

Current microbiological challenges in drinking water

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ABSTRACT

Drinking water (DW) is paramount to human health, serving as a cornerstone of public health worldwide. However, DW is not a sterile product and can harbor a large diversity of microorganisms, including pathogens. This comprehensive review addresses the critical importance of DW for human health and the ongoing challenges posed by microbial pathogens and biofilms in water distribution systems. It further analyzes the growing challenges driven by contemporary factors such as antimicrobial resistance, biofilms, climate change, and micropollutants. Biofilms, in particular, remain underestimated in conventional water treatment processes despite their significant contribution to microbial contamination, the formation of disinfection by-products, and disinfectant resistance. Emerging challenges, such as climate change and micropollutants, have become significant concerns due to their profound impact on microbial communities and their role in shaping biofilm formation. This review highlights the key microbiological threats to DW distribution systems, focusing on the role of biofilms and the emerging challenges posed by climate change. It explores the factors promoting biofilm development, including water composition, pipe materials, and treatment strategies. It also explores the limitations of current water treatment strategies, which often fail to address biofilms effectively, and highlights the need to integrate microbiological considerations into water quality management. This review aims to underscore the urgent need to reassess water treatment and management practices to address current microbiological challenges and ensure the delivery of safe and sustainable DW.

1. Introduction

Water, the essence of life, is a fundamental resource indispensable for human survival and well-being [1]. However, its contamination by microbial pathogens presents a persistent and frightening challenge to public health worldwide [2]. Within the vast array of pathogens that inhabit water sources, emerging and reemerging pathogens stand out as particularly concerning entities [3]. These pathogens, spanning bacteria, viruses, fungi, and parasites, have the potential to incite widespread illness and outbreaks if not effectively monitored and controlled [3]. Notifiable diseases associated with waterborne transmission, underscore the critical importance of robust disease surveillance and intervention strategies [4].

Drinking water distribution systems (DWDS), while designed to

deliver potable water, often harbor complex microbial communities, namely biofilms [5–7]. These biofilms, consisting of microbial cells embedded in a self-produced extracellular matrix, adhere to pipe surfaces and significantly impact water quality. Some negative effects on DWDS include biocorrosion and increased tolerance to disinfection [8]. A potential consequence of the interplay between these issues is the formation of disinfection by-products (DBPs). Specifically, corrosion products from the piping network and organic matter, including biofilm molecules, can significantly affect the effectiveness of disinfectants and promote the formation of DBPs [9–11]. While evidence of this relationship is still emerging, current models predicting the formation of DBPs during water treatment often underestimate the role of biofilms [12,13].

Adding to these challenges, climate change exacerbates microbial

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proliferation in aquatic environments. Rising temperatures, altered precipitation patterns, and extreme weather events create favorable conditions for biofilm formation and the dissemination of antimicrobial resistance (AMR) [14]. Also, micropollutants have increasingly become a major concern in DWDS. These trace contaminants, originating from diverse sources, persist through conventional treatment processes and can significantly impact biofilm formation, alter microbial diversity, and facilitate the spread of antimicrobial resistance genes [15,16]. Among them, microplastics serve as novel surfaces for microbial colonization, further promoting biofilm development and potentially harboring opportunistic pathogens [17]. These interrelated issues demand a deeper understanding of the environmental and infrastructural factors driving microbial dynamics in DWDS.

This review addresses the multifaceted challenges associated with microbiological stability and safety in DW, with a particular focus on the role of biofilms. It explores biofilm formation and persistence, their contributions to biocorrosion and DBP production, and their function as reservoirs for opportunistic pathogens. Recognizing the complexity of these issues, the review emphasizes the need for interdisciplinary collaboration and adaptive management strategies to safeguard water quality and public health amid evolving environmental pressures. By synthesizing current knowledge and identifying research gaps, this review seeks to inspire innovative approaches and technological advancements to ensure the sustainable provision of safe DW for all.

2. Drinking water and public health

2.1. Definition, quality criteria, and treatment

Water is the essential component of life, covering over two-thirds of the Earth's surface. However, <3 % of this water is freshwater, and only about 1 % of it is accessible for use [18]. Given the growing demand for water and its limited availability, sustainable water use has become critical to ensure access to safe drinking water (DW) for present and future generations [19]. The United Nations recognizes access to safe DW as a human right [1], and the World Health Organization (WHO) emphasizes the importance of maintaining high standards for DW quality to safeguard public health [20]. By 2020, 5.8 billion people were using "safely managed" DW services, yet 771 million people still lacked basic services, particularly in low-income or informal urban settlements [2]. Addressing these disparities is central to the WHO/UNICEF Joint Monitoring Program's Sustainable Development Goal 6, which aims to ensure the availability and sustainable management of water and sanitation for all by 2030 [21].

Safe DW is generally defined as water that does not pose significant health risks over a lifetime of consumption [22]. WHO guidelines set comprehensive quality criteria across chemical, microbiological, and physical parameters to ensure the safety of DW [20]. Chemical criteria limit contaminants such as heavy metals, pesticides, and organic pollutants, while microbiological criteria focus on preventing pathogens that can cause waterborne diseases. Physical parameters, including colour, taste, and turbidity, ensure that water is aesthetically acceptable. The WHO guidelines also stress the importance of monitoring and surveillance to maintain high standards of water quality and prevent risks to public health [20].

DW treatment plants (DWTPs) and DWDS function collaboratively to ensure the chemical and biological safety of DW, safeguarding public health [23]. DWTPs utilize a multi-barrier approach to remove contaminants and inactivate pathogens through a sequence of physical, chemical, and biological processes [23]. The treatment process typically begins with coagulation and flocculation, where chemical coagulants such as aluminum sulfate or ferric chloride are added to destabilize suspended particles and microorganisms, forming larger aggregates called flocs [24]. These flocs are then removed in the sedimentation stage, where gravitational settling eliminates a significant portion of particulate matter, including microbial contaminants [24]. Filtration

follows, using materials like sand, activated carbon, or membranes to trap remaining particles and microorganisms, further enhancing water clarity and safety [25]. The final and most critical step is disinfection, which employs chemical agents such as chlorine, chloramines, or ozone, as well as non-chemical methods like ultraviolet (UV) light or advanced oxidation processes (AOPs), to inactivate bacteria, viruses, and protozoa [26]. Once treated, water enters the DWDS, where maintaining its chemical and biological integrity is vital to delivering safe water to consumers. Residual disinfectants, such as free chlorine or monochloramine, are maintained throughout the distribution system to prevent microbial regrowth and protect against contamination [27]. However, while water treatment processes are effective in ensuring the safety and quality of DW, several limitations and drawbacks challenge their efficacy and sustainability. Table 1 highlights the challenges and limitations at each stage of water treatment and distribution. These limitations highlight the need for continuous innovation in water treatment technologies, coupled with effective monitoring and maintenance strategies, to address the growing challenges for water safety and quality.

2.2. Challenges of microbial etiology in DW

Each year, 1.5 million people worldwide die from waterborne diseases, with the majority being children who die due to dehydration caused by diarrhea [2]. According to the WHO, approximately 1.7 billion cases of diarrhea occur in children annually, being the second leading cause of death in children under 5 years of age [2].

Microorganisms are normally present in DWDS at relatively high concentrations (10^3 to 10^6 cells/mL) but without compromising public health [28]. This safety is ensured by a combination of water treatment processes, stringent regulatory oversight, the predominantly non-pathogenic nature of these microorganisms, competitive microbial ecology that inhibits pathogen growth, and the robustness of the human immune system [20,29–31]. Nevertheless, DWDS must be microbiologically safe and stable. In other words, a microbiologically stable DWDS must have a constant concentration and bacterial community composition, from the point of DW production to the tap [28]. However, unwanted bacterial growth inside the pipes can be responsible for adverse changes in DW and DWDS quality. The presence of some microorganisms may be responsible for some deterioration of the organoleptic properties of the water [28], which represents the main consumer complaint to DWDS companies [32]. The water distribution system itself can also be affected by the presence of bacteria, for example, the presence of biofilms in pipe walls can accelerate its corrosion [33]. In addition, biofilms can also harbor pathogenic microorganisms that can pose a danger to human health [8].

The quality of water is inherently shaped by its geographic and geological context, including factors like temperature, precipitation, leaching, and the release of elements from the crust of Earth [34]. Nevertheless, human activities have a significant impact on surface water bodies globally [34]. In rural regions of developing nations, individuals facing economic challenges frequently rely on untreated surface water for bathing, laundry, cooking, and occasionally even drinking [34,35]. Similarly, in both developed and developing countries, surface waters serve purposes such as recreational swimming, fishing, and irrigation [34,36]. Waterborne diseases can be caused by a variety of pathogens including protozoa, parasites, bacteria, and viruses [37,38]. Due to the impracticality of testing for all pathogens in all locations, monitoring efforts for polluted water often prioritize the detection of specific indicator organisms that signal the potential presence of pathogens [38,39]. One of the most employed indicators is faecal coliform bacteria [39]. While most faecal coliform bacteria are not inherently harmful, they are typically associated with human and animal waste. Furthermore, elevated levels of these bacteria often indicate the presence of harmful pathogens [20,40]. Numerous countries have acknowledged the correlation between faecal coliform bacteria and health risks, leading them to develop guidelines dictating permissible

Table 1
Limitations and drawbacks of water treatment processes in DWDS, highlighting the challenges associated with each stage of treatment and distribution. Ver este artigo e completar com info de lá (Microbial ecology of drinking water from source to tap).

Stage	Description	Limitations and drawbacks	References
Coagulation and Sedimentation	Coagulation involves adding chemical coagulants (e.g., aluminum sulfate) to destabilize and aggregate particles, followed by sedimentation to remove flocs	Generates significant quantities of sludge requiring proper disposal, posing environmental and logistical challenges. Effectiveness can be reduced by fluctuations in water chemistry (e.g., pH, temperature)	Prest et al. [28]
Filtration	Filters, such as sand and activated carbon, are used to physically remove remaining particles and microorganisms	Sand and activated carbon filters may become clogged or fouled over time, reducing efficiency and requiring frequent maintenance. Membrane filtration is costly and prone to fouling and damage, increasing operational costs	Prest et al. [28]
Chemical Disinfection	Chemical agents like chlorine, chloramines, and ozone are used to kill pathogens and provide residual protection	Chlorine has reduced effectiveness against protozoan cysts (<i>Cryptosporidium</i> , <i>Giardia</i>) and reacts with natural organic matter, forming harmful DBPs. Chloramines are less effective as primary disinfectants and produce nitrosamines. Ozone lacks residual activity, making water vulnerable to recontamination and bromate formation	Oliveira et al. [26]
Non-Chemical Disinfection	UV light or AOPs are used to inactivate microorganisms through non-chemical means	UV disinfection inactivates microorganisms but does not provide residual protection, leaving water susceptible to regrowth and contamination. AOPs have high energy demands and operational costs, limiting their widespread adoption.	Abkar et al. [27]
Residual Disinfection	Disinfectant (e.g., chlorine) is added to maintain a residual concentration (0.2–2 mg/L, max. 5 mg/L) to inhibit microbial regrowth	The effectiveness of residual disinfectants can be influenced by changes in water quality, such as fluctuations in pH or temperature, and interactions with biofilms, reducing their long-term efficacy. Formation of DBPs. Does not fully prevent microbial growth and alters microbial community structure.	Abkar et al. [27]

levels of these bacteria in water sources. Evaluating the degree of pathogen pollution requires a comparison of the levels of faecal coliform bacteria against established safety benchmarks [34]. The WHO has established these benchmarks, drawing upon water quality standards from 17 countries (Table 2). However, throughout the different phases of DW systems, complex microbial communities thrive, engaging in intricate networks of interactions [41]. Additionally, as previously mentioned, existing regulations governing the microbial safety of DWDS primarily target the probability of faecal contamination. These regulations rely on conventional culture-based methodologies to detect and quantify coliforms, particularly *Escherichia coli*. Despite their century-long application, these techniques have remained largely unaltered. Critically, these conventional indicators present several limitations: the vast majority of bacteria, over 99 %, evade culturing [42,43]; the emergence of non-faecal pathogens in DW; and the correlation between total coliforms and other pathogenic markers is weak [40,44,45].

The microbiome of DW is influenced by factors like source water quality, treatment processes, flow dynamics, temperature, and the presence of biofilms in the distribution system, among others [46]. Abkar et al. [27] reviewed key studies that elucidate how operational conditions shape microbial communities within DW systems. Lautenschlager et al. [47] provided foundational insights into the effects of stagnation and low flow rates, showing that these conditions promote microbial regrowth and biofilm formation, particularly in dead-end zones of the distribution network. They also observed that prolonged stagnation fosters microbial communities with increased metabolic activity, which can degrade water quality. Prest et al. [28] emphasized the selective pressures exerted by disinfectant residuals, demonstrating how different concentrations of chlorine and chloramine impact microbial

diversity, often favoring resistant taxa like *Nitrospira* and opportunistic pathogens. Fish et al. [48] further explored the effects of temperature increases, linking higher temperatures to accelerated microbial activity, enhanced biofilm growth, and shifts in microbial community composition. The study highlighted how temperature-driven changes can increase the prevalence of *Proteobacteria* and other biofilm-associated taxa, raising concerns about potential pathogen proliferation. Additionally, Abkar et al. [27] highlighted studies focusing on taxa-specific responses, such as the dominance of *Pseudomonas* and *Acinetobacter* under nutrient-rich and low-disinfectant conditions, contributing to infrastructure corrosion and antimicrobial resistance. Collectively, these studies reveal the complex interplay between physical, chemical, and environmental factors in DW systems, stressing the need for dynamic management strategies that address microbial proliferation while maintaining water quality. All of the aforementioned studies underscore the critical importance of investigating the microbiome of DW. Such research not only enhances the understanding of microbial dynamics within DW systems but also holds significant implications for public health by elucidating factors influencing microbial abundance, diversity, and community structure. The microbiome of DW encompasses a diverse array of microorganisms that can significantly influence water quality, treatment processes, and human health. Understanding these changes is crucial for water utilities to ensure safe DW by comprehending their impact on microbiomes and pathogen prevalence. By elucidating the microbial ecology of water systems, one can identify potential sources of contamination, track the spread of pathogens, and develop targeted strategies for water treatment and distribution. Moreover, studying the DW microbiome can also shed light on the ecological relationships within aquatic ecosystems, contributing to broader efforts in environmental conservation and resource management [46].

Table 2
Classification of pathogen water pollution based on river concentrations of faecal coliform bacteria, utilizing benchmarks derived from water quality standards of 17 countries.

Classification for water pollution	Faecal coliform (CFU/100 mL)	Explanation
Low pollution	≤200	Generally suitable for contact (e.g. swimming and bathing)
Moderate pollution	200 < x ≤ 1000	Appropriate only for contact during irrigation and fishing activities
Severe pollution	>1000	Unsuitable for contact

Adapted from UNEP [34].

3. Waterborne pathogens

3.1. Pathogens of concern in drinking water

It is estimated that there are 1407 human-infecting pathogens, with 177 being considered emerging or reemerging [49]. These pathogens encompass bacteria (538 species), viruses (208 types), fungi (317 species), parasitic protozoa (57 species), and helminths (287 species) [50]. Water, wastewater, food, and soils can harbor infectious agents, potentially contaminating DW [50]. Some potentially harmful bacteria

that could be present in DW include *Vibrio cholerae*, *Salmonella Typhi*, *Shigella* spp., *Campylobacter jejuni*, *E. coli*, *Yersinia enterocolitica*, *Legionella* spp., *Aeromonas* spp., various species of *Mycobacterium* (e.g. *M. avium*, *M. tuberculosis*, *M. fortuitum*, *M. abscessus*, *M. chelonae*, *M. kansasii*, *M. marinum*, *M. xenopi*), and *Pseudomonas aeruginosa* [51,52]. One important factor for disease development is the minimal infective dose (MID) which varies widely with the type of pathogen [50]. The MID of these waterborne pathogens are presented in Table 3.

Some of these previous mentioned bacteria are also included in the list of notifiable infectious diseases, requiring healthcare providers to report cases to public health authorities. Notifiable diseases, particularly those associated with waterborne transmission, pose significant challenges to public health worldwide [53]. These diseases can rapidly spread through contaminated water sources, leading to outbreaks and widespread illness [53]. Examples of such waterborne diseases include cholera caused by *V. cholerae*, typhoid fever caused by *S. typhi*, shigellosis caused by *Shigella* spp., listeriosis caused by *Listeria monocytogenes*, and campylobacteriosis caused by *C. jejuni*. Timely reporting of notifiable diseases is vital for effective disease surveillance, enabling public health authorities early detection of outbreaks and implementation of targeted interventions to control transmission [4]. By monitoring and promptly notifying health authorities of suspected or confirmed cases, it becomes possible to implement targeted interventions, such as water treatment and sanitation improvements, to prevent further spread and protect community health [4].

Despite the remarkable success of water treatment and sanitation programs in improving public health, sporadic cases and point-source outbreaks of waterborne diseases continue to occur [54]. According to the Centers for Disease Control and Prevention (CDC), a waterborne disease outbreak (WBDO) is an incident in which two or more epidemiologically-linked persons experience a similar illness after exposure to the same water source and epidemiologic evidence implicates the water as the likely source of the illness [55]. Table 4 shows the WBDO, with bacterial etiology, that occurred in Europe in the period between 2000 and 2023. A search in SCOPUS (November 2024) filtered using the keywords “Waterborne”, “Outbreak” and “Europe” in the title, keywords and abstract; limited to English and the period between 2000 and 2023, displayed 88 document results. Of these, 64 correspond to studies on viruses (17), chemicals (2), climate change (6), parasites (19), other continents (4) and other subjects (study tools, control strategies, projections, non-waterborne, etc.) (29). Only 11 were directly related to waterborne bacterial outbreaks. Of the 11 records found, three were associated with the genus *Campylobacter*, three with *L. pneumophila*, one with *Salmonella kottbus*, one with *S. typhi* and three with multiple aetiologies. In general, cases of campylobacteriosis and Legionnaires’ disease occurred in milder seasons. Similar to what was previously described for *Campylobacter* spp., infections tend to occur more frequently in warmer months, which corresponds to the ideal temperature range of 25 to 42 °C for *Legionella* [56]. Studies have observed that temperature, rainfall, and river systems play significant roles in

influencing the incidence of legionellosis in various locations [57–59]. In 2006, cases of *S. kottbus* infections on Gran Canaria (Spain) were documented [60], predominantly affecting children under the age of one, necessitating hospitalization. Instances of outbreaks attributed to this specific *Salmonella* species are infrequent in the literature, with only five reported outbreaks since 1959 [61–65]. Additionally, the outbreak on Gran Canaria marked the first instance of *S. kottbus* associated with commercially bottled water in Europe. Also, in non-endemic countries, occurrence of typhoid fever are scarce and typically sporadic [66], resulting in limited public health outcomes. Typhoid fever, a severe systemic illness, is caused by *Salmonella enterica* subspecies enterica, serovar Typhi [66]. Its transmission usually occurs through faecal contamination of food or water and is prevalent in regions with inadequate sanitation [66]. On April 2022, a significant outbreak of typhoid fever emerged in the Netherlands among asylum seekers housed on a renovated vessel. Ooms et al. [67] reported that the contamination originated from a leakage of the wastewater tank into the DW tank supply.

3.2. Emerging and reemerging pathogens in drinking water

Emerging and reemerging pathogens in DW refer to two distinct yet interrelated categories. Emerging pathogens are those that have only recently been identified as waterborne threats, either because they are newly discovered or because advancements in detection methods have revealed their presence and significance. These may include previously unknown microorganisms or pathogens that were not previously recognized as a concern in DW but have gained attention due to their public health implications. In contrast, reemerging pathogens are those that were once considered controlled or of limited concern but have resurged, leading to an increase in infections or an expansion in their geographical distribution [3]. In recent years, waterborne pathogens have come to light, encompassing both known pathogens originating from faecal contamination and novel pathogens arising from environmental reservoirs [54]. Various factors contribute to the resurgence or emergence of these waterborne pathogens, including shifts in human demographics, alterations in human habits, deterioration of public health infrastructures, microbial evolution, and modifications in agricultural methods [68]. For instance, urbanization and population growth can lead to increased contamination of water sources, while changes in agricultural practices such as irrigation techniques can introduce pathogens into water reservoirs. Additionally, climate change may influence the distribution and survival of pathogens in water environments, further complicating efforts to mitigate the risks associated with waterborne diseases [3,69].

The emerging and reemerging pathogenic bacteria highlighted herein (Table 5) have the potential to be transmitted through DW; however, they do not necessarily correlate with the presence of *E. coli* or other commonly used DW quality indicators. Typically, existing microbiological indicators used to assess DW quality may not reliably detect the presence of these emerging pathogenic bacteria, posing challenges for their identification and monitoring [54]. The species listed in Table 5 stand out for their remarkable proliferation in water systems. Their ability to form biofilms is a critical factor in their resistance to disinfection efforts and environmental stresses, enhancing their persistence in water systems. Climate change, human activities such as urbanization, agricultural runoff, and improper waste management, further contribute to the spread and resilience of these pathogens [70]. The increasing prevalence of these bacteria in water sources underscores the need for improved water quality monitoring, effective disinfection practices, and comprehensive strategies to mitigate their impact on public health [44].

Table 3
MID for harmful waterborne pathogens.

Pathogen	Minimal infective dose (MID) (CFU/mL)
<i>Vibrio cholerae</i>	10 ³
<i>Salmonella</i> spp.	10 ⁴ –10 ⁷
<i>Shigella</i> spp.	10 ¹ –10 ²
<i>Campylobacter jejuni</i>	≈500
<i>Escherichia coli</i>	10 ⁶ –10 ⁸
<i>Escherichia coli</i> O157:H7	<100
<i>Yersinia enterocolitica</i>	10 ⁶
<i>Legionella</i> spp.	Unknown
<i>Aeromonas</i> spp.	10 ⁴
<i>Mycobacterium avium</i>	10 ⁴ –10 ⁷
<i>Pseudomonas aeruginosa</i>	Unknown

Based on Bitton [50], and Teunis and Figueras [168].

Table 4
Bacterial-related outbreaks caused by waterborne transmission in Europe between 2000 and 2020.

Year	Country	Pathogenic bacteria	Number of cases	Source	Reference
2000	France	Multiple	202	Groundwater system contamination	[169]
2002–2003	Sweden	<i>Campylobacter jejuni</i>	101	Communal water	[170]
2006	Spain	<i>Salmonella kottbus</i>	41	Bottled water	[60]
2007	Norway	<i>Campylobacter</i> spp.	105	Vulnerability of the DW system	[171]
2007	Denmark	Multiple	140	DW	[210]
2010	Belgium	Multiple	222	DW	[172]
2012	United Kingdom	<i>Legionella pneumophila</i>	21	Spa pool device	[173]
2014	Portugal	<i>Legionella pneumophila</i>	550	Industrial wet cooling systems	[174]
2019	Norway	<i>Campylobacter jejuni</i>	2000	Municipal DW system	[175]
2020–2021	Finland	<i>Legionella pneumophila</i>	12	Tap water	[176]
2022	Netherlands	<i>Salmonella Typhi</i>	72	Wastewater leak in DW tank	[67]

4. Biofilms in drinking water systems

4.1. Definition and biofilm-associated problems

Biofilms are well-organized structures of microorganisms formed through a sequence of events, starting with the adhesion of planktonic cells to a surface, followed by subsequent replication and production of an extracellular matrix of EPS [71]. However, not all biofilms develop in the same manner, especially within living organisms, in medical and industrial settings, and in nature. In these environments, biofilms can be observed as unattached aggregates [72–74]. A clear example is the formation of clusters of bacteria, commonly called granules, observed in wastewater treatment plants (WWTPs) [75]. Similarly, in marine, lake, and river ecosystems, these biofilms are often termed “marine snow” [76]. Fig. 1 illustrates an adaptation of the biofilm formation model proposed by Sauer et al. [74], where the steps described above are incorporated.

The presence of biofilms in DW presents a multitude of challenges, particularly for water disinfection. Bacteria in biofilms are remarkably more resistant to conventional disinfection methods [77]. Assaidi et al. [78] studied the persistence of *L. pneumophila* in water systems, focusing on the role of biofilms and the effectiveness of sodium chloride disinfection. Assessing various concentrations of sodium chloride on both planktonic and sessile cells, they found that while planktonic cells showed susceptibility to sodium chloride, with survival up to 2 % concentration at 20 °C, biofilms were resilient, surviving and regrowing even after exposure to a sodium chloride shock. Interestingly, this resilience was more pronounced in older biofilms compared to the younger ones.

Moreover, biofilms serve as reservoirs for various microorganisms, including pathogens, raising concerns about microbial contamination and associated health risks [79]. For instance, research on *L. pneumophila* biofilms has shown that they protect the colonizer cells from disinfection [5,7,78]. Andreozzi et al. [80] used bacterial cultures and advanced molecular biology techniques to investigate how biofilms protected the replicative form of *L. pneumophila*, focusing on *mip* gene expression. The *mip* gene and its encoded protein, MIP, in *Legionella* is crucial for understanding its role as a virulence factor, elucidating host-pathogen interactions, developing diagnostic markers, and identifying potential therapeutic targets for Legionnaires' disease [81]. Results showed an up-regulation of *mip* gene under nutrient depletion, with biofilms preserving *Legionella* in the replicative form. Other studies have shown that biofilms in DWDS can also serve as reservoirs for other opportunistic pathogens [7,81].

However, disinfection tolerance and pathogen protection are not the only issues associated with the presence of biofilms in water systems. Biofilms also promote biocorrosion, accelerating the degradation of the piping infrastructure [8]. Biocorrosion, driven by biofilm-forming microbes like bacteria, archaea, and fungi, accelerates pipe material degradation, compromising water quality and infrastructure [82,83]. Microbes such as iron-oxidizing bacteria (IOB), sulfate-reducing bacteria

(SRB), and manganese-oxidizing bacteria (MOB) contribute to corrosion by altering local chemistry. IOB oxidize ferrous iron, forming ferric hydroxide deposits that create anaerobic conditions for SRB, which produce corrosive sulfides [84,85]. MOB oxidize manganese, depositing oxides that further corrosion [86]. Biofilms, stabilized by an EPS matrix, trap nutrients and corrosion products, establish redox gradients, and create differential aeration cells, exacerbating corrosion [11,87]. These processes accelerate disinfectant decay, destabilize water quality, and increase harmful DBP production, posing health risks [11]. This topic will be further discussed in Section 5.1, as it is considered one of the emerging key issues associated with biofilm formation.

4.2. Biofilms as a source of waterborne microbiological diseases

Biofilms are believed to be the primary source of microorganisms in DWDS, with an estimated 95 % of the total biomass in the water being attached to the pipe walls, while only 5 % is in the water phase [6]. In fact, after treatment procedures like chlorination, bacteria levels in water tanks are at their lowest, but they increase in tap water due to biofilms in plumbing systems [88]. The levels of colony forming units (CFU) vary, ranging from up to 10⁶ CFU/mL in groundwater to between 10² and 10⁵ CFU/mL in household tap water [88,89]. Fig. 2 presents the bacterial concentrations at key stages of the water treatment process. These stages encompass the water source, the treated water immediately post-disinfection within the holding tank, the storage tank before distribution, and finally, the water as it reaches the tap for consumption. Moreover, it provides a breakdown of the predominant and commonly found bacterial classes and genera identified within a household environment across various locations. Each area exhibits distinct microbial compositions, reflecting localized environmental conditions and usage patterns. In fact, bacterial diversity is influenced by factors like disinfectant type, temperature, flow rate, and building materials [90]. For example, in lower parts of plumbing systems, Alphaproteobacteria are dominant over Betaproteobacteria in biofilms, especially in systems with regular water consumption and high flow rates [91]. This dominance was also observed in copper pipes [91], while cross-linked polyethylene (PEX) pipes, particularly in conjunction with hot water, tend to favor Betaproteobacteria, Gammaproteobacteria, Actinobacteria, and Bacilli [89,91,92].

The lack of reporting and surveillance on waterborne outbreaks associated with biofilms represents a significant deficiency in public health monitoring. Recent findings from the CDC underscore the magnitude of this issue, with their report on “Surveillance of Waterborne Disease Outbreaks Associated with Drinking Water” in the United States revealing alarming statistics. Between 2015 and 2020, 87 % of reported outbreaks were linked to biofilms, accounting for 187 cases out of 214. These outbreaks were predominantly attributed to *Legionella*, comprising 98 % of biofilm-related cases, with isolated instances involving nontuberculous mycobacteria and *Pseudomonas* spp. [93]. Such data highlights the urgent need for enhanced surveillance and reporting mechanisms globally to effectively address biofilm-associated

Table 5
Emerging and reemerging waterborne pathogenic bacteria of public health concern.

Bacteria	Prevalence in water sources	Resistance to disinfection	Biofilm formation	Impact of climate change	Human activities impact	Emerging or reemerging status	Health effects	References
<i>Acinetobacter</i> spp.	28 % of tested DW samples	Resistant to chlorine	Biofilm formation enhances resistance to disinfectants and antibiotics	Increased survival and dissemination in warmer water	Antibiotic overuse, improper waste disposal, untreated wastewater	Emerging pathogen due to multidrug resistance (MDR) and survival in diverse environments	Nosocomial infections; meningitis, pneumonia, endocarditis, bacteremia, skin and urinary tract infections in immunocompromised patients	[99,177–180]
<i>Arcobacter</i> spp.	35 % of surface water samples	Moderate resistance to chlorine and ozone	Biofilm formation enhances resistance to disinfectants and environmental stresses	Increased risk of waterborne transmission due to altered water temperatures and precipitation patterns	Intensive livestock farming, inadequate wastewater treatment, and agricultural runoff	Emerging pathogens due to rising detection in water sources and resistance to common disinfection methods	Diarrhea	[181–184]
<i>Aeromonas hydrophila</i>	18 % of DW samples	Resistant to chlorination and oxidative stress	Biofilm formation enhances resistance to environmental stresses and disinfectants	Increased presence and virulence in warmer waters	Agricultural runoff, urbanization, industrial discharges	Reemerging pathogen due to persistent water presence and resistance to disinfectants and antibiotics	Gastroenteritis	[44,185,186]
<i>Campylobacter</i> (including <i>C. jejuni</i> , <i>C. coli</i> , and related species)	Surface water, groundwater, rural watershed runoff, marine water, rainwater, rivers, and lakes	Sensitive to chlorination but persists in biofilms and low-nutrient environments	Biofilm formation enhances resistance to chlorination and environmental stresses	Higher temperatures and intense rainfall increase cases, while heat waves and winter precipitation decrease cases	Spread by improper waste management, agricultural runoff, recreational water activities, and occupational exposure in poultry farms	Emerging pathogens due to increasing incidence, environmental adaptability, water source presence, and climate and human activity impacts	Acute gastroenteritis	[69,190,191,187–189,209,211]
<i>Helicobacter pylori</i>	30 % of tested DW samples	Survives in water for extended periods, resistance to chlorine is uncertain	Biofilm formation protects against environmental stresses and disinfection	Increased survival in warmer waters due to climate change	Improper wastewater management and contaminated water supplies	Emerging pathogen due to association with waterborne transmission and resilience in environmental conditions	Gastrointestinal disease including chronic active gastritis; peptic and duodenal ulcer disease; gastric cancer	[44,192,208]
<i>Legionella pneumophila</i>	High prevalence in water systems	Resistant to standard disinfection, requires thermal treatment	Biofilm formation provides protection against disinfectants	Increased risk due to higher water temperatures	Use of cooling systems, inadequate water system maintenance, urbanization	Emerging pathogen due to association with modern water systems and rising incidence	Legionnaire's disease and Pontiac fever	[56,191,193,194]
<i>Mycobacterium avium</i> complex	40 % of municipal water samples	Resistant to chlorine and common disinfectants	Biofilm formation provides a protective niche for persistence and growth	Enhanced survival in warmer water	Urbanization, agricultural runoff, treated water system use	Emerging pathogens due to increasing incidence in immunocompromised populations and resilience in treated water systems	Pulmonary disease	[44,195,196]
<i>Listeria monocytogenes</i>	25 % of tested DW sources	Resistance to chlorine and other disinfectants	Biofilm formation enhances survival and resistance to environmental stresses and disinfectants	Increased prevalence due to warmer temperatures and extreme weather events	Spread by improper wastewater management, agricultural runoff, and food processing environments	Emerging pathogen due to its resilience in various environments, biofilm formation, and increasing detection in water sources	Listeriosis, abortion, human meningitis, infection during the perinatal period, granulomatosis infantiseptica, sepsis, diarrhea, pyelitis, and “flu-like” symptoms	[197–199]
<i>Pseudomonas aeruginosa</i>	42 % of hospital water systems	Resistant to chlorine and other disinfectants	Biofilm formation enhances resistance to antibiotics and disinfectants	Increased prevalence in warmer water and higher rainfall	Antibiotic misuse, poor hospital sanitation, improper wastewater treatment	Emerging pathogen due to high resistance to antibiotics and disinfectants, association with healthcare-associated infections	Infections in immunocompromised patients and patients with underlying diseases (i.e. wound and urinary tract infections and	[191,200,201]

(continued on next page)

Table 5 (continued)

Bacteria	Prevalence in water sources	Resistance to disinfection	Biofilm formation	Impact of climate change	Human activities impact	Emerging or reemerging status	Health effects	References
<i>Stenotrophomonas maltophilia</i>	30 % of hospital water sources	Resistant to chlorine and several disinfectants	Biofilm formation enhances resistance to disinfectants and environmental stresses	Facilitated spread and persistence in changing water availability	Antibiotic overuse, inadequate water treatment	Emerging pathogen due to increasing prevalence in hospital settings and MDR	pneumonia); tracheobronchitis in cystic fibrosis patients Nosocomial infections, mainly respiratory tract infections	[202–204]
<i>Yersinia enterocolitica</i>	27 % of surface water samples	Resistant to chlorine, especially in cold water	Biofilm formation enhances survival in cold and chlorinated water	Increased persistence in cold water due to climate variability	Agricultural runoff, untreated wastewater, and improper waste disposal	Emerging pathogen due to cold water persistence and moderate resistance to disinfection	Gastrointestinal infections	[69,191,205–207]

waterborne illnesses and mitigate public health risks.

4.3. Impact of materials and treatments on biofilm formation

Biofilm formation in DW systems is highly variable and influenced by numerous factors, including water composition, materials in use, treatment methods, and the presence of residual disinfectants.

The chemical and microbiological composition of water plays a pivotal role; high levels of organic matter, nutrients (e.g., carbon, nitrogen, phosphorus), and ions such as calcium and magnesium often promote biofilm growth, providing substrates for microbial metabolism [6]. In contrast, low-nutrient conditions may limit biofilm density but favor microorganisms adapted to oligotrophic environments [94]. For instance, a study by Dang et al. [95] highlights that nutrient availability not only influences biofilm biomass but also alters microbial community composition, with oligotrophic environments favoring slower-growing, resource-efficient organisms. Organic carbon is often the most critical growth-limiting nutrient due to its role as the primary energy source for heterotrophic bacteria. Bioavailable organic matter (BOM), comprising simple sugars, organic acids, and complex humic substances, represents only a fraction of the dissolved organic carbon (DOC) in water, typically between 1 and 30 % [96]. Even small concentrations of BOM, such as 1 µg C/L, can support bacterial growth, highlighting the sensitivity of microbial communities to nutrient availability [97]. Additionally, the balance of inorganic nutrients like nitrogen and phosphorus is essential, as deficiencies in these elements can limit bacterial proliferation even in the presence of sufficient organic carbon [98]. These nutrient dynamics shape microbial communities, where competition for substrates influences the diversity and resilience of bacteria in water systems.

The materials used in distribution systems also significantly impact biofilm formation. For example, rough or porous surfaces like unlined cast iron facilitate microbial adhesion more than smoother materials such as polyethylene or stainless steel [99]. Polyvinyl chloride (PVC) pipes generally show reduced biofilm formation compared to copper pipes, where ion release may affect microbial communities [100]. However, material choice alone does not determine biofilm growth; factors such as pipe ageing, maintenance, and operational conditions also play critical roles. Poorly maintained systems can exacerbate biofilm formation, leading to aesthetic issues (e.g., discoloration, odor), technical problems (e.g., pipe corrosion), and potential public health risks from waterborne diseases. Studies indicated that high-density polyethylene (HDPE) and unplasticized polyvinyl chloride (PVC-U) have been increasingly preferred in modern networks due to their resistance to corrosion and cost-effectiveness, though biofilm growth on these materials can still occur under certain conditions [101]. Yet, biofilm composition and biomass vary across materials. Despite its smooth surface, research has shown that stainless steel can support significant biofilm growth under low-nutrient conditions, although electropolished surfaces reduce initial bacterial adhesion [102]. Copper pipes, known for releasing antimicrobial ions, typically harbor fewer bacteria, especially when combined with chloramine disinfection [103]. Conversely, plastic materials like PVC-P may exhibit higher biomass and microbial diversity compared to PVC-C, which is more effective in minimizing bacterial accumulation [104].

Treatment methods, such as filtration and coagulation, play a vital role in reducing organic loads, which can help mitigate biofilm formation. However, disinfection methods, such as chlorination, while effective against planktonic microorganisms, can inadvertently alter microbial community structures and promote the selection of resistant species, potentially contributing to biofilm persistence [105]. Advanced disinfection techniques, such as ozonation and UV irradiation, effectively inactivate microorganisms but lack residual disinfection capacity, leaving systems susceptible to biofilm regrowth and recontamination [106]. The choice of disinfection strategy significantly impacts biofilm formation and composition. Conventional disinfectants like chlorine and chloramine have been shown to lower biofilm diversity while selecting

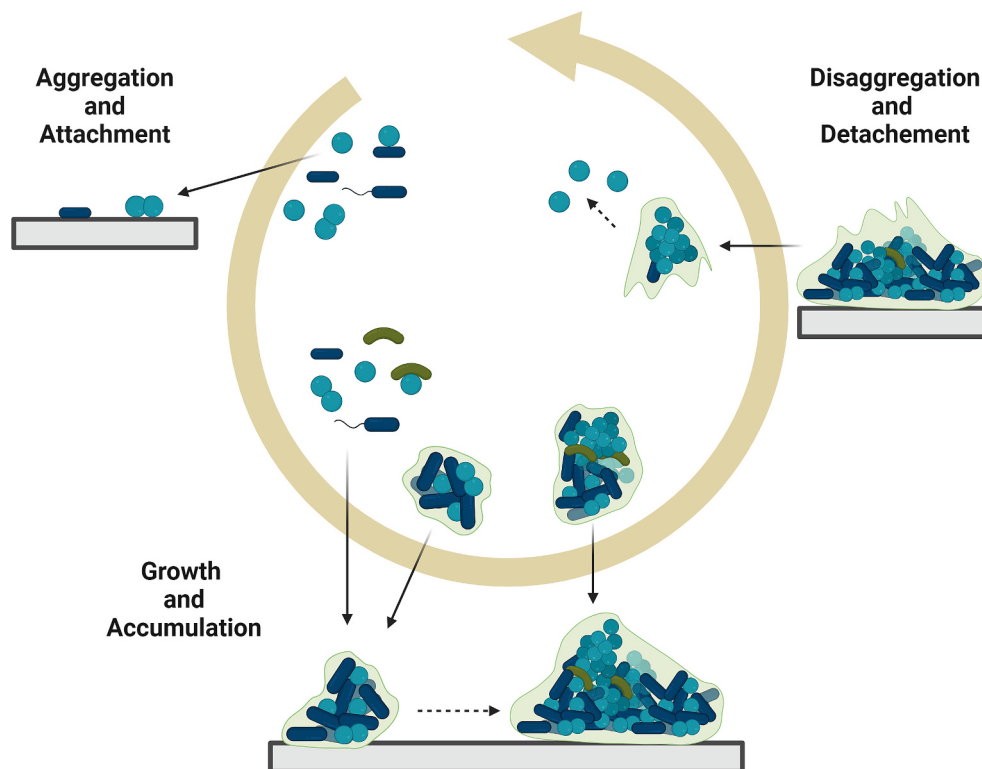


Fig. 1. Schematic representation of biofilm formation highlighting bacterial aggregation. In the “Aggregation and Attachment” phase, planktonic single cells, autoaggregates, or coaggregates adhere directly to the surface or coadhere with primary colonizers, later participating in the coadhesion process. During the “Growth and Accumulation” phase, active microbial growth and the recruitment of secondary colonizers lead to microcolony formation and EPS excretion. This phase also involves the formation of coaggregation/coadhesion bridges between early colonizers. In the “Disaggregation and Detachment” phase, bacteria can leave the biofilm as aggregates or single cells, depending on the mechanism, and may integrate into other biofilms.

Based on Sauer et al. [74].

for opportunistic pathogens, highlighting the importance of maintaining effective residual concentrations to inhibit biofilm-associated risks [26]. However, the inability of current strategies to fully eradicate biofilms underscores the need for innovative approaches. Emerging technologies, including multi-barrier disinfection, bacteriophages, and nanoparticle-based treatments, offer promising solutions to limit biofilm development and control microbial regrowth [26]. Furthermore, the interaction between residual disinfectants and pipe materials, along with the degradation of chloramines, poses additional challenges for biofilm management. Addressing these factors through optimized treatment protocols and advanced materials, such as carbon quantum dots and metal-organic frameworks-based systems, could significantly improve biofilm prevention [107].

Residual disinfectants, particularly chlorine and chloramines, are essential for maintaining microbial control within DWDS. Free chlorine is the most widely used residual disinfectant worldwide, whereas monochloramine is favored in some systems due to its ability to limit disinfection by-product (DBP) formation in high organic load environments [108]. However, countries such as the Netherlands, Germany, Austria, and Switzerland avoid residual disinfectants due to concerns about DBPs, which are increasingly recognized for their potential carcinogenicity [108,109]. Interestingly, research indicates that systems using chlorine residuals, like those in the USA, report ten times more total coliform failures compared to systems without residuals, such as in the Netherlands, after adjusting for population differences [110]. This suggests that factors beyond disinfection residuals, including infrastructure age and water quality, significantly affect water safety outcomes. Although chlorine generally attenuates bacterial biofilm cell concentrations [111–113], biofilm-bound microorganisms exhibit greater resistance to disinfectants compared to planktonic cells, largely

due to the protective EPS matrix. Fish et al. [48] investigated how residual chlorine influences biofilm ecology and overall water quality. The study highlighted that while residual chlorine is effective in controlling planktonic bacteria, it can inadvertently select for chlorine-tolerant species within biofilms, potentially altering microbial community structures and reducing treatment efficacy. The EPS layer not only shields biofilm cells but also plays a role in mechanical stability and influences DBP formation [114–116]. The study of Xu et al. [117] examined the interplay between biofilms and chlorine decay, showing that biofilms not only contribute to the depletion of residual chlorine but also facilitate the formation of DBPs such as trihalomethanes (THMs). Additionally, studies have shown that increasing chlorine concentrations do not necessarily reduce EPS or other biofilm components, meaning risks to water quality persist if biofilms are mobilized [116].

Therefore, biofilm formation in DW systems is shaped by water composition, materials, and treatment methods, highlighting the need for integrated strategies that address microbial dynamics, material interactions, and the limitations of current disinfection approaches.

4.4. Role of metals in biofilm formation

In water systems, biofilm formation is not only influenced by organic nutrients, microbial communities, and water composition but also by the accumulation of inorganic deposits such as metals like iron (Fe), manganese (Mn), and aluminum (Al). For example, discolored water events remain a persistent challenge for the DW industry, often leading to customer dissatisfaction [118]. Ginige et al. [119] investigated the contribution of pipe wall biofilms toward Fe and Mn deposition, and discolored water events. Using biofilm reactors and field biofilm monitors designed identically, the study compared Fe and Mn accumulation

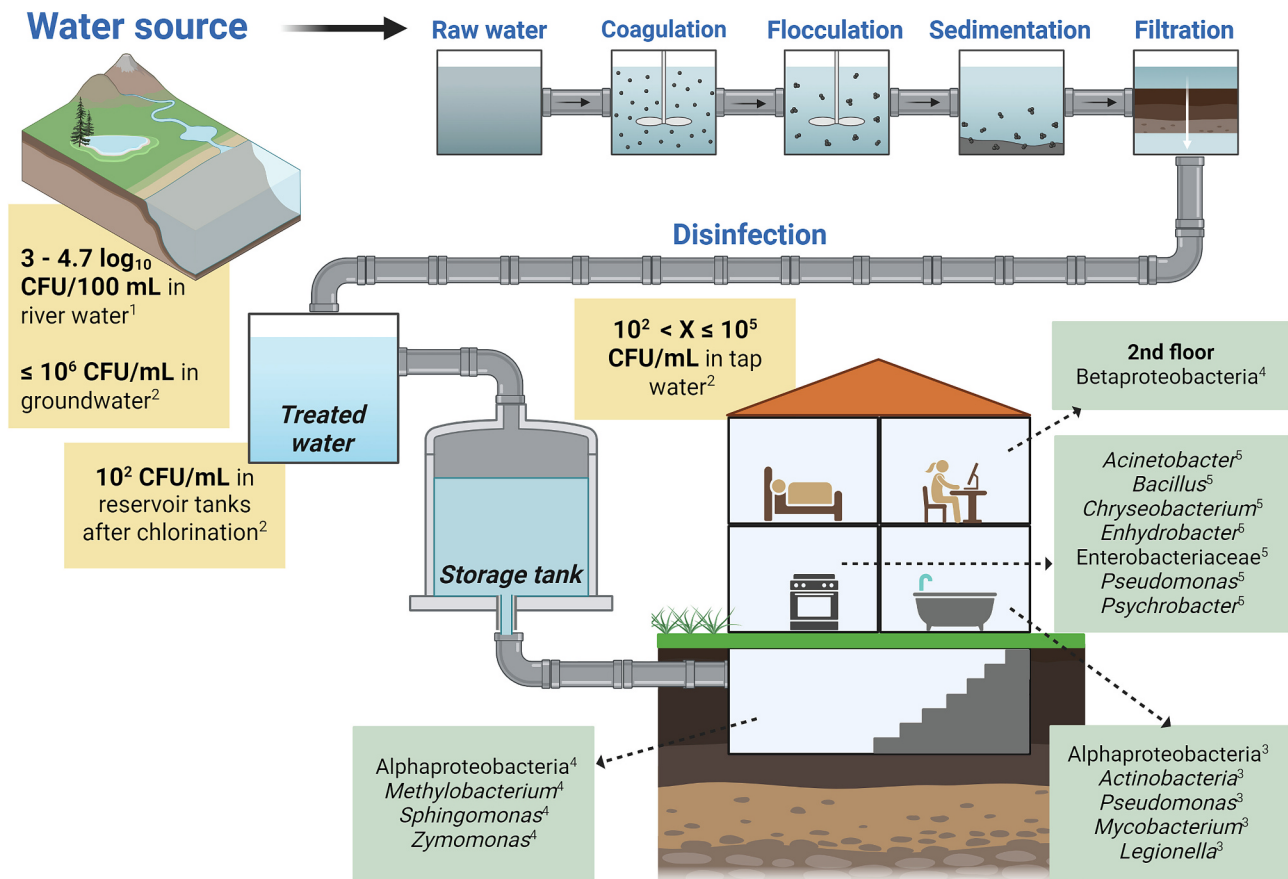


Fig. 2. DW microbial dynamics from the source to household consumption. ¹Pinar-Méndez et al. [166]; ²Kormas et al. [88]; ³Moat et al. [92]; ⁴Inkinen et al. [91]; ⁵Moen et al. [167].

on glass surfaces in the presence and absence of biofilms. In non-chlorinated reactors, biofilms significantly enhanced Mn deposition, with biofilm-covered surfaces accumulating 33 times more Mn than those exposed to lower Mn concentrations. Conversely, Fe deposition was only about twice as high under high Fe conditions, suggesting that Mn accumulation is more influenced by biofilm activity. Chlorinated reactors exhibited a 95 % reduction in biofilm biomass, yet Mn deposition remained 17 times higher in reactors with elevated Mn concentrations, indicating abiotic deposition mechanisms. Upon chlorination of previously biofilm-rich systems, Fe and Mn concentrations in effluent water increased six-fold, demonstrating that biofilm detachment could lead to metal release and potential water discoloration events. Aluminum, often introduced through water treatment processes or the use of aluminum-based coagulants [120], can also influence biofilm formation. It has been shown to promote microbial adhesion to surfaces and strengthen the structural stability of the biofilm matrix by interacting with EPS and bacterial cell surfaces [121]. Cui et al. [121] examined the effects of low concentrations of residual Al(III) on biofilm formation, exploring mechanisms potentially relevant to biofouling in WTPs and reuse systems. At concentrations below 2.0 mg/L, Al(III) stimulated bacterial growth by approximately 3.7 times and enhanced EPS production, promoting biofilm development. Al(III)-induced flocculation facilitated bacterial aggregation, leading to rapid biofilm formation, with biofilm thickness increasing to $103.0 \pm 7.1 \mu\text{m}$ and $112.0 \pm 5.7 \mu\text{m}$ at 0.6 and 2.0 mg-Al/L, respectively, compared to $61 \pm 4.24 \mu\text{m}$ in control conditions. The zeta potential of bacterial cells decreased with increasing Al(III) concentrations, indicating charge neutralization and reduced electrostatic repulsion, thereby enhancing microbial adhesion. Notably, Al(III) interacted strongly with bacterial outer membranes, causing structural damage, protein leakage, and ATP

depletion by up to 55.91 %, leading to a distinct double-layer biofilm structure with inactivated cells and proteins at the bottom and polysaccharides with active cells at the top. The presence of inorganic deposits can also alter the microbial composition within biofilms, promoting the growth of specific metal-tolerant microorganisms that can thrive in the altered chemical environment. Recently, Li et al. [122] investigated the impact of trace metals on water quality and microbial risks by conducting a 168-hour experimental simulation, where biofilms developed on glass beads in tap water supplemented with Fe(III) and Al(III), followed by metagenomic analysis. The study revealed that Fe(III) significantly increased turbidity, while both Fe(III) and Al(III) promoted biofilm growth and enhanced the production of EPS. Bacterial communities, dominated by *Pseudomonadota*, exhibited adaptive mechanisms in response to metal-induced stress, with Al(III) having a more pronounced effect on bacterial proliferation than Fe(III). The presence of Al(III) also increased the relative abundance of pathogenic bacteria, such as *S. enterica* and *P. aeruginosa*, raising concerns about potential health risks. Moreover, Fe(III) facilitated the co-occurrence of antibiotic resistance genes and pathogens, increasing the overall risk of antibiotic-resistant bacteria in DW systems.

These studies collectively underscore the significant role of inorganic deposits in shaping biofilm formation and stability by not only enhancing the accumulation of metals but also contributing to their remobilization. The findings highlight how biofilms facilitate metal deposition and influence metal release during disinfection, as well as how accelerate biofilm formation through microbial adhesion, EPS production, and charge neutralization. Furthermore, the synergistic effects of these inorganic deposits promote biofilm development while increasing the abundance of pathogenic bacteria and antibiotic resistance genes. Additionally, the redox processes associated with metal

oxidation and reduction contribute to the establishment of electrochemical gradients within biofilms, further influencing microbial activity and community composition. For example, the oxidation of iron can create anaerobic conditions within the biofilm, which may favor the growth of SRB and other anaerobic microorganisms, contributing to biocorrosion and other biofilm-associated problems [123].

5. Rising challenges

5.1. DBPs formation

Biofilms contribute to the formation of DBPs, some of which are carcinogenic, and cause taste and odor issues in DW [11]. Zhang et al. [124] investigated biofilm-related DBPs in DWDS, focusing on changes in biofilm-originated halogenated DBP formation potential (biofilm DBP-FP) under sequential UV and chlorine disinfection (UV-Cl₂) treatments. The study measured the formation of trihalomethanes (THMs), haloacetic acids (HAAs), and total organic halogen (TOX) produced by biofilms, finding that total organic chlorine formation potential (TOCl-FP) was minimized with a UV dose of 80 mJ/cm², correlating with reduced amounts of protein and polysaccharides in EPS. Bacterial community analysis revealed shifts in dominant bacteria and metabolic functions post-UV-Cl₂ disinfection, influencing biofilm properties and ultimately biofilm DBP-FP.

Shi et al. [125] examined how corrosion inhibitors impacted the physical structure and biochemical composition of biofilms in premise plumbing systems, focusing on chlorine decay and DBP formation. The study evaluated biofilms grown in simulated DW amended with silicate, phosphate, and phosphate blends, finding that phosphate-based inhibitors led to the lowest formation of THMs and HAAs. Chlorine decay rates varied among different biofilm types, with phosphate blends exhibiting the fastest decay. Modelling based on experimental data predicted an increased formation of biofilm-derived THMs and HAAs with higher chlorine and total organic carbon (TOC) concentration. Phosphate-based inhibitors reduced DBPs formation but increased bacterial release, potentially elevating the risk of exposure to opportunistic pathogens in DW, while silicate biofilms exhibited higher DBP formation yield but less biomass and bacterial release.

Similarly, Wang et al. [126] evaluated how biofilms influence DBPs formation and decay in chlorine (Cl₂) or monochloramine (NH₂Cl) disinfected reactors mimicking DWDS conditions. At low disinfectant residuals (0.5 mg/L), neither Cl₂ nor NH₂Cl effectively removed biofilms, but as residual levels increased, Cl₂ eradicated biofilms while NH₂Cl did not, even at the highest allowable dose (4 mg/L). Low DBP formation occurred at the minimum recommended Cl₂ residual due to limited Cl₂ reactions with biofilms and DBP volatilization and biodegradation. However, at higher Cl₂ residuals (2 mg/L), DBP concentrations sharply increased, mainly trihalomethanes and haloacetic acids, temporarily due to biofilm removal. NH₂Cl disinfection resulted in much lower DBP levels with minor variations across residual levels. These studies highlight that while UV treatment combined with chlorine disinfection can mitigate biofilm-related DBP formation potential, phosphate-based corrosion inhibitors may reduce DBP formation in biofilms but increase bacterial release. Additionally, chlorine disinfection effectively eradicates biofilms but can lead to increased DBP concentrations, whereas monochloramine disinfection shows promise in controlling DBP levels with less variability.

An additional perspective is that corrosive by-products from the piping network can accelerate disinfectant decay [127]. Since biofilms are major contributors to biocorrosion, this relationship should not be overlooked. The corrosive by-products of the piping network facilitate disinfectant decay, severely jeopardizing water quality stability and escalating DBP production, thereby amplifying health risks [11]. Given the ubiquity of natural organic matter (NOM) in DW sources and its resistance to conventional treatment processes, it reacts with residual chlorine to generate DBPs [128]. Moreover, metabolites from microbial

life within biofilms, secreted EPS, and the organisms themselves increase water organic content [129,130]. With residual chlorine present, these organic materials become DBP precursors, increasing both the quantity and toxicity of DBPs [11]. However, studies explicitly linking biofilm formation, biocorrosion, and DBP formation are limited. Wang et al. [131] used two annular reactors (ARs) with different disinfection methods—UV/Cl₂ and Cl₂ alone—to examine whether biocorrosion influences NOM and DBP removal. In the initial stage, higher corrosion rates were observed, particularly in the UV/Cl₂ AR, leading to greater NOM and DBP removal due to UV light controlling bacterial regrowth and enhancing chlorine reactivity with iron, promoting corrosion. In later stages, UV/Cl₂ disinfection reduced bacterial load and altered the bacterial community, reducing dehalogenation-related bacteria and decreasing corrosion rates, thereby lowering NOM and DBP removal efficiency. Similarly, Chen et al. [132] compared water quality risk and microbial ecology between new and old cast iron pipe systems, finding that older pipes had higher corrosion rates and biofilm accumulation, leading to increased DBP formation and microbial risks. These findings underscore the need for infrastructure renewal and optimized disinfection strategies to manage biocorrosion and minimize DBP formation for safe drinking water.

5.2. Climate change

Climate change is increasingly recognized as a significant factor influencing microbial proliferation in aquatic environments and, consequently, AMR [14] (Fig. 3). Aquatic environments serve as reservoirs and transmission routes for AMR, with antibiotics entering these systems through various pathways, including human and animal waste, improper disposal of unused drugs, and direct contamination from pharmaceutical manufacturing [133,134]. Climate change exacerbates these issues by altering environmental conditions that favor microbial growth and the spread of resistance. Rising temperatures, changes in precipitation patterns, and increased frequency of extreme weather events disrupt ecosystems, leading to heightened interactions between humans, animals, and environmental reservoirs of resistant bacteria. This contact elevates the risk of zoonotic and vector-borne diseases, potentially escalating antimicrobial use and driving resistance further [135,136]. Additionally, temperature is a critical driver of microbial evolution and the spread of antibiotic-resistance genes (ARGs). For instance, higher temperatures have been linked to an increased prevalence of carbapenem-resistant *P. aeruginosa*, with a 1.02-fold increase in resistance for every 0.5 °C rise in temperature [137]. Similarly, *E. coli* has shown comparable responses to elevated temperatures and antibiotic exposure, underscoring the importance of physiological adaptations to heat in promoting resistance [138].

The increase in global temperatures significantly drives AMR in aquatic environments by influencing bacterial physiology, genetic exchange, and stress responses. Elevated temperatures disrupt bacterial membrane structures, reducing antibiotic permeability and increasing resistance to drugs like polymyxins [139,140]. Warmer conditions also accelerate bacterial growth, metabolic activity, and the repair of antibiotic-induced damage, promoting resistance development [141,142]. These physiological changes are compounded by increased horizontal gene transfer rates, enabling the rapid spread of resistance genes such as beta-lactamases, especially in warmer regions [143–145] *bur*. Prolonged exposure to high temperatures enhances these effects, driving the gradual evolution and dominance of antibiotic-resistant bacteria [146]. Moreover, heat stress activates bacterial heat shock responses that overlap with antibiotic resistance mechanisms, equipping bacteria with cross-protective strategies against both antibiotics and thermal stress [138,147]. These responses include the upregulation of genes involved in cell wall biosynthesis and protein folding, which increase resistance to beta-lactams and aminoglycosides [148]. For instance, *E. coli* and *P. aeruginosa* exposed to heat exhibit enhanced survival and antibiotic resilience due to shared defense mechanisms

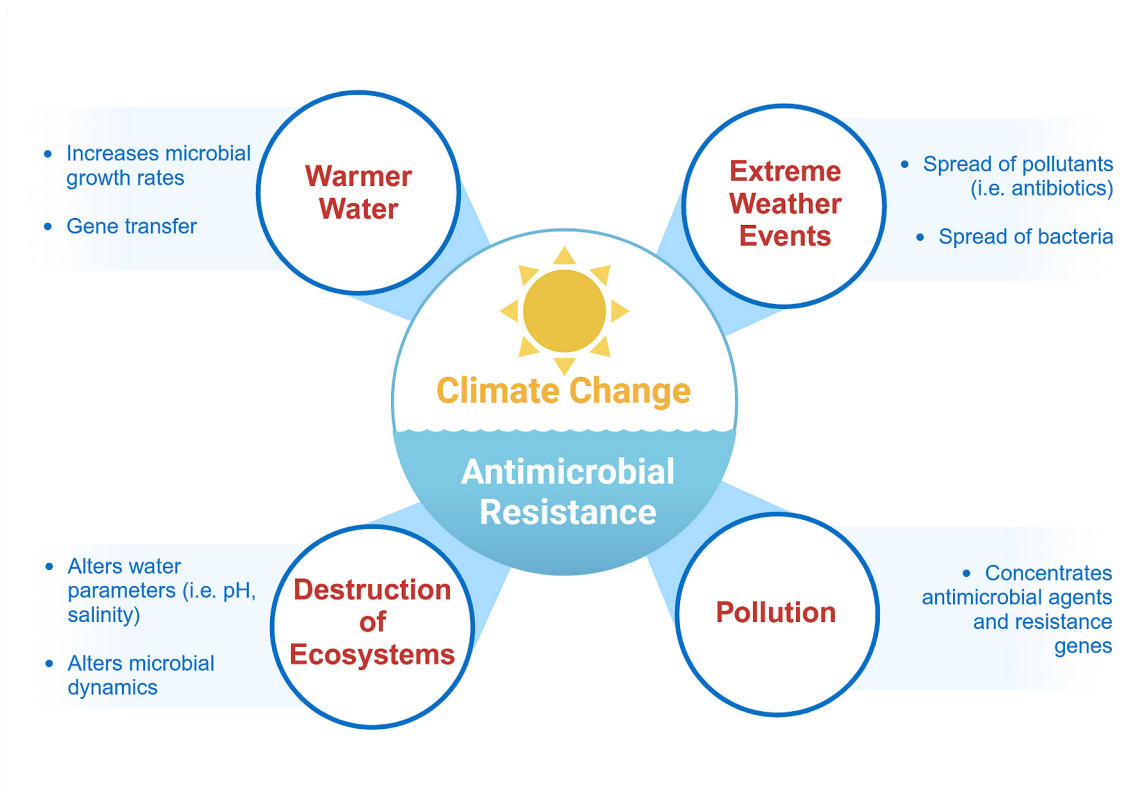


Fig. 3. A schematic representation of how climate change influences microbial proliferation and the development of AMR in aquatic environments.

[149,150]. Additionally, the heat shock response accelerates integron-mediated gene recombination, further facilitating the spread of multi-drug resistance [143,151].

Additionally, more frequent extreme weather events, such as heavy rainfall and prolonged periods of drought, may influence the availability of safe DW. Heavy rainfall can overwhelm combined sewer systems (CSS), causing overflows that discharge untreated wastewater into surface water, spreading pathogens and contaminants [152]. Stormwater runoff has been identified as a primary source of coliform bacteria, further increasing water quality risks [153]. Increased temperatures may enhance pathogen survival in aquatic environments, potentially raising public health concerns, although clear evidence is still emerging. Historical data shows extreme rainfall events are often linked to waterborne disease outbreaks, particularly in regions with private water supplies or insufficient infrastructure [154]. Furthermore, rising temperatures and ultraviolet radiation may alter organic matter in water, stimulating bacterial activity and compounding risks to water quality [155]. Also, in cold climates, projected changes in hydrology, contaminant transport, and water quality are expected to be intense and rapid. For example, in high-latitude regions like Canada, low river flows and increased risks from floods, frazil ice, microorganisms, and chemical contaminants highlight the complex challenges to drinking water systems [156]. These climate-related threats not only compromise water supply security but also elevate socioeconomic and public health risks, underscoring the need for adaptive risk management and resilience strategies to safeguard drinking water under changing climate conditions [156].

In short, climate change has far-reaching impacts on the entire potable water supply chain, from source to tap. Alterations in precipitation patterns, increased runoff, and changing surface water quality pose significant challenges to water availability and safety. These changes can lead to contamination risks, increased waterborne pathogens, and fluctuations in water quality, making it more difficult to provide clean and reliable DW. Additionally, extreme weather events

such as heavy rainfall, prolonged droughts, and heatwaves exacerbate these issues, placing greater stress on urban water systems and natural ecosystems.

5.3. Micropollutants

Micropollutants have emerged as critical contaminants in DW systems, with significant implications for biofilm development and water quality [17,157,158]. Commonly known as emerging contaminants, these pollutants can be categorized into several groups such as pharmaceuticals, microplastics, personal care products, steroid hormones, pesticides, and industrial chemicals [15,16]. The EU FP7 project elaborated a comprehensive list of 242 chemicals, of which approximately 70 % are pharmaceuticals and personal care products [159]. The remaining 30 % comprises industrial substances, including perfluoro compounds, pesticides, herbicides, and food additives. These pollutants originate from various sources, including agricultural runoff, industrial discharge, and household waste, entering the environment through leaching, atmospheric deposition, and wastewater effluents [160]. Often found in water at trace concentrations (ng/L to µg/L) and weight varies (i.e. pharmaceuticals between 150 and 500 Da), these pollutants challenge detection and complicate water treatment [17]. Conventional WWTPs are not specifically designed to eliminate micropollutants, allowing many to persist and enter aquatic ecosystems [16].

These pollutants can influence the growth and behavior of microorganisms and biofilms in distribution systems, as they may act as additional substrates for microbial growth or stressors that alter biofilm composition. Recently, Gross et al. [161] investigated the influence of microplastics (MPs) concentration, size, and composition on AMR by exposing *E. coli* to various MPs, including polyethylene, polystyrene, and polypropylene, at sizes ranging from 3 to 500 µm. The study revealed that biofilm formation on MPs significantly increased MDR, with *E. coli* showing up to a 100-fold increase in resistance to antibiotics such as ciprofloxacin, doxycycline, and streptomycin after 10 days of exposure.

MPs, particularly polystyrene, enhanced biofilm formation and resistance due to their hydrophobic nature and higher surface adsorption capacities compared to controls like glass. Additionally, bacteria passaged with MPs exhibited stronger biofilm formation even after the MPs were removed, linked to impaired motility. On a different study, Sabatino et al. [162] demonstrated the significant impact of MPs on biofilm formation in freshwater environments. Their study revealed that after 43 days of exposure in Lake Maggiore, biofilms on synthetic materials like polyethylene terephthalate, polypropylene, and biodegradable Mater-Bi had a higher proportion of potentially pathogenic bacteria compared to natural materials like wood and cellulose. While bacterial richness across the surfaces was similar, biofilms on polyethylene terephthalate, polypropylene, and Mater-Bi exhibited a higher abundance of antibiotic resistance genes, particularly *tetA*, which was significantly more abundant on polyethylene terephthalate and polypropylene than on the control cage, cellulose, and wood. Beta diversity analysis revealed that 77.0 % of the variation in microbial community composition within biofilms could be explained by the material. Furthermore, *Flavobacterium*, *Pseudomonas*, and *Brevundimonas* were the most abundant genera in the biofilms, with *Flavobacterium* being the dominant genus on both microplastics and wood. Another study on MPs occurrence but in wastewater found that biofilm formation was highest after three weeks of exposure, peaking at an optical density (OD) value of 1.77, before declining at week 5 (OD = 1.1). Biofilms were predominantly formed on MPs exposed to dark conditions, mesophilic temperatures (25 °C), and aerobic environments, where positive correlations were observed between biofilm formation and NH_4 ($r = 0.824$) and NO_2 concentrations ($r = 0.1$), while a negative correlation was noted with nitrate NO_3 levels ($r = -0.673$). Again, polyethylene particles supported the highest biofilm formation, and the microbial communities were predominantly composed of Proteobacteria, Bacteroidetes, and Planctomycetes, with *Methylotenera* being the most abundant genus.

In terms of micropollutants from pharmaceutical and personal care products, Borreca et al. [163] examined the dissipation and impact of metformin, metolachlor, and terbutryn on prokaryotic communities in sediment-water microcosms, introducing these micropollutants individually or in a mixture. Metformin and metolachlor dissipated within 70 days, while terbutryn persisted, with no significant difference in dissipation between individual and mixed exposures. Prokaryotic community shifts were primarily driven by matrix composition and incubation time, but micropollutants also influenced community structure. Bdellovibrionota, a predatory phylum, was markedly enriched in the mixed exposure (fold change in micropollutant mixture = 6253) compared to individual treatments, suggesting a role in reducing certain taxa. The enrichment of Campilobacterota, which includes human pathogens, highlighted potential health risks. Investigating micropollutant interactions, the study identified additive effects as predominant (33–57 %), while antagonistic interactions (34–52 %) were also frequent. Synergistic effects were less common (5–15 %) but notable for certain taxa, such as WS4 in sediment and Micrarchaeota in water. A ranking of micropollutant contributions showed that metolachlor and terbutryn were the primary drivers of additive effects, while metformin had a minor impact, possibly due to its faster dissipation. Pereira et al. [164] investigated the impact of methylparaben and chlorine, on the architecture of *S. maltophilia* biofilms, an emerging pathogen. Using Mesolens widefield epi-fluorescence mesoscopy, the study found that methylparaben exposure at both environmental (15 µg/L) and in-use (15 mg/L) concentrations led to significant architectural changes in biofilm morphology. Methylparaben exposure induced the formation of more complex internal structures, evidenced by a higher number of channel-like structures, particularly in the colony center, as shown by GFP intensity profiles and peak analysis. Statistically significant differences were observed, with biofilms exposed to 15 mg/L MP displaying an average of 297 channel structures compared to 193 in the control. Lipid distribution was also altered, with MP exposure shifting lipids toward the colony periphery at 15 µg/L, while at 15 mg/L, lipids

concentrated in the center. Chlorine treatment at 5 mg/L further modified biofilm architecture, particularly in the presence of MP, affecting colony diameter and roundness. Biofilms exposed to both MP and chlorine showed a significant decrease in colony roundness (0.1 ± 0.04) compared to control (0.5 ± 0.2).

Additionally, microbial consortia, such as biofilms, have emerged as a promising biotechnological strategy for the removal of micropollutants, as microorganisms within biofilms can effectively degrade or adsorb these environmental contaminants. Understanding the factors influencing biofilm efficiency, such as environmental conditions and biofilm composition, is crucial for optimizing their potential in micropollutant removal. For example, Horemans et al. [165], investigated not only the impact of linuron, an herbicide, on bacterial multispecies biofilms in aquatic ecosystems but also how the biofilm itself affects pollutant removal. They focused on a bacterial consortium composed of *Variovorax* sp. WDL1, *Comamonas* sp. WDL7, and *Hyphomicrobium* sp. WDL6, which degrades linuron and its metabolites. Results showed that biofilms formed at higher concentrations (1000 µg/L) exhibited large, organized biomass mounds, while lower concentrations (100 and 10 µg/L) resulted in thinner biofilms. Linuron removal efficiency decreased with lower pollutant concentrations, from 85 % at 100 and 1000 µg/L to 30 % at 10 µg/L. When additional carbon sources such as citrate and dissolved organic matter were provided, biofilm biomass increased, but linuron degradation remained low. The study highlighted the significant impact of micropollutants on biofilm structure, revealing that while supplementary carbon sources can stimulate bacterial growth and increase biofilm biomass, they do not significantly enhance linuron degradation at lower pollutant concentrations. This suggests that the presence of additional carbon sources has a limited effect on degradation processes when pollutant levels are low. These findings are crucial as they demonstrate that micropollutants can alter biofilm formation and influence its pollutant-degrading capacity, with biofilms displaying varying structural responses depending on pollutant concentration. This indicates that strategies involving supplementary carbon sources may not be effective in enhancing biodegradation in environments with low micropollutant levels. Furthermore, the study suggests that biofilms can exhibit resilience to low-level contamination, which is important for understanding their persistence in aquatic systems even in the presence of minimal pollutants.

The previous studies collectively underscore the significant role of micropollutants in promoting biofilm formation and the selection of AMR bacteria, posing environmental and healthcare challenges. Micropollutants act as persistent substrates that not only enhance biofilm formation but also contribute to the spread of pathogens and resistant bacteria. These findings highlight the need for comprehensive strategies to mitigate biofilm-related risks, particularly in wastewater treatment and DWDS. Additionally, the combined impact of micropollutants and chlorine on biofilm architecture emphasizes the potential risks posed by emerging contaminants, underlining the necessity for improved treatment methods to control biofilm-related contamination in aquatic environments.

6. Conclusions and perspectives

The presence of biofilms in DW systems poses multifaceted challenges that extend beyond microbial contamination, including increased disinfection resistance, biocorrosion, and the formation of harmful DBPs. Despite advancements in water disinfection, sporadic outbreaks of waterborne diseases and the emergence of novel pathogens persist, highlighting the need for enhanced disease surveillance and innovative control strategies. Biofilms reduce the efficacy of disinfection and contribute to the formation of DBP precursors, establishing a critical nexus between biofilms, corrosion, and water quality degradation.

Addressing these challenges requires a comprehensive and interdisciplinary approach:

1. Research and innovation: continued exploration of biofilm dynamics, microbial interactions, and advanced disinfection technologies.
2. Optimized treatment strategies: multi-barrier approaches that integrate physical and chemical disinfection, optimize residual disinfectant concentrations, and account for pipe material interactions are vital to maintaining microbial control without compromising water quality.
3. Climate adaptation and pollution control: climate change and micropollutants exacerbate microbial proliferation and antimicrobial resistance, necessitating adaptive water management strategies and improved pollution mitigation efforts.
4. Enhanced surveillance systems: strengthened monitoring systems for biofilm-associated outbreaks and the development of standardized testing protocols are imperative for timely intervention and prevention.

Collaboration among public health agencies, water utilities, and research institutions is crucial to safeguarding global water quality. By prioritizing innovation, ecological understanding, and interdisciplinary efforts, the water sector can address biofilm-associated challenges and ensure the delivery of safe DW in the face of evolving microbial threats and environmental changes.

CRedit authorship contribution statement

Ana C. Afonso: Writing – original draft, Visualization, Formal analysis, Conceptualization. **Maria J. Saavedra:** Writing – review & editing, Visualization, Validation, Supervision, Methodology, Conceptualization. **Inês B. Gomes:** Writing – review & editing, Visualization, Methodology, Investigation, Conceptualization. **Manuel Simões:** Writing – review & editing, Visualization, Validation, Supervision, Resources, Methodology, Investigation, Funding acquisition, Conceptualization. **Lúcia C. Simões:** Writing – review & editing, Visualization, Validation, Supervision, Methodology, Formal analysis, Conceptualization.

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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