DETECTION OF BIOFILM PRODUCTION AMONG THE BACTERIAL ISOLATES FROM WATER PLUMBING SYSTEMS USING DIFFERENT SCREENING METHODS

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Bacterial biofilms are clusters of bacteria that are attached to a surface and embedded in a self-produced matrix. The biofilm formation of bacteria in water plumbing systems causes persistent infections because the bacteria are resistant to antibiotic treatment. Therefore, biofilm bacteria are a severe threat to public health increasing morbidity and mortality rates. This study aims to screen the bacteria associated with biofilm production in water plumbing systems. The bacterial colonies were isolated from a total of 80 samples collected from both distribution lines of scrapings and drinking water, drain lines of scrapings, and wastewater from two different locations; kitchen and washroom outlets. A total of 189 bacterial isolates were tested for biofilm production using the standard methods including Congo red agar, Tissue culture plate, and Tube method. Among the screened bacteria, the biofilm producers detected in the distribution line were found to be 80% in scrapings and 35.71 % in drinking water in the samples collected from the kitchen outlets whereas 61.11 % of biofilm producers were detected in the scrapings and 46.15% in drinking water in the samples collected from the washroom outlets. Furthermore, 67.64 % of biofilm producers were detected in the scrapings and 85.71 % in the wastewater in the samples collected from the drain line of the kitchen outlets whereas the biofilm producers were found to be 58.53 % in the scrapings and 78.57 % in the wastewater in the samples collected from the drain line of the washroom outlets. The results confirmed the presence of biofilm-producing bacteria in samples collected from kitchen and washroom outlets in both distribution and drain lines. However, the highest prevalence was observed in the wastewater samples of the drain lines compared to the distribution lines while the highest prevalence was recorded in the scrapings of the distribution lines compared to the drain lines. In conclusion, 66.13 % of biofilm producing bacteria were detected in all the samples screened for the present study. Therefore, this study emphasizes the importance of regular screening and maintenance of water plumbing systems to ensure the quality of drinking water and the safe removal of wastewater.

Key words- Biofilm detection methods, Biofilm producing bacteria, Water plumbing systems

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INTRODUCTION

It is estimated that over two billion people globally drink from water sources contaminated with microbes, putting them at risk of waterborne diseases such as diarrhea, cholera, dysentery, typhoid, and polio. Biofilms are bacterial accumulations attached to surfaces and prevalent in water plumbing systems including water distribution lines and drain lines, especially in old pipes. Biofilm cells express specific phenotype characteristics that confer adaptability to environmental change and higher resistance to adverse conditions, such as limited nutrient availability, desiccation, antibiotics, biocides, shearing force, heavy metals, pH fluctuations, osmotic shock, UV light, and predation (Mahapatra *et al*., 2015; Agbabiaka *et al*., 2021). Drain lines in plumbing systems provide a conducive environment for biofilm growth due to a steady nutrient supply and moisture. Deteriorated drain lines and defective drainage systems are the primary sources for introducing biofilm bacteria into the environment (Papciak *et al*., 2019). *Acinetobacter* spp., *Pseudomonas* spp., *Klebsiella* spp., *Escherichia coli*, *Staphylococcus aureus*, and *Aeromonas* spp. are commonly isolated biofilm bacteria in water plumbing systems (Agbabiaka *et al*., 2021). Bacteria in biofilms exhibit resistance to antibiotics, posing challenges to treatment. Antibiotic resistant bacterial infections are more complex and expensive to treat, leading to higher mortality and morbidity rates, particularly in developing countries (Ullah *et al*., 2021). Various factors such as disinfectant concentration, temperature, hydrodynamic conditions, and pipeline material affect biofilm formation. The corrosion of pipeline materials and the presence of phosphates and carbon sources accelerate biofilm formation leading to the deterioration of pipelines. Moreover, persistent and chronic bacterial infections caused by biofilms lead to lengthy hospital stays and increased health expenses. Therefore, research on biofilm detection in water plumbing systems is essential for public health and safety. This study emphasizes the urgent need for comprehensive measures to address biofilm formation in water plumbing systems, including effective biofilm management strategies (Ullah *et al*., 2021; Papciak *et al*., 2019).

METHODOLOGY

(i) Sample Collection

A total of 189 bacterial colonies were isolated from 80 samples collected from both distribution lines of scrapings and drinking water and drain lines of scrapings and wastewater from two different locations: kitchen and washroom outlets in and around the Colombo area, including individual residences, apartments, and university hostels.

(ii) Detection of Biofilm Production of Bacterial Isolates

The detection of biofilm production was carried out using methods widely used for the phenotypic identification of biofilm production, such as the Tube method, Congo red agar method, and Tissue culture plate method. The selected strains, *Staphylococcus aureus* ATCC 35556, *Pseudomonas aeruginosa* ATCC 27853, *Escherichia coli* ATCC 35218, and *Staphylococcus epidermidis* ATCC 12228, were used as the reference samples to be used as controls.

a) Tube Method

A qualitative method of biofilm production was used using the technique of Hassan *et al*., 2011. A loopful of bacterial isolates was inoculated in trypticase soy broth in test tubes. After incubation, the tubes were stained with crystal violet and dried in an inverted position. The scoring was done by comparing the strength of the visible film on the test tube of the sample with the control strain.

b) Congo Red Agar (CRA) Method

A simple qualitative method to detect biofilm production described by Hassan *et al*., 2011, was carried out using Congo red agar medium. The CRA medium plates were inoculated with the bacterial isolates and incubated at 37^0 C. Black colonies with a dry crystalline consistency were considered positive for biofilm production.

c) Tissue Culture Plate (TCP) Method

A standard quantitative test method described by Hassan *et al*., 2011 was carried out for biofilm detection. Bacteria isolated from fresh agar plates were inoculated in trypticase soy broth. Individual wells of sterile 96 well-flat bottom polystyrene tissue culture treated plates were filled with bacterial isolates. Biofilm formed by bacteria adherent to the wells was stained by crystal violet. The optical density (OD) of stained adherent biofilm was obtained by using **a** micro-ELISA auto reader at wavelength 570 nm. The interpretation of biofilm production was done according to the criteria described by Stepanovic *et al*., 2007 (Hassan *et al.*, 2011; Stepanovic *et al*., 2007).

RESULTS

Number of Isolated Bacteria	**Number of Biofilm Producing Bacteria**	**Percentage of Biofilm Producing Bacteria**
189	125	66.13 %

Table 1: Total number of biofilms producing bacteria in water plumbing systems

Source		**Number of Isolated Bacteria**	**Number of Biofilm Producing Bacteria**	**Percentage of Biofilm Producing Bacteria**
Kitchen distribution line	Scrapings	20	16	80 %
	Water	14	05	35.71 %
Washroom distribution line	Scrapings	11	06	61.11 %
	Water	18	13	46.15 %

Table 2: Biofilm producing bacteria in the distribution line

Source		**Number of Isolated Bacteria**	**Number of Biofilm Producing Bacteria**	**Percentage of Biofilm Producing Bacteria**
Kitchen drain line	Scrapings	34	23	67.64 %
	Water	21	18	85.71 %
Washroom drain line	Scrapings	41	24	58.53 %
	Water	28	22	78.57 %

Table 3: Biofilm producing bacteria in the drain line

DISCUSSION

Biofilms are typically considered a primary source of microorganisms in any water plumbing system especially in old pipes. Bacterial contamination associated with biofilm is one of the most profound aspects of water quality as it can cause severe diseases (Blaustein *et al*., 2015). The present study indicates the existence of biofilm bacteria in the water plumbing systems.

125 bacteria were confirmed as biofilm producers among the total of 189 bacterial isolates from different sources including both distribution lines of scrapings and drinking water and drain lines of scrapings and wastewater from two different locations, kitchen and washroom outlets.

Among those, 20 bacterial isolates obtained from scrapings and 14 bacterial isolates from drinking water samples, only 16 and 5 bacterial isolates were positive for biofilm production respectively in the water distribution line collected from the kitchen outlets. Accordingly, 80 % of biofilm production was in the scrapings and 35.71 % in the drinking water samples.

Moreover, a total of 18 bacterial isolates were obtained from scrapings and 13 bacterial isolates from drinking water samples, only 11 and 6 bacterial isolates were positive for biofilm production respectively in the water distribution line collected from washroom outlets. Accordingly, it resulted in 61.11% of biofilm production in the scrapings and 46.15% in drinking water samples among the screened bacterial isolates.

Furthermore, a total of 34 bacterial isolates obtained from scrapings and 21 bacterial isolates from wastewater samples, only 23 and 18 bacterial isolates were positive for biofilm production respectively in the drain line collected from the kitchen outlets. Accordingly, 67.64 % of biofilm production was in the scrapings and 85.71 % in the wastewater samples.

In addition, a total of 41 bacterial isolates obtained from scrapings and 28 bacterial isolates from wastewater samples, only 24 and 22 bacterial isolates were positive for biofilm production respectively in the drain line collected from washroom outlets. Accordingly, 58.53% of biofilm production was in the scrapings and 78.57 % in the wastewater samples.

According to the results, the highest prevalence of biofilm-producing bacterial colonies was isolated from the wastewater from the drain line from the kitchen. The reason may be food waste is deposited in the kitchen. Thus, bacteria get constant nutritional sources to feed on in the kitchen drain line. Also, a considerable amount of biofilm-producing bacterial colonies was isolated from wastewater samples of the drain line from the washroom. The washroom drain line contains body oil, hair, shampoo, etc. Therefore, the drain line may provide the bacteria with a steady supply of nutrients and a comfortable, moist environment. Further, the highest prevalence of biofilm bacteria was recorded in the scrapings of the distribution line from the kitchen compared to the drain line. Moreover, 35.71 % of biofilm bacteria were identified in the kitchen drinking water, which correlates with the study of Mahapatra *et al*. (2010) which indicates that 19 % of isolated bacteria in the kitchen drinking water were biofilm producers, raising concerns about the safety of the drinking water. Based on the findings of this study, it becomes necessary to add bacterial biofilm counts to the indicators of water quality as they could indicate poor water quality and the presence of virulent bacteria in water.

Based on the morphological and biochemical tests, most biofilm producing bacteria

exhibited biochemical characteristics similar to coliform group bacteria (including *Escherichia coli*), *Pseudomonas aeruginosa* and *Staphylococcus aureus*.

Among the total of 189 bacterial isolates, 133 bacteria were found to be biofilm producers using the Congo red agar and Tissue culture plate methods whereas, only 125 bacterial isolates were found to be biofilm producers by using the Tube method. The methods, used for the present study almost showed **a** good correlation with each other when detecting biofilm production. The similar results obtained by Congo red agar and Tissue culture plate methods indicate the reliability of the results of the presence study. The results of the Tissue culture plate could be considered as an accurate method as it provides numeric value for the biofilm production, suggesting the accuracy and reliability of the research output.

CONCLUSIONS / RECOMMENDATIONS

The present study revealed the presence of biofilm producers in high numbers in the water plumbing system, including the distribution lines and drain lines from both the washroom and kitchen. The highest number of biofilm bacteria was detected in the drain line compared with the distribution line. However, the study confirmed the presence of biofilm-producing bacteria in a considerable amount even in the distribution line. Identifying biofilm bacteria in water plumbing systems emphasizes the need for further studies on biofilm bacteria that would reduce public health risks associated with waterborne infection. Screening the biofilm bacteria in the water before consumption is extremely important and it is also essential to establish effective prevention measures to avoid the entry of biofilm bacteria into the water plumbing system. Also, wastewater needs to be safely removed through drain lines without contaminating the surrounding area with biofilm bacteria.

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