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Molecular Characterization of Biofilm-Producing Bacteria Isolated from Water Stored in Polyvinyl Chloride (PVC) Tanks

Arah, U. E*., Amadi, L. O. and Peekate, L. P.
Department of Microbiology, Rivers State University,
Nkpolu-Oroworukwo, Port Harcourt, Nigeria
***Corresponding Author:** *arahuyoyou@gmail.com*

ABSTRACT

Development of biofilm in water storage tanks deteriorates the quality of drinking water in homes. The aim of this study was to identify bacteria that produce biofilm in water storage polyvinyl chloride tanks (WSPT). Water samples were obtained from WSPT of 70 households in Obio/Akpor and Port Harcourt Local Government Areas (OBALGA and PHALGA) in Rivers state, Nigeria. Samples were analyzed for total heterotrophic bacteria (THB), total and faecal coliform, *Vibrio*, and *Salmonella*/*Shigella* species which were screened for biofilm production using standard bacteriological procedures. Isolates positive for biofilm production were identified through conventional and molecular methods. THB count in samples from PHALGA and OBALGA ranged from $3.0\pm0.1\times10^4$ to $1.04\pm0.01\times10^6$ CFU/ml and $2.2\pm0.3\times10^4$ to $2.30\pm0.03\times10^5$ CFU/ml respectively; total coliform count ranged from $1.5\pm0.7\times10$ to $6.4\pm0.1\times10^2$ CFU/ml and $1.6\pm0.3\times10^2$ to $2.26\pm0.04\times10^3$ CFU/ml respectively; faecal coliform count ranged from 0 to $1.6\pm0.3\times10^2$ CFU/ml and $8.0\pm0.0\times10$ to $1.54\pm 0.03\times10^3$ CFU/ml respectively; *Shigella* count ranged from 5±0.0 x 10 to $1.1\pm0.1\times10^2$ CFU/ml and $8\pm0.1\times10$ to $3.5\pm0.1\times10^2$ CFU/ml while *Salmonella* counts ranged from 1±0.0 x10 to 4±0.01x10CFU/ml and 1±0.0 x10 to 9±0.1 CFU/ml for samples from PHALGA and OBALGA respectively. Ten genera among the bacterial isolates were positive for biofilm production and they include *Proteus mirabilis* LC728309, *Shigella flexneri* LR738991, *Enterococcus faecium* AB512765, *Klebsiella aerogenes* SGRCBNR5-D11, *Serratia aquatilis* NR_147771, *Streptococcus algalactiae* CP010867, *Bacillus subtilis* NR_102783, *Enterobacter cloacae* NR_102794, *Alcaligens viscolactis* AY911519, and *Escherichia coli* ETEC MF919609. The genera of *Proteus*, *Shigella*, *Enterococcus*, and *Klebsiella* were observed to be highest producers of biofilm. The presence of these bacteria particularly *Escherichia coli* ETEC MF919609 is worrisome and may constitute health hazard in water storage tanks. It is therefore recommended that the interior of WSPT should be cleaned regularly with a plan put in place to determine the frequency of cleaning.

Keyword: Polyvinyl chloride tanks, stored water, biofilm producing bacteria, *E. coli*, *Klebsiella aerogenes*.

Introduction

Water storage has been identified as a source of contamination of domestic water (Nnaji *et al*. 2019). Water pathogens that cause various illnesses and diseases are present in storage tanks. The risk of spreading waterborne illnesses and the potential for numerous infectious outbreaks are increased (Khan and AlMadani, 2016; Manga *et al.*, 2021). It has been reported that the development of biofilm on the inside surface of storage vessels provides an ideal environment for the growth of microorganisms, which in turn deteriorates the quality of drinking water in homes.

Research has revealed that stored drinking water contains high levels of feacal coliforms and heterotrophic bacteria, far above the limits recommended for human consumption (Budeli *et al.*, 2018). A tiny number of microorganisms in water may also be able to multiply under the correct circumstances, enabling them to grow and proliferate in the storage containers. According to a study (Merve *et al.*, 2013), microorganisms attached to the surface wall of storage containers during storage multiplied at the expense of low carbon concentrations in the water. There is a direct relationship between the degree of bacterial regrowth on drinking water storage vessels and the storage components as well as the input water's quality.

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These include elements like temperature, turbidity, concentrations of organic nutrients, and dissolved organic carbon (Budeli *et al.,* 2018; LeChevallier *et al.,* 2007).

Biofilm is a community of microorganisms irreversibly attached to a surfaces and produce Extracellular Polymeric Substances (EPS) (Sadekuzzaman *et al* 2015). Biofilms can be formed on various surfaces such as medical devices, food processing equipment, and natural environments (Okafor *et al.,* 2005). In aquatic environments, microorganisms have the ability to adhere to solid surfaces and form biofilms due to different unfavourable conditions that required adaptations. The matrix contains polysaccharides, proteins, glycoproteins, glycolipid and DNA. The matrix allows the microbes to stick more stably to the surface and protects them from antimicrobial agents (Suma *et al.,* 2014).

The formation of biofilm by bacteria involves several stages. Initially, the bacteria attach to a surface using specialized adhesion molecules. They then multiply and produce Extracellular polymeric substances (EPS) which are organic polymers involved in bacterial cells' interactions with their environment, and help to anchor the bacteria and provide protection against external factors such as antibiotics and the host immune system. The biofilm structure provides a favorable environment for the bacteria to thrive and communicate with each other through a process known as quorum sensing (Suma *et al.,* 2014).

Biofilm increase the opportunity for gene transfer among bacteria (Okafor *et al.,* 2005). Bacteria that are resistant to antibiotics may transfer the genes for resistance to neighboring susceptible bacteria (Sadekuzzaman *et al.,* 2015). Also, gene transfer could convert a previous virulent commensal organism into a highly virulent pathogen (Lewis 2012).

According to Zhao *et al*. (2017), the most common way that microorganisms grow in drinking water distribution and storage systems is through biofilms. It is commonly known that one of the main issues with drinking water distribution systems is biofilm (Duarte *et al.,* 2022; Prest *et al.,* 2016). Bacterial cells have the ability to adhere to the inner surfaces of piping systems, form biofilm there, and then separate into the bulk water.

This can lead to biocorrosion of the pipes, unfavorable changes in the water's color, taste, turbidity, and odors, as well as a decrease in the efficiency of heat exchange (Prest *et al*., 2016). More specifically, sulfate-reducing, sulfur-oxidizing, iron-oxidizer, iron-reducer, and manganese-oxidizer bacteria are the main biofilm-producing bacteria that are known to promote the corrosion of metals (Karaguler *et al.,* 2017). These bacteria are also known to negatively impact water pipelines and storage tanks, as well as the safety of drinking water (Muhammad *et al.,* 2020). Biofilm producing bacteria are associated with various impacts and challenges. They can cause persistent infections that are difficult to treat, as the bacteria within the biofilm exhibit increased resistance to antibiotics. Biofilms are also implicated in the contamination of water systems, leading to outbreaks of diarrheal diseases. The aim of the study was to determine the antibiogram of biofilm producing bacteria from water-storage PVC tanks.

Materials and Methods

Study Area

This study was carried out in Obio/Akpor Local Government Area (OBALGA) and Port Harcourt Local Government Area (PHALGA) in Rivers State, Nigeria. These Local Government Areas are highly populated about 464,789 and 1,865,000 respectively. Majority of the residents depend on groundwater from extraction boreholes stored in Polyvinyl chloride (PVC) tanks for drinking and domestic use.

Sample Collection

Seven locations in OBALGA and PHALGA were visited for sample collection. The name of the locations and their geo-coordinates are: It is located between latitudes 4°45'N and 4°65'N and longitudes 6°50'E and 8°00'E Within these locations, 70 water samples were collected from households that have Water Storage Polyvinyl chloride Tanks (WSPT). The water samples were collected by opening the tap connected to the WSPT, and allowing the water to flow for about one minute before collecting about 600 ml water using sterile bottles (Kpormon *et al.,* 2023). The water bottles were appropriately labeled, placed in iced-packed containers, and transported to the Microbiology Laboratory of Rivers State University, Port Harcourt for bacteriological analysis.

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Bacteriological Analyses

Aliquot of 0.1 ml of the water samples were cultured on plates of nutrient agar (NA) for isolation and enumeration of total heterotrophic bacteria (THB); on plates of MacConkey agar (MA) for isolation and enumeration of total coliforms; on plates of Eosin methylene blue agar (EMBA) for isolation and enumeration of feacal coliform (*Escherichia coli*); on plates of Thiosulfate Citrate Bile-salts Sucrose agar (TCBSA) for isolation and enumeration of *Vibrio*; and on plates of *Salmonella-Shigella* agar (SSA) for isolation and enumeration of *Salmonella* and *Shigella*. Inoculated NA and MA plates were incubated at 37 ºC for 24 hours; inoculated EMBA plates at 44.5 ºC for 24 hours; and inoculated TCBSA and SSA plates at 37 ºC for 48 hours.

After incubation, colonies on NA plates were counted and used to calculate the THB population; pink colonies on MA plates were counted and used to calculate the total coliform population; black colonies with metallic sheen on EMBA plates were counted and used to calculate the feacal coliform and *Escherichia coli* population; yellow colonies on TCBSA plates were counted and used to calculate the *Vibrio* population; and colourless colonies and colonies with black centres on SSA plates were counted and used to calculate *Shigella* and *Salmonella* populations respectively.

Discrete bacterial colonies that grew on the respective media were sub cultured using streak plate method onto freshly prepared nutrient agar and incubated at 37 °C for 24 hours in order to obtain pure cultures. The pure bacteria cultures were then stocked and maintained according to the method adopted by Amadi *et al.* (2014) using ten percent (v/v) glycerol.

Identification of Bacterial Isolates

Pure bacterial cultures were subjected to Gram staining and microscopic examination, and the following biochemical/physicochemical tests: catalase, oxidase, , coagulase, citrate utilization, indole production, Methyl Red, Vogues Proskauer, starch hydrolysis, Triple sugar iron agar test, and fermentation tests. The tests were carried out as described in Cheesbrough (2006) and Peekate (2022). Stock cultures of identified isolates were sent for identification through bio-molecular means.

Molecular Identification of Isolates

DNA Extraction

About 5 ml of an overnight broth culture of the bacterial isolate in Luria Bertani (LB) was spun at 14000rpm for 3 min. The cells were re-suspended in 500ul of normal saline and heated at 95^0C for 20 min. The heated bacterial suspension was cooled on ice and spun for 3 min at 14000rpm.

The supernatant containing the DNA was transferred to a 1.5ml micro-centrifuge tube and stored at -20^oC until required for DNA quantification, sequencing and phylogenetic analysis (Morange and Michel, 2016).

DNA Quantification

The extracted genomic DNA was quantified using the Nanodrop 1000 spectrophotometer set at 500 wavelength. The software of the equipment was launched by double clicking on the Nanodrop icon.

The equipment was initialized with 2µl of sterile distilled water and blanked using normal saline. Two microlitre of the extracted DNA was loaded onto the lower pedestal; the upper pedestal was brought down to contact the extracted DNA on the lower pedestal. The DNA concentration was measured by clicking on the “measure” button.

16S rRNA Gene Amplification

The 16s rRNA region of the rRNA genes of the isolates were amplified using the 27F: 5'-AGAGTTTGATCMTGGCTCAG-3' and 1492R: 5'-CGGTTACCTTGTTACGACTT-3’ primers on a ABI 9700 Applied Biosystems thermal cycler at a final volume of 50 microlitres for 35 cycles (Olsen and Morrow, 2012).

Sequencing

Sequencing was done using the BigDye Terminator kit on a 3510 ABI sequencer by Inqaba Biotechnological, Pretoria South Africa.

The sequencing was done at a final volume of 10ul; the components included 0.25 µl BigDye terminator v1.1/v3.1, 2.25ul of 5 x BigDye sequencing buffer, 10uM Primer PCR primer, and 2-10ng PCR template per 100bp.

The sequencing conditions were as follows: 32 cycles of 96°C for 10s, 55°C for 5s and 60°C for 4min (Sadekuzzaman *et al*., 2015).

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Phylogenetic Analysis

Obtained sequences were edited using the bioinformatics algorithm Trace edit, and compared with sequences stored in the National Center for Biotechnology Information (NCBI) data base using BLASTN. Similar sequences were aligned using ClustalX, and the evolutionary distances were inferred using the Neighbor-Joining method in MEGA 6.0.

Determination of Biofilm Producing Bacteria

Bacteria isolated were screened for biofilm production using the Congo red method (Bose *et al*., 2009). In the Congo red method, bacterial isolates were inoculated on a complex medium containing Congo red. The composition of the medium is as follows: Brain heart infusion broth (37 g/L), sucrose (5g/L), agar (10 g/L), and Congo red dye (0.8 g/L). Inoculated plates were incubated at 37 °C for 24 to 48 hours. Black colonies with dry crystalline consistency indicated biofilm production (Douglas *et al.*, 2022).

Statistical Analyses

Analyses of variance (ANOVA) of the data obtained were carried out using one way ANOVA.P-value of less than 0.05 was considered to be statistically significant (<0.05).

Results

Populations of the different investigated bacterial groups in water samples from PVC tanks in the different locations within PHALGA are presented in Tables 1. The total heterotrophic bacterial count (THBC) of the samples ranged from $3.0\pm0.1\times10^4$ to $1.04\pm0.01\times10^6$ CFU/ml with water sample from PVC tank in location E1 having the least count while the highest count was recorded in water sample from PVC tank in location A1.

Vibrio was not detected in all the water samples from PVC tanks in PHALGA. The coliform count ranged from $1.5\pm0.7\times10$ to $6.4\pm0.1\times10^2$ CFU/ml with water sample from PVC tanks in locations E1 and D1 having the least and highest count respectively. Fecal coliform was not detected in water samples from PVC tank in location E1, while water samples from PVC tank in location C1 had the highest fecal coliform count ($1.6\pm0.3\times10^2$ CFU/ml). The count of *Shigella* ranged from 5±0.01x10CFU/ml $1.1\pm0.1x10^2$CFU/ml in the samples for samples B1and G1 respectively while *Salmonella* counts ranged from 1±0.01x10CFU/ml to 4±0.01x10CFU/ml for samples C1 and G1 respectively.

Table 1: Bacterial Population in Water Samples from the PVC tanks in PHALGA

Locations	THB ($\times10^5$) CFU/ml	*Vibrio* sp CFU/ml	Total Coliform ($\times10^2$) CFU/ml	Feacal Coliform ($\times10^2$) CFU/ml	*Shigella* sp. ($\times10^2$) CFU/ml	*Salmonella* sp ($\times10$) CFU/ml
A1	10.4 ± 0.03^{e}	0	1.4 ± 0.2^{b}	1.2 ± 0.1^{bc}	0.6 ± 0.1^{a}	0
B1	0.50 ± 0.1^{a}	0	2.5 ± 0.1^{c}	1.2 ± 0.3^{bc}	0.5 ± 0.0^{a}	0
C1	1.6 ± 0.3^{b}	0	3.6 ± 0.3^{d}	1.6 ± 0.3^{c}	0.7 ± 0.1^{a}	1 ± 0.1^{a}
D1	2.6 ± 0.01^{d}	0	6.4 ± 0.1^{e}	1.1 ± 0.1^{b}	1.0 ± 0.1^{a}	3 ± 0.1^{a}
E1	0.30 ± 0.1^{a}	0	0.15 ± 0.07^{a}	0	0.10 ± 0.0^{a}	2 ± 0.0^{a}
F1	1.8 ± 0.1^{bc}	0	2.1 ± 0.1^{c}	1.3 ± 0.1^{bc}	0.7 ± 0.1^{a}	0
G1	2.2 ± 0.3^{cd}	0	0.50 ± 0.1^{a}	0.10 ± 0.0^{a}	1.1 ± 0.1^{a}	4 ± 0.1^{a}

*****Means with similar superscript down the group share no significant difference (P>0.05)

Populations of the different investigated bacterial groups in water samples from PVC tanks in the different locations within OBALGA are presented in Tables 2. The THBC of the samples ranged from $2.2\pm0.3\times10^4$ to $2.30\pm0.03\times10^5$ CFU/ml; the highest count was in water samples from PVC tanks in location B2, while the least was recorded in water samples from PVC tanks in location A2. *Vibrio* was not detected in all the water samples from PVC tanks in OBALGA. The total coliform count ranged from $1.6\pm0.3\times10^2$ to $2.26\pm0.04\times10^3$ CFU/ml with water Samples from PVC tank in location E2 having the least count, and the highest count in water samples from PVC tank in location G2. Similar trend was observed in locations E2 and G2 for fecal coliform; fecal coliform count ranged from $8.0\pm0.0\times10$ to $1.54\pm0.03\times10^3$ CFU/ml. The count for *Shigella* was lowest in water samples from PVC tanks in location D2, and highest in water samples from PVC tanks in location G2; *Shigella* count ranged from $8\pm0.0\times10$ to $3.5\pm0.1\times10^2$CFU/ml and *Salmonella* counts ranged from 1±0.2 CFU/ml (B2) and 9±0.1 CFU/ml (G2).

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Table 2: Bacterial Population in Water Samples from the PVC Tanks in OBALGA

Locations	THB ($\times10^5$) CFU/ml	*Vibrio* sp CFU/ml	Total Coliform ($\times10^2$) CFU/ml	Feacal Coliform ($\times10^2$) CFU/ml	*Shigella* sp. ($\times10^2$) CFU/ml	*Salmonella* sp ($\times10$) CFU/ml
A2	0.22 ± 0.03^{a}	0	5.7 ± 0.1^{c}	2.9 ± 0.1^{b}	1.3 ± 0.4^{a}	4 ± 0.4^{a}
B2	2.3 ± 0.03^{g}	0	7.3 ± 0.4^{d}	3.3 ± 0.2^{b}	1.2 ± 0.2^{a}	1 ± 0.2^{a}
C2	1.8 ± 0.01^{f}	0	9.2 ± 0.3^{e}	3.5 ± 0.4^{b}	2.0 ± 0.3^{a}	4 ± 0.3^{a}
D2	1.1 ± 0.01^{e}	0	4.5 ± 0.5^{b}	2.3 ± 0.1^{b}	0.8 ± 0.1^{a}	3 ± 0.1^{a}
E2	0.53 ± 0.01^{c}	0	1.6 ± 0.3^{a}	0.80 ± 0.0^{a}	2.0 ± 0.3^{a}	5 ± 0.3^{a}
F2	0.47 ± 0.01^{b}	0	1.97 ± 0.1^{f}	1.28 ± 0.07^{c}	2.3 ± 0.5^{a}	7 ± 0.5^{a}
G2	0.83 ± 0.01^{d}	0	2.26 ± 0.4^{g}	1.54 ± 0.3^{d}	3.5 ± 0.1^{a}	9 ± 0.1^{a}

*****Means with similar superscript down the group share no significant difference (P>0.05)**.**

The identity of the bacterial isolates and their relatedness is presented in Table 3 and the figure 1 respectively. The 16S rRNA sequence of the isolate ISO, had 100% pairwise identity with *Serratia aquatilis* strain 2015-2462-01 which has NCBI accession number, NR_147771.1. The 16S rRNA sequence of the isolate, ISO2 had 100% pairwise identity with *Proteus mirabilis* which has NCBI accession number LC728309.1. The 16S rRNA sequence of the isolate ISO3 had 100% pairwise identity with *Shigella flexneri* which has NCBI accession number LR738991.1. The 16S rRNA sequence of the isolate ISO4 had 100% relatedness with *Enterobacter cloacae* strain ATCC13047 which has the NCBI accession number, NR10274.2. The 16S rRNA sequence of the isolate ISO5 had 100% relatedness with *Enterococcus faecium* with the NCBI accession number, AB512765.1, the 16S rRNA sequence of the isolate ISO6 had 100% pairwise identity *with Streptococcus agalactiae* with the NCBI accession number, CP010867.1, 16S rRNA sequence of the isolate ISO7 had 100% relatedness with *Alcaligene viscolactis* S-2 with the NCBI number, AY911519.1 , the 16S rRNA sequence of the isolate ISO8 had 100% relatedness with *Bacillus subtilis* strain 168 with the NCBI accession number of NR_102783.2, the 16S rRNA sequence of the isolate ISO9 had 100% pairwise identity with *Klebsiella aerogenes* strain SGRCBNR5-D11and the 16S rRNA sequence of the isolate, ISO10 had 99 % pairwise identity with *Escherichia coli* ETEC which has NCBI accession number MF919609.1**.**

The percentage frequency of biofilm production ability of the identified bacterial isolates is presented in Figure 2.

All (100%) of the isolates of *Proteus*, *Shigella*, *Enterococcus*, *Klebsiella* were positive to biofilm production while 90% of *Serratia*, 60% of *Enterobacter*, 80% of *Streptococcus*, 60% of *Alcaligenes*, 72% of *Bacillus* and 60% of *Escherichia coli* were observed to be positive for the production of biofilm.

Table 3: Accession Number and Molecular Identity of Bacteria Species Isolated from the Drinking Water Sources within OBALGA and PHALGA

Isolate code	Phenotypic Identity	Genotypic Identity	Accession No.	% Identity
ISO 1	*Serratia* sp	*Serratia aquatilis*	NR_147771.1	100
ISO 2	*Proteus* sp	*Proteus mirabilis*	LC728309.1	100
ISO 3	*Shigella* sp	*Shigella flexneri*	LR738991.1	100
ISO 4	*Enterobacter* sp	*Enterobacter cloacae*	NR_102794.2	100
ISO5	*Enterococcus* sp	*Enterococcus faecium*	AB512765	100
ISO 6	*Streptococcus* sp	*Streptococcus agalactiae*	CP010867.1	100
ISO 7	*Alcaligenes* sp	*Alcaligenes viscolactis*	AY911519.1	100
ISO 8	*Bacillus* sp	*Bacillus subtilis*	NR_102783.2	100
ISO 9	*Klebsiella* sp	*Klebsiella aerogenes*	SGRCBNR5-D11	100
ISO 10	*Escherichia coli*	*Escherichia coli ETEC*	MF919609.1	99

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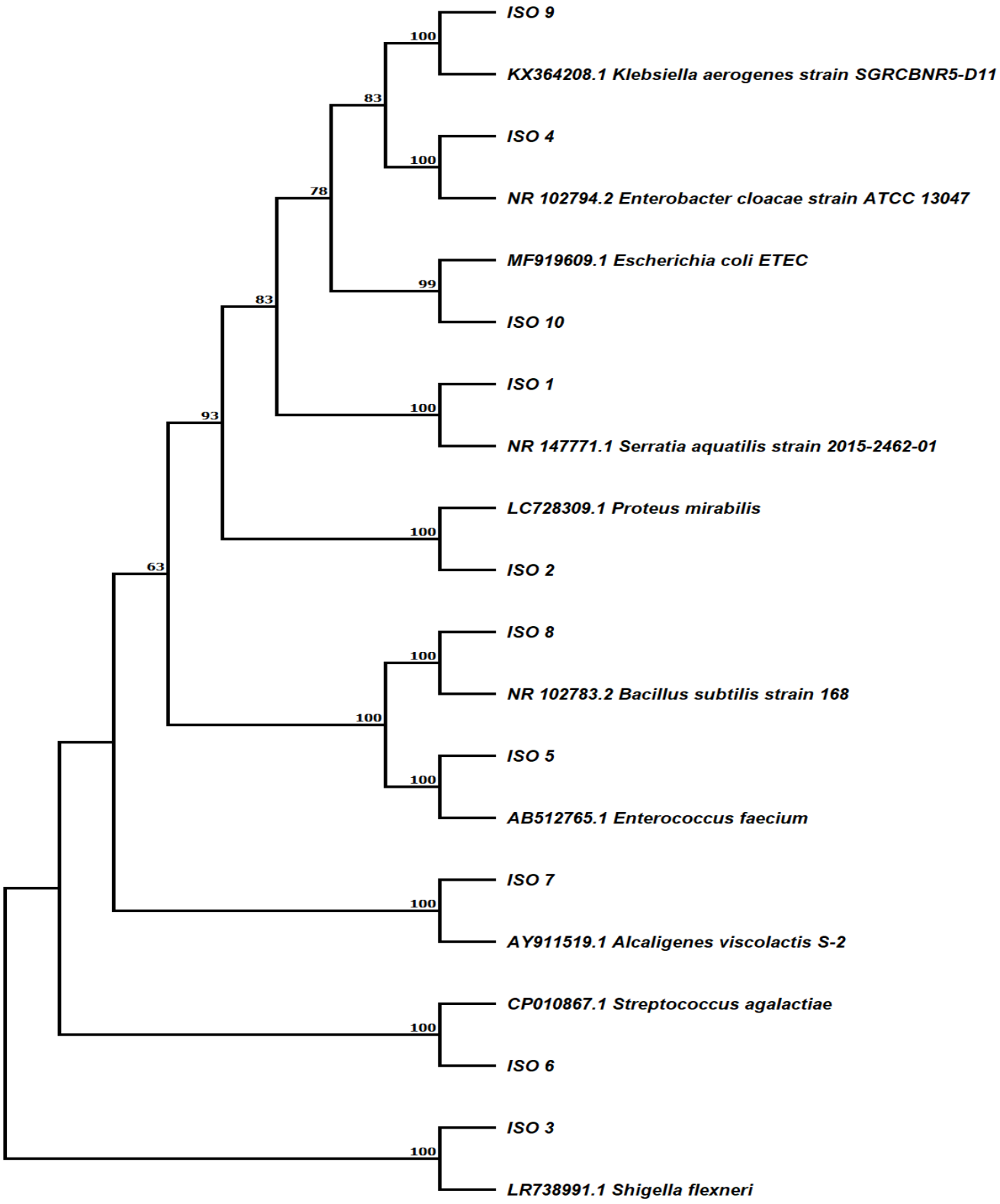

Figure 1: Evolutionary Distances between the Bacteria Isolated from PVC Stored Water Samples

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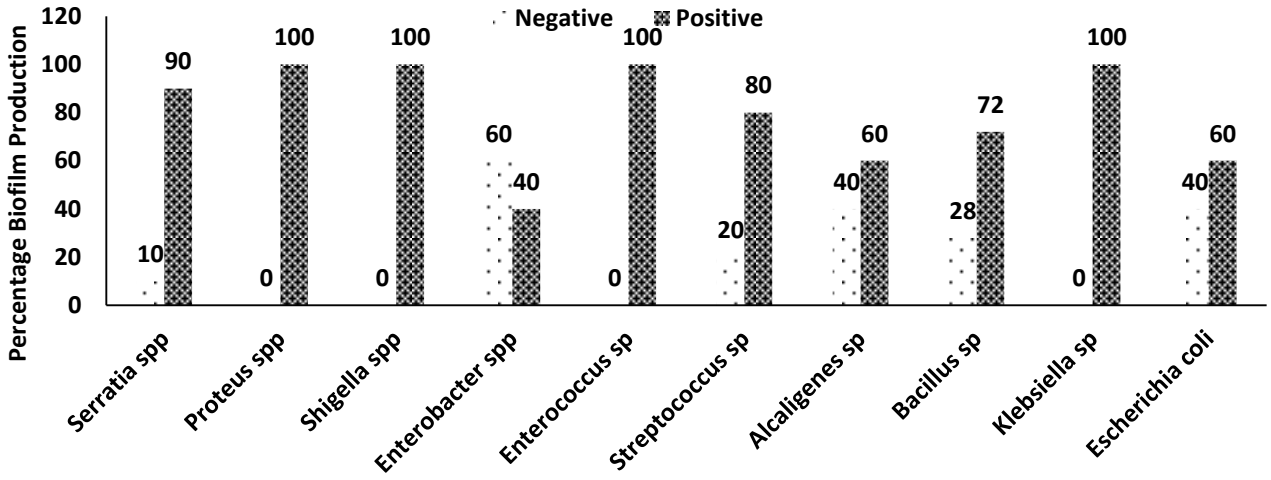

Figure 2: Percentage of Biofilm Production by Bacteria Isolated from PVC Stored Water Samples

Discussion

The results of the T. test analysis showed that there are significant differences between the microbial counts obtained in the water samples stored in the Polyvinyl Chloride (PVC) Tanks from the different points of PHALGA and OBALGA at P>0.05. The counts obtained in this study, especially the feacal coliform counts were very high compared to the standard recommended by (WHO, 2010; NSDWQ, 2008). These could be attributed the storage condition of the water. This result is in line with the report of Sunday *et al.* (2016) where the authors obtained a high level of bacterial counts in water samples stored in plastic container from Abakaliki area of Abia State, Nigeria.

Generally, bacteriological counts of the water in this study were above the recommended standard by World Health Organization and Nigeria Standard for Drinking Water Quality (WHO 2010, NSDWQ 2008). The count of heterotrophic bacteria is in consonant with the findings of Akin-Osainaiye *et al.* (2018) which reported the heterotrophic bacterial mean count of 6.96 Log CFU/ml from house hold water distribution tank and the study of Orogu *et al*. (2017) which also recorded heterotrophic bacteria count of 0.5×10^4CFU/ml - 6.6×10^9CFU/ml observed in the microbiological study of pipe borne chlorinated water and untreated water. Water of good quality must have a low total bacterial count fewer than 100 CFU/mL, whereas World Health Organization (WHO) and National Environmental Standards and Regulation Enforcement Agency (NESREA) standards demands that, drinking water should have a total heterotrophic bacteria count of <1CFU/mL Therefore, these water samples are above the required standard of WHO and NESREA. The count of coliform which ranged from $1.5\times10\pm0.07$CFU/ml - $6.4\times10^2\pm0.1$CFU/ml and $1.6\times10^2\pm0.3$CFU/ml - $2.26\times10^3\pm0.1$ CFU/ml for samples from PHALGA locations and OBALGA locations respectively. The count of coliform count as recorded is lower than those recorded in the study of Alotaibi and Burkhari *et al.* (2021) which recorded count of 5.50LogCFU/ml for coliform and the study of Orogu *et al*. (2017) which recorded the count of 3.5×10^2 - 1.8×10^4 CFU/ml. Coliform in water is in an indication of faecal contamination and potent, pathogenic microorganisms. The result of the coliform in this study however exceeded the standard of zero coliform stipulated by WHO and NSDWQ limit of 10CFU/ml. The count of Fecal coliform in the sample from OBALGA is also in line with the study of Orogu *et al*. (2017) which reported the presence of enteric bacteria from untreated and treated pipe borne water.

No count of *Vibrio* species was recorded in the all the samples from both PHALGA and OBALGA as recorded in this study. This finding is similar to the findings of Akin-Osanaiye *et al.* (2018) which reported 0±0.00LogCFU/ml count of *Vibrio* in the portable water and pipeborne water under storage duration. The count of *Shigella* ranged from 5.0×10 ±0.00 - $4.4\times10^2\pm0.1$CFU/ml recorded in this study is lower than the count recorded in the study of

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Akin-Osanaiye *et al.* (2018) which reported the count of 1.3×10^5_5.0×10^5CFU/ml of Shigella and Salmonella in the samples of pipeborne water stored. The predominant bacteria isolated were; *Klebsiella sp*, *Enterococcus sp*, *Escherichia sp*, *Alcaligens sp*, *Bacillus sp*, *Enterobacter sp*, *Shigella sp*, *Streptococcus sp*, *Proteus sp* and *Serratia sp*. With the bacteria, *Klebsiella sp* showed the highest frequency and Serratia sp being the least occurred bacteria. The isolated bacteria from the sample are similar to those isolated in the study of Orogu *et al*. (2017) and the study of Babalola *et al*. (2021).

Water for consumption by human should be devoid of potentially harmful microorganisms. However, some may result from factors lead to the contamination of water stored in containers or tanks. Water stored in PWST can be contaminated due to poor handling, unclean containers, unhygienic domestic water handling practices, and natural contamination from the ambient domestic environment into PWST which may also result in biofilm formation (Van der Merwe *et al.*, 2013). Microbes form biofilms in response to many factors, which may include cellular recognition of specific attachment sites on a surface, nutritional cues or exposure of cells to sub-inhibitory concentrations of antimicrobials (Karatan *et al.*, 2009). When a cell switches to the biofilm mode of growth, it undergoes a phenotypic shift in behaviour in which large suites of genes are differently regulated (Wei *et al.*, 2013). Motility and flagella play a vital role in adhesion, biofilm formation and colonization of several pathogenic bacteria (Igbinosa *et al.*, 2013).

Although all the different bacterial genera of the identified bacteria showed the potential to produce biofilm, there was difference in percentage in terms of each genus. From the results of the study, all (100%) of the isolates of *Proteus*, *Shigella*, *Enterococcus*, *Klebsiella* were positive to biofilm production while 90% of *Serratia*, 60% of *Enterobacter*, 80% of *Streptococcus*, 60% of *Alcaligenes*, 72% of *Bacillus* and 60% of *Escherichia coli* were observed to be positive for the production of biofilm. This result corroborates with the report Lewis (2012) in which similar bacterial genera isolated from water samples were reported to possess the potential to produced biofilm. It was also reported in the study of Wei *et al.* (2013) that more bacterial genera with this potential were isolated from water stored in the polyvinyl chloride (PVC) containers in comparison to other types of storage containers.

As shown in the result of this study, more than 50% all the genera produce biofilm however, all (100%) the isolates of *Proteus, Shigella, Enterococcus, Klebsiella* were observed to produce biofilm hence their protection from adverse condition which might be effect of antimicrobial activity (Wei *et al.*, 2013). This in line with the report of Alotaibi and Bukhari (2021) in which similar organisms were reported for biofilm production.

The formation and structure of biofilm can be influenced by various environmental factors that control these ecosystems; physical (light penetration, temperature and water current), chemical (pH, nutrient availability and toxicant effects), as well as biological factors, including community composition (bacteria, algae and fungi), relative contribution of autotrophs and heterotrophs biomass thickness and grazing (Alotaibi and Bukhari, 2021). Biofilm formation in water stored for drinking has also been reported by LeChevallier (2007). A biofilm formation can be attributed to the long period of time the bacteria survives in the water (Amadi-Ikpa *et al.*, 2020). Biofilm-producing bacteria have been shown to be associated with numerous human diseases and capable of colonizing a wide range of environments. In aquatic environment, microbial adhesion initiates biofilm formation, exacerbates contamination, reduces the aesthetic quality of the water body, and reduces microbiological safety through augmented survival of pathogens (Igbinosa *et al.*, 2013).

In conclusion, the result of this study showed that the microbial quality of water samples from water-storage tanks in PHALGA and OBALGA were above the required standard for potable drinking water both for World Health Organization (WHO, 2010) and Nigeria Standard for Drinking Water Quality (NSDWQ, 2008). The predominant bacteria isolated from the sample include *Escherichia coli*, *Streptococcus* sp, *Klebsiella* sp, *Bacillus*, *Enterobacter*, *Shigella*, *Alcaligens*, *Serratia*, *Proteus* and *Enterococcus*. All the bacterial genera isolated from the water samples in storage tank showed the potential to produce biofilm however, the genera, *Proteus, Shigella, Enterococcus* and *Klebsiella* were observed to produce the highest percentage of biofilm production and this could be a buildup during the storage. Water stored in the tank have been shown to have ability of microbial contamination and biofilm production which results in their increased antibiotic resistance.

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Hence, water in storage containers are not recommended for long term storage of water as they have the potential to support biofilm formation, which may be detrimental to public health. Many microorganisms in water in water-storage PVC tanks can produce biofilm for attachment to the inner surface of the tanks. The biofilm produced enables the attachment of more microorganisms, thereby reducing the quality of the water supplied through the tanks. It is therefore recommended that the interior of water-storage PVC tanks should be cleaned regularly, and a plan put in place to determine the frequency of the cleaning.

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