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Research Article

INVESTIGATION OF BIOFILM FORMING BACTERIA FROM POTABLE WATER SAMPLES

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ABSTRACT

A prospective study was undertaken to evaluate the water quality and bacteria involved in biofilm production in potable water samples. Potable water samples were collected and assessed for isolation and enumeration of total coliforms by spread plate method and membrane filtration technique and identified by streak plate method. Through literature review and present investigation, the water samples were assessed for biofilm forming potential of water borne bacteria. *Klebsiella pneumoniae* was the only isolate that was successful in biofilm formation in harvested rainwater samples. Regular water quality investigations need to be approached at every secondary water storage utilities.

Keywords: Potable water, Total coliforms, Membrane filtration, Streak plate, Biofilm

1. INTRODUCTION

Water is the most common and important chemical compound on earth. Only 2.6% of global water amount of $1.4x10^9$ km^3 however is freshwater and consequently available as potential drinking water. The availability of drinking water has been the most critical factor for survival throughout the development of all life. In drinking water distribution systems, basically all existing interfaces are covered by biofilms, from the waterworks through the distribution system to all house installations. The existence of these biofilms was reported and documented several decades ago. These descriptions of drinking water biofilms were mostly based on microscopic or electron microscopic examinations of pipe materials and encrustations from pipe surfaces [1].

Although universal access to safe and piped drinking water is an important long-term solution, it is very costly and exigent to implement in developing countries in the short time. Drinking Water Distribution Systems are complex engineering systems consisting of pipes, storage vessels, fitting valves etc. made of a range of different materials such as cast iron, PVC and polyethylene that interact with mass of water. The water consumed by consumers at the tap has travelled possibly for long distances and period through the distribution network after leaving from the water treatment plant might undergo deterioration in quality. This deterioration in water quality will be influenced by factors such as decay of disinfectant residual, temperature, hydraulic regime, water residence time and bacterial regrowth [2, 3].

Microorganisms have the ability to adhere to the solid surfaces and form biofilm in aquatic environment [4]. Generally most water distribution systems are character-rized by the presence of biofilm, regardless of purity, the brand of pipe material used for distribution or the presence of a disinfectant [5]. Biofilms are the bacterial communities embedded in a polysaccharide matrix, which enables to resist destruction by antibodies, environmental stress, biocides and detergents. Bacterial regrowth in the distribution system may result from the detachment of biofilm bacteria, which increases the risk of infection in humans when water is consumed [6]. Adverse heath affects in humans, especially in children is known to occur due to the corrosion of plumbing materials made of lead and this favors lead contamination in tap water [7].

The water purifier system is susceptible to the formation of glue biofilms or microorganisms as it produces, stores and then circulates water under contextual conditions and this can be a source of unwanted levels of living microorganisms in effluent water. Thus recent studies have revealed the biofilm formation in the piping area of all bigger purification systems. This biofilm is able to spread microorganisms within the water distributing system and contribute to enhance microorganism's quantity. In the pharmaceutical industry, contamination

will affect the whole process, also in hospital setting as these systems will be requiring frequent sanitation and microbiological nursing that ensures water with suitable microbiological quality (at the point of use with microbial limit) [8]. Thus with an eminent interest in isolation and identification of bacterial biofilm, the present work was undertaken.

Therefore, biofilm formation is of major concern for most municipal supply agencies and communities as it results in the deterioration of the quality of drinking water.

2. MATERIAL AND METHODS

2.1. Study area

Mysuru is one of the largest city in Karnataka, a southern Indian state, of India. Mysuru is located at 12.30°N 74.65°E and has an average altitude of 770 m (2,526 ft). The city's average rainfall is 804.2 mm (31.7 in).

2.2. Collection of water samples

Potable water samples were collected from different sources of drinking water in Mysuru city. Isolation and identification of biofilm forming bacteria was executed to the water intended for human consumption. Chemicals required for water analysis was procured from Hi-media laboratories Pvt. Ltd., Mumbai, India.

2.3. Bacteriological water analysis

Isolation and identification of bacterial network associated with biofilm formation: The term heterotrophic bacteria include all bacteria that use organic nutrients for growth. The bacteria are universally present in all types of water, food, soil, vegetation and air. *Escherichia*, *Klebsiella*, *Enterobacter*, *Citrobacter* and *Serratia* are some of the pathogens included as primary and secondary heterotrophic bacteria. In drinking water including aquatic systems comprise water based bacteria (autochthonous) and low-nutrient media like R_2A was established as better media for enumeration of heterotrophic bacteria. There is no one single method to enumerate by recovering all bacteria in water analyzed. Incubation temperatures like low-temperature (20˚C-28˚C) incubating with a longer incubation time of about (5-7 days) would support water based bacteria to grow profusely [9]. Spread plate method was performed by aseptically inoculating with 0.1 ml of undiluted water sample and spread using a sterile spreader for an even distribution of the inoculum on entire agar surface. The R_2A agar plate was incubated at 20 ˚C-28 ˚C for 5 to7 days [10].

A prospective study was undertaken to evaluate the water quality and bacteria involved in biofilm production in potable water samples. Potable water samples were collected and assessed for isolation and enumeration of total coliforms by membrane filtration technique [11] and samples of water were filtered through 0.22 µm cellulose nitrate filters. Then the filters were placed on HiCrome Chromogenic coliform agar media for detection of bacterial diversity. The plates were incubated at 44.5˚C for 3 to 5 days, observed after incubation and results were noted.

3. RESULTS AND DISCUSSION

Potable water samples were collected and assessed for isolation and identification of biofilm forming bacteria. Isolation and enumeration of biofilm forming bacteria was detected by Heterotrophic plate count method [9] and by membrane filtration method [11].

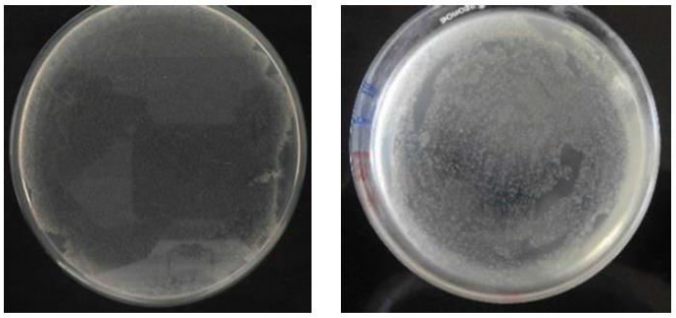

Fig. 1: R_2A agar plates with heterotrophic bacteria

Stages of Biofilm bacterial growth showing mushroom like structure by *Klebsiella pneumonia* on CCA by Membrane filtration method and streak plate method In the present study, artificial medium R_2A agar was used to isolate bacteria from drinking water. This medium has been shown to isolate a higher number of bacteria from low nutrient (oligotrophic) environments [10]. Too numerous to count (>1000 cfu/0.1 ml) colonies were developed on R_2A medium (Fig.1). The importance of microorganisms in concern with health and disease to people and massive impact with the pure culture method devised by Robert Koch and others has logically led to an attitude in microbiological research that emphasizes the study of microorganisms in pure culture. This approach has revealed that the biofilm research work has been neglected prominently from a long time until microbiologists revived these captivating communities [12]. From the present study, Membrane filtration

method and streak plate method gave a significant, strong biofilm production with formation of mushroom like (Figs. 2 and 3) structures [13]. Studies of biofilms that was grown in flow cells has provided a familiar image of a submerged biofilm entailing of pillars and mushroom-like structures separated by water-filled channels [14].

Fig. 2: Stages of bacterial growth on membrane filter

Fig. 3: Stages of bacterial growth by streak plate method

Among all the pure cultures isolated and identified from potable water samples, *K. pneumoniae* was the only bacteria isolated from harvested rainwater thrived well in biofilm formation. *Klebsiella* isolate formed a moderate consistent biofilm at 44.5˚C than 35˚C as measured by membrane filtration and streak plate method. The potential to disperse through detachment is the only last advantage left to the biofilm mode of growth. It is well known that microcolonies are capable to exist in separate mushroom-shaped structures. The detachment process is mediated in a direction of mechanical fluid shear that detaches the microcolonies or detachment is mediated by a genetically responding programme [15]. Present work has shown the formation of mushroom like structures to the water sample assessed by membrane filtration technique and by streak plate method. The pathogenic bacteria can survive in a safe harbor biofilm as it will become resistant to diverse disinfectants compared to other genera [16].

Diverse bacterial species along with *Pseudomonas aeruginosa* association in biofilm have been noticed within water distribution systems in countries which have added progressive water-treatment facilities [17, 18]. Turbidity is caused by biofilm when a pathogenic bacteria enters into a water distribution network and stay for a longer time producing foul smell and bad taste. Formation of biofilm within the pipes increases the rate of corrosion making physical damage as microbiologically induced corrosion (MIC) increases huge loss to distribution channel towards its maintenance. Many recalcitrant infections are caused by biofilm producing bacteria and are problematic notoriously to eradicate. By various methods resistance to antibiotics is expressed such as biofilm's restriction to penetration of antibiotics, by expressing resistant genes and reduced growth rate. There are many methods to detect biofilms. In the present study two types of isolates namely *Klebsiella pneumonia* and *Escherichia coli* were detected successfully, screening by two methods to check the ability to form biofilms [13]. Analysis of biofilm forming bacterial network was undertaken to potable water samples. Bacterial isolates obtained from the work was investigated to assess the biofilm forming potential in water. The H_2S bottle inoculated with harvested rainwater sample showed a change in colour from clear dark blue to bluish purple with turbidity after incubation, specifying the presence of *Klebsiella/ Enterobacter* sp. contamination, and a bluish green with turbidity indicating *E. coli /Streptococcus/ Shigella* sp. Contamination to groundwater sample (Himedia, H_2S bottle-K022). Thus, the presence of H_2S producing pathogenic bacteria was determined by H_2S test.

Further, biofilm forming potential of bacteria were analyzed by membrane filtration and streak plate method. Water samples were filtered through membrane filter (0.22µm pore size) and filter was inoculated on HiCrome chromogenic coliform agar (M1991I) (Fig. 2). Here, *Klebsiella pneumoniae* from harvested rainwater sample was the only sample which showed mushroom like structures indicating positive to biofilm formation. This method confirmed that *Klebsiella* isolate from harvested rainwater sample formed a reasonable and steady biofilm at 44.5˚C by membrane filtration and streak plate method which confirms biofilm activity in harvested rainwater sample (Figs. 1 and 2). Klebsiella isolate formed a moderate consistent biofilm at 44.5˚C than 35˚C as measured by CRA and Tube method [19]. On Congo red agar media plate, development of Black crystalline colonies were measured to be positive isolate, whereas colourless colonies as negative to biofilm production [20]. Diverse bacterial species along with *Pseudomonas aeruginosa*-association in biofilm have been noticed within water distribution systems in countries which have added progressive water-treatment facilities [17, 18].

4. CONCLUSION

Water is known to be one of the significant resources for all organisms, to all utilities on the earth involving plants, microbes and animals. Thus, the present study is prompting to study the quality of water and its availability. In this planet water is the most precious and indispensable natural resources. The inherent quality of potable water in Mysuru city of Karnataka, India is quite low at some drinking water sources, a proper socioeconomic policy and environment to improve and maintain water quality is lacking. Anthropogenic activities have had disturbances and continue to exert an impact on potable water sources at the study area. Most of the groundwater samples were found to be of potable quality except south zone of the city and this was mainly due to close proximity of sewage water treatment plant and groundwater sampled site. Some harvested rainwater samples also revealed bacterial contamination since these water sources are devoid of disinfection. The majority of drinking water sources were highly contaminated by *Enterobacteriaceae* members. *Klebsiella pneumonia* could produce biofilm expressing ability of resistance to many kinds of antibiotics with rising concern on infection to consumers in Mysuru.

This condition may pose potential health problems to those using the water from such unhygienic surroundings. Regular bacteriological water quality control mechanisms need to be effectively initiated to ensure bacteriological safety of drinking water in the city.

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