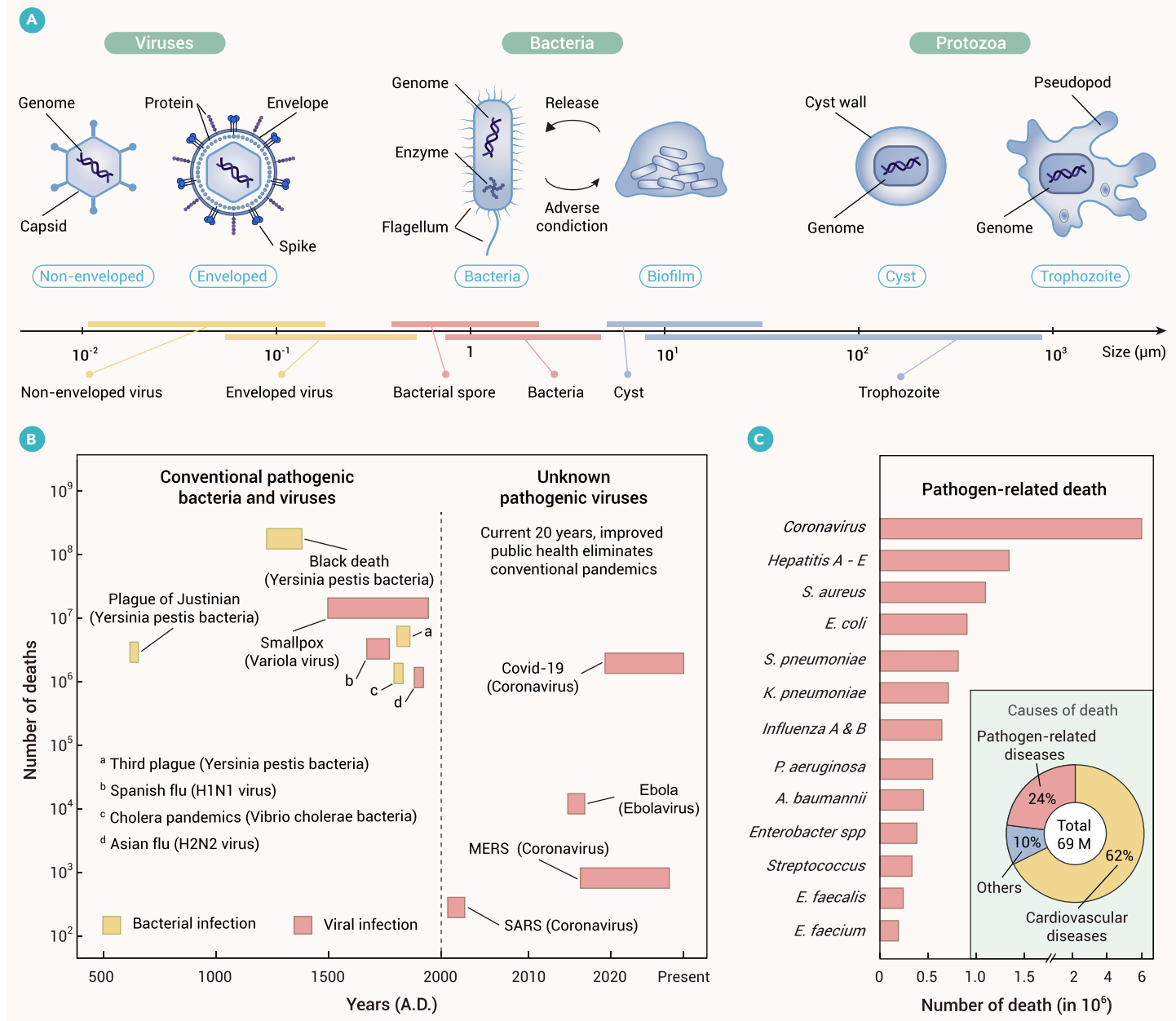


Figure 1. Pathogen characteristics, historical trends in pathogen outbreaks, and current status of pathogen-related mortality (A) Schematics of different types of pathogens (e.g., viruses, bacteria, and protozoa), size ranges, and their self-protective structures. (B) Historical trends in pathogen outbreaks. The emergence of high-risk pathogens, such as new mutant viruses, may cause future pathogen-related epidemics. (C) Current status of pathogen-related mortality, including the types of pathogens responsible for most deaths and the proportion of total mortality caused by pathogen infections.

SARS-CoV-2 wild-type (~2), ultimately leading to a worldwide epidemic with 130 million people infected.⁷⁷

Pathogens directly cause 24% of all deaths worldwide (Figure 1C).⁷⁸ Current epidemiological data have revealed the most high-risk environmental pathogens to be the mutating viruses and persistent infectious bacteria. Since 2020, COVID-19 has become the leading cause of death from pathogens (>6 million deaths) as a result of the emergence of Delta and Omicron mutants of high infectious capacity, followed by the easily transmissible hepatitis viruses as the second cause (>1.2 million deaths).^{79,80} Pathogenic bacteria with the ability to form biofilms under adverse environmental conditions, such as *Staphylococcus aureus*, *Escherichia coli*, *Streptococcus pneumoniae*, and *Pseudomonas aeruginosa*, exhibit high resistance to medical or hygienic treatment and have also become the main reason for pathogen-related mortality.^{81,82}

The significant public health challenge posed by environmental pathogens stems their transmission through complex, multiscale networks that link infection sources to high-risk locations and end users—networks that are intricately intertwined with human activity (Figure 2A). While some human pathogens,

such as SARS-CoV-2, *Mycobacterium tuberculosis*, and influenza viruses, are primarily airborne, most environmental pathogens are mainly transmitted via water. This is because microorganisms can metabolize and reproduce in water while remaining active. The infection network of the pathogen typically begins in a rural environment near rivers, lakes, and reservoirs with sparsely distributed populations and decentralized livestock production. In the absence of high-standard collection and treatment facilities, farm manure and untreated sewage containing fecal matter can introduce a variety of pathogens into water sources, thereby posing a threat to nearby people (Figure 2B).^{83,84} Pathogen-laden water can further compromise the safety of farmland through irrigation. The contaminated farmland, in turn, introduces pathogens to livestock and people through product intake and to water environments through agricultural runoff.⁸⁵

As the river traverses rural regions and enters urban areas, potential fecal contamination in the water can threaten the drinking safety of people living in downstream cities. Typically, a municipal disinfection treatment can disinfect pathogens with >99.9% inactivation but fails to eliminate all existing pathogens.^{86,87} Thus, the disinfection method becomes a selection process with

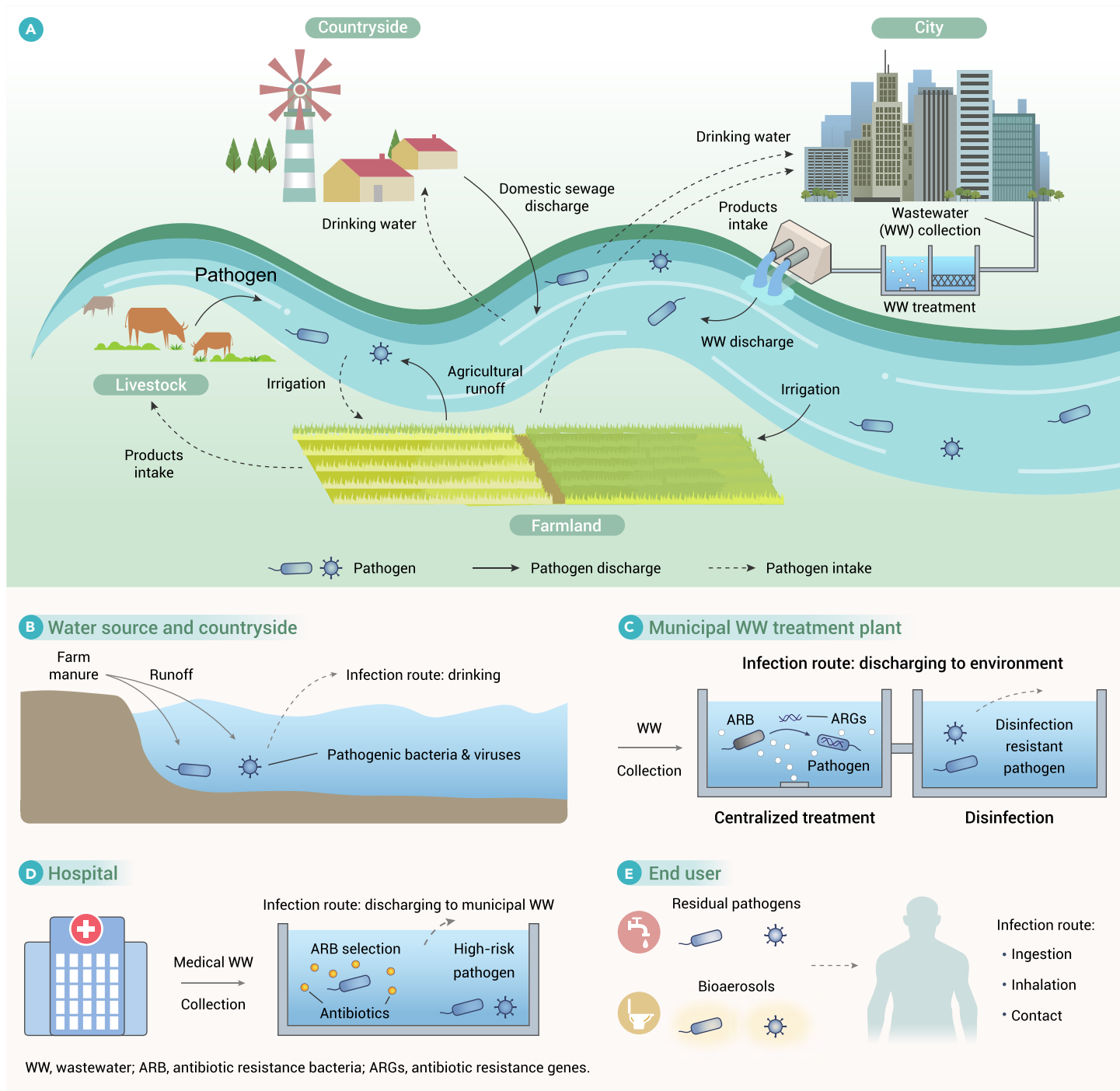


Figure 2. Characteristics of multiscale pathogen transmission networks in the environment and typical high-risk sites for pathogen infection (A) Schematic of pathogen transmission in the environment through multiple media from the primary source to the end user. (B–E) Typical high-risk sites for pathogen transmission and infection, including water sources in the countryside contaminated by farm manure (B), municipal wastewater (WW) treatment plants collecting large numbers of microorganisms such as antibiotic-resistant bacteria (ARB) that may result in antibiotic-resistant genes (ARGs) transfer to pathogens (C), hospital wastewater introducing pharmaceuticals and high-risk pathogens (D), and end users (E) likely to be infected by ubiquitous pathogens in their daily lives.

surviving pathogens showing strong resistance to disinfectants (Figure 2C).^{88,89} Additionally, urban settings with advanced drainage and wastewater treatment infrastructure collect large volumes of pathogen-laden effluent efficiently—but this also creates hotspots for pathogen accumulation and evolution.⁹⁰ When coexisting with antibiotic-resistant bacteria (ARB), the pathogen can capture the released antibiotic-resistant genes (ARGs) from ARB through a process of horizontal gene transfer.^{91,92} Pathogens with ARGs represent an extremely high risk as a result of their high resistance to medical treatment (Figure 2C).^{93,94}

In addition to the general pathogen transmission pathway of urban water systems in municipal wastewater, hospital wastewater is a critical high-risk hotspot

in the city region, which is typically enriched with high levels of pathogenic microorganisms and pharmaceuticals (Figure 2D). Specifically, pathogens such as *Legionella pneumophila* and *Pseudomonas aeruginosa* can be introduced by accidental contamination from patients through drinking water, utensils, and medical equipment, leading to hospital-acquired infections.⁹⁵ The coexistence of multiple types of antibiotics can lead to multi-drug resistance. As an example, *Acinetobacter baumannii*, which is resistant to β -lactams, quinolones, and sulfonamides, is found in hospital environments and medical wastewater. Additionally, after the disinfection process in the hospital, some pathogenic bacteria may survive and tend to persist in municipal water systems after being discharged from the hospital.⁹⁶

Another critical hotspot for pathogen transmission in urban areas is reclaimed water, which can serve as an essential urban water supply after a high level of municipal treatment but may pose a potential pathogen risk to residents. During the water distribution process, both drinking and reclaimed water may contain residual pathogens that have survived the disinfection process due to their significant resistance. These resistance pathogens typically form a protective biofilm containing excessive EPS (e.g., polysaccharides, lipids, and DNA), which adheres to the inner surfaces of distribution pipes and makes the pathogens difficult to control. Furthermore, unfavorable conditions (e.g., low temperature and poor nutrients) and the presence of emerging contaminants (e.g., pharmaceuticals and chemicals) in the reclaimed water can stimulate some pathogens to form biofilms. When external conditions change, such as turning on the tap water (i.e., change in hydrodynamic conditions) and heating (i.e., change in temperature), the attached biofilm containing pathogens may partially detach and be released into the water, thereby remaining a persistent risk of infection.⁹⁷

End users may face threats from airborne pathogens, such as SARS-CoV-2, *Mycobacterium tuberculosis*, and influenza viruses, due to the high infection capacity of these human pathogens and the poor ventilation characteristics associated with indoor spaces. Humans with pathogen infections can release micrometer-sized aerosols containing the pathogenic bacteria and viruses into the air, which can lead to the infection of nearby individuals. Additionally, fecal pathogens from human waste can be introduced into bioaerosols through toilet flushing, resulting in potential infection through accidental inhalation and skin contact (Figure 2E).^{98,99}

In summary, water has emerged as a critical vector for pathogen transmission. From the source of infection to the end user, waterborne pathogens can infect nearby people through drinking, inhalation, and even contact, thereby making infection ubiquitous and difficult to control. Additionally, the water environment provides opportunities for the convergence of pharmaceuticals, pathogens, and harmful genes (i.e., antibiotic-resistant and high-pathogenic genes), leveraging the emergence of high-risk pathogens, such as biofilm-protected and multidrug-resistant pathogens, posing a mounting threat to public health. Successfully identifying high-risk species, such as emerging viruses and resistant bacteria, also demonstrates the limitations of current methods for detecting and controlling environmental pathogens.

DETECTION OF ENVIRONMENTAL PATHOGENS AND PROSPECTS FOR FUTURE DEVELOPMENT

Low-level, high-risk pathogens that are present on complex waterborne matrices pose significant challenges for accurate and real-time detection (Figure 3A). With the significant developments in public health in the last decade, people are less likely to suffer from exposure to high levels of pathogens due to poor sanitation. For this reason, current high-pathogenicity microorganisms commonly exist in the environment at low-abundance levels and have a controllable infection potential. However, these low-abundance pathogens (typically <1,000 per m³ of water), in particular emerging viruses and resistant bacteria that have potential to lead to future epidemics and have been identified above, pose a challenge for detection due to the limited sensitivity of current conventional detection methods. Additionally, as a critical vector for pathogen transmission, water environments typically contain organic matter (1–3 mg-C/L of total organic carbon) and inorganic ions (100–400 mg/L), thereby making it difficult to concentrate the pathogen for accurate detection.¹⁰⁰ As an example, the PCR detection method in a water environment is often hindered by the high levels of background substances, leading to inaccurate results.¹⁰¹

Improved sanitation maintains waterborne pathogens at relatively low levels. For example, high-risk pathogenic viruses, such as rotavirus, norovirus, and hepatitis A, are typically present at low concentrations in water environments (<100 per m³ of water; Figure 3B).^{102–104} Some pathogenic bacteria, such as *Legionella* and *Salmonella*, have lower pathogenicity compared with the above viruses, and are present at higher concentrations (100–1,000 per m³ of water; Figure 3B).¹⁰⁵ Other opportunistic pathogenic bacteria, such as *Enterococcus faecalis* and *Escherichia coli*, can cause infection in susceptible children or elderly people and are generally present in water at higher levels (>1,000 per m³ of water).¹⁰⁶ Therefore, high-risk and low-level pathogens, in particular viruses, need special attention and, more importantly, accurate detection.

However, existing microbial detection technologies, such as cultivation and PCR techniques, struggle to provide accurate, real-time detection of low-abundance pathogens in complex aquatic environments. For example, the cultivation method can provide relatively accurate and cost-effective detection for live bacteria; however, it suffers from the drawbacks of being time intensive (typically requiring 24–48 h) and reliant on professional expertise (Figure 3C).¹⁰⁷ Furthermore, the cultivation method is only feasible for cultivable bacteria and several bacterial phages (e.g., coliphages MS2 and Φ X174), while it cannot specifically identify human viruses, which are often among the most concerning high-risk pathogens.

In contrast, the technology of PCR provides sensitive detection of all pathogens, including high-risk human viruses, with a reduced operating time (1–3 h; Figure 3C). In particular, quantitative PCR (qPCR) is able to detect both DNA (bacteria and viruses) and RNA (viruses).¹⁰⁸ The digital droplet PCR method is particularly effective at detecting low levels (several copies per L) of high-risk viruses with high sensitivity, such as SARS-CoV-2.¹⁰⁹ However, PCR relies on an amplification process to achieve accurate detection of the low-level target genes. One amplification cycle involves a series of previously controlled temperatures, and hundreds of cycles are needed to amplify enough genes for measurement. For this reason, the amplification process becomes the limiting factor in terms of speed, which makes PCR unsuitable for field deployment and miniaturization (Figure 3C). Additionally, a sample with a large volume of water (>1 L) is needed that contains sufficient pathogens for accurate PCR-based measurement. To be appropriate for PCR measurement, the collected water sample (several L) that contains the pathogens should be concentrated and reduced to less than 1 mL, and the pathogen genome needs subsequent extraction with a final volume of ~100 μ L (Figure 3D). This pretreatment process to achieve a >1,000-fold concentration of water can be time-consuming. The organics and ions present in the complex water matrix can potentially affect the PCR's accuracy and sensitivity.¹¹⁰

To address the low sensitivity and time-consuming limitations of environmental pathogen detection, emerging molecular biology techniques represent a promising route to improve the detection process. For example, the merging CRISPR-based gene editing method, is able to trim the target gene segment from the comprehensive genome of a low-level pathogen with improved sensitivity and increased signal for further detection (Figure 3E, left panel).¹¹¹ CRISPR can cleave non-target nucleic acids (i.e., trans-cleavage) as short single-stranded reporter oligos that can separate the fluorophore from the quencher, typically labeled at opposite ends of the reporter molecule, and generate measurable fluorescence signals without further amplification for fast detection.^{112–116} Compared with PCR's amplification-driven detection method, amplification-free CRISPR assays offer several key advantages: precise target recognition, programmable guide RNAs, and the ability to discriminate single-nucleotide mutations. These features enable the detection of pathogens with high specificity and minimal sample processing in complex water matrices. Furthermore, the loop-mediated isothermal amplification (LAMP) and recombinase polymerase amplification (RPA) methods can accelerate the gene amplification, relying on one fixed temperature (Figure 3E, right).^{117,118} Supported by the gene editing (CRISPR) and isothermal amplification (LAMP and RPA) methods, the enhanced molecular detection achieves a more accurate (as low as one copy/L) and rapid (30 min) detection of environmental pathogens in a complex water matrix (Figure 3F).^{117–122} However, while the techniques outlined above can only decode the high-risk genes of pathogens, they are unable to fully identify the infectious capacity at the protein level.

In contrast, multi-omics approaches, including metagenomics, metatranscriptomics, and metaproteomics, provide a comprehensive evaluation of pathogen infectious capacity (Figure 3G).^{123,124} By investigating the active pathogenic genes and proteins, multi-omics approaches have become indispensable as a route to understanding the pathogenicity and evolution of high-risk pathogens, such as SARS-CoV-2, norovirus, and hepatitis A.^{125–127} Additionally, the high-throughput evaluation of multi-omics approaches provides complete information on the bioactivity of the entire microbial community, which may reveal any undiscovered pathogenic dynamics.¹²⁸ However, multi-omics methods require sophisticated instrumentation, complex workflows, and skilled personnel, thereby limiting their potential application to comprehensive detection for low-cost, on-site applications throughout the pathogen transmission network. Therefore, portable devices that provide accurate, sensitive, and

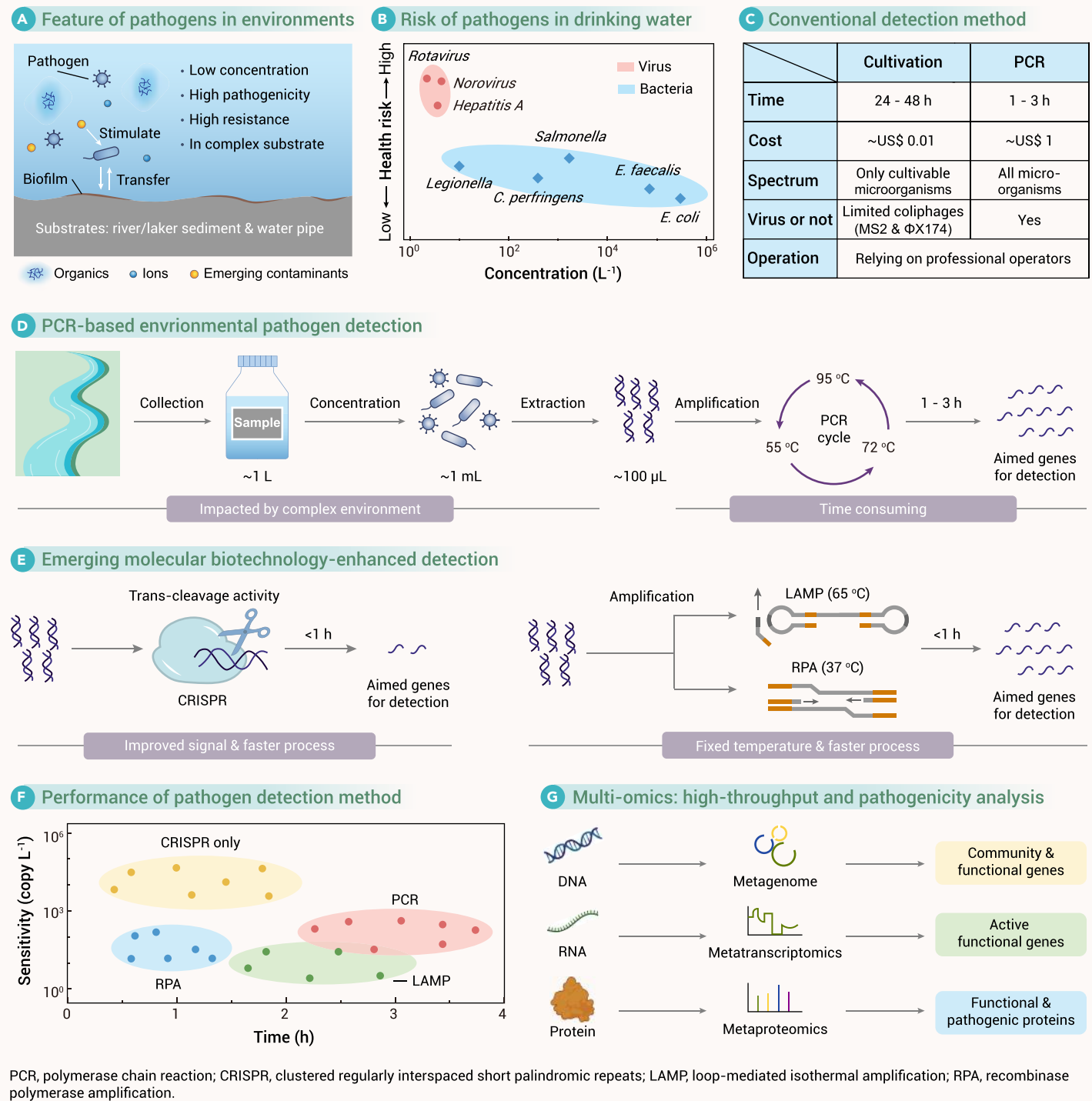


Figure 3. Limitations of current detection technologies for environmental pathogens and prospects for future detection (A) Characteristics of pathogens in the environment posing challenges for rapid and accurate detection. (B) Risk and concentration of typical pathogens in drinking water. (C) Limitations of conventional detection methods for environmental pathogens. (D) Schematic of the PCR-based method for environmental pathogen detection and identification of its potential inaccuracy and time-consuming reasons. (E) Schematic of emerging gene editing (CRISPR) and amplification (loop-mediated isothermal amplification [LAMP] and recombinase polymerase amplification [RPA]) methods designed to enhance pathogen detection. (F) Performance summary of detection methods for environmental pathogens. (G) Characteristics of multi-omics approaches for analysis of environmental pathogens.

real-time detection are essential for on-site applications, in particular in decentralized high-risk locations. For example, portable PCR and rapid lateral flow detection devices can effectively identify pathogenic bacteria and viruses, including *Escherichia coli*, SARS-CoV-2, Zika virus, and dengue virus.^{24,35,129} Additionally, fluorescent and electrochemical sensors are emerging as promising technologies for real-time, on-site pathogen detection, reducing the reliance on laboratory-based investigation.¹³⁰ The two most commonly used types of sensors are biosensors and microfluidic sensors. Both are highly sensitive and specific to pathogens, enabling the direct detection of low levels of bacteria

and viruses without pretreatment. These portable, miniaturized sensors can be integrated with digital technologies, such as smartphones, making them highly accessible for non-experts and technicians to use—particularly in rural areas, where infectious diseases are prevalent.¹³¹

In summary, the future of environmental pathogen detection requires a comprehensive system that enables accurate, real-time monitoring throughout the transmission network. The molecular technology-enhanced PCR method, with its improved sensitivity and speed, is necessary for routine, accurate, broad-spectrum pathogen monitoring. A set of rapid, low-cost, portable

detection devices for real-time, on-site pathogen detection is also essential at each high-risk location. The multi-omics approaches that are able to decode high-risk active genes and functional proteins are also critical to fully understanding the infectious capacity of environmental pathogens. Therefore, combining continuous, on-site monitoring data, such as pathogen concentration, with metagenomic sequencing information, including pathogen infectious capacity, is a promising approach to evaluate the risk posed by environmental pathogens. However, research in this area is currently limited.

CONTROL OF ENVIRONMENTAL PATHOGENS FROM A TECHNO-ECOLOGICAL PERSPECTIVE

Disinfection remains the cornerstone of public health defense against high-risk, low-level, and easily transmissible environmental pathogens. Current disinfection technologies, including both conventional and emerging techniques, are feasible methods for inactivating microorganisms and are fit for specific application scenarios (Figure 4A). For example, chlorination is the most common disinfection method due to broad-spectrum efficacy, cost-effectiveness, and long-lasting residual activity during the subsequent water distribution process. The free chlorine can effectively disrupt the microbial outer structure (e.g., bacterial cell walls/membranes and viral capsids) and inclusions (e.g., proteins and genome) through a series of oxidation reactions.⁴⁴ This broad-spectrum disruption, directed at multiple pathogen targets, ensures the effective inactivation of bacteria (Gram-positive and Gram-negative) and viruses (even envelope protected).

However, in addition to pathogen inactivation, the dosed chlorine will inevitably react with complex substrates in the water environment, such as humic acid, pharmaceuticals, and other coexisting trace chemicals, thereby resulting in the formation of harmful disinfection byproducts (DBPs).^{43,44,132} The generated chlorine-based DBPs, including trihalomethanes, haloacetic acids, and haloacetamide, exhibit carcinogenic or toxic properties and have been strictly controlled by drinking water standards worldwide (concentration commonly less than hundreds of $\mu\text{g/L}$).¹³² Additionally, chlorination use is primarily centralized, relies on continuous chemical inputs, and is sensitive to operating conditions and water quality, such as temperature, pH, and organic matter concentration. These limitations hinder the use of chlorination to ensure environmental pathogen control throughout the transmission network, in particular at any decentralized location.

In contrast, UV disinfection, which typically uses a low-pressure mercury lamp to generate 254 nm UV light, can induce dimerization between two adjacent thymine bases in the same DNA strand to inhibit microbial replication and proliferation (Figure 4A).⁴⁵ UV disinfection is highly effective against a broad spectrum of pathogenic bacteria (Gram-positive and Gram-negative). Furthermore, since this process does not use any external chemical input, the physics-based UV disinfection involves no DBP formation. However, UV disinfection can stimulate the treated bacteria in a viable but non-culturable (VBNC) state.^{133,134} After UV disinfection (254 nm), some treated bacteria in the VBNC state may recover by repairing the dimerization of thymine in the presence of a favorable environment (e.g., light and rich nutrients) during subsequent distribution or discharge to natural water. Additionally, UV disinfection exhibits a relatively lower efficacy against envelope-protected viruses, and the use of 254 nm UV light is also less effective against RNA viruses due to the absence of thymine in the RNA strand. To address the inherent limitations of 254 nm UV disinfection, researchers have designed novel light sources based on emitting alternative wavelengths, such as 222, 260, and 280 nm. In addition to DNA, these wavelengths can disrupt other key microbial components (e.g., RNA, lipids, and proteins), thereby effectively suppressing bacterial reactivation and improving viral disinfection efficiency.¹³⁵ Another challenge for UV disinfection is that water turbidity and suspended particles can shield pathogens from radiation exposure and inhibit disinfection efficiency. As a result, turbid or particle-rich water may require pretreatments, such as coagulation and filtration, to ensure effective UV performance.

Ozonation is another conventional disinfection method that demonstrates broad-spectrum operation and superior efficacy against bacteria and viruses by using highly oxidizing ozone (O_3) molecules (Figure 4A).¹³⁶ The O_3 molecule is able to damage various microbial components, including peptidoglycans (the main component of the cell wall), lipids, proteins, and genomes. In particular, ozonation can effectively inactivate low-abundance and high-risk species in wa-

ter matrices, such as highly pathogenic viruses and biofilm protected or ARB. However, despite its superior disinfection performance, ozonation is energy intensive and is primarily suited to centralized applications. Additionally, the O_3 molecule is able to oxidize trace bromide ions in natural water and form bromate, a carcinogenic and harmful DBP, which is subject to strict regulation ($<10 \mu\text{g/L}$) by drinking water standards.¹³⁷

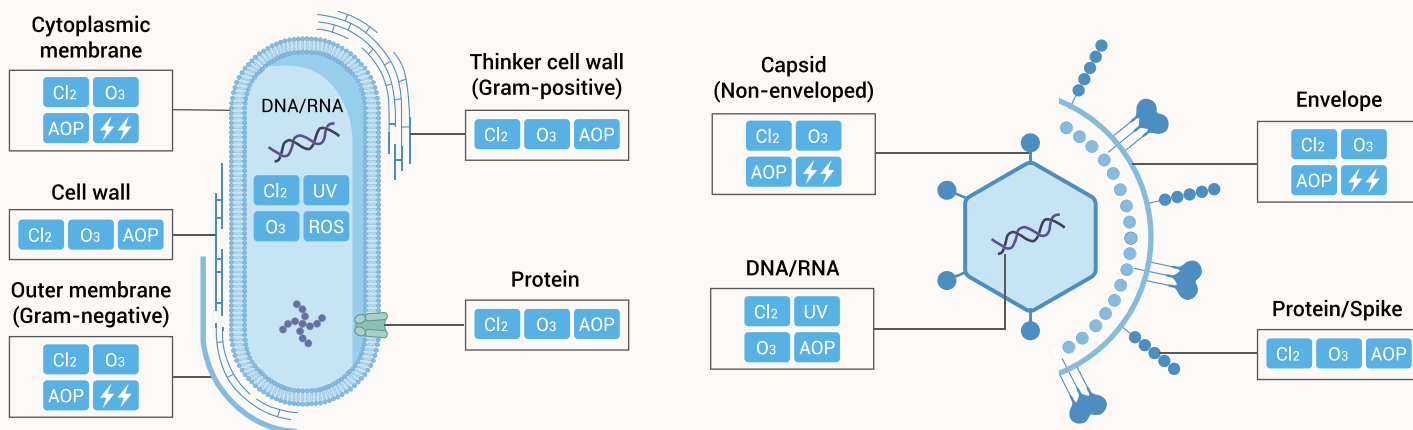
Conventional disinfection methods demonstrate efficacy for pathogen inactivation under certain conditions (Figure 4B). However, these disinfection processes, which are primarily designed for centralized applications, face the challenge of needing to control all pathogens throughout the whole transmission network at any decentralized and high-risk contamination sites. For example, agricultural drainage pollution from point sources containing considerable pathogens and ARB can have a significant impact on the raw wastewater. Additionally, the complex water substrate (e.g., organics and ions) potentially compromises the conventional disinfection performance (Figure 4B). A compromised disinfection efficacy can result in the survival of high-risk, resistant pathogens (e.g., enveloped viruses and biofilm protected or ARB), further threatening public health.

Accordingly, emerging AOPs are feasible for decentralized application scenarios. AOP disinfection methods employ a peroxy-, photo-, or electrified-based process to generate highly reactive oxygen species (ROS), such as hydrogen peroxide (H_2O_2), hydroxyl radicals ($\cdot\text{OH}$), and singlet oxygen ($^1\text{O}_2$).^{138,139} The formation of *in-situ*-produced ROS can induce extensive oxidative damage to a range of bacterial and viral components in small facilities or reactors. Thus, AOP-based disinfection provides broad-spectrum microbial inactivation against high-risk and resistant pathogens and is potentially feasible for decentralized applications. However, AOP-based disinfection methods require an external chemical or electrical input and suffer from relatively high operation costs, making their use for decentralized applications at each high-risk site impractical. Additionally, coexisting ions and organics in the complex water matrices may impact the AOP disinfection performance through competitive reactions with the generated ROSs. These limitations result in insufficient efficiency for near-complete microbial inactivation due to any unexpected water quality fluctuations that often occur during the decentralized treatment (Figure 4B).

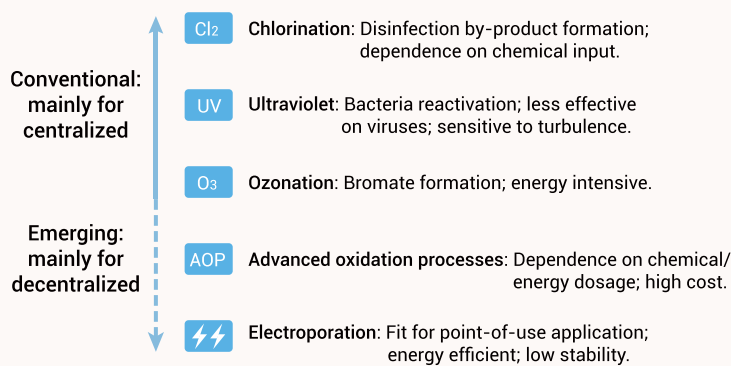
Electroporation can provide reliable water disinfection by generating strong electric fields ($>10^7 \text{ V/m}$) to disrupt bacterial membranes and viral capsids for broad-spectrum disinfection (Figure 4B).¹⁴⁰ The physics-based electroporation method can effectively reduce the required external voltage (as low as 1 V) by exploiting one-dimensional (1D) nanomaterials (e.g., nanowires and nanorods) to generate strong local electric fields at their tips.¹¹² Therefore, the process of nanomaterial-assisted electroporation enables complete disinfection coupled with extremely low energy consumption (as low as $10^{-4} \text{ kWh m}^{-3}$), which can utilize off-grid energy sources such as solar panels, wind turbines, and nanogenerators, without any reliance on grid electricity.^{21,141} Additionally, reaction-free electroporation that relies solely on the use of strong electric fields exhibits superior performance over a wide range of water qualities. For this reason, the application of chemical-free and energy-efficient electroporation is ideally suited for decentralized disinfection at each high-risk location throughout the pathogen transmission network of complex water matrices. However, the use of 1D nanomaterials on electroporation electrodes may raise concerns with regard to the release of harmful materials into water. Therefore, the fabrication of stable and scalable nanomaterial-assisted electrodes is the primary concern for the practical application of electroporation.

In addition to environmental conditions and specific application scenarios that affect disinfection efficacy, current disinfection methods suffer from inaccurate evaluation due to their reliance on indicating microorganisms (Figure 4C). The most commonly used indicators, such as total coliform and *Escherichia coli*, are able to indicate the potential presence of enteric bacteria, including *Salmonella* and *Shigella*, in particular when fecal pollution originates from human or animal sources. While these "indicator" bacteria primarily represent the potential bacterial risk from fecal contamination, they are unable to reflect a broader indication of water/foodborne and respiratory pathogens for particular viruses (e.g., norovirus and adenovirus) and protozoan (e.g., *Cryptosporidium* and *Giardia*).¹⁴² The evaluation of disinfection performance based solely on cultivable fecal bacteria leads to an underestimation of the overall pathogenic threat to human health. Therefore, there is an urgent need for

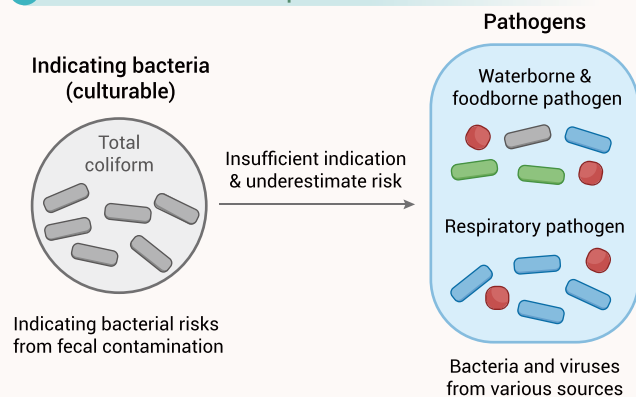
A Disinfection of microorganisms: covered by various mechanisms



B Disinfection methods: fit for purpose is the best



C Current indicator: unrepresentative and inaccurate



D Pathogen control: techno-ecological synergies in multiscale transmission networks

1. Source water protection

- Evaluation**
- Indicator for pathogen
 - Indicator for viruses
 - Scenario-based indicator



2. Agriculture runoff control



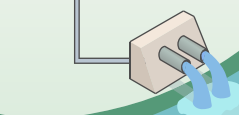
3. Enhancing ecology resilience

- System control**
- Ecology protection
 - From source to end user multi-barrier protection

4. High-standard wastewater collection and treatment



- Technology**
- Effective & cost-effective
 - Safe & broad-spectrum
 - Fit for multiple scenarios



5. End control as last defense



Figure 4. Environmental pathogen control from a techno-ecological synergistic perspective (A) Structure and components of bacteria (left) and viruses (right) primarily associated with pathogenic disease. Disinfection methods susceptible to each microbial component are summarized. (B) Summary of conventional and emerging disinfection methods and their application scenarios and challenges. (C) Schematic of current indicating bacteria representing fecal contamination, but inadequate for broader indication of water/foodborne and respiratory pathogens. (D) Schematic of environmental pathogen control throughout the transmission network by bridging ecological resilience to reduce pathogen introduction and the development of decentralized disinfection as multiple safety barriers.

more scientific microbial or molecular indicators that are directly relevant to human health to accurately evaluate disinfection performance to serve as an early warning signal of known pathogens, or even emerging or unrecognized microbial threats.

As the water environment is a critical vector for pathogen transmission, the control of pathogens in water must fully consider the complex transmission network, from sources of infection to high-risk locations and end users, thereby providing multiple safe barriers. Although current disinfection methods provide broad-spectrum disinfection for various application scenarios, their application throughout the complete pathogen transmission network has challenges due to their initial centralization design. For this reason, emerging decentralized disinfection methods are necessary for high-risk locations. However, reliance on disinfection alone cannot fully eliminate pathogen containment, and a resilient ecosystem with improved ecological function and self-cleaning ability can ultimately reduce pathogen involvement and growth.

Here, we propose a techno-ecological synergistic perspective for environmental pathogen control in a systemic aspect that can ultimately eliminate pathogen risk (Figure 4D). Our techno-ecological protection approach effectively bridges ecological resilience to reduce pathogen introduction and fit-for-purpose disinfection systems to achieve multi-barrier pathogen inactivation. The protection system begins at the water source, where farm manure and untreated fecal sewage can introduce pathogens. Through feasible farming and domestic wastewater collection, the protection of the source water can minimize the introduction of pathogens at the very beginning of the water environment and ensure water safety for people living downstream. Pathogen control systems, including farm waste management, enhanced disinfection for safe wastewater irrigation, and construction of viable drainage systems, enable appropriate management of agricultural pollution, in particular from farm fertilizers or wastewater irrigation.

Ecological restoration of the entire water environment is essential for pathogen control, which can enhance the ability of the ecosystem to self-clean for filtering and neutralizing pathogens (Figure 4D).¹⁴³ Techniques such as developing riparian buffers (i.e., transitional areas between land and water to block pathogen involvement) and constructing wetlands contribute significantly to pathogen attenuation. These ecological remediation methods can reduce nutrient levels (e.g., organic matter, nitrogen, and phosphorus) in the watershed, which limits the bioactivity of surviving pathogens. In particular, plants in the constructed wetland produce antimicrobial allelochemicals that eliminate bacteria and algae, reducing the microbial concentration of the source water and lowering the disinfection burden on the drinking water treatment system. The riparian buffers also effectively block turbidity in source water, ensuring the efficiency of UV disinfection in drinking water treatment.

In urban areas, diverse and massive pathogen sources, such as municipal and hospital wastewater, can introduce pathogens and increase the risk of infection due to their close association with human activities. Therefore, an effective wastewater collection and high-standard treatment system is critical for the subsequent disinfection treatment of any pathogens in the water (Figure 4D). Once the collected pathogens are in the municipal wastewater treatment plant, high-efficiency centralized disinfection technologies, such as high-performance UV and ozonation treatments, can inactivate pathogens to eliminate the risk of infection.¹⁴⁴ However, complex and energy- or chemical-intensive disinfection methods are predominantly designed for centralized treatment and cannot interrupt the entire pathogen transmission network at each high-risk site.

At high-risk locations and end users, the use of decentralized disinfection using emerging methods (i.e., AOP and electroporation disinfection) can serve as the last line of defense against waterborne pathogens just before drinking or use (Figure 4D). Without any reliance on external chemical or energy inputs, the ideal decentralized disinfection technologies (e.g., electroporation) are feasible for multiple point-of-use applications that range from community to personal use, such as water tanks, tap water filters, and portable disinfectant bottles. The successful integration of such decentralized systems into the broader disinfection framework offers a critical layer of protection, effectively bridging the gaps left by centralized infrastructure and enabling comprehensive coverage throughout the pathogen transmission network. The efficacy of the

techno-ecological synergistic system can be assessed by examining infection rates among residents and expenditure on diarrheal medication.

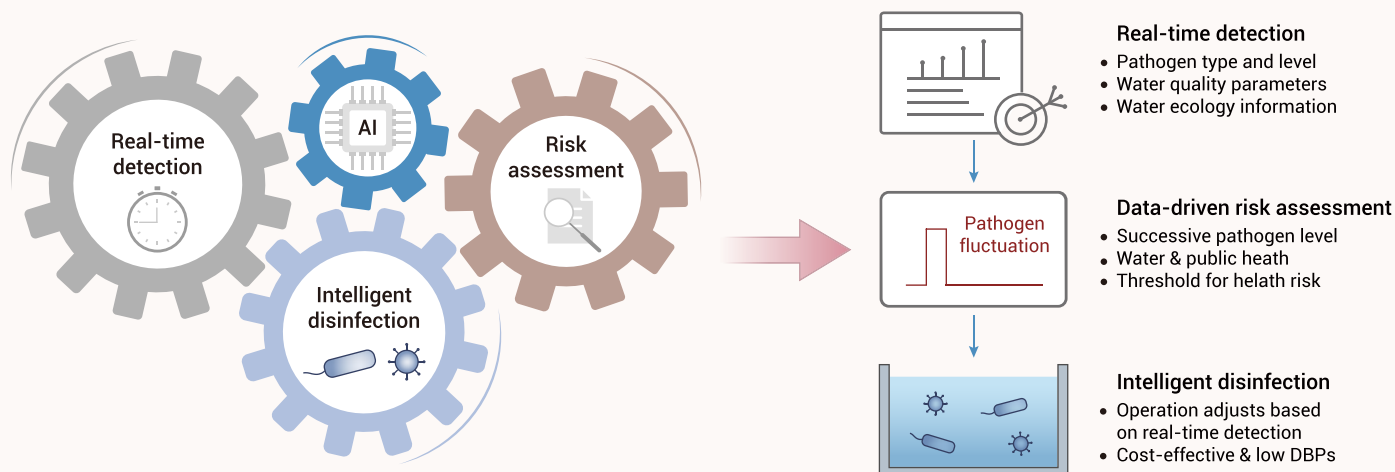
In summary, our techno-ecological perspective promises an innovative pathway to protect against environmental pathogens. However, it requires an improvement and adaptation of current pathogen assessment and disinfection methods to meet the new needs of this synergistic approach to pathogen control (Figure 4D). First, there is a need to introduce more scientific and accurate indicators related to human health directly, in particular pathogenic viruses, which is essential for risk evaluation. As a result of the relatively stable containment sources and environmental conditions, a scenario-based indicator could be selected within one specific application situation, allowing for accurate targeting of pathogens for early warning detection. Additionally, pathogen disinfection methods must be highly efficient, cost-efficient, and, most importantly, versatile across a range of applications to address various microbial threats, which include agricultural runoff, wastewater discharges, and emerging pathogens in urban wastewater systems. Implementing novel decentralized disinfection, such as AOPs and electroporation, could provide more flexible and robust protection for point-of-use applications. Combining decentralized methods with conventional municipal disinfection can also provide a multi-barrier protection system to mitigate pathogens from their source to the eventual end users. Furthermore, ecological protection through developing riparian zones and constructing wetlands is also necessary to eliminate any pathogens introduced into the environment and mitigate against residual pathogen levels before eventually reaching any end users.

DATA-DRIVEN PATHOGEN CONTROL AND CLIMATE CHANGE EPIDEMIC CONCERNS

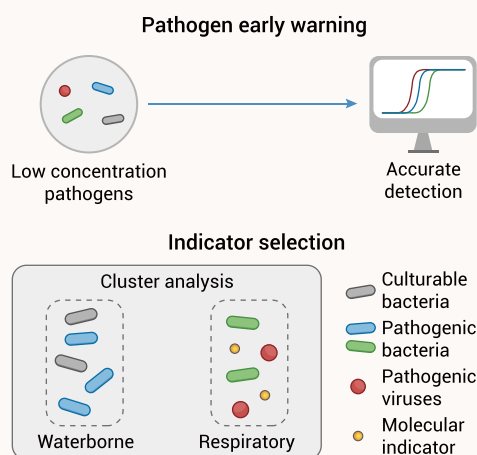
Current discrete detection methods can impede real-time pathogen risk assessment, thereby creating a disconnect between the detection and disinfection processes. This forces the disinfection process to rely on empirical approaches, rather than any scientific guidance from real-time surveillance data. Recent advances in data-driven technologies offer new opportunities to achieve intelligent, detection-guided disinfection (Figure 5A). The principle of data-driven pathogen control first involves regular baseline sampling (e.g., several times per year) using cultivation, qPCR, and multi-omics methods to determine pathogen species, general concentration, and infection capacity. After identifying locations where pathogens are likely to occur and transmit, point-specific and emerging detection technologies such as molecular technology-enhanced PCR and portable sensors are deployed to collect high-frequency data on pathogen abundance and activity within 30 min. The application of data-driven technologies, including artificial neural networks and machine learning approaches, is then able to convert these discrete, high-frequency detection events into a continuous temporal profile, thereby enabling rapid identification of baseline deviations caused by any pathogen containment.^{145,146} This integrated system aligns monitoring frequency with risk level, minimizing unnecessary disinfection and enhancing responsiveness by involving computational decision-making tools. For example, point-of-use disinfection can be activated automatically when pathogens are detected.

Real-time, continuous pathogen detection, made possible by data-driven technology, can optimize disinfection operations (Figure 5A). These data-driven technologies can guide disinfection processes by using successive pathogen risk assessments to dynamically adjust operating parameters, including chemical dosage, UV intensity, and contact time.¹⁴⁷ Disinfection performance can provide further feedback to optimize operation and, thus, this enhanced feedback capability can achieve real-time decision-making for reliable and robust pathogen inactivation. Data-driven algorithms can also continuously refine operational strategies based on historical data and real-time feedback, allowing the disinfection system to better adapt to environmental variability. Furthermore, an intelligent, detection-guided disinfection system can also minimize chemical and energy use by avoiding the overuse of disinfectants during low-risk periods. Therefore, a detection-guided disinfection system is ideally suited for a wide range of needs, in particular where conventional disinfection methods are unable to provide complete protection. For example, UV disinfection can automatically increase irradiation levels or extend treatment times in response to increased turbidity in the influent. Similarly, in point-of-use applications where water conditions and treatment requirements fluctuate considerably, the data-driven feedback system can adjust operating conditions in real time based on

A Data science-driven environmental pathogen control



B Data science-driven water surveillance



C Climate change-induced high-risk pathogens

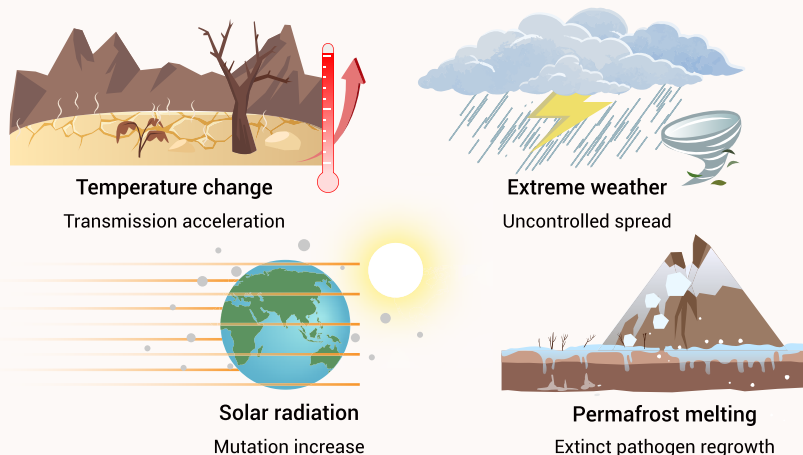


Figure 5. Prospects for pathogen detection and control using data-driven technologies and highlights for emerging high-risk pathogens due to climate change (A) Schematic of pathogen control systems using detection-guided disinfection supported by discrete data fitting and real-time decision-making based on data-driven technologies. (B) Prospects for data-driven water surveillance for pathogen early warning and accurate indicator selection. (C) Highlights of emerging high-risk pathogens due to climate change for potential epidemics.

the detection of water quality and pathogens in both the source water and the effluent.

Data-driven technologies can also improve microbial surveillance in water, with transformative potential to leverage the comprehensiveness and accuracy of pathogen detection and the specificity of indicator selection (Figure 5B). Conventional water surveillance approaches rely on low-frequency sampling and limited microbial targets, thereby making detecting low-level emerging pathogens inadequate. Since pathogens are continuously entering the water environment through multiple pathways, the complex transmission network contributes to a mixture of microorganisms of varying abundance, infectivity, persistence, and host specificity. Within this network, the early detection of pathogens is essential, particularly to identify any high-risk viruses and resistant bacteria present at concentrations below the regulatory thresholds, but may still pose a significant health threat.¹⁴⁸ For this reason, data-driven technologies are able to capture the metadata of the microbial community and ecosystem to accurately and agilely identify any potential high-risk pathogen and, more importantly, trace the source of the contaminant for early control. Accurately identifying high-risk pathogen can significantly contribute to policy-making regarding where to apply suitable point-of-use disinfection with optimized operational conditions. Moreover, algorithmic signal processing allows water surveillance to move beyond conventional fixed health thresholds to a continuous risk model based on pathogen fluctuation trends, enabling more scientific and accurate pathogen control.¹⁴⁹

Furthermore, the judicious use of clustering and classification algorithms is also promising for scientific and accurate selection of pathogen indicators by incorporating the metadata of pathogen bioactivity, transmission dynamics, ecological information, and public health (Figure 5B). In addition to conventional indicators (i.e., culturable fecal bacteria), clustering and classification algorithms are able to accurately identify any indicators (e.g., related bacterial species) of the pathogenic bacteria, such as *Salmonella* and *Legionella*. The readily detectable indicating bacteria that are selected typically coexist with the target pathogenic bacteria, with a strong correlation, and can represent the relative level and risk of the high-risk bacteria. For example, *Clostridium perfringens* is a widespread pathogen that causes waterborne and foodborne diseases. It is strongly associated with fecal contamination from animals and can be detected through selective cultivation, allowing for a representative indicator of enteric diseases (e.g., dysentery, diarrhea, and enteritis).

In contrast to pathogenic bacteria with a complete cell structure and bioactivity, pathogenic viruses, such as enteric and respiratory viruses, are difficult to identify using indicating bacteria. Accordingly, data-driven technologies can further enhance the selection of viral indicators based on microbial and ecological information, in particular in a specific application scenario with a relatively stable source of pathogen contamination. Furthermore, the clustering of readily detectable biomarkers linked

to pathogen metabolism (e.g., endotoxins) and the host response (e.g., procalcitonin and interleukin-6) also enables accurate and early warning detection of pathogens.¹⁵⁰ These biomarkers, if detected in water, can serve as an early warning for emerging pathogenic activity, even in the absence of known genomic signatures (i.e., unknown environmental pathogens). Although the newly identified indicator offers the advantage of rapid detection, it must be closely linked to specific diseases and environments. In practice, both new and conventional indicators are necessary in the initial stage for cross-validation. Only when the new indicator exhibits a more accurate and specific correlation with the targeted diseases can its future application be considered feasible. In summary, data-driven technologies are able to select pathogen indicators that are directly relevant to human health, thereby overcoming the unrepresentative limitations of current fecal bacterial indicators and allowing for real-time evaluation and precise early control.

Anthropogenic climate change has become a major driver in the evolution, mutation, and global spread of high-risk pathogens, reshaping the epidemic potential of recent decades (Figure 5C).^{48,151} In particular, global warming can accelerate the transmission of environmental pathogens by leveraging the microbial replication and infection ability by enhancing the host-pathogen interaction.¹⁵² This requires intensive disinfection for comprehensive protection. Our warming climates also enable the geographic expansion of waterborne pathogens into previously unsuitable regions, thereby increasing population exposure. With a higher transmission efficiency and persistent colonization, an increase in temperature also influences biofilm formation, antibiotic resistance expression, and the viability of enteric pathogens in water environments.¹⁵³

More frequent extreme weather events, another consequence of human-induced climate change, can significantly accelerate the transmission of pathogens and threaten public health. Extreme weather events, such as intense rainfall, flooding, and hurricanes, disrupt the domestic water treatment facilities and ecosystem, leading to the uncontrolled spread of pathogens across environmental and infrastructure boundaries. For example, intense rainfall can overwhelm the combined sewer system, causing runoff to mix with untreated sewage and discharge directly into the water environment, requiring highly efficient pathogen prevention technology, especially for point-of-use application.¹⁵⁴ Therefore, future pathogen control systems must adapt to emerging, climate-induced pathogen threats. This requires incorporating temperature fluctuations, extreme weather conditions, and local health surveillance data from precise weather forecasts, hydrological models, and public health databases. Additionally, extreme weather events have the potential to reduce ecological resilience as a result of damage to vulnerable ecosystems.

Climate change-induced stratosphere O₃ depletion may also increase the intensity of solar radiation, resulting in higher microbial mutation rates.¹⁵⁵ The increased solar radiation levels elevate the potential of viral adaptive evolution and cross-species transmission. Furthermore, it can induce stress responses and genetic recombination in bacteria, leveraging horizontal gene transfer capacity. Additionally, permafrost thawing due to climate change has the potential to release long-dormant or previously extinct pathogens, posing a significant threat by reintroducing pathogens with unknown virulence or antibiotic/disinfection resistance profiles into contemporary ecosystems.¹⁵⁶ Documented cases of *Bacillus anthracis* and poxvirus reactivation in thawing regions underscore the public health implications of permafrost degradation as a result of global warming scenarios.

PROSPECTS

Promoting the rational detection and disinfection of environmental pathogens will contribute to universal and equitable access to a safe and disease-free environment for humankind. However, pathogens in our environment exhibit considerable diversity in terms of species and size ranges, with an ability to form protective structures, thereby resulting in complex and difficult-to-block transmission pathways. Human activities and environmental pollution have further accelerated the emergence and spread of high-risk pathogens, making them ubiquitous in a range of waterborne matrices. As a result, it is critical to implement effective detection and control strategies that are able to target

the entire transmission network of low-level, high-risk pathogens within complex environmental matrices. Conventional pathogen detection methods are currently time-consuming, labor intensive, and rely on manual procedures, making them inadequate for real-time and on-site monitoring throughout the complex transmission network. Additionally, current microbial disinfection technologies are predominantly centralized, are insufficient to inactivate pathogens at each high-risk location, and are therefore unable to eliminate the continuous spread of pathogens.

In this review, we propose a real-time, on-site detection and synergistic techno-ecological protection system that is designed to mitigate against environmental pathogens throughout multiscale transmission networks. By exploiting accurate and rapid detection methods (e.g., emerging molecular technologies and on-site sensors), researchers are able to assess the level and bioactivity of pathogens in complex environments (Table 1). When combined with conventional centralized disinfection methods, the emerging, highly efficient, and cost-effective point-of-use disinfection technologies are able to provide multi-barrier protection from a range of sources, high-risk locations, and end users. Furthermore, the development of a resilient ecosystem with improved ecological function and self-cleaning ability can mitigate pathogen involvement and growth. The techno-ecological protection system that bridges multi-barrier protection with ecological resilience achieves a synergistic effect to enable comprehensive and reliable pathogen control, particularly in locations closely linked to human activities.

Additionally, data-driven technologies enable successive, accurate water surveillance for early disease warning and intelligent detection-guided disinfection for highly effective pathogen inactivation. Advancements in data-driven methods can also select pathogen indicators that are directly relevant to human health, thereby overcoming the current unrepresentative limitations of the fecal bacterial indicator. Finally, we highlight concerns related to the most likely causes of potential pandemic outbreaks due to the emergence of mutant viruses and resistant bacteria that are likely induced as a result of climate change.

However, implementing accurate, real-time detection and techno-ecological protection throughout the environmental pathogen transmission network still remains challenging. The challenge of optimizing the trade-off between portable on-site detection and laboratory quantification of active pathogenic genes/proteins requires further exploration: while on-site detection is necessary to investigate real-time pathogen levels, it cannot fully replace PCR and multi-omics approaches that can understand pathogenic infection information. Additionally, current disinfection facilities are typically implemented in isolation, without sharing treatment efficacy information, making it difficult to achieve accurate disinfection throughout the overall system. As a result, a metadata-sharing platform is needed to communicate all the disinfection facilities throughout the transmission network. If the upstream disinfection is insufficient for complete inactivation, the downstream disinfection will increase the operational dose for additional protection. The disinfection effectiveness information platform takes full advantage of multi-barrier protection and is able to reduce disinfectant dosage with lower carcinogenic, harmful byproduct formation while maintaining complete pathogen inactivation. Finally, techno-economic and life cycle analyses can examine the costs and environmental impacts of proposed pathogen detection and control methods and help design the most sustainable integrated system. As a result, more effort is needed to provide financial support and knowledge transfer of techno-ecological pathogen control methods to low- and middle-income countries to address the global challenge of pathogen spread and infection.

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AUTHOR CONTRIBUTIONS

Z.-Y.H. and F.W. developed the concept. M.T., Z.-Y.H., and Z.Z. prepared the figures and co-wrote the paper. M.X., C.R.B., and Z.Y. revised the manuscript. X.W., Q.W., W.Z., and G.L.

Table 1. Summary of detection and control technologies for environmental pathogens

Prevention methods	Mechanisms	Advantages	Disadvantages	Point of use?
Detection				
culturing method	detection of cultivable fecal bacteria and coliphages	cost-efficient easy operation	unrepresentative time intensive operator dependent	primarily no
conventional PCR	detection targeted genes of pathogens	sensitive broad spectrum	cost intensive impacted by water quality operator dependent	no
isothermal amplification (LAMP and RPA)	gene editing/amplifying for enhanced PCR detection	rapid and sensitive broad spectrum	Cost intensive operator dependent	no
multi-omics method	study DNA, active RNA, and functional proteins of pathogens	high-throughput evaluation active infectious capacity	time/cost intensive operator dependent	no
biosensor	fluorescence, electrochemistry, and paper-based detection	easy operation real-time and on-site cost-efficient	impacted by water quality limited throughput	yes
Disinfection				
chlorination	free chlorine disrupts the microbial components	broad spectrum cost effective persist inactivation	DBP formation impacted by water quality	primarily no
UV	DNA damage (254 nm) lipid and protein disruption (222, 260, and 280 nm)	rapid no DBP formation	induce VBNC bacteria reactive after treatment less effective to viruses energy dependent	primarily no
ozonation	O ₃ oxidizes various microbial components	high efficacy broad spectrum	energy/cost intensive bromate formation	no
AOP disinfection	producing ROS to damage microbial components	high efficacy broad spectrum feasible for various applications	chemical or energy dependent impacted by water quality	yes
electroporation	local electric fields to disrupt bacterial membranes and viral capsids	high efficacy broad spectrum no DBP formation	potential material release	yes
Ecological restoration				
riparian buffer	transitional areas between land and water to block pathogen involvement	eco-friendly broad spectrum energy/chemical-free	N/A	no
constructed wetland	ecological function and self-cleaning ability to eliminate pathogen growth	eco-friendly broad spectrum energy/chemical-free	N/A	no

PCR, polymerase chain reaction; LAMP, loop-mediated isothermal amplification; RPA, recombinase polymerase amplification; DBP, disinfection byproduct; VBNC, viable but non-culturable; AOP, advanced oxidation process; N/A, not applicable.

provided comments on the manuscript. All authors contributed to the manuscript and approved the final version.

DECLARATION OF INTERESTS

The authors declare no competing interests.

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