Diversity of Biofilm-producing Bacteria in a Drinking Water Distribution System in a Suburban Community in South-South Nigeria

Ochi P, *Otokunefor K, Abu G

ABSTRACT

Reports of biofilms and associated bacterial communities in drinking water distribution systems (DWDS) have been made from various parts of the world, often in association with several negative implications. Such awareness data is not widespread in Nigeria. Knowledge of this would be essential in improving the quality of drinking water. This study thus set out to explore bacterial diversity in a DWDS and determine the biofilm-forming potential of the bacterial isolates. Standard plating and biochemical test methods were used to isolate and identify bacteria present in a DWDS, and biofilm-forming potential of the bacteria determined using the Congo Red Agar (CRA) method. Nine different potentially pathogenic bacterial species were detected, with Staphylococcus aureus and Serratia sp being predominant. A higher number of species was however associated with water samples than swab samples. Using the CRA method, 24.3% of the bacteria were found to be possible biofilm producers, with Klebsiella sp as the predominant organism. The presence of potentially pathogenic bacterial species in this DWDS is considered an early warning signal of public health concern. The biofilm-forming ability of some of the isolates further highlights the public health risk considering the association between biofilm formation and increased virulence, pathogenicity and drug resistance of microbes with such potential.

Keywords: Biofilm, Congo, Red, Agar, Nigeria

INTRODUCTION

Biofilm is a term which refers to a complex microbial community existing within a slime layer matrix, attached to surfaces in a solid-liquid heterogeneous system including aqueous environments. Biofilms have been described as the predominant way microbes exist in various settings.[1,2] Biofilm formation is a complex process which occurs over some time and involves bacterial collaboration. This process first begins with the initial attachment of bacterial cells to a surface, followed by the development of macrocolonies and finally, the maturation step whereby the biofilm architecture develops.[1,3] Biofilms are not a fixed static structure but rather are dynamic whereby the whole assembly is in a continuous state of flux with the different stages occurring simultaneously.

These biofilms have been widely described in drinking water distribution systems, where up to 95% of bacteria have been reported contained within these biofilms.[4,5] Bacteria within biofilms are protected by the biopolymer matrix, making them resistant to disinfection, environmental stresses and xenobiotics.[6] This could result in an increased ability of the organisms to persist, as well as serve as a source of secondary contamination to the water system. Other negative effects on the water quality associated with biofilms include pipe corrosion and the breakdown of residual disinfectants.[7] Additionally, biofilms could negatively impact water aesthetics, contributing to the generation of tastes, odours and discolouration.[8,9]

Several studies have reported on biofilms in DWDS from around the globe. A research group in the United Kingdom monitoring biofilm composition in DWDS for 1 year, noted that while the microbial composition had a degree of variability over the period, a core community of bacteria was present in all of the biofilms.[10] A similar report was made from a group in the United States of America[11], who noted the presence of several groups of bacteria in all samples. Liu and colleagues further reported DWDS biofilms comprised several possible pathogens[12], while

Department of Microbiology, Faculty of Science, University of Port Harcourt. PMB 5323 Port Harcourt, Nigeria.

*Corresponding author: komeotokunefor@uniport.edu.ng.

in general, *Pseudomonas* sp has been reported as the most prominent bacteria associated with biofilms.[10,13] Variations have been described in the nature and type of biofilm formed based on the composition of the surface.[14]

Differences may exist in the composition and construction of drinking water distribution systems (DWDS) across the globe; there may also be climatic differences. Few studies have nevertheless explored biofilms in DWDS in a typical metropolis in Nigeria.[15] Hence, the scope of the problem is not well characterized. Knowledge of the specific bacterial communities associated with biofilms in DWDS in our locale would be essential as future control strategies could then be specifically aimed at these specific organisms. This study, therefore, set out to explore bacterial diversity in a DWDS in a suburban community in South-South Nigeria and determine the biofilm-forming potential of these bacteria.

MATERIALS AND METHODS

Sample Collection and Processing

Ten water and swab samples were aseptically and respectively collected from taps and water storage tanks in a tertiary institution in Rivers State, Nigeria. The total of 20 samples was immediately transported to the microbiology laboratory of the University of Port Harcourt for processing. Bacteria isolation was carried out using the standard spread plate method on nutrient agar. In brief, this involves spreading a 1ml aliquot of the sample on the surface of the solid media, resulting in distinct colonies following incubation.[16] Isolates were then identified using standard biochemical tests which include, Gram staining, oxidase, coagulase, catalase, citrate utilization, sugar fermentation, methyl red, Voges Proskauer, indole production, urease and triple sugar iron agar tests.[17,18]

Determination of Biofilm-producing Bacteria

Biofilm producing bacteria present in the samples were identified using a previously described method[19]. This method involves the inoculation of pure cultures of the bacterial isolates onto Congo red agar plates. Following a 24-hour incubation at 37°C, biofilm producers showed up as black colonies with a dry crystalline consistency, while non-biofilm producers present as red colonies.

RESULTS

Bacterial Diversity

In this study, a total of 9 different bacteria types were detected in both samples. A higher number of bacterial types were however associated with the water samples (Table 1) than the swab samples.

Table 1: Diversity of bacteria in the samples

Organism	Water Sample	Swab Sample
Serratia sp	+	+
Staphylococcus aureus	+	+
Escherichia coli	+	+
Micrococcus sp	+	-
Klebsiella sp	+	+
Bacillus sp	+	+
Enterobacter sp	+	-
Shigella sp	+	+
Salmonella sp	+	-

Staphylococcus aureus and *Serratia* sp were the predominant organisms detected in this study (Figure 1). *S. aureus* was the more commonly occurring bacteria associated with the water samples while *Serratia* sp was the more commonly occurring in the swab samples. From the swab samples, *E. coli* was the third most commonly occurring isolate along with members of the *Bacillus* sp while a low occurrence (4.8%) was noted in the water sample.

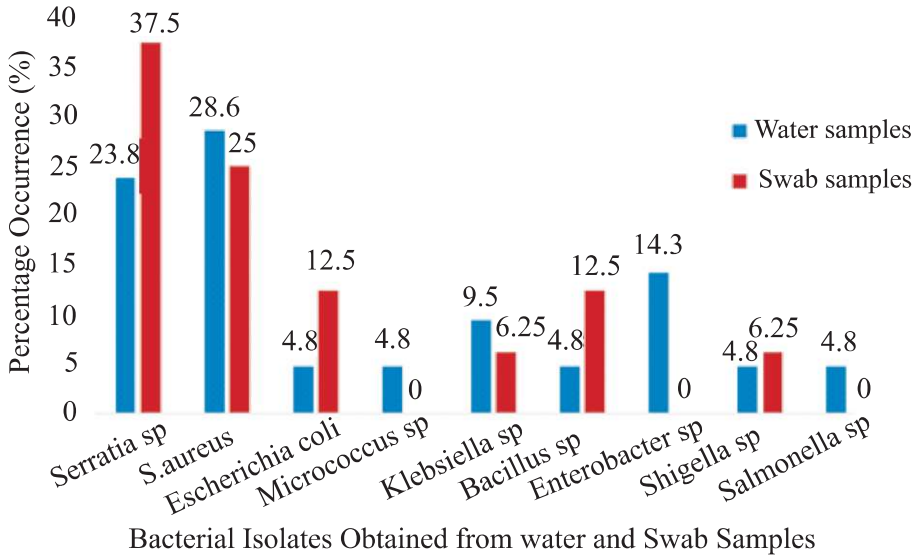

Figure 1: Occurrence of bacterial isolates in water and swab samples

The Occurrence of Biofilm-producing Bacteria

The assay for biofilm production revealed a 24.3% (9/37) occurrence among bacteria isolated in this study (Table 2). A higher rate of biofilm-producing bacteria was detected in the water samples. Of the nine different bacterial types initially identified, six were found to be biofilm-producing (Table 3). Four of these were identified from the water samples specifically. These four bacterial groups comprised only a minority of the total bacteria initially identified from these samples (7/21, 33.3%), but showed a high association with biofilm production (6/7, 85.7%). In general, *Klebsiella* sp was the predominant bacteria associated with biofilm production followed by *Enterobacter* sp.

Table 2: Biofilm producing bacteria according to sample type

Sample Type	Biofilm Producers, n (%)
Water (n=21)	6(28.6)
Swab (n=16)	3(18.8)
Total (n=37)	9(24.3)

Table 3: Distribution of biofilm producers by bacteria type

Bacteria type (total number isolated)	Biofilm producers by bacteria type n
Water Sample	
Escherichia coli (1)	1
Micrococcus sp (1)	1
Klebsiella sp (2)	2
Enterobacter sp (3)	2
Swab Sample	
Staphylococcus aureus (4)	1
Klebsiella sp (1)	1
Serratia sp (6)	1

DISCUSSION

Drinking-water distribution systems comprise a complex network of pipes, fittings, storage tanks and tap outlets which connect the water treatment plants with the consumer.[20] The World Health Organization

recommends that water in the DS must be both microbiologically safe and have biological stability.[21] However, while the water is normally at an acceptable standard at the source, the DWDS may sometimes serve as reactors resulting in the degradation of the water quality during the process of transportation, negatively impacting on the delivery of safe clean water. Such deterioration of water quality has been seen as an increase in turbidity, cell numbers and specific indicator organisms.[12,22] A 2003 study by Lee and Kim reported a consistently higher number of bacteria in water obtained from the DWDS outlet in contrast with water sampled at the inlet.[23] This deterioration could have adverse health effects on consumers. Of particular concern is deterioration associated with the presence of pathogenic bacteria capable of resulting in disease conditions in susceptible individuals. Biofilms have been implicated as one of the major contributors to this process acting both as a protective environment for bacteria, as well as serving as a continuous source of bacteria.

Results of this study reported the presence of nine different bacterial types associated with a DWDS in Rivers State, Nigeria. More bacterial types were however associated with the water samples than the biofilm samples. This is similar to previous reports which noted a higher diversity in planktonic communities as opposed to the biofilm communities in a DWDS.[20,23] More recent studies analyzing microbial communities in DWDS have made use of molecular techniques which identify both viable and non-viable bacteria based on variations in their 16sRNA sequences. These studies have found a preponderance of a wide variety of bacterial classes such as the mycobacteriales, methylomonas, betaproteobacteria, proteobacteria and alphaproteobacterial.[7,24,25,26] In some cases, several possibly pathogenic genera were detected, such as *Pseudomonas*, *Acinetobacter*, *Escherichia*, *Clostridium*, *Streptococcus*, *Legionella*, *Vibrio* and *Mycobacterium*. Despite the current use of molecular techniques to detect bacteria in biofilms, culture-based techniques remain the standard. Studies using these culture-based techniques have reported the presence of a wide variety of pathogenic species key of which were the *Pseudomonas*, *Staphylococcus* and the *Acinetobacter*.[23,27] Similarly, we report the presence of potentially pathogenic bacteria genera such as *Serratia* sp, *Staphylococcus aureus*, *Escherichia coli*, *Micrococcus* sp, *Klebsiella* sp, *Bacillus* sp, *Enterobacter* sp, *Shigella* sp and *Salmonella* sp. These organisms have been associated with various diseases ranging from mild skin conditions to more life-threatening gastrointestinal disorders. While drinking water is not expected to be free from microorganisms, it is expected to be free from bacteria which might constitute a risk to human health.[27] The detection, therefore of potential pathogens in drinking water systems by this study indicates a possible public health problem and highlights a need for further study to ascertain if indeed these bacteria isolated are pathogenic species or mere commensals. The study by September and colleagues (2007) which identified pathogenic species especially *Aeromonas* and *Pseudomonas* went on to further ascertain using molecular techniques that while the *Pseudomonas* isolates obtained in their studies were not related to pathogenic strains, the *Aeromonas* isolates were.

The presence of *Escherichia coli* in water could often be used as an indicator of recent faecal contamination. This study noted a low occurrence of this organism in the water sample (4.8%). This is similar to a previous study which noted a 2% occurrence of coliforms in general and a 0.08% occurrence of *E. coli* specifically in a drinking water system.[28] Like our study, however, this study failed to ascertain whether the coliforms represented were faecal or non-faecal. The most predominant species reported in this study (both in the water and swab samples), were *Serratia* sp and *S. aureus*. *Serratia* sp, a member of the enterobacteriaceae family, which was initially considered a harmless group of bacteria, is presently known to be an opportunistic pathogen causing healthcare-associated infections particularly pneumonia and bloodstream infections[29]. Therefore, while the presence of this bacteria in the

DWDS sample poses a minimal threat to the general population, it would be more problematic if this DWDS supplies a healthcare institution. *S.aureus*, on the other hand, is a known pathogen, one of the most notorious in clinical microbiology. This organism has been associated with a wide variety of disease conditions such as boils, food poisoning, cellulitis and toxic shock syndrome[30]. Additionally, certain strains of *S.aureus* are associated with multidrug resistance, particularly the methicillin-resistant *S.aureus* (MRSA) strains. Hence the detection of these organisms in the DWDS in this study raises public health concerns.

The potential to form biofilms is often counted as a major virulence factor associated with bacteria.[31] Additionally, bacteria with the ability to produce biofilms have been associated with higher levels of pathogenicity and multidrug resistance.[32] In this study, biofilm-forming potential was determined using the affordable, simple phenotypic Congo Red Agar (CRA) method. While this method often results in the detection of fewer bacteria with biofilm-forming potential as opposed to the microtiter plate (MTP), it has been reported to detect biofilm-forming potential up to 73.1% of MTP positive cases.[31,33] This study reports a 24.3% occurrence of bacteria with biofilm-forming potential in DWDS. These could have negative health implications.

CONCLUSION

Potentially pathogenic bacterial species were detected in a tertiary institution in a sub-urban community DWDS in Rivers State. Some of these organisms exhibited the ability to form biofilms which can lead to increased virulence, pathogenicity and drug resistance. Additionally, this study reports on the use of a culture-based, the simple and non-costly method to identify organisms with biofilm-forming potential; this method might be of great importance in a resource-limited setting like Nigeria.

CONFLICT OF INTEREST

The authors declare that no conflict of interest exists

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