Bacterial biofilm significance and implication in the epidemiology of waterborne disease

By
Marium Momin
19376002

A thesis submitted to the Department of Mathematics and Natural Sciences in partial fulfillment of the requirements for the degree of Master of Science in Biotechnology

Department of Mathematics and Natural Science
BRAC University
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Declaration

It is hereby declared that

- The thesis submitted is my/our own original work while completing degree at Brac University.
- The thesis does not contain material previously published or written by a third party, except where this is appropriately cited through full and accurate referencing.
- The thesis does not contain material which has been accepted, or submitted, for any other degree or diploma at a university or other institution.
- I/We have acknowledged all main sources of help.

Student's Full Name & Signature:

Marium Momin

_____

Student Full Name

19376002

Student ID

Approval

The thesis/project titled "Bacterial biofilm significance and implication in the epidemiology of waterborne disease" submitted by

Marium Momin (19376002)

of Fall, 2021 has been accepted as satisfactory in partial fulfillment of the requirement for the degree of Masters in Biotechnology on [04-09-2021].

Examining Committee:

Supervisor: (Member)	Iftekher Bin Naser Associate Professor, Mathematics and Natural Science Department BRAC University
Departmental Head: (Chair)	A F M Yusuf Haider Professor and Chairperson, Mathematics and Natural Science Department BRAC University

Ethics Statement

This is an entirely new piece of work that has never been published before. It is based on my own honest and thorough study and analysis. All of the sources used in the study are appropriately credited (correct citation).

Dedicated to my beloved Parents

Acknowledgement

My Master's thesis would not have been completed without the continual blessings of Almighty in every stage of my life. I'd like to thank my mother for everything and absolutely everything, as well as my father for always being there for me.

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Abstract

In the epidemiology of WBDOs, assessing the possible risk of biofilm and studying it is crucial. In this study, we look at the epidemiology of waterborne diseases as well as the diseases altogether. WB disease outbreaks are common, and bacterial biofilm is one of the major reasons. The paper also provides a broad overview of the role of bacterial biofilm in the epidemiology of WBDO by providing a general overview of the nature, life cycle, and mechanism of bacterial biofilm, as well as how infectious diseases associated with bacterial biofilm cause waterborne disease outbreaks around the world. Later, the implication of bacterial biofilm in WBDO is highlighted by depicting biofilm formation in the water distribution system. There are numerous nations on the globe today where water shortages, rather than water quality, are the most serious health concern. Access to enough safe drinking water should be considered a basic human right. Bacterial biofilm development in water distribution networks is the underlying cause of WBDOs. To draw a conclusion on the review paper, certain cases of waterborne diseases induced by bacterial biofilm formation were mentioned in the concluding part. One of the objectives of this review is to discuss the vast range of unique issues impacting waterborne diseases in terms of biofilm formation and development, which are often overlooked in the epidemiology of waterborne diseases for disease control. Furthermore, we feel that once the reader understands them better, it becomes more crucial to evaluate the past and recent occurrence of outbreaks and their epidemiology of waterborne diseases. Finally, this study demonstrates that bacterial biofilm is present in many, if not all, chronic infections. This information is vital for the creation of successful WBDO epidemiological initiatives.

Key words

Biofilm, Waterborne disease, Bacterial biofilm, Waterborne disease outbreaks, Drinking water system, Epidemiology.

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List of Acronyms

WBDOs	Waterborne Disease Outbreaks
WB	Waterborne
DW	Drinking Water
DWDS	Drinking Water Distribution Systems
WHO	World Health Organization
CDC	Centers for Disease Control and Prevention
WBDOSS	Waterborne Disease and Outbreak Surveillance System
NIH	National Institutes of Health
EPS	Extracellular Polymeric Substances
LPS	lipopolysaccharide
NVE	Native Valve Endocarditis
NBTE	Non-Bacterial Thrombotic Endocarditis
MAC	Mycobacterium Avium Complex
CVEC	Cells Containing Environmentally Viable Conditions
HUS	Hemolytic Uremic Syndrome
STEC	Shiga Toxin-producing E. coli
EAEC	Enteroaggregative E. coli

- ## Introduction:

The worldwide health hazard is significant with regard to waterborne illnesses, particularly those caused by uncertain drinking water. Understanding disease pathogens can help us create better strategies for preventing illnesses and controlling them internationally. Epidemiological research reveals links between illness and potential risk factors. The major causative component, presumably the main reason for epidemics of watery diseases, is bacterial biofilm. Structural diversity and dynamism of biofilms (Hall-Stoodley et al., 2004). 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 (Costerton et al., 1987). The worldwide health hazard is significant with regard to waterborne illnesses, particularly those caused by uncertain drinking water. Understanding disease pathogens can help us create better strategies for preventing illnesses and controlling them internationally. Epidemiological research reveals links between illness and potential risk factors. The major causative component, presumably the main reason for epidemics of watery diseases, is bacterial biofilm. Structural diversity and dynamism of biofilms (Hall-Stoodley et al., 2004). 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 (Costerton et al., 1987). 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 (McDougald et al., 2011). The second cyclic-di-GMP messaging (Jenal et al., 2017), nitric oxide (Barraud et al., 2009) and quorum sensing signals are included (Rice et al., 2005).

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 (Friedrich, S., AG). A major potential concern for public health is the presence in drinking water of biofilms carrying pathogens such as Aeromones spp, Escherichia coli, and Pseudomonas spp. Biofilms can include a huge number of microbial cells that can lead to illnesses if swallowed, up to 1.0 to 109 per category (Reuben et al.,2019). 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.

We have underlined the importance and implications of bacterial biofilms and the need to recognize them in aquatic illness epidemiology in this comprehensive analysis. This enables readers to have a more mindful view of the relevance of WBDOs worldwide in biofilms. The epidemiology of WBDO and how it is caused by bacterial biofilm. This work includes representations and brief explanations of basic concepts to help us grasp abstract topics. This study was therefore organized to invite scientists in the field of biofilm from many areas of expertise.

Firstly, we review some background material in the first portion of the report on waterborne disease and the waterborne disease epidemiology. How often WB illness produces outbreaks and bacterial biofilm is one of the primary causes. In the second section, we give to you a broad picture of the importance of bacterial biofilm in wildlife epidemiology through an overview of how the bacterial biofilm is causing waterborne illnesses globally by infectious diseases linked with bacterial biofilm. The third portion of the paper emphasizes the impact of bacterial biofilm on WBDO, illustrating the production of biofilm in the water distribution system and its causative variables and the environment. In today's world, there are many nations where water shortages are the primary health problem, rather than quality. Appropriate and secure access to DW should be a fundamental human right. The fundamental cause of the WBDOs is the growth

of biofilms in the system of water distribution. A few aquatic illnesses caused by the formation of bacterial biofilms were mentioned in the concluding part to conclude the review subject.

One of our objectives is to address the vast range of unique aspects that are generally overlooked with regard to waterborne illnesses for biofilm development and biofilm formation in the epidermis. In epidemiology for WBDOs, the assessment and research of the potential risk of biofilm is also essential.

In addition, we think the reader will become more skeptical once they have a better grasp of this when assessing the earlier and more current prevalence of and epidemiological epidemics of waterborne diseases. The emergence and progression of bacterial biofilms and their illnesses could only address WBDO-related concerns. Therefore, a short explanation of the basic benefits and constraints of infectious aquatic diseases associated with bacterial biofilms and their relevance in epidemiology might be of greater benefit when it comes to reducing the dangers posed by waterborne disease outbreaks.

- **Goal of the Study:**

The goal of this paper is to examine what we know about existing bacterial biofilm literature and information on its importance in relation to waterborne disease epidemiology and on the impact of biofilm on outbreaks of waterborne illness.

1.2 Specific Aims:

This study is intended to collect all the facts concerned and give suitable advice based on the existing information from many notable publications. This study collects material from Elsevier, Nature, Springer and other leading publications, online articles and guidelines on bacterial biofilms and waterborne disease outbreaks. The data obtained has been cited correctly and provides a thorough understanding of bacterial biofilm, waterborne diseases, outbreaks of waterborne conditions, WBDO-related variables and their epidemiology, and of the importance of biofilm in WBDOs. Certain WBDO cases involved in the formation of bacterial biofilms are also presented here.

- **Background Information**

In this part, the specifics of waterborne diseases and waterborne disease epidemiology are examined, along with the frequency of waterborne disease outbreaks and the driving forces behind the waterborne disease outbreak. The following are discussed. In addition, how biofilm and one of the primary sources of these WBDOs are linked.

2.1 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 (Stuttgart, 2016).

Waterborne diseases are any diseases induced by the use of DW polluted with human or animal excrement, including pathogens or chemical compounds. Waterborne pathogens include bacteria, protozoa, viruses, and helminths that can be transferred to people when 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 Francisella tularensis). If these pathogens are not disinfected and reach the consumer's tap, they can trigger disease outbreaks within the community (Chaves Simões & Simões, 2013).

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 safer drinking water. In the developing world, % of all fatalities are 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 (N. Gilbert, 2012).

2.2 Epidemiological methods and Waterborne Disease

In research from (Percival et al., 2000), Epidemiological investigation described as an essential to the understanding of the waterborne disease. Epidemiology is a branch of medicine that specifies the study of the incidences and transmission of diseases in population. The assessment of risk in human populations relies heavily on the science of epidemiology.

In 1854, Jhon Snow created epidemiology concurrently and demonstrated that drinking water might spread disease. Since then, the significance of water in many infectious diseases has been emphasized in epidemiological research. Epidemiology is important for the research of waterborne disease epidemics. In addition, epidemiological research finds links between diseases and potential risk factors.

They argue the link between water and disease in a number of epidemiological studies. In general, these investigations are split into descriptive and analysis. A logical method is used to investigate a waterborne outbreak or any outbreak, and this approach is –

- Preparation for the outbreak
- Detection of an outbreak
- Confirmation that an outbreak is occurring
- Description of the outbreak
- Generation of a hypothesis as to the cause of the outbreak
- Implementation of an initial control measure
- Testing the validity of the hypothesis
- Implementation of further control measures if necessary
- Learning the lessons for the future

This is always followed by the best epidemiological research. The study shows that a fundamental grasp of these concepts is crucial since epidemiology builds on a full understanding of health risk.

2.3 Outbreak of waterborne diseases

Chaves Simões & Simões (2013) highlight the incidence of outbreaks of waterborne diseases in various countries in a review article. They indicated that an epidemic of waterborne disease is typically characterized as having at least two people suffering from a comparable condition following water exposure when evidence reveals a plausible source of water. The incidence of waterborne disease outbreaks is not just in impoverished nations; it is equally influenced by rich ones. Certain disease outbreaks have been caused in various areas of the world in the last 150 years by water contamination.

While gastroenteritis is the most common in developed nations, waterborne outbreak diseases include many more: cholera, typhoid fever, meningitis, encephalitis, dysentery, hepatitis, legionellosis, lung disease, poliomyelitis, leptospirosis, giardia, and salmonellosis. There are several diseases in other developing countries that are the most common.

Although waterborne diseases in developed countries are infrequently deadly, some authors argue that intake of DW caused by pathogenic bacteria, protozoa, and viruses raises a series of health problems. DW treatment plants and distribution networks which did not maintain a sufficient quantity of disinfectant to prevent pathogens from developing and/or retaining pathogens created various public health risks.

In this millennium, there have been reported waterborne outbreaks in Canada, France, Italy, the United Kingdom, Switzerland, Northern Ireland, Northern Ireland, Norway, Belarus, New Zealand, Poland, and the United States due to Escherichia coliO157: H7, Helicobacter pylori, P. aeruginosa, Salmonella Para typhi, Cryptosporidium parvum, Giardia, and viruses (i.e., norovirus, calicivirus, enterovirus). Despite the enormous number of outbreaks recorded, these statistics are very probably understated since not all of them are discovered, investigated, or reported. These health-related occurrences continue to occur much too frequently in the United States: 56–5831 in 2000–2001 and 30 in 2003–2004.

Between 1920 and 2002, at least 1870 DW outbreaks were recorded in the United States, with an average of 22.5 outbreaks per year and 883806 infections, or 10648 cases each year. Virus (norovirus, hepatitis A virus), protozoa (Cryptosporidium spp., Giardia spp., Naegleria fowleri), and hazardous bacteria pollution (Salmonella typhimurium, Vibrio cholerae, Legionella spp., Shigella spp., E. coli O157:H7, and Campylobacter jejuni).

The World Health Organization (WHO) report shows that between 2000 and 2007 there have been 354 waterborne disease outbreaks of DW-related illness, leading to over 4,7617 diseases in 14 European countries (Belgium, Czech Republic, Croatia, Estonia, Greece, Finland, Norway, Italy, Latvia, Lithuania, Slovakia, Spain, Sweden and the United Kingdom). All the nations involved have, on the basis of a legislative framework, a systematic mechanism for waterborne disease monitoring.

Bacteria (Campylobacter, Aeromonas spp., and Shigellasonnei) were the most prevalent causal agents, accounting for 163 (44.9 percent) outbreaks and 33.3 percent of disease cases. Viral agents were found to be responsible for 136 outbreaks (37.5%) and 49.4 percent of disease cases, whereas protozoa were responsible for 17 outbreaks (4.7%) and 9.9% of disease cases. Chemical contamination was responsible for ten instances (0.2%), whereas an unknown microbiological agent was found to be responsible for 37 cases (7.1%). Parasitic protozoans were responsible for at least 325 waterborne outbreaks globally.

Pathogen control in DW appears simple, but poverty mixed with water shortage is a lethal combination. Appropriate clean water sources, along with proper sanitation and increased hygiene standards, would greatly reduce the prevalence of waterborne illnesses (Chaves Simões & Simões, 2013).

2.4 A Glimpse of WBDOs cases in U.S. during 2005-2006

Since 1971, the Centers for Disease Control and Prevention (CDC), the U.S. The Environmental Protection Agency (EPA) and the Council of State and Territorial Epidemiologists have collaborated on the Waterborne Disease and Outbreak Surveillance System (WBDOSS) to collect and report data on the occurrences and causes of waterborne disease outbreaks (WBDOs) and cases of waterborne disease. This monitoring system is the major source of information in the United States about the breadth and consequences of waterborne illness. The monitoring

system contains information on WBDOs connected with recreational water, drinking water, water not meant for drinking (excluding recreational water), and water usage with unclear intent.

The data submitted includes 28 WBDOs from January 2005 to December 2006, as well as four previously unnotified WBDOs from 1979 to 2002. Between 2005 and 2006, fourteen Member States reported 28 WBDOs: Twenty were connected to drinking water, six to WNID, and two to WUI.

The 20 WBDOs related to drinking water sickened an estimated 612 people and killed four. Etiologic agents were found in 18 (90.0 percent) of the drinking water-associated WBDOs. Twelve (66.7%) of the 18 WBDOs with pathogens found were connected with bacteria, three (16.7%) with viruses, two (11.1%) with parasites, and one (5.6%) was a mixed WBDO with bacteria and viruses. In two WBDOs where the etiology could not be determined, norovirus was suspected.

Ten (50%) of the 20 WBDOs involving drinking water were outbreaks of acute respiratory illness (ARI), nine (45%) were outbreaks of acute gastrointestinal illness (AGI), and one (5.0%) was an epidemic of hepatitis.

All ARI WBDOs were caused by Legionella, and this is the first reporting period in which the proportion of ARI WBDOs has exceeded that of AGI WBDOs since Legionella WBDO reporting began in 2001.

A total of 23 deficiencies were cited in the 20 WBDOs associated with drinking water; 12 (52.2 percent) were classified as NWU/POU (deficiencies occurred at areas not under water supply competence or at a point of use); and 10 (44.5 percent) were classified as SWTD (deficiencies occurred at areas not under water supply competence or at a point of use) (source water contamination, treatment facilities, etc.). Legionella spp. was part of a drinking water system with 10 of the 12 NWU/POU flaws (83.3 percent).

The most commonly mentioned SWTD inadequacies were related to a lack of treatment (n = four [40.0 percent]) and untreated ground water (n = four [40.0 percent]. Three of the four WBDOs with inadequate treatment relied on ground water. Approximately half (52.2 percent) of the drinking water inadequacies occurred outside of a water utility's authority. The bulk of these WBDOs were linked to Legionella spp, indicating that more emphasis should be paid to

decreasing Legionella spp-related disease risks. Almost majority of the WBDOs linked with SWTD deficits occurred in ground water systems (Yoder J et al., 2005-2006).

2.5 Factors that produce 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 (Ingerson-Mahar M et al., 2012) and when repaired, patentees can enter (Hunter P.R et al., 2005). Defection leading to outbreaks of Campylobacter, Salmonella, Shigella, E. coli O157: H7, Cryptosporidium, Giardia, and Norovirus (Hunter P.R et al., 2005) (Dore M.H et al., 2015). Deficiencies of water treatment, such as insufficient or no surface water filtration and insufficient or discontinued groundwater disinfection, led to 14% of WBDOs between 2001 and 2002 (Craun M.F. et al., 2006); (c) (Blackburn B.G et al., 2001-2002). 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. (Ingerson-Mahar M et al., 2012).

A waterborne pathogen's goal, like any other creature's, is to reproduce and spread. The technique 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 (Costerton et al., 1999).

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 (Percival et al., 2016, p. 4).

- **Significance of bacterial biofilm in waterborne disease**

This section explains why bacterial biofilm is important in the epidemiology of waterborne diseases. As we all know, biofilm is a critical component of WBDOs in both developed and developing nations. The more precise features of the biofilm and their properties must also be acknowledged. As a result, this section provides some background information on bacterial biofilms that are related with WBDO.

3.1 Definition of Biofilms

The notion of biofilm has evolved during the last 25 years. Marshall discovered "very thin extracellular polymer fibrils" attaching surface bacteria in 1976. Costerton et al. identified bacteria colonies in aquatic settings enclosed in a "glycocalyx" matrix confirmed to be polysaccharide in composition.

According to Costerton et al., biofilm is made up of single cells and microcolonies that are all immersed in a highly hydrated, mainly anionic exopolymer matrix. In 1990, Characklis and Marshall went on to identify other distinguishing features of biofilms, including as geographical and temporal heterogeneity, and the participation of inorganic or abiotic components bound together in the biofilm matrix. In 1995, Costerton et al. underlined those biofilms might attach to surfaces, interfaces, and each other, including microbial aggregates and floccules, as well as adhering populations inside porous medium pore spaces, in their definition.

Thus, a new definition for Biofilm must take into account not only the characteristics easy to observe, that is the cells irreversibly attached, in a matrix of extracellular polymer substances produced by these cells and including their non-cellular or abiotic components, but also other physiological characteristics such as these organisms and the characters involved.

The new definition of biofilm is a sessile community with microbial derived characteristics defined by cells irreversibly linked to a substratum or interface or to each other, embedded in an extracellular polymeric material matrix, and exhibiting a modified phenotype in terms of growth rate and gene transcription. This definition will be beneficial since certain bacterial populations that met the previous biofilm requirements, which included matrix formation and surface growth,

did not really assume the biofilm phenotype. These "nonbiofilm" populations, which include bacteria colonies growing on the surface of agar, behave like planktonic cells "stranded" on a surface and lack the intrinsic resistance properties of genuine biofilms. We can now speak about biofilm cells within matrix-enclosed fragments that have broken off from a biofilm on a colonized medical device and are now circulating in bodily fluids with all of the parent community's resistance features (Donlan & Costerton, 2002).

3.2 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 N 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 M, et al.,2017).

3.3 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. 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 (Muhammad et al.,2020).

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.

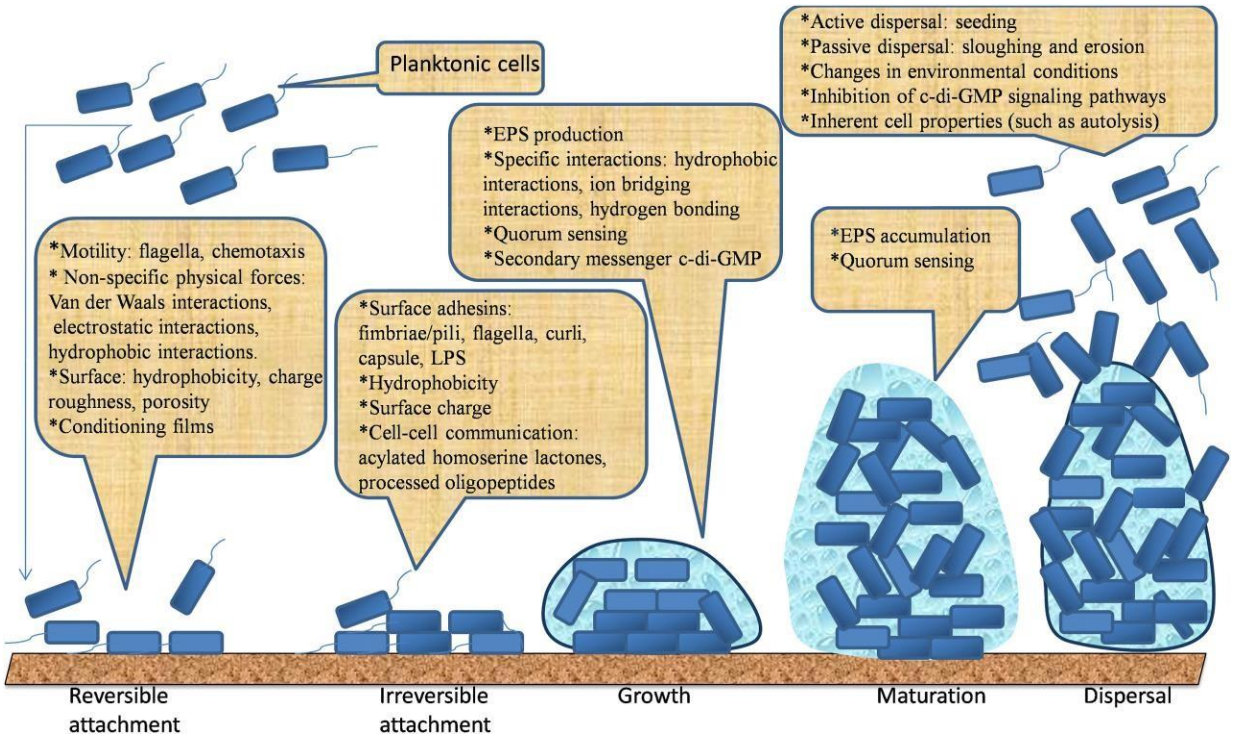

Figure 1: The five main phases leading to the development and dispersal of biofilm. [collected from (Muhammad et al.,2020)]

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

- reversible attachment phase, where bacteria non-specifically attach to surfaces;
- irreversible attachment phase, which involves interaction between bacterial cells and a surface using bacterial adhesins such as fimbriae and lipopolysaccharide (LPS);
- production of extracellular polymeric substances (EPS) by the resident bacterial cells;
- biofilm maturation phase, in which bacterial cells synthesize and release signaling molecules to sense the presence of each other, conducing to the formation of microcolony and maturation of biofilms; and
- 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.

3.4 Infections/Diseases associated with Bacterial Biofilm

Biofilms may contribute in a number of ways to a disease's etiology. Around 65% of all bacterial illnesses are thought to be linked with bacterial biofilms (Lewis K, 2001). These include illnesses linked with both the device and non-device.

Data have been assessed on device-related infections for various devices, including: 2% for breast implants; 2% for joint prostheses; 4% for mechanical heart valves; 10% for ventricular shunts; 4% for pacemakers and defibrillators, and around 40% for ventricular-aided devices. The interaction of microorganisms with the vascular endothelium and pulmonic valves of the heart causes native valve endocarditis (NVE). Streptococci, staphylococci, gram-negative bacteria, and/or fungal infections are the most common causes. Microbial cells acquire access to the heart and circulation in this disease via the gastrointestinal system, urinary tract, and/or oropharynx. A non-bacterial thrombotic endocarditis (NBTE) develops at the injury site as the intact valve endothelium is damaged by the microorganisms that attach to it, even after the bacteria have been cleared by the immune system. As a result, a thrombus forms, which is a condition in which platelets, red blood cells, and fibrin are aggregated (Jamal M, et al.,2017).

The bacteria's resistance to the body's defensive mechanisms and anti-microbial therapies is increased by biofilm development, therefore increasing persistent infections. Biofilms can also operate as an ecosystem in which various bacterial species and bacterial populations can accumulate in a particular area. Due to concentrated, sequent and synergistical actions of the current bacteria, this might lead to detrimental effects on host cells. The presence of biofilm can relate, for example in chronic rhinosinusitis and cystic fibrosis, to the severe and prognostic illness (Vestby, L. K et al.,2020).

3.5 Bacterial biofilm associated with WBDOs

In 2002, the USEPA released an issue paper on the topic of "Health Risks from Microbial Growth and Biofilms in Drinking Water Distribution Systems," which summarized the existing evidence. In this research, the microorganisms linked with WBDOs are identified. A quick overview of the gathered data is provided here to demonstrate the importance of the bacterial biofilm in the WB disease epidemiology.

Shigella, Salmonella, Yersinia enterocolitica, Campylobacter jejuni and Escherichia coli O157 were among the principal intestine bacterial infections transmitted by water. They have the ability to adhere to biofilms, and limited development in some conditions cannot be ruled out.

- Helicobacter pylori, a potentially waterborne pathogen, was shown to persist for at least 192 hours on stainless steel coupons (inserts used to detect biofilm formation) in a chemostat (Mackay et al., 1998).
- H. pylori has been found in biofilms on drinking water mains, according to Park et al. (2001).
- In another research, two non-pathogenic E. coli strains were put into a pilot distribution system with a biofilm (20°C) and developed somewhat before dying out (Fass et al., 1996).
- The building plumbing system can either function as a direct route for infections sloughed off the distribution system biofilms or as a pathogen amplifier.
- Weeks after a probable source-contaminated water meters and major breakdowns-were replaced or repaired, waterborne disease outbreaks of E. coliO157 (Swerdlow et al., 1992).
- Although a biofilm was not involved, biofilms have the ability to prolong the life of some bacteria.
- Salmonella typhimurium was able to grow for a brief duration at 24°C in non-sterile tap water in another investigation. (Armon et al., 1997).

Much more information on the prevalence of opportunistic bacterial pathogens is now available. Table 1 shows some of the aquatic and soil bacteria linked to distribution system biofilms and illness. However, strain diversity exists within each of the microorganisms listed. The distribution system may consist of purely ambient strains and clinical strains of the same genus or species. The clinically important strains are opportunistic infectious pathogens. Infective dosage tests on healthy people and animals via the oral or intranasal route show that extremely large doses (106-1010 cells) are required for infection or illness, at least in healthy people (Rusin et al., 1997).

Acinetobacter was found at levels as high as 109/cm2 on the surface layer of a mortar walled pipe in one research (Olson, 1982). While this research focused on healthy people and animals, there is limited information on infective doses for more vulnerable groups.

The clinically significant strains of bacteria listed in Table 1 may cause diseases ranging from mild to severe, including pneumonia and septicemia (invasion of the blood). The outcomes are sometimes fatal (Toder, 1998; Inderlied et al., 1993; Jarvis et al., 1985; Pier, 1998; Hardalo and Edberg, 1997; Thomas et al., 1977).

Table 1 Opportunistic Bacterial Pathogens Detected in the Distribution System and/or Biofilms [collected from ("Us epa," 2002)]

Opportunistic pathogens	Health Effects	Information on:			WBDO[1]	CCL[2]
		Disease	Presence in DS	Biofilm Presence		
Acinetobacter calcoaceticus	pneumonia, meningitis, infections of urinary tract, septicemia	Davis et al., 1973, Horan et al., 1988	Geldreich, 1990	LeChevallier et al., 1987; Geldreich, 1990		
Aeromonas hydrophila	sepsis, gastrointestinal illness, respiratory tract infections	Davis et al., 1973	Geldreich, 1990	Reasoner, 1991, van der Kooij and Hijnen, 1988		X
Citrobacter spp.[3]	septicemia, pneumonia	Keusch and Acheson, 1998	Geldreich, 1990	Geldreich, 1990		
Enterobacter spp.[3]	septicemia, pneumonia	Keusch and Acheson, 1998	Geldreich, 1990	Geldreich, 1990		
Flavobacterium spp.	septicemia, meningitis	Davis et al., 1973	Geldreich, 1990	Geldreich, 1990		
Klebsiella pneumoniae[3]	septicemia, pneumonia	Keusch and Acheson, 1998	Geldreich, 1990	Geldreich, 1990		
Moraxella spp.	pneumonia, conjunctivitis, septicemia, otitis, urethritis, meningitis, bronchitis, sinusitis	Benenson, 1995, Davis et al., 1973, Walker, 1998	LeChevallier, 1987	LeChevallier, 1987		
M. avium complex	chronic diarrhea, chronic lung disease	Schaechter et al. 1998	Geldreich, 1990	Norton et al., 2000	X	X
Pseudomonas cepacia	foot infections	Tally, 1998	Geldreich, 1990	LeChevallier et al., 1987		
Pseudomonas aeruginosa	infections when severe burns, cancer patients, lungs when cystic fibrosis, pneumonia, meningitis, others	Toder, 1998	Geldreich, 1990	Geldreich, 1990		
Serratia marcescens[3]	septicemia, pneumonia	Schaechter et al. 1998	Geldreich, 1990			

[1] Documented waterborne disease outbreak in U.S.
[2] Pathogen is on EPA's Contaminant Candidate List (CCL) of March 1998
- Some species are coliforms

Because opportunistic pathogens afflict vulnerable persons, including some hospitalized patients, the percentage of hospital-acquired (nosocomial) infections caused by these organisms may provide some insight into their total effect. Infants, young children, pregnant women, the elderly, and those with a highly impaired immune system or other significant impairment of host defense are among the people who are vulnerable to opportunistic bacterial infections that are widespread in biofilms. L. pneumophila, MAC, and P. aeruginosa are the most concerning opportunistic bacterial infections found in biofilms, although there are others. Waterborne disease and damage

may arise from ingestion, aerosol inhalation, or cutaneous exposure, depending on the organism (e.g., through wounds). There is a connection between nosocomial or community illnesses due to such pathogens and potable water ("Us epa," 2002).

- **Bacterial Biofilm Implication in DWDS system and WBDOs**

Biofilms account for 95% of bacteria in DWDS. A key source of high microbial populations is the presence of biofilms adhering to DWDS's inner tube surface. This is extremely important for public health since it impacts the quality and wholesomeness of drinking water. Despite the variety of circumstances (nutritional and physicochemical) in DWDS, biofilms have evolved ways to live and thrive. This section provides a brief overview of the consequences of bacterial biofilm in DWDS.

4.1 Biofilms in the Drinking Water Distribution System

Paul R. Hunter, consultant medical microbiologist and director of the Chester Public Health Laboratory, as well as honorary professor of epidemiology and public health at the University of Central Lancashire, presented World Health Organization data showing high morbidity and death rates worldwide due to consumption of unsafe drinking water in a panel on ‘Emerging Infectious Diseases.'

From 1990 to 1998, 127 outbreaks of drinking water were recorded in the United States, most of which were connected with groundwater systems. Mark W. LeChevallier, director of research at American Water Works Service Company, spoke on the same panel about health concerns about biofilms in the drinking water distribution system. Biofilms are organic and inorganic coverings on pipes that can shelter, protect, and facilitate the spread of microorganisms such as Legionella and Mycobacterium avium complex (MAC).

Factors that impact biofilm bacterial growth include water temperature, type of disinfectant and residual concentration, assimilable organic carbon content, biodegradable organic carbon levels, corrosion levels and features of the processing and distribution system. Chloramine is far more efficient than chlorine at controlling Legionella in biofilms, probably because it is more stable and hence less reactive than chlorine, allowing it to enter the biofilm more deeply.

One major element in the contamination of the distribution system and the growth of bacteria in biofilms is the transient variations in water pressure, which generate pressure in the distribution system through pipelines. A significant volume of the polluted water (> 1 gallon per minute) can be drawn from the outside into pipes via a minor breach during the negative part of the pressure wave. When wastewater lines are put near water pipelines, this problem is exacerbated.

Dr. LeChevallier noted that several outbreaks of waterborne disease have been associated with inadequacies in the distribution system. MAC is among the agents of watery nosocomial infections. It thrives in water, is resistant to water treatment (greater than Giardia cysts) and is produced by biofilms in pipes (Hunter et al., 2001).

Biofilms are the main form of microbial development in the distribution networks of potable water. Biofilm is one of the biggest issues in drinking water distribution systems, which is widely documented. Contaminated water intake with pathogenic biofilms is connected to human and waterborne diseases. P. aeruginosa, Campylobacter jejuni, Legionella pneumophila, Mycobacteria, Aeromonas hydrophila, and Klebsiella pneuminiae are the primary biofilm forming bacteria in drinking water (Prest et al., 2016; Chan et al., 2019).

Bacterial cells can attach to and from biofilms on the inner surfaces of piping systems, from which cells can be detached and released into the bulk water, resulting in pipe biocorrosion, undesirable water quality changes affecting color, taste, turbidity, and odors, and a reduction in heat exchange efficiency (Prest et al., 2016).

The primary biofilm generating bacteria known to cause metal corrosion include sulfate-reducing bacteria, sulfur-oxidizing bacteria, iron-oxidizers, iron-reducers, and manganese-oxidizers (Kip and van Veen, 2015). Overall, biofilms can have an impact on the safety of drinking water as well as water pipes.

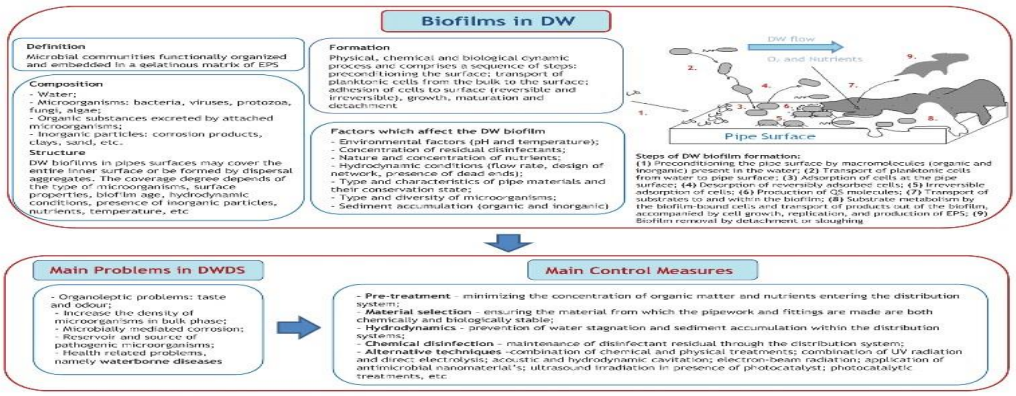

Fig. 3 Aspects of biofilm formation in DWDS: problems, development and control.

microorganisms need a C : N : P (carbon, nitrogen and phosphorous) ratio of 100 : 10 : 1, where carbon is the growth-limiting nutrient. Thus, restricting the carbon concentration will decrease the propensity for microbial growth.[110,111] Another preventative and promising strategy suggests the control of biofouling with an aqueous suspension of silver nanoparticles as a pre-treatment in water systems prior to the main treatment units, such as membrane filtration.[112] It was not meant as a treatment for the eradication of existing or mature biofilms or as a disinfection process. The pre-treatment with molecularly-capped silver nanoparticles presented in this study was able to control or retard biofilm formation on pipe surfaces.[112]

4.2 Material selection

Other preventative strategies have attempted to identify materials that do not promote or can even suppress biofilm formation.[113] Different materials (ethylene-propylene, natural latex, stainless steel (SS), mild steel, polypropylene, polyethylene (PE), chlorinated polyvinyl chloride (PVC) and unplasticized PVC) were ranked according to their biofilm growth propensity, which unfortunately led to the conclusion that there is hardly any material that does not allow biofilm formation.[113] There is, however, a considerable inhibitory effect (respiratory chain inhibition) of copper when compared to biofilm growth on other materials (high density PE, PVC, silicon, SS and glass).[114–116] Concentrations of copper ions relevant to DWDS seem to induce a viable but non-culturable state in *P. aeruginosa* accompanied by a loss of culturability and cytotoxicity.[117]

The type and stability of the material used in DWDS is an important factor that can influence biofilm proliferation. There is a distinct development rate and microbial community structure of biofilms in different types of pipe.[84,118] Bacteria are able to leach nutrients from the materials.[113] A report stated that iron pipes can support 10 to 45 times more growth than plastic pipes.[105] Also, iron pipes are more reactive with disinfectants and quench their antimicrobial effects.[119] Thus, the type of material can also affect the disinfectant efficiency of biofilms. Biofilms grown on copper, PE, PVC and cement-lined ductile iron were inactivated with a much lower amount of free chlorine or monochloramine than those grown on unlined iron surfaces.[120–122] This was explained by the interaction of chlorine with iron. In cement-lined ductile iron, the cement provides a layer of protection for the iron against attack by chlorine. The pipe service age is another important factor influencing chlorine decay and this effect decreases in the following order: cast iron > steel > cement-lined cast iron

Figure 2Aspects of biofilm formation in DWDS: problems, development and control. [collected from (Chaves Simões & Simões, 2013)]

Biofilms in drinking water distribution systems (DWDS) have received a great deal of attention in recent decades. Therefore, a broad range of evaluations on this issue, including the sanitary quality of DW and its evolution, is published.

4.2 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:

- Entry through the source water (e.g., treatment breakthrough)
- Entry through broken or leaking pipes, valves, joints and seals
- Entry through cross-connections and backflow

- Entry through contamination of finished water storage vessels
- Entry through Improper Treatment of Materials, Equipment or Personnel in Contact
- with Finished Water
- 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 (Kirmeyer et al., 2001). Table 2 summarizes these findings. While possible sources of contamination entrance are recognized, the method by which microorganisms in distribution systems enter is yet unknown (Gauthier et al., 1999).

Table 2: Some Pathways Through Which Pathogens Can Enter the Distribution System

Risk Level	**Pathway**
High	Treatment breakthrough, intrusion, cross-connections, main repair/break
Medium	Uncovered water storage facilities.
Low	New main installation, covered water storage facilities, growth and resuspension, purposeful contamination.

Generated based on information from expert panel ranking in Kirmeyer et al., 2001.

4.3 Factors that Influence Pipe Biofilm Development

They address a number of physical, chemical and biological variables affecting the growth of biofilm pipes in the USEPA problem paper ("Us epa," 2002). 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. The following factors are addressed in this document:

- Environmental factors
- Presence of nutrients
- Microbial interactions
- Distribution system materials
- System hydraulics
- Presence of distribution system residual
- Sediment accumulation

- **Some WBDOs cases**

Some examples of waterborne illnesses due to bacterial biofilm have been shown in this section. Bacterial pathogens-generated biofilms can transfer such infections via the water into various communities, causing epidemics of waterborne diseases. In numerous nations, a representative illustration was given of this phenomenon. Bangladesh, for example, is a cholera endemic location. Cholera bacteria are further introduced into the adjacent brackish ecosystems in Bangladesh, colonized with V-cholera in form of biofilm particles and planktons in the sea water of Bengal. (Faruque, S. M.et al.,2006)

5.1 Vibrio cholerae

Water polluted by pathogenic vibrio cholera is recognized for the outbreak of cholera diarrheal. O1 or O139 Serogroup toxigenic vibrio cholerae are the causal agents of cholera and form part of a group of organisms with a large aquatic habitat (Colwell R. R.,1992). Cholera epidemics are common in many Asian, African and Latin American developed nations and correspond with an increasing frequency of the V pathogenic strain in the aquatic environment (Khan M. U.,1984).
Several theories were presented to explain the way in which pathogenic *V. cholerae* persists in the aquatic environment and to imply that the real frequency of pathogen might be significantly greater than that found under normal techniques of cultivation.
A research done by Faruque; S. M. et al. (2006) indicates that surface water containers (biofilms) of partly dormant cells containing environmentally viable conditions (CVEC) of pathogenic V.

cholerae which are resistant to culture by traditional procedures. This CVEC may be recovered by inoculating water in the intestines of rabbits as full-virus bacteria. In addition, when V. cholerae are injected into cholera feces, cells display comparable features to CVEC that indicate that CVEC is the infectious form of V. in water, and CVEC in nature may be generated from human cholera stools. We also found that cholera feces contain a heterogeneous mixture of bio filmed clumps and V. cholerae planktonic free-swimming cells. The relative infectivity of various types of V. cholerae cells has been estimated to be primarily attributable the existence in vivo clumps of cells, with a high dosage of the disease. The increased infectiveness of V. cholerae shed in human stuffs. The results of this work suggest a cholera transmission model that enhances the contagious diseases of pathogenic V. cholerae in vivo-fashioned biofilms (Faruque, S. M.et al.,2006).

Islam et al. (2007) did another investigation to explain the role of biofilm as a habitat for plankton-associated V. cholerae in the aquatic environment of Bangladesh. The role of biofilm as a microenvironment of plankton-associated Vibrio cholerae was studied in this work, utilizing plexiglass as a bait. A total of 72 biofilm samples were examined utilizing conventional culture, direct fluorescent antibody (DFA), and molecular methods. In the tributaries of the Meghna River in Bangladesh, the study found that algae containing V. cholerae, as well as free floating V. cholerae from the water column, move to the surface of the plexiglass disc and form biofilm communities in which a complex interaction between the algal and V. cholerae communities takes place. Biofilm has also been shown to provide V. cholerae with a microenvironment in which rugose varieties can survive.

Yildiz et al. found that rugged versions are more able to build biofilms than smooth variants, as they can generate extra-cellular polysaccharide in a laboratory experiment. Rugose forms of V. cholerae were also identified from biofilm samples in this field research. The presence of txA, tcpA, and ace genes in V. cholerae O1 isolates suggests that V. cholerae O1 retains its virulence when it develops biofilm in the aquatic environment. The results of this research are also in line with the results of an earlier investigation. It was also discovered that various clones of V. cholerae O1 survive in the aquatic environment of Bangladesh.

In the current investigation, the isolation from the samples of biofilm of V. cholerae O1 of the same clone (same ribo type model) shows that the formation of biofilm in the aquatic environment of Bangladesh may offer an advantage to a specific V. cholerae clone O1. The

recurrent isolation of the R2 V cholerae non-O1, non-O139, ribo type revealed that this specific ribo type is more likely than the other ribo types to be connected to the surface. Dramatic resistance gains in both T and TMP-SMZ clinical isolates of V. cholerae O1 were reported in the 1991 and 1992 periods, ranging from two to 90% in T and from 18 to 90% in TMP-SMZ.

Other studies by Sack et al. similarly found that T resistance between El Tor strains rose significantly from 1.9% in 1990 to 7.6% in 1991, 61.1% in 1992 and 85.4% in 1993. The current investigation found that V. cholerae O1 El Tor isolated from biofilm is resistant to TMP-SMZ, S, NA, and FR. The resistance of environmental strains of V. cholerae O1 to TMP-SMZ is consistent with earlier clinical strain results. It was also discovered that all El Tor V. cholerae O1 were resistant to VSC, indicating that biofilm might function as a reservoir of VSC-and antibiotic-resistant V. cholerae in Bangladesh's aquatic environment. Islam and colleagues (2007)

5.2 *Escherichia coli*

The development of new infections poses a significant public health risk, producing widespread epidemics in vulnerable populations. The Escherichia coli O104: H4 strain involved in a 2011 epidemic in northern Germany was responsible for the greatest frequency of hemolytic uremic syndrome (HUS) and fatalities ever reported in a single E. coli outbreak. As a result, it has been claimed that this strain is more virulent than other pathogenic E. coli strains (e.g., E. coli O157: H7).

The E. coli O104: H4 outbreak strain has virulence factors from both Shiga toxin-producing E. coli (STEC) and enteroaggregative E. coli (EAEC), however the mechanism of pathogenesis remains unknown. Safadi RA et al. (2012) show that E. coli O104: H4 produces a stable biofilm in vitro and that in vivo virulence gene expression is highest when E. coli O104: H4 overexpresses genes required for aggregation and exopolysaccharide production, both of which are characteristics of bacterial cells that reside within an established biofilm. Interrupting exopolysaccharide synthesis and biofilm development may thus be useful strategy for dealing with future E. coli O104: H4 infections.

- ## Conclusion

Biofilm is produced in its own unique method by many species. Although biofilms may be home to a diverse range of micro-organisms, the primary responsibility for laying the foundation stones for the microbial town is that of the bacteria. The ecosystem is affected beyond risk by bacterial biofilms. Bacteria's capacity to colonize surfaces and create organic films is seen as major problems and has been connected with adverse effects in various food, water, pharmaceuticals and healthcare sectors.

This review of the literature demonstrates that bacterial biofilm has enormous relevance and consequences for the epidemiology of waterborne disease outbreaks. A growing number of waterborne illnesses have been linked to biofilms. Biofilms can contribute in a number of ways to a disease's etiology. Biofilm development enhances bacteria resistance to the body's defensive mechanisms as well as antimicrobial treatments, encouraging persistent infections.

Biofilms can also work as an ecosystem that collects diverse kinds of bacteria and bacterial populations in some places. Due to concentrated, sequential, and/or synergetic actions of the existing bacteria, this might have detrimental consequences for cells. Biofilm is a major danger to public health in developing countries since it is an example of a defensive mechanism in many bacteria. The area of biofilm research has come a long way. However, the capacity of pathogens in DWDS to build biofilms, as well as their interaction within a multispecies microbial community composed of aquatic bacteria, which promotes disease survival and dissemination, has to be explored further. Future research should focus on multispecies biofilms in aquatic ecosystems to reduce pollution in these settings.

As new methodologies are discovered, employing them for biofilm research will create new information and lead to new fields of biofilm study. To conclude, our increased knowledge of biofilm-building bacteria's nature and characteristics is the cornerstone of waterborne disease epidemiology. It also works well for treating persistent biofilm infections. Nevertheless, the present knowledge employed is not enough. There is no question that further development will be based on comprehensive efforts in basic, application and clinical research on WBDO-related bacterial biofilms.

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