International Journal of
Entrepreneurial Development, Education and Science Research
p-ISSN: 2360-901X | e-ISSN: 2360-9028

IJEDESR

February, 2026
Vol. 9, No. 1

Biofilms in Waterborne Disease Transmission, Control and Management Strategies

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Article DOI:
10.48028/iiprds/ijedesr.v9.i1.09

Abstract

A biofilm is a community of microorganisms, like bacteria, that adhere to a surface, embedding themselves in a protective matrix of extracellular polymeric substances (EPS). This "slime" acts as a shield, making biofilms resilient to antibiotics and immune responses. The formation of a biofilm is a developmental process in which a quorum sensing signal molecule functions to induce the secretion of EPS. The materials used in the drinking water network are significantly affected by the disinfection method used to produce the biofilm that adheres to them. Studies suggest that materials based on plastics (such as PVC and HDPE) tend to be more effective in controlling biofilm formation or removal than those based on metals (such as stainless steel), which have been found to be less effective in some instances. Chlorine-resistant strains are becoming more common in drinking water networks, resulting in the occurrence of diseases such as typhoid and cholera.

Keywords:
Biofilms, Waterborne disease, Biofilms life cycle and Confocal scanning laser microscopy

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Background to the Study

Access to safe and controlled drinking water remains inadequate despite its essential role in sustaining life. Waterborne bacterial infections, such as gastroenteritis, are a common cause of preventable illness and death, affecting many who lack safe water sources (Ekundayo *et al*., 2021). Additionally, chemical contaminants present significant health risks, resulting in vomiting, skin diseases, lung irritation, dizziness, and, in extreme cases, fatalities. Fully purifying drinking water is not necessary, as it may contain harmless and varied microorganisms. These are frequently derived from dispersed and detached biofilm cells and fragments. However, consuming contaminated drinking water or participating in unsafe water activities such as swimming, sailing and other water sports can lead to waterborne diseases caused by bacteria and viruses. Land-based activities, such as land use and the disposal of faecal waste, are the primary factors contributing to the spread of pathogenic species in water. In the absence of adequate treatment, waterborne diseases will remain a significant public health concern (Hemdan *et al*., 2021). The presence of biofilm in drinking water systems is the root cause of the spread of enteric pathogens and the contamination of drinking water. Biofilm is the primary cause of the increase in microbial contamination in drinking water systems. Human health is at risk due to three factors: ingestion or inhalation of water droplets while showering and skin contact during swimming or showering (Speight *et al*., 2019). Drinking water treatment plants deliver microbiologically safe drinking water to consumers through numerous miles of pipelines. In several instances, the pipes within the water distribution system are coated with biofilm, which, according to the scientific community, may impact water quality by introducing bacteria into the drinking water.

Biofilm formation can be viewed as a survival mechanism for bacteria; biofilm can provide protection from toxic compounds, such as antibiotics, thermal stress, and predation (Zhu *et al*., 2023). Biofilms in drinking water containing *Escherichia coli*, *Aeromonas,* and *Pseudomonas* spp., harbor potential risk to human health if pathogenic forms are present. The number of cells in a biofilm may reach as high as 1.0×10^{9} cells per clump which, in most cases, can comprise an infectious dose of a pathogen (Wang *et al*., 2023). However, survivability of bacterial cells and retention of their potential to cause disease when present in a biofilm remains to be fully elucidated (Hemdan *et al*., 2021). The major causative component, presumably the main reason for epidemics of watery diseases, is bacterial biofilm, Structural diversity and dynamism of biofilm. Diversion and separation of Extracellular Polymer Substances and the development of microcolonies follow the creation of biofilms in phases beginning with the attachment to the surface of cells. Channels allow nutrition and cellular waste to be exchanged between the microcolonies. A dispersal drive, although not restricted to environmental indications and bacterial signals, is the final phase of the biofilm life cycle. The second cyclic-di-GMP messaging (Jenal *et al*., 2017), nitric oxide and quorum sensing signals are included. In contrast to plankton and other non-biofilms, it is clear that the connection between infection and biofilm leads to prolonged life and persistence. A key factor in biofilm longevity might be due to improved resilience to environmental stress and biological film disinfection. Considerably more life in all major water disinfection procedures is seen compared to

their planktonic equivalents following biological treatment. Clearly, cells that have separated biofilms have retained better resistance. However, resistance to disinfection is lost when the detached cells have been suspended. Detailed biofilms demonstrate resistance phenotypes which allow the survival of biofilm cells to start downstream. The chronic nature of biofilm-related illnesses and their great resistance to antibiotics and antimicrobial compounds thus pose a cost-effective burden on global health systems.

Waterborne Disease

Waterborne diseases are caused by a diversity of pathogens in both developed and developing nations, and they are linked to a considerable burden. While an evaluation of the unfavorable impact of climate change on infectious illnesses on human health focuses mostly on vector-borne diseases, the frequency of waterborne diseases and patterns of transmission can also be affected as a result of environmental change (Wang *et al*., 2023). Waterborne diseases are any diseases induced by the use of drinking water polluted with pathogens include bacteria, protozoa, viruses, and helminths that can be transferred to people when human or animal excrement, including pathogens or chemical compounds. Waterborne they consume water that is untreated or inadequate. The pathogens that may be transmitted through contaminated DW are diverse in characteristics, behavior, and antimicrobial resistance. Bacteria are generally the group of pathogens that are most sensitive to disinfection. However, non-tuberculous mycobacteria in the environment, however, exhibit great resistance to chlorine, while others show moderate resistance (*Pseudomonas aeruginosa*, *Staphylococcus aureus*, and *Francisellatularensis*). If these pathogens are not disinfected and reach the consumer's tap, they can trigger disease outbreaks within the community (Hemdan *et al*., 2021). In drinking water distribution systems (DWDS), a number of harmful bacteria can live through stable biofilms and therefore constantly disseminate their population across the dynamic water bodies of the system. A wide range of diseases and well-being challenges might result from the ingestion of pathogen-contaminated water. These waterborne diseases are a major risk for newborns, pregnant women, and those with substantially low immunity. In the last two decades, approximately two million people, primarily children, have died unnecessary annually from water-related diarrhea. More than a billion people do not have access to safe drinking water. In the developing world, percentage of all fatalities is thought to be caused by polluted potable water. More than 80% of the world's waste is not collected or cleaned, which kills millions of people every year in the developing world from diseases linked to diarrhea (Reuben *et al*., 2019).

Factors that Produce Waterborne Drinking Outbreaks (WBDOs)

Multiple variables are responsible for outbreaks. Even if microbial populations in drinking water systems cause outbreaks, the infrastructure, chemical pipe cover and system architecture may boost or hinder the development of bacteria. Pathogens can enter the system in the case of breakages or leaks (Vestby, *et al*., 2020) and when repaired, patentees can enter. Defection leading to outbreaks of *Campylobacter*, *Salmonella*, *Shigella*, *E. coli* O157: H7, *Cryptosporidium*, *Giardia*, and Norovirus (Garner *et al*., 2019). Deficiencies of water treatment, such as insufficient or no surface water filtration and insufficient or

discontinued groundwater disinfection, led to 14% of WBDOs (Garner *et al*., 2019). The weather is another major contributing element to outbreaks as it adds pollutants by rinsing from heavy rain or flooding into water sources. Temperature variations can also affect the microbial dynamics in pipes, as planktonic bacteria may become trapped in biofilms, while biofilm pathogens may be discharged into flowing water (Garner *et al*., 2019).

A waterborne pathogen's goal, like any other creatures, is to reproduce and spread. The techniques of dispersion as well as the site of propagation have important effects. Some viruses spend the most of their lives in the water and only come into touch with a host by chance. They are often adapted to low nutrient concentrations as well as the physical, chemical, and biological conditions present in water. Water is their natural habitat, and they may breed in both water and on the host. In the last 20 years, it has been revealed that biofilms contain a substantial quantity of microbial life (Luo *et al*., 2021).

Biofilms may also be thought of as a place where various species come into close proximity, allowing communication, genetic material transfer, and even ingestion of smaller microorganisms (bacteria and perhaps viruses) by protozoan predators feeding on biofilms. Waterborne pathogens are unlikely to be an exception, as biofilm congregation and integration might provide significant benefits. Pathogens in biofilms are difficult to sample and identify, yet their ecology and survival are crucial (Garner *et al*., 2019).

Formation of Biofilm in Aquatic Environments

Bacteria in biofilms in an aquatic environment are rarely planktonic, instead are associated with surfaces that can include living tissues, indwelling medical devices, or industrial or potable water system piping, attachment helping the bacteria from being swept away (Jamal *et al*., 2018). Production of alginate, an exopolysaccharide present as an extracellular matrix in *Pseudomonas aeruginosa* biofilm, is induced upon the contact of the cells with a surface. Laboratory microcosm experiments suggest cells of *Vibrio cholerae* form biofilms on biotic and abiotic surfaces, thereby protecting themselves with this exopolymer barrier (Reuben *et al*., 2019). Other factors, such as less soluble or metabolizable large organic compounds, including humic acids that are adsorbed onto aquatic surfaces and chitinous exoskeletons of crustaceans provide nutrition directly to the attached bacteria. Ca^{2+}, which is abundant in marine and brackish water ecosystems, plays an important role in the formation of biofilms by directly stabilizing intercellular interactions, as has been shown for *P. aeruginosa* and *Streptococcus downei*, presumably by forming intercellular salt bridges. Biofilms in seawater similarly can involve chromium, nickel, and molybdenum, used as alloying elements to resist corrosion of stainless-steel bodies of marine vessels. Biofilms on those surfaces, however, do become corroded over time. Interestingly, the correlation between sea surface height in the Bay of Bengal and cholera epidemics in Bangladesh, can be explained by seawater, carrying particulates and plankton colonized with cholera bacteria in the form of biofilms inundating adjacent estuaries, and ultimately introducing the cholera bacteria to brackish ecosystems (Garner

et al., 2019). *Vibrio cholerae* serogroups O1 and O139 have been shown to survive year-round, mostly in a nonculturable state, within clusters of biofilm in the ecosystem adjacent to the coast of the Bay of Bengal, accounting for the endemic cholera in that geographic region. Biofilm-like, multicellular clumps of *V. cholerae* are found in human stools (Zhu *et al*., 2023), and a recent epidemiological and ecological study offered firm evidence that bodies of water serving as drinking water sources harbored biofilm-bound, nonculturable *V. cholerae* O1 cells between epidemics occurring in the coastal villages of the Bay of Bengal seasonally. Subsequent studies also showed that nonculturable *V. cholerae* O1 cells naturally present in biofilms were more adapted to the aquatic ecosystem, compared to *V. cholerae* shed in cholera stools and discharged into the aquatic ecosystem. The latter were found to remain infective for a relatively shorter period. *Vibrio cholerae* cells within naturally occurring biofilms in the aquatic ecosystem, a significant role in the ecology and seasonal epidemics of cholera in Bangladesh.

The Nature of Bacterial Biofilm

Biofilms are prevalent in nature because bacteria produce them as part of their survival strategies. A biofilm is a bacterial colony that lives in a matrix of extracellular polymeric material that they create. Proteins (for example, fibrin), polysaccharides (for example, alginate), and eDNA make up the biofilm matrix. In addition to the matrix's protection, bacteria in biofilms can use a variety of survival tactics to get beyond the host's defenses. Microbial cells attach to one another and to a static surface to form a biofilm (living or non-living). Bacterial biofilms are often harmful, and may lead to nosocomial infections. Bacterial biofilms are resistant to antibiotics, chemicals, phagocytosis, and other parts of the body's innate and adaptive inflammatory response. For instance, it is well recognized that biofilm development is responsible for the persistence of staphylococcal infections linked to foreign substances. Chronic Pseudomonas lung aeruginosa infections are also produced by biofilm developing mucosal strains in cystic fibrosis patients. Nutrient and oxygen gradients exist from the top to the bottom of biofilms, and bacterial cells in nutrient-deficient regions have lower metabolic activity and higher doubling times. As a result, some of the antibiotic tolerance is attributed to these latent cells. An increase in the number of mutations is linked to the formation of biofilms. Bacteria in biofilms interact via chemicals that activate genes involved in the synthesis of virulence factors and, to a degree, biofilm structure. This is known as quorum sensing, and it is based on the concentration of quorum sensing molecules in a niche, which is determined by the number of bacteria there. Antibiotic prophylaxis or early aggressive antibiotic therapy can prevent biofilms, while persistent suppressive antibiotic therapy can treat them. Compounds that disintegrate the biofilm matrix and quorum sensing inhibitors that enhance biofilm susceptibility to antibiotics and phagocytosis are two promising methods (Høiby *et al*., 2011).

According to the National Institutes of Health (NIH), biofilm formation is connected to 65 percent of all microbial infections and 80 percent of chronic diseases. The development of a biofilm comprises a number of steps, starting with attachments to a living or non-living surface that led to the development of a micro-colony, which then creates a 3-dimensional

structure and leads to separation after maturity. During biofilm formation, several types of bacteria communicate with one another via quorum sensing. Bacterial biofilms are often immune to both human and antibiotic immune systems. Because the biofilm can cause diseases through both device-related and non-device-related infections, health concerns are loud and clear. To summarize, understanding bacterial biofilm is critical for controlling and/or eliminating biofilm-related diseases (Jamal *et al*., 2017).

Biofilm Life Cycle

In reaction to environmental stressors including UV radiation, desiccation, nutrition restriction, extreme pH, extreme temperature, high salt content, high pressure and antimicrobial chemicals, bacteria create biofilms. Bacteria develop (Wang *et al*., 2023). The events that lead to the development of bacterial biofilms are complicated here. Biofilm production is typically considered to start with a reversible bacterium attachment on the surface, followed by an irreversible attachment which is generally supported by bacteria's adhesive structures and short-range interactions. Their reversible connection has been advanced by the manufacture of EPS. Subsequently they become a structured structure, imprisoned inside an EPS matrix. In the end, bacteria can escape the mature biological film and spread to the environment to establish new niches (Reuben *et al*., 2019). These steps of the development of biofilms are shown in figure 1. The following are five major stages leading to the evolution of free-living planktonic life into a sedentary "biofilm" existence.

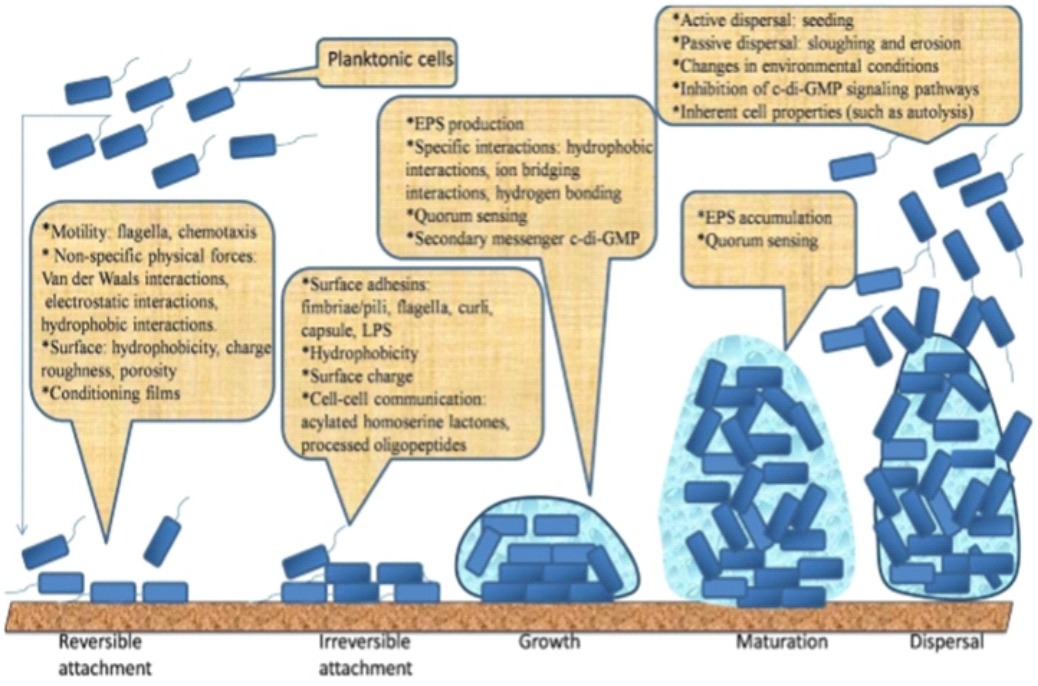

Figure 1: The five main phases leading to the development and dispersal of biofilm.
Source: Wang *et al*. (2020).

So, bacterial biofilm formation is a complex process and can be described in five main phases:

i. Reversible attachment phase, where bacteria non-specifically attach to surfaces
ii. Irreversible attachment phase, which involves interaction between bacterial cells and a surface using bacterial adhesins such as fimbriae and lipopolysaccharide (LPS)
iii. production of extracellular polymeric substances (EPS) by the resident bacterial cells
iv. Biofilm maturation phase, in which bacterial cells synthesize and release signaling molecules to sense the presence of each other, conducing to the formation of micro colony and maturation of biofilms
v. Dispersal/detachment phase, where the bacterial cells depart biofilms and comeback to independent planktonic lifestyle. Biofilm formation is detrimental in healthcare, drinking water distribution systems, food, and marine industries, etc.

Routes Through Which Pathogens Can Enter the Water Distribution System

Pathogens can enter the distribution system via a variety of pathways and become entrained in the biofilm for later release. The pathways are:

i. Entry through the source of water (e.g., treatment breakthrough)
ii. Entry through broken or leaking pipes, valves, joints and seals
iii. Entry through cross-connections and backflow
iv. Entry through contamination of finished water storage vessels
v. Entry through Improper Treatment of Materials, Equipment or Personnel in Contact with Finished Water
vi. Entry through inadequate distribution system security

The extent to which possible health implications of pollution in the distribution system are caused is partly dependent upon the route of entrance. A panel of experts has assessed many methods of disease entrance into distribution systems with the possible health implications, taking into consideration disease severity, risk of waterborne disease breakout, contaminated volume of incursion and frequency of intrusion (Jamal *et al*., 2017).

Factors that Influence Pipe Biofilm Development

There are numbers of physical, chemical and biological variables affecting the growth of biofilm pipes. The interaction between these elements is complicated and changeable for each given system, which frequently makes forecasts problematic. A number of studies have thrown light on these factors and relationships, and numerous full reviews have been published. The majority of data on the variables that impact biofilm development are based on changes in total viable counts (e.g., heterotrophic plate count) or changes in the growth of particular coliforms (Wang *et al*., 2023). The following factors are majored

i. Environmental factors
ii. Presence of nutrients

iii. Microbial interactions
iv. Distribution system materials
v. System hydraulics
vi. Presence of distribution system residual
vii. Sediment accumulation

Analysis of Biofilms and Detection of the Organisms

Microscopy continues to be the most widely used tool in the analysis of biofilms. Confocal scanning laser microscopy (CSLM) captures top-down images of mature biofilms that can be viewed in a qualitative manner. Additionally, those images can be sectioned along the xz axis and reconstructed to give sagittal or orthogonal profiles, for which the depth, and subsequently volume, of the biofilm can be quantified. There are a number of software packages, such as COMSTAT or IMARIS, that can assist in this type of analysis. Phase contrast light microscopy and epifluorescence microscopy are used primarily to examine early development of biofilms, that is cell attachment and migration to form microcolonies, and in those cases where CSLM is not available. Occasionally, scanning and transmission electron microscopy are used (Muhammad *et al*., 2020).

Nonmicroscopic methods for the analysis of biofilm biomass include staining with crystal violet (absorbance at 570 nm), Syto9, fluorescein diacetate (FDA), resazurin, tetrazolium salts (CTC, XTT), and dimethyl methylene blue (DMMB). These assays principally work the same, except they stain in different ways; e.g., cells and biofilm matrix, viable cells, and/or the polysaccharide matrix. Attached cells are incubated with the staining agent, rinsed to remove unattached cells, and then quantified, based on fluorescence absorbance. For bacterial enumeration, the biofilm is first mechanically disrupted (glass beads), serially diluted and colony-forming units determined by plate count (Hemdan*et al*., 2021).

Culture Conditions

Flow cell biofilm reactors, derivatives of chemostats, have been used primarily for studying biofilms. Most are driven by a peristaltic pump and include single pass systems and recycling reactors. The biofilm bait can be silicone tubing, such as is used in catheters, or glass capillary (Høiby *et al*., 2011). Many less expensive alternatives to flow cells have been described. In one study, researchers used a chambered cover glass system (Nunc Lab-Tek), originally designed for tissue culture. Borosilicate slide cover glasses submerged in media in 50 ml conical centrifuge tubes were employed with good results by Liu *et al*. (2021). For all culture methods, the resultant biofilm can be assayed by microscopy. When non-microscopic methods are used, the biofilm can be cultured in polyvinyl chloride or polystyrene microtiter plates or borosilicate glass tubes.

Role of Biofilms in Causing Disease and Disease Transmission

It has been long known that bacteria can adhere to solid surfaces and form a slimy, slippery coat (i.e. biofilm). Furthermore, it has been suggested that biofilms play a significant role in the transmission and persistence of human disease. For human pathogenic bacteria, biofilms offer protection to the bacteria from the host immune system

and allow those bacteria to withstand killing doses of antibiotics. For example, in the medical community, one of the greatest concerns has been microbial biofilms on indwelling medical devices (e.g. prosthetic heart valves) or other devices used in the healthcare environment that can harbor biofilms. In humans, *V. cholerae* biofilms in the gut of cholera patients may be protected from acid pH and/or antibiotic, thereby serving as a vehicle for fecal-oral mode of transmission of the disease. In the natural environment, biofilms can also provide a safe haven for pathogenic bacteria, protecting them from a variety of physiochemical stresses, including UV light, oxidative stress, dehydration, and biocidal agents. Additionally, since bacteria in the natural environment are subject to predation by protozoa, bacterivorous microorganisms (e.g. *Bdellovibrio* spp.), and bacteriophages, it is probable that biofilms provide a mechanism for the persistence of bacterial-forming bacterial pathogens in the environment (Reuben *et al*., 2019).

There is an increasing realization of the importance of the world's oceans as a source of potentially pathogenic microorganisms. Human bacterial pathogens associated with the marine environment and the diseases they cause have been reviewed. Several bacterial pathogens found in the marine environment produce biofilms. While progress is being made in elucidating molecular and genetic mechanisms by which these bacteria produce biofilms, its role in disease transmission, by far the most detailed work has been done on *V. cholerae*, the causative agent of cholera (Lu, *et al*., 2022). Biofilm formation in *V. cholerae* is a multistep developmental process controlled by several regulatory pathways. In particular, recent evidence suggests that two main systems are responsible for biofilm formation and regulation in *V. cholerae*, namely, the matrix exopolysaccharide and quorum sensing. *Vibrio cholerae* has been shown to undergo phenotypic (phase) variation, generating two morphologically distinct variants, smooth and rugose. This phase variation has been implicated in its adaption to variation in its natural aquatic ecosystem. In particular, the rugose variant has an enhanced capacity to form biofilms, a phenotype mediated, in part, by increased production of exopolysaccharide VPS (Vibrio polysaccharide synthesis). New data suggest that the gene, rbmA (rugosity and biofilm structure modulator A), is required for rugose colony formation and integrity of the biofilm structure in *V. cholerae* (Garner *et al*., 2019). Bacteria can engage in social behavior in which they coordinate expression of certain genes or phenotypes using quorum-sensing regulation.

Essentially, individual cells detect the density of cells around them and activate expression of target genes when the density is high. It has been established that V. cholerae employs cell-to-cell communication, namely quorum sensing, to control biofilm formation. Specifically, quorum sensing and 30, 50-cyclic diguanylic acid (c-di-GMP) act reciprocally to control biofilm formation. In V. cholerae, quorum sensing represses biofilm formation and c-di-GMP, an intracellular second messenger, activates biofilm formation. Recent genetic evidence shows that a protein, HapR, which is produced at high cell density, represses biofilm formation by two distinct mechanisms (Garner *et al*., 2019). First, HapR controls transcription of 14 genes encoding a group of proteins that synthesize and degrade c-diGMP, resulting in a net reduction in cellular c-di-GMP levels at high cell

density and, thereby, a decrease in biofilm production. Second, HapR binds directly to and represses the expression of the biofilm transcriptional activator, vpsT, also leading to decrease in biofilm formation.

Biofilm formation by pathogens in the environment can play a role in the transmission of those pathogens. For example, an obvious mechanism by which microbial pathogens active in biofilms because disease is by the seeding and dispersal of a large number of cells that subsequently initiate an infection. This phenomenon was shown to be the case for *V. cholerae* in surface waters in a cholera-endemic area in Bangladesh, where large numbers of toxigenic *V. cholerae* in the viable but nonculturable state were detected in biofilms. Since it had been shown that cholera patients shed high concentrations of *V. cholerae* in vivo-formed biofilms, shedding clumps of cells appears to be an efficient way of delivering an infectious dose of organisms. Thus, biofilm formation by *V. cholerae* can play a major role in its ecology and in the epidemiology of cholera and, very likely, other water-borne bacterial pathogens can do so as well (Zhu *et al*., 2023).

Control and Management Strategies of Biofilms Microbial Chlorine Resistance in Drinking Water Distribution Systems (DWDS)

The removal of biofilm in drinking water distribution systems is based on both preventive and restorative methods, such as reducing the nutrient content of the tap water implementing various disinfection measures or flushing and replacing the water. However, these procedures often fail, primarily because of the cohesive nature of the biofilm, attributed to the physicochemical properties of the exopolymeric matrix (Reuben *et al*., 2019). Effective cleaning procedures must disrupt the matrix and/or modify the properties of bacterial biofilms. Chlorine and chlorine-based methods are widely used for the disinfection of water in DWDSs for both households and industries. These methods are popular due to their easy accessibility, affordability, and safety. Chlorination is the prevalent approach to combat biofilm. Chlorine has an exceptionally broad-spectrum effect against most microorganisms. Additional factors contributing to the widespread use of chlorination include its ability to inactivate a significant proportion of microbiological populations through chlorine residues, as well as its powerful sterilization ability, low investment, and operating costs, comparatively lower organic carbon reactivity when compared to other disinfectants, and the long-term maintenance of biostability in treated water. A chlorine-based disinfection process is advantageous due to its lack of bacteriostatic effect and need for continuous application, in contrast to UV and ozone treatments.

Problems with Microbiological Chlorine Resistance (MCR)

However, the efficiency of the chlorination method has declined due to the emergence of microbiological chlorine resistance (MCR), resulting in an increased risk of waterborne diseases among the population. Chlorine-resistant strains are increasingly prevalent in drinking water networks, leading to the occurrence of diseases including typhus, cholera, shigellosis, salmonellosis, giardiasis, cryptosporidiosis, campylobacteriosis, and Hepatitis A virus infection. MCR is a state in which the previously established

concentration of chlorine or chlorine-based disinfectant/procedure no longer has a destructive effect on the microorganism during drinking water and wastewater treatment. This state encompasses the survival of microorganisms following chlorine disinfection and their subsequent or repeated growth despite the physiological and genetic destructive effects of chlorine. The mechanisms of MCR comprise in situ cell aggregation, clumping, and structural modification of the microbial cell surface, EPS production, the formation of resistant spores due to re-proliferation, and good adhesion to surfaces within the biofilm matrix (Gaudreau *et al*., 2018). MCR poses several technical problems in both residential drinking water and wastewater treatment. These include issues with management and technical aspects related to inadequate chlorine dosing, problems due to fluctuating flow rates, hazards arising from unstable free chlorine residuals, improper design, and process selection, as well as a lack of quality control in the chosen process. The aforementioned issues collectively contribute to the emergence of pathogenic microorganisms such as protozoa, fungi, and viruses in drinking water. Biofilm removal using chlorination should be compared with alternative techniques to determine its effectiveness. Within an artificially created drinking water network, a biofilm in drinking water was matured for two months on the inner surface of a pipe with high polyethylene content, and the efficacy values of two biofilm removal methods were compared. In the case of one method, a significant level of chlorine disinfectant was applied by increasing the hydrodynamic shear stress on the wall of the drinking water network. The other method involved exerting continuously increasing pressure on the inner surface of the HDPE to mechanically remove the biofilm. Based on the results, more than half of the bacteria were removed with both procedures. During exposure to increasing hydrodynamic shear stress, biofilm volume decreased. With chlorination, 75 % of the cells could be removed and the volume of the biofilm was also reduced (Zhu *et al*., 2023).

Chlorine-Resistant Bacteria

Bacteria that are highly resistant to chlorine disinfection, capable of survival or even reproduction, are classified as chlorine-resistant bacteria (CRB). Chlorine disinfection does not fully control the risks of CRB, such as pathogenicity, antimicrobial resistance, or microbial growth. In most cases, the attention of researchers is focused on pathogenic and antibiotic-resistant bacteria, and in several locations, the presence of non-pathogenic bacteria is allowed to a certain extent in drinking water networks (Chan *et al*., 2019). Several researchers have confirmed the presence of the following CRB bacteria in drinking water networks: *Mycobacterium*, *Pseudomonas*, *Staphylococcus*, *Acinetobacter*, *Bacillus*, *Acidovorax*, and *Sphingomonas*. Subsequent tests are required with these bacteria to determine the chlorine concentration at which their presence in drinking water networks can be minimized (Høiby *et al*., 2011). It is also worth dealing with the presence of non-pathogenic CRBs in drinking water networks, as these bacteria can also cause problems. Their presence can lead to pipeline corrosion, increased biofilm formation, nitrification, and affect the sensory quality of drinking water (i.e., colour, smell, taste). Additionally, the presence of CRBs in the biofilm can also lead to the appearance of pathogens in the biofilm, they are better able to adhere to surfaces as they are protected against the disinfectant. The

chlorine resistance mechanism of CRB is non-specific (while it is specific for antibiotic resistance). As a rule, chlorine reacts with various cell components, such as the cell wall or cell membrane, resulting in damage, and the inactivation of microorganisms. Consequently, the primary source of chlorine resistance can be attributed to the permeability barrier and chlorine consumption capacity of the biofilm matrix (cells and EPS). A protective permeation barrier formed by cell membranes, cell walls, spore membranes, and EPS serves as a defense mechanism against chlorine. Additionally, chlorine disinfection triggers the activation of various functional genes, including those responsible for managing oxidative stress, facilitating DNA repair, secreting antioxidant enzymes, regulating pore proteins, and repairing cell walls The development of chlorine resistance in bacteria can be attributed to three primary factors: increased efficiency of the efflux system, activation of the bacterial self-repair mechanisms, and an increased capacity to absorb nutrients (Garner *et al*., 2019).

Conclusion

Biofilms play a significant role in waterborne disease transmission, and their control and management are crucial for public health. Biofilms form on water distribution system surfaces, harboring pathogens like *Legionella*, *Pseudomonas*, and *Mycobacterium*, biofilms also contribute to waterborne disease outbreaks, including Legionnaires' disease, cholera, and typhoid fever, exhibit increased resistance to antimicrobial agents, complicating treatment and control. Effective control strategies are Chlorination and disinfection, Pipe material selection and maintenance, Temperature control and biofilm-disrupting agents.

Recommendations

1. Foster collaboration among water utilities, public health officials, and researchers.
2. standardized protocols for biofilm detection, monitoring, and control should be developed.
3. The public should be educated on the risks associated with biofilms in water distribution systems.

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