



BIOFILM FORMATION POTENTIAL OF BACTERIA RECOVERED FROM WATER TAPS AND SINKS

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ABSTRACT

Biofilms are bacterial communities embedded in a polysaccharide matrix, resistant to antibiotics, environmental stress, biocides and could cause increase in the prevalence of diseases worldwide. This study elucidates the biofilm forming abilities of bacterial isolates from water taps and sinks. Water taps and sinks swabs samples were collected from Federal University of Agriculture, Abeokuta (FUNAAB), Isolu, Oshiele and Federal Medical Centre (FMC), Abeokuta. Isolation and identification of bacteria were carried out using standard microbiological methods and tube method was employed for biofilms assay. The isolated bacteria species include *Escherichia*, *Klebsiella*, *Salmonella*, *Proteus*, *Pseudomonas* and *Citrobacter*. The highest bacterial count of 9.8×10^5 (cfu/mL) was obtained from Oshiele tap samples while the lowest count was gotten from FMC sink samples (1.2×10^3 cfu/mL). Out of 108 bacteria isolated, 36 bacteria were biofilm formers. The isolated bacteria from FUNAAB hostel tap exhibited the highest biofilm potential followed by OSHIELE samples while FMC sink samples had highest bacteria biofilms. This study, thus, revealed that bacterial biofilm formers could be found in taps and sinks and presence of these diverse bacterial biofilms in the tap outlets and sinks could pose a serious threat to life-forms.

Keywords: Biofilms, Taps, Sinks, Bacterial counts, Polysaccharide matrix

INTRODUCTION

Complex organic materials and a very extensive microbial flora could be found in water that passes through distribution pipes (Ayansina et al., 2023). Surface-attached cells of microbial groups that are lodged solidly in a self-produced extracellular polymeric matrix are known as biofilms (Muhammad et al., 2020). Bacterial polysaccharides are the major component in the formation and stabilization of biofilms and also function as an intermediary between most cell-to-surface and cell-to-cell interactions (Flemming & Wingender, 2010).

Garnett & Matthews (2012) documented that bacterial biofilms are ubiquitous in the environment and can be found on almost any hydrated non-shedding surface including stagnant pools, rivers, man-made and also biological materials. Biofilms can form mushroom and tower-like structures surrounded by fluid filled channels and has been implicated in different types of bacterial infections in both humans and animals, such as, urinary tract infections, osteo articular infections, orthopedic and cardiac implant-associated infections, cystic fibrosis and periodontal diseases (Olsen, 2015).

The formations of slime layers and unique features like high population densities, high complex extracellular polymeric, chemical, physical and metabolic heterogeneities and their ability to form association with living or inert surface differentiate biofilms from their planktonic counterparts (Mirghani et al., 2022). Burmolle et al. (2010) added that many persistent and chronic bacterial infections have a link from biofilm. Biofilms are not only an issue in the medical field and the food industries but have also been found to be problematic in homes as they colonize surfaces of sanitary installations such as toilet bowls, showerheads or household devices like refrigerators and washing machines (Gattlena et al., 2010).

Community organization in biofilm is one of the major pathogenic strategies generally exercised by disease-causing microorganisms due to the resistance it renders to antibiotics and biocides, as well as to the body's natural immune system (Hoiby et al., 2011). Barroco et al. (2019) reported that

bacteria cells in biofilm interact strongly with each other and express their genes when changes such as pH, oxygen, nutrient availability, carbon source, cell density and presence of a solid surface condition take place.

The great significance of biofilms is the production of massive matrix with elevated hydrated matrix of polymeric substances produced outside the cell that also contains polyuronic acids, nucleic acids, proteins, lipids and polysaccharides (Mangwani et al., 2012). Generally, most water distribution systems are characterized by the occurrence of biofilms, regardless of purity, the type of pipe material used for distribution or the addition of a disinfectant (Mulamattathil et al., 2014). Due to the paucity of research on biofilm formation in both taps and sinks samples as well as the negative implications of bacterial biofilms, this study investigates the detection of bacteria biofilms in taps and sinks using the simple screening assay.

MATERIALS AND METHODS

Samples Collection

Swab samples were aseptically collected from 30 different water taps at the Federal University of Agriculture (FUNAAB), Isolu, and Oshiele, respectively; while 20 sink swab samples were collected from the Federal Medical Centre and the Federal University of Agriculture laboratories in Abeokuta, Ogun State. Separate swab sticks were rotated to pick samples from the mouths of the taps, and sinks. After sampling, the swabs were returned to their pre-labeled tubes and transported in ice-packs to the laboratory for analysis.

Preparation of Swab Samples

Two millimeters of sterile peptone water in each swab was labelled as the stock homogenate.

Isolation of Bacteria

Aliquot (0.1ml) of each of the serially diluted stock homogenate samples were aseptically transferred into sterile, already prepared petri-dishes separately containing nutrient agar, macconkey and eosin methylene blue agar. Inoculated plates were incubated at 30 °C for 24 h and 48 h. pure colonies

were obtained through repeated subculturing and distinct colonies on the plates were counted and recorded (Nehra et al., 2015).

Screening of Bacterial Isolate For Biofilm Formation

Isolates were screened for biofilm formation using tube method as described by Hassan et al. (2011). Brain heart infusion broth (5mls) containing the colonies were supplemented with 2% glucose in tubes. Cultured tubes contents were incubated for 18-24 hours at 37 °C and aspirated; the control tube was examined unstained and cultured tubes were stained with crystal violet. Appearance of visible stained film lining the wall of the tube indicated a positive result while absence of film indicated a negative result.

Identification of Bacterial Isolates

Bacterial isolates were identified using standard microbiological methods. Shape, pigmentation, elevation, size, appearance and motility were used for the morphological characterization. Biochemical tests, including Gram staining; catalase, oxidase, indole, nitrate, urease, coagulase and motility tests were conducted following the method of Karim et al. (2018).

Data Analysis

Statistical Package for Social Sciences (SPSS) was used to analyse the data. Duncan multiple range test was used for descriptive statistics

RESULTS AND DISCUSSION

The eventual occurrence of biofilms in tap outlets can be challenging to regulate as bacteria can grow and multiply when water is transported via a distribution system, particularly in unfavorable environments (Ayansina et al., 2023). Improved water distribution and hygiene as well as effective handling of water resources can strengthen a nation’s social-economic development and promote poverty alleviation (Okere et al., 2024). The variations in bacterial population of samples from diverse micro-habitats often depends on moisture and other environmental conditions. Table 1 illustrates the bacterial population of bacteria from taps and sinks.

The highest bacterial count was observed in the tap swabs from Oshiele with 9.8×10^5 (cfu/mL) while the lowest count was 1.2×10^5 (cfu/mL) on nutrient agar from FUNAAB hostels. The highest count of 8.4×10^5 (cfu/mL) on macconkey agar was obtained from FUNAAB hostel while the lowest was 2.9×10^5 (cfu/mL) from Oshiele. On the EMB agar, 7.4×10^5 (cfu/mL) was the highest count recorded from FUNAAB hostel while the lowest count was 2.1×10^5 (cfu/mL) from Isolu (Table 1).

Table 1: Total Bacterial Count from Taps Sample

Sample	Bacterial counts in cfu/mL on nutrient agar (10^5)	Bacterial counts in cfu/mL on macconkey agar (10^5)	Bacterial counts in cfu/mL on eosin methylene blue agar (10^5)
Os1	1.4 ± 0.8	5.2 ± 0.5	3.1 ± 1.0
Os2	5.7 ± 1.3	3.5 ± 1.6	2.5 ± 0.6
Os3	9.8 ± 1.0	3.0 ± 1.1	5.7 ± 1.2
Os4	1.5 ± 0.8	6.4 ± 1.5	3.0 ± 0.8
Os5	7.8 ± 1.2	2.9 ± 0.8	3.0 ± 1.5
Os6	1.4 ± 0.6	3.8 ± 1.0	4.3 ± 0.8
Os7	2.6 ± 0.9	3.3 ± 0.6	3.0 ± 0.7
Os8	4.0 ± 1.2	4.0 ± 1.7	6.0 ± 2.4
Os9	1.8 ± 0.4	3.2 ± 1.4	5.6 ± 1.2
Os10	3.6 ± 1.7	4.4 ± 1.9	4.8 ± 1.6
FHs1	1.9 ± 0.6	7.2 ± 2.8	3.0 ± 0.8
FHs2	1.7 ± 0.2	5.0 ± 1.3	5.0 ± 2.1
FHs3	8.2 ± 1.3	4.6 ± 1.2	4.8 ± 1.7
FHs4	4.6 ± 1.5	6.3 ± 1.8	7.1 ± 2.6
FHs5	7.8 ± 1.6	5.6 ± 2.5	4.9 ± 0.4
FHs6	8.4 ± 2.3	8.4 ± 1.0	5.2 ± 2.0
FHs7	7.0 ± 3.8	4.0 ± 1.6	5.3 ± 1.5
FHs8	1.2 ± 0.4	3.5 ± 1.4	4.1 ± 1.8
FHs9	9.2 ± 1.1	6.8 ± 3.2	5.5 ± 1.3
FHs10	1.8 ± 0.2	7.6 ± 2.4	7.4 ± 0.6
Is1	9.7 ± 2.3	6.4 ± 1.2	4.5 ± 1.2
Is2	1.8 ± 0.5	6.0 ± 0.6	5.0 ± 1.9
Is3	1.6 ± 0.2	5.7 ± 1.8	3.2 ± 1.0
Is4	8.5 ± 1.9	6.9 ± 2.4	5.1 ± 1.7
Is5	7.2 ± 3.1	8.0 ± 1.1	4.1 ± 0.9
Is6	6.1 ± 1.2	5.1 ± 1.6	4.7 ± 1.1
Is7	9.3 ± 2.5	6.8 ± 2.3	2.1 ± 0.8
Is8	8.1 ± 3.8	7.2 ± 1.4	6.0 ± 2.3
Is9	1.6 ± 0.9	6.3 ± 1.5	3.5 ± 1.7
Is10	1.3 ± 0.4	5.6 ± 2.7	4.2 ± 1.3

KEY: Os= OSHIELE TAP SWAB; Is = ISOLU TAP SWAB; FHs = FUNAAB HOSTEL TAP SWAB

Table 2 shows the count for sink samples using the different agar. On nutrient agar, the highest recorded count was 6.4×10^5 (cfu/mL) while the lowest count was 2.0×10^5 (cfu/mL), both from FMC. The highest 4.2×10^5 (cfu/mL) and the lowest count of 1.8×10^5 (cfu/mL), on the macconkey agar were also both recorded in the samples

from FMC. On the eosin methylene blue agar, the highest and lowest counts obtained were 3.6×10^5 (cfu/mL), and 1.2×10^5 (cfu/mL) from FLS and FMCS. The dispersion of bacteria found in this study suggested that the water from selected sinks and taps could be less portable and might even be dangerous for human health.

Table 2: Total Bacterial Count from Sink Samples

Sample	Bacterial counts in cfu/mL on nutrient agar (10^5)	Bacterial counts in cfu/mL on macconkey agar (10^5)	Bacterial counts in cfu/mL on eosin methylene blue agar (10^5)
FMCS1	4.0 ± 1.2	2.9 ± 0.5	3.0 ± 1.0
FMCS2	3.6 ± 0.8	4.2 ± 0.6	2.1 ± 0.6
FMCS3	2.5 ± 1.0	2.0 ± 0.4	3.2 ± 0.8
FMCS4	4.7 ± 1.4	3.1 ± 1.0	3.4 ± 1.3
FMCS5	6.4 ± 1.5	1.8 ± 0.2	1.2 ± 0.2
FMCS6	3.6 ± 0.8	3.6 ± 1.9	3.1 ± 1.0
FMCS7	4.3 ± 1.9	3.2 ± 1.0	2.2 ± 1.0
FMCS8	4.0 ± 1.4	3.8 ± 0.6	2.6 ± 1.0
FMCS9	3.9 ± 1.2	3.4 ± 0.8	3.1 ± 0.5
FMCS10	2.0 ± 0.4	3.7 ± 1.4	2.0 ± 0.4
FLS1	4.6 ± 2.1	3.4 ± 1.6	3.6 ± 0.8
FLS2	2.8 ± 1.3	2.5 ± 1.2	2.7 ± 0.6
FLS3	3.0 ± 1.1	2.0 ± 0.7	2.0 ± 0.2
FLS4	2.4 ± 1.0	3.7 ± 1.1	2.2 ± 0.4
FLS5	2.9 ± 0.8	4.0 ± 1.0	3.0 ± 0.9
FLS6	5.0 ± 1.3	3.1 ± 1.4	2.5 ± 0.6
FLS7	4.8 ± 1.9	2.2 ± 0.6	3.2 ± 1.0
FLS8	3.4 ± 1.7	3.0 ± 0.8	2.7 ± 0.8
FLS9	3.2 ± 1.4	3.1 ± 0.6	3.0 ± 1.2
FLS10	5.6 ± 2.3	2.0 ± 0.9	2.1 ± 0.4

KEY; FMCLS = FEDERAL MEDICAL CENTRE LABORATORY SINK SWAB; FLS = FUNAAB LABORATORY SINK SWAB

Plate 1 shows the percentage occurrences of different bacterial species from taps and sinks samples. The bacteria isolated were *Salmonella*, *Proteus*, *Pseudomonas*, *Klebsiella*, *Citrobacter*, *Escherichia* and *Proteus* species. *Salmonella*

species were the dominant bacteria followed by *Pseudomonas* species while *Proteus mirabilis* and *Citrobacter* sp had the least occurrences of 4.76% each from tap samples (Plate 1).

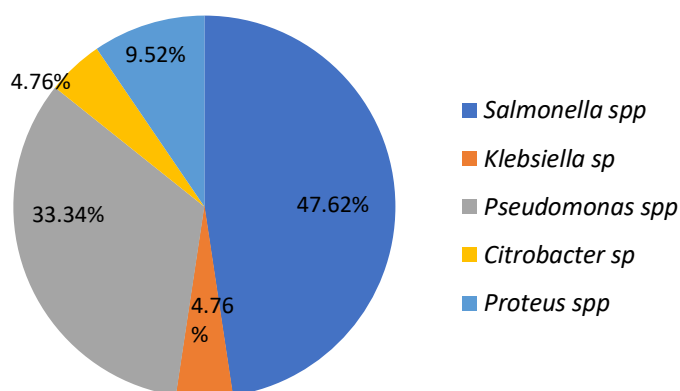


Figure 1: Percentage Occurrences of Bacterial Isolates from Tap Samples

Pseudomonas species (35.71%), followed by *Escherichia* (28.57%) were the dominant species while *Proteus* had the least occurrence of (14.29%) for sink samples in Plate 2. Among the different isolates identified, *Salmonella arizona*, a subspecies of *Salmonella enterica* was the dominant sp. The bacterium mainly infects humans, especially infants or immune-compromised individuals and causes gastroenteritis, peritonitis and osteomyelitis. The high bacterial counts also indicated that water taps and sinks could allow growth and formation of biofilms that could cause infections and water-borne diseases.

The prevalence of these bacteria in the taps and sinks could likely be due to human activities and interactions including poor hygiene (Enogiomwan & Ibeh, 2018). This study agrees with the work of Ayansina et al. (2023) who also identified *Pseudomonas*, *Proteus*, *Klebsiella*, *Escherichia*, *Citrobacter*, *Enterobacter* and *Salmonella* spp from taps. Kinge et al. (2012) opined that the presence of enteric bacteria of the genera *Escherichia*, *Salmonella* and *Klebsiella* in water distribution systems is a major threat to human health and are causative agents for many diseases. The isolation of *Pseudomonas*, *Klebsiella* and *Citrobacter* from sink samples

and their biofilms formation abilities in our study corroborates with the work of Franco et al. (2020).

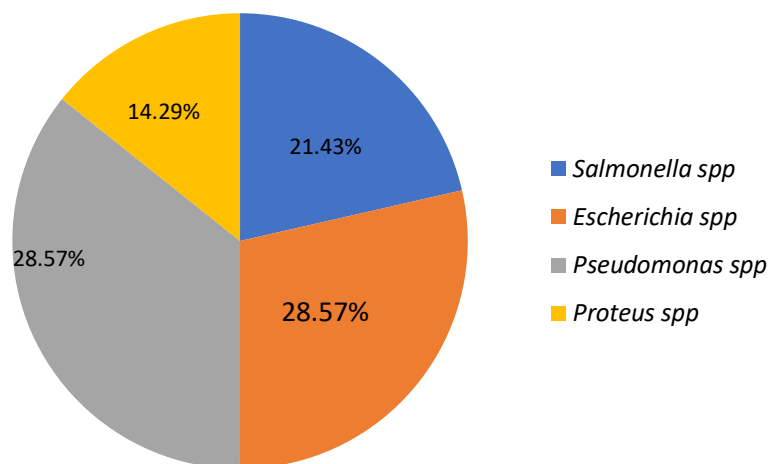


Figure 2: Percentage Occurrences of Bacterial Species Identified From Sink Samples

Our results indicated that tap water pipes harbored larger number of bacteria species compared to the laboratory sinks. This can be attributed to favorable conditions in the pipes, including increased nutrient availability which aid biofilm attachment at various stages (Rather et al., 2021). Majority of the bacteria from the laboratory sinks might have emanated from cultures used by the personnel in the laboratory. Biofilms in distribution systems have been found to serve as reservoir for pathogens. The biofilm formers recorded in the study did not corroborate with the work of Mathur et al. (2006) where 53.95 % were positive and 46.05 % were negative. Table 3 shows the biofilm-forming abilities of the

tap samples and results indicated that the biofilm-forming isolates were mostly from tap swabs of 20.37 %. It was observed that about 75% of the microorganisms from cold water taps and laboratory sink swabs are potential human pathogens (*Pseudomonas sp*, *Proteus sp* and *Salmonella sp*). In a healthy person, an infection with an opportunistic pathogen is controlled by the immune system, however, opportunistic pathogens are the main cause for morbidity and mortality in immune compromised individuals (Achermann et al., 2014). This corroborates with the work of Hausner et al. (2012) who reported that biofilms could be found in water distribution system such as taps and could lead to potential risks from waterborne pathogens.

Table 3: Bacteria Biofilm Formers from Tap Samples

ISOLATE CODE	DEGREE OF BIOFILMS
FHs21	+++
FHs32	+++
FHs71	+++
FHs83	+++
FHs91	+++
FHs51	+++
FHs62	+++
Os7 ³	+++
Os4 ⁵	+++
Os5 ³	++
Os8 ⁶	+++
Os9 ²	+++
Os10 ²	+++
Is11	+++
Is32	+++
Is35	++
Is46	+
Is73	++
Is92	++
Is92	+++
Is48	+++
Is67	+++

KEY; + = LOW BIOFILM FORMERS; ++ = MODERATE BIOFILM FORMERS; +++ = HIGH BIOFILM FORMERS
 Os= OSHIELE TAP SWAB; Is = ISOLU TAP SWAB; FHs = FUNAAB HOSTEL TAP SWAB; ISOLATE CODE:

The letters represent the sample site and the sub-superscript represents the isolate under each isolate number, i.e, the sub-isolate number.

Biofilms formation from sink samples was demonstrated in 12.96% of the 108 bacterial isolates and majority of the sink swab samples with high biofilms were from FMCS (Table 4). The biofilm-forming bacteria isolated from these micro-habitats might have developed mechanisms which supported

their growth within biofilms. The presence of bacterial biofilm formers in our studies agrees with the report of Patil et al. (2012) who suggested that an effective cleaning of the water pipes and taps will significantly reduce the incidence and occurrence of the bacteria. Ayansina et al. (2023) also reported that poor personal hygiene of the water treatment plant workers and environmental hygiene contributes significantly to the level of contamination.

Table 4: Bacteria Biofilm Formers from Sink Samples

ISOLATE CODE	SLIME POSITIVITY
FMCS1 ₁	+++
FMCS2 ₂	++
FMCS4 ₅	+++
FMCS5 ₈	+++
FMCS7 ₁₀	+++
FMCS8 ₁₂	+++
FMCS9 ₁₅	+++
FMCS9 ₁₈	+++
FLS1 ₁	+++
FLS3 ₂	+
FLS4 ₃	+
FLS4 ₄	+++
FLS5 ₇	+++
FLS8 ₉	+++

KEY: + = LOW BIOFILM FORMERS; ++ = MODERATE BIOFILM FORMERS; +++ = HIGH BIOFILM FORMERS
FMCS=FEDERAL MEDICAL CENTRE LABORATORY SINK SWAB; FLS = FUNAAB LABORATORY SINK SWAB.
ISOLATE CODE:

The letters represent the sample site and the sub-superscript represents the isolate under each isolate number, i.e, the sub-isolate number.

CONCLUSION

In this study, it was observed that bacterial counts from tap samples had higher counts than the sink samples which could be because sinks are easily recognize when dirty and may be washed more often than the mouths of taps. Early detection of these bacteria and removal from equipment where they are not needed is of beneficial effect both medically and environmentally because these isolates may harbor virulence gene manifesting their ability to cause human diseases.

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