Population of Microbes Associated With Stored Drinking Water in Some Diobu Homes, Port Harcourt, Nigeria

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***Abstract*: The microbial evaluation of drinking water stored in homes have become necessary as the water could be contaminated by pathogens. Questionnaire administration was done, followed collection of one hundred and eighty stored drinking water samples from homes having no toilet/water facilities and homes with toilet/water facilities. Microbial analyses reported a mean and standard deviation counts of heterotrophic bacteria, *Salmonella, Shigella, Vibrio,* heterotrophic fungi, Staphylococcal, Pseudomonads, fecal coliform and total coliform bacterial as $1.6 \pm 5.8 \times 10^3$, $2 \pm 1.0 \times 10^2$, 0 ± 0.0, $1 \pm 1.2 \times 10^2$, 0 ± 0.0, $1 \pm 1.1 \times 10^2$, 0 ± 0.0, $2 \pm 2.8 \times 10^2$, $6 \pm 3.2 \times 10^2$ CFU/ml respectively for homes with toilet/water facilities, while for homes without toilet /water facilities, the counts were $2.8 \pm 9.8 \times 10^3$, $4 \pm 1.2 \times 10^2$, 0 ± 0.0, $3 \pm 4.6 \times 10^2$, $1 \pm 1.2 \times 10^2$, $6 \pm 8.6 \times 10^2$, 0 ± 0.0, $3 \pm 1.5 \times 10^2$, $1.2 \pm 9.2 \times 10^3$ CFU/ml for heterotrophic bacteria, *Salmonella, Shigella, Vibrio,* heterotrophic fungi, Staphylococcal, Pseudomonads, fecal coliform and total coliform bacterial respectively. A total of seventy (70) isolates belonging to five (6) genera namely: *Staphylococcus aureus, Vibrio* spp, *Salmonella* spp, *Escherichia coli, Klebsiella* spp and *Candida* spp were isolated and identified biochemically. The isolates with their prevalence from water samples from homes with water and toilet facilities are *Staphylococcus aureus* 34%, *Vibrio* spp 3.4%, *Salmonella* spp 20.6%, *Escherichia coli* 31%, *Klebsiella* spp 10.3%, and *Candida* spp 0%, while *Staphylococcus aureus* 34.1%, *Vibrio* spp 9.7%, *Salmonella* spp 21.9%, *Escherichia coli* 26.8%, *Klebsiella* spp 4.8%, and *Candida* spp 2.4% were noted for homes without toilet and water facilities. Thus, stored water is challenged by poor storage containers, unhygienic sanitary practice and ignorance, as the water samples did not meet the WHO permissible bacteriological limits for drinking water. It is recommended that households develop an altitudinal interest in water security through the practice of good hygiene.**

Keywords: **Microbes, Population, Stored Drinking Water, Homes.**

I. INTRODUCTION

The assessment of homes in terms of (i) the nature of the building as regards the construction materials used, the facility and ventilation provisions (ii) the environment of the building as regards the immediate surroundings, air, ground, space and sanitary condition and (iii) the people living in the home as par their perception and disposition to hygienic practices (Thakadu *et al.,* 2018), interplay to affect the sensitivity and ability of the home occupant to eliminate and control water-borne microbes in the home where they live (WDTR, 2019).

There are two types of recognized homes in Nigeria, the makeshift homes commonly known as "batcher" in local parlance in Nigeria, do not have toilet facility within their abode (Yilrwang, 2019); the reason for which Yilrwang (2019) reported that majority of the inhabitants sometimes, defecate in open drainage. These practices however, sometimes do not take place or happen amongst people living in permanent (sandcrete built) homes or, buildings which have a well built toilet facility within the home (Yilrwang, 2019). According to Haruta and Kanno (2015) microbes have become more common in home drinking water due to some anthropogenic factors, some of which directly affect the water source.

Microorganisms commonly enter the homes where people live via humans, food, water and pets (Passmore and Robson, 1973). Some microbes also can gain entry into the home via air from where they express their virulence properties.

Tritely, pests such as housefly, cockroach, rodents and rodents destroy or contaminate food substances and water in the homes. When pest get in contact with food stuffs or water, they introduce harmful organisms or substances into the food or water (Rather *et al.*, 2017). In the same vein, food substances and water can be destroyed or contaminated indirectly by pests and people who unknowingly bring virulent organisms or dangerous substance into the food and water at home (Rather *et al.*, 2017).

Relatively, humans have been reported to be the principal point or source of microbes into the homes (Luby *et al.*, 2001). Luby *et al.*(2001) reported that human hands are one of the commonest medium by which food and water in the homes are contaminated, especially unclean or dirty hands are breeding ground for bacteria and other microorganisms (Luby *et al.*, 2001). Most times, changes in water quality or state may not be easily noticed or pose any concern to people because there may be no immediate problem arising from the consumption of such water. Thus, it is only when the consumer's body begins to experience worrisome conditions or signals after drinking some water that the thought about water quality can become seriously of interest and concern

(Adna, 2014). Owing to the devastating impact of human activities, some communities on every continent of the world are experiencing declining water quality (Gupta and Quick, 2006). The immediate environment of some homes especially the makeshift and other temporary homes and the activities of the occupants or residents which may be bereft of sanitary and hygienic measures, need to be examined, as these factors could affect the quality of the stored water used for drinking (Duru *et al*., 2013) and in turn it may translate into health problems or challenges for the consumers of such water.

The challenges faced-by some homes especially makeshift homes or shanties in Port Harcourt and its environs are enormous. The challenges include: lack of or no toilet facility, poor sanitation, congested room-homes with poor ventilation, unavailable water and dirty home surroundings and the entire environment. The problem of unavailable water for domestic and other uses including for drinking is a common feature of shanties and shanty towns/settlements. Even with a few irregular and seasonal alternatives such as unsuspecting water from commercial water-borne tankers and rain water that sometimes only temporarily alleviate or mitigate the problem (Farley, 2018).

The importance of this study lies in its focus on the issues surrounding the availability of quality and standard stored drinking water or otherwise in the homes. Further, poor water quality has effect on what happens to the health of the occupants of the homes and to the healthcare delivery approach of health practitioners and authorities alike, in the area of study.

Issues covered clearly buttresses the report that a safe and wholesome drinking water is essential for the good health of humans (Ohaka, 2007). This research was aimed at evaluating the microbial population of stored drinking water in some homes, via creating awareness and cautioning or re-directing households who practice unhygienic exercises or act to be mindful of stored drinking water in the home.

II. MATERIALS AND METHODS

Study Area

The study area Diobu of this work is a community in Rivers State, Nigeria. The area has a topography of a flat plains with networks of rivers and tributaries including: New Calabar, Orashi, Bonny, Sombreiro and Bartholomew Rivers with a vast area of arable land. The inhabitants practice farming and fishing occupations, while a few engage in trading. The area is known for its economic hub, with the infux of people, who construct homes without toilet and water facilities.

Administration of Questionnaire and Water Sample Collection

Copies of a questionnaire on some challenges of water quality were administered to one hundred (100) persons in the study area for completion. As the respondents fill in the questionnaire and the information/data retrieved, water samples were collected right away from them. Water samples collected were classed into permanent and temporary/makeshift home samples for the purposes of this study. Water samples obtained from permanent homes with toilet and water facilities were classified and marked permanent homes samples while water samples obtained from makeshift homes (batcher houses) without toilet and water facilities were classified and marked temporary/makeshift home samples.

A total of one hundred and eighty (180) stored drinking water samples were collected aseptically from homes with toilet and water facilities, and homes without toilet/water facilities. The samples were collected in three (3) batches of sixty samples per batch. After collection samples were transported in a ice-block cooler to the laboratory, for microbiological analyses.

Enumeration of Microbial Population

Selective, non selective and differential agars media were prepared and used for enumerating the bacterial population. The investigation employed the spread plate technique, which involved using a sterile pipette to inoculate 0.1ml of the undiluted sample potion into various prepared media plates and again in another plate, a 0.1ml volume of the serially diluted potion of the water sample inoculated onto a freshly prepared media. With a sterile bent glass rod, the inoculums were spread over the plate, and the plates incubated at a temperature of 37^{o}C for 24 hours. After incubation, bacteria colonies were counted and recorded accordingly.

Maintenance of Pure Cultures

Discrete bacterial colonies on the media were purified by sub-culturing onto nutrient agar media and incubated at 37^{o}C for 24 hours. The pure cultures were preserved in a bjou bottle containing a sterile 10% glycerol. The bjou bottle and its content were then kept in a refrigerator at -4 ^{o}C for further identification.

Biochemical Identification of Isolates

Biochemical reaction aspect of characterizing the isolates required the preparation and use of reagents such as Oxidase, Methyl red, Voges-Proskauer, Citrate, Sucrose, Manitol, Lactose, Indole, Glucose, Catalase, and Coagulase assay as described by Wemedo *et al.,* (2016).

Statistical Analysis

Data collected were analysed and presented in tabular and graphic forms as employed by Okolie (2007). The data analyses consisted of several statistical methods namely: (i) measure of central tendency (ii) measure of variability (iii) percentage determination, and (iv) descriptive analysis.

The analyses and presentation of the data using the method of measure of central tendency involved summarizing the data obtained in terms of the mean, while the measure of variability involved determining the deviation of the data. It detects differences in the degree of variances of data obtained from both the permanent and temporary/makeshift homes.

Basically, the unpaired t-test (two sample t-test) statistical tool was employed to determine the statistical difference of the results obtained with a statistical test established at a significance level of 0.05%; that is, a chances of 5 in 100, that the null hypothesis would be rejected.

In using percentage determination approach, the data obtained were processed and presented in tabular form to determine the relative standing of the different variables studied.

The descriptive analysis approached involved description of the research results using bar charts. The data recovered from the questionnaire were analysed using the Statistical Package for Social Science (SSPC) soft ware.

III. RESULTS

Survey Analyses Report

Consequently, in the survey analyses report in figure 1 shows that, households of temporary and permanent homes living in one room have a higher potential to introduce microbes in their drinking water than the two rooms' household occupants. In yet another analysis in figure 2, the male gender in temporary homes has a higher microbial input rate in water than the female gender, thus an opposite report in the permanent homes. The survey analyses report on figure 3 showed that the permanent home household's perception on drinking water were concerned about their drinking water than temporary home occupants. However, the survey report revealed that the temporary home occupants were never concerned on the state of their drinking water.

Survey report as shown in figure 4 has it that 1-3 persons contributed less to the contamination of their water than 10 persons while figure 5 showed that the ages grades under 17 years old in temporary homes were reported to be associated with high input of microbes in water than age grades between 18 and 29 years old.

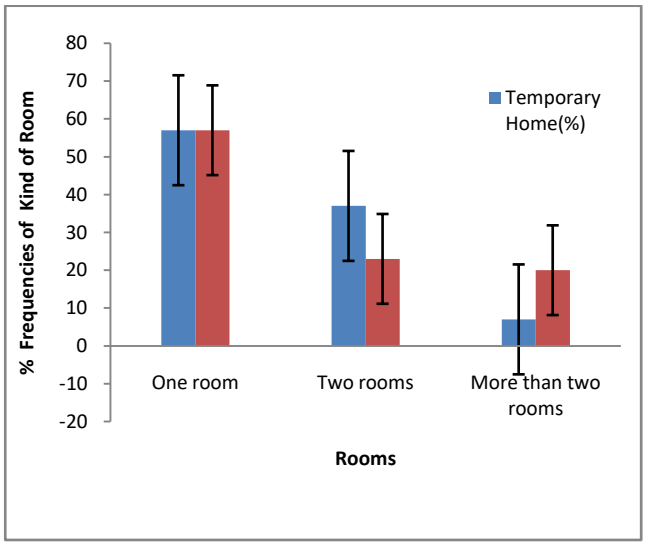

Figure 1: Distribution of Rooms Occupied by Inhabitants

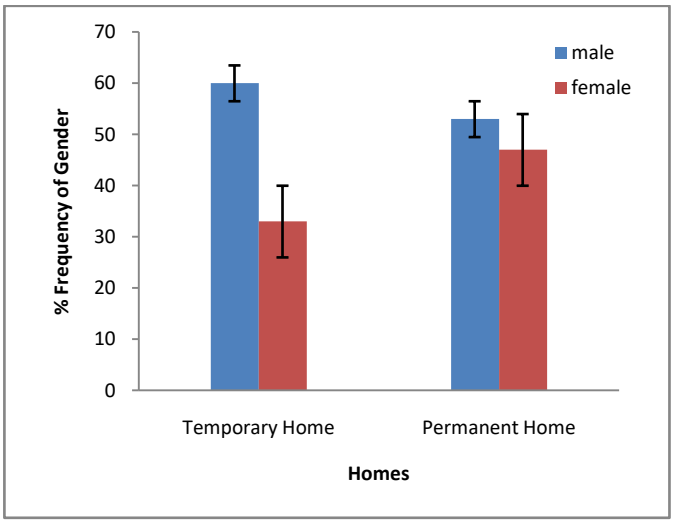

Figure 2: Household Distribution of Gender

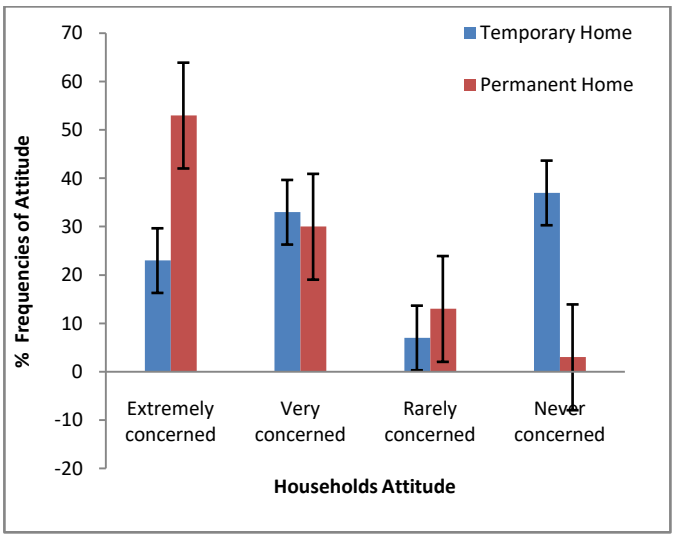

Figure 3: Distribution of Households Attitude

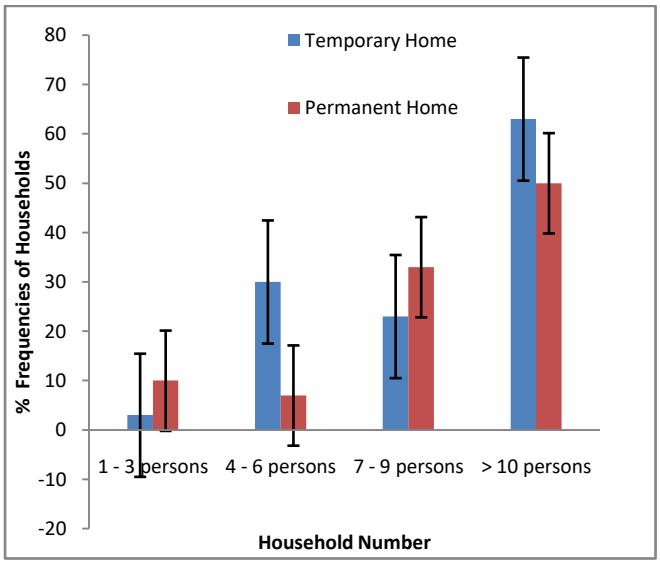

Figure 4: Percentage of Households

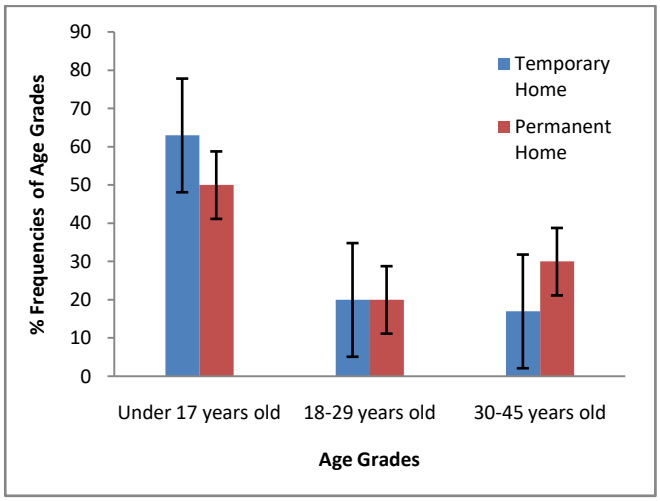

Figure 5: Age Grade Distribution

Enumeration of Bacteria Population

Results in table 1 showed evidently, heterotrophic bacteria had mean ± standard deviation counts of 1.6 ± 5.8 x 10^3 CFU/ml and 2.8 ± 9.8 x 10^3 CFU/ml for water samples from permanent and temporary/makeshift homes respectively, with the temporary/makeshift homes water samples having a lower count; significantly different from the water samples from the permanent homes at a probability value less than 0.05. *Salmonella* had a mean ± standard deviation counts of 2±1.0 x 10^2 CFU/ml and 4 ±5.5 x 10^2 CFU/ml for water samples from permanent and temporary/makeshift homes respectively. The counts showed no significant difference at a probability value less than 0.05 between the water samples from the homes. *Vibrio* bacteria had viable mean ± standard deviation counts of 1 ±1.2 x 10^2 CFU/ml and 3±4.6 x 10^2 CFU/ml for water samples from permanent and temporary/makeshift homes respectively. This showed that the water samples from the temporary/makeshift homes had higher counts that were not significantly different from the counts obtained from the permanent homes at probability level greater than 0.05. Staphylococcal load from the water samples had mean ± standard deviation counts of 1 ±1.1 x 10^2 CFU/ml for water samples from permanent homes while water samples from the temporary/makeshift homes accounts for 6 ± 8.6 x 10^2 CFU/ml. The presumed Staphylococcal mean cell counts obtained from the water samples from the permanent homes were significantly lower than that obtained from the water samples in the temporary/makeshift homes at a probability level less than 0.05. Furthermore, the mean ± standard deviation counts, of 6 ±3.2 x 10^2 CFU/ml was obtained for total coliform bacteria in water samples derived from permanent homes. This counts were thus lower and significantly different at a probability value less than 0.05 from that obtained in temporary/makeshift homes, which had a total coliform 1.2± 9.2 x 10^3 CFU/ml. Additionally, the mean ± standard deviation counts of 2 ± 2.8 x 10^2 CFU/ml was derived for fecal coliform in water samples sourced from permanent homes, and the counts were lower and not significantly different at a probability level greater than 0.05 from the counts obtained in temporary/makeshift homes which had a fecal count of 3 ± 1.5 x 10^2 CFU/ml.

Table 1 Variation of Microbial Loads in Water Samples Obtained from Permanent and Temporary Homes

S/no	Microbial Isolates	Permanent Homes (CFU/ml)	Temporary/ Makeshift Homes (CFU/ml)	T-test	WHO (2017) Standards
I	Total Heterotrophic Bacteria	1.6±5.8 x 10^3	2.8±9.8 x 10^3	$P <$ 0.05	≤ 100
Ii	Total Heterotrophic Fungi	0±0	1±1.2 x 10^2	$P <$ 0.05	≤ 100
Iii	*Salmonella* Counts	2±1.0 x10^2	4±5.5 x10^2	$P >$ 0.05	≤ 100
Iv	*Vibrio* Counts	1 ± 1.2 x10^2	3 ± 4.6 x10^2	$P <$ 0.05	≤ 100
V	*Staphylococcal* Counts	1.±1.1 x10^2	6 ± 8.6 x10^2	$P <$ 0.05	≤ 100
Vi	*Pseudomonads* Counts	0±0	0±0	$P >$ 0.05	≤ 100
Vii	Total Coliform Counts	6±3.2 x10^2	1.2±9.2 x10^2	$P <$ 0.05	≤ 2
Viii	Fecal Coliform Counts	2±2.8 x10^2	3±1.5 x10^2	$P >$ 0.05	≤ 0

Key;

Values are mean of triplicates determinations ± Standard Deviations (SD).

CFU/ml= Coliform Forming Unit per ml, WHO= World Health Organization, *P* =Probability.

Each variable was used independent of the statistically significant and insignificant variables presented as $P<0.05$ and $P>0.05$. Where < = Less Than and > = Greater Than.

Table 2: Biochemical Characterization of the Isolates

Iso	Ind	Met	Vog	Cit	Lac	Man	Oxi	Glu	Suc	Cat	Coa	G.Sta	Probable Bacteria
I	-	+	-	-	-	+	-	+	-		-	-	*Salmonella* spp
Ii	-	+	+	+	+	+	-	+	+	+	+	+	*Staphylococcus .aureus*
Iii	-	-	+	+	+	+	-	+	+	+	-	-	*Klebsiella* spp.
Iv	+	+	-	-	+	+	-	+	-	+	-	-	*Escherichia Coli*
V	+	+	-	+	-	+	+	+	+	+	-	-	*Vibrio* spp

Keys;

Ind=	Indole	G.Stain=	Gram Stain
Vog=	Voges-Proskauer	*Spp*=	Species
Glu=	Glucose	+ =	*Positive*
Cat=	Catalase	-=	Negative
Oxi=	Oxidase	*Met*=	Methyl Red
Lac=	Lactose	*Cit*=	Citrate
Man=	Manitol	*Suc*=	Sucrose
		Coa=	Coagulase

Macroscopic Identification of the Fungi Isolates

The fungi isolate was identified macroscopically as *Candida* spp, it had a creamy coloured appearance, surrounded by a white background. The colony is circular in shape with an elevated center

Table 3: Frequency / Percentage Occurrence of Bacterial Isolates Recovered from Drinking Water Samples Sourced from Permanent and Temporary/makeshift Homes

S/no	Probable Isolates	Permanent Homes n(%)	Temporary/ Makeshift Homes n(%)
I	*Staphylococcus* spp	10 (34.4%)	14 (34.1%)
Ii	*Vibrio* spp	1 (3.4%)	4 (9.7%)
Iii	*Salmonella* spp	6 (20.6%)	9 (21.9%)
v	*Escherichia coli*	9(31%)	11 (26.8%)
Vi vii	*Klebsiella* spp *Candida* spp	3(10.3%) 0(0%)	2 (4.8%) 1 (2.4%)

IV. DISCUSSIONS

The load of heterotrophic bacteria in the water samples obtained from temporary/makeshift homes as reported, were significantly different and higher than that obtained in permanent homes. Thus, suggests an increased microbial re-growth in the storage vessels of most temporary/makeshift homes as earlier indicated by Amanidaz (2015). This probably, may be due to continuous refilling of storage containers or vessels without thorough cleaning exercise (Amanidaz, 2015). Similarly, the elevated levels of heterotrophic bacteria counts according to Amanidaz (2015), indicates more likelihood of the dangers which the bacterial isolates pose to its host's body with respect to the homes. Moreso, the counts obtained from both homes, do not satisfy the permissible counts of heterotrophic bacteria in water according to the World Health Organization report (WHO 2017). Thus, WHO (2017) reports that heterotrophic bacteria counts in potable water should not exceed 100 CFU/ml. Moreover, the significant difference and the decrease in heterotrophic fungi count in water samples obtained from permanent homes over that from temporary/makeshift homes may be due to inadequate protection of temporary/makeshift homes' stored water, as well as possible fungal pollution of indoor environment (Haleem *et al.,* 2012). However, the low load in stored drinking water samples from permanent homes most of which have direct water supply facilities agreed with the work of Haleen *et al*. (2012), who in their study reported that the number and diversity of fungi are generally low in tap-water. Thus, this report satisfies WHO (2017) report on permissible heterotrophic fungi in potable water. WHO (2017) reports that heterotrophic fungal counts in potable water should not exceed 100 SFU/ml. *Salmonella* counts which showed no significant difference in counts at P values greater than 0.05 between the homes (permanent and makeshift) may suggest animal droppings that found their way into home water (Rusin *et al.,* 1997) as this result confirms report from the questionnaire where both homes rear domestic or food animals such as birds, goats, dogs etc in their abode. *Vibrio* counts, which were also not significantly different in the water samples from both homes may have resulted to the fact that *Vibrio* is considered a natural dweller of aquatic environment, were it monitors the aquatic ecosystem (Ashbolt, 2015). Although, the result of this study revealed the presence of high *Vibrio* cells in temporary/makeshift home stored water sample than that in permanent homes, this of course could be explained by the absence of toilet facilities in makeshift homes. The absence of Pseudomonads counts in the stored drinking water samples from both homes agreed with report by Mena (2009), where it was reported that *Pseudomoas aeruginosa* were not found in drinking water obtained from tap water, having it that the source of water from this homes are generally bore-hole. The significant difference in the population of Staphylococci isolates in stored drinking water samples from temporary homes, were higher than that from permanent homes and this may be explained by good hygiene practices including hand washing, which are most times ignored in the temporary homes, whereby *Staphylococcus aureus*, a normal flora of the skin could be introduced into drinking water bottles or containers during refill (Mark *et al.*, 1980). The high prevalence of 34.4% and 34.1% for *Staphylococcus aureus* in both homes water samples may not agree with LeChevallier and Seidier (1980) who reported that 6% of coagulase positive *Staphylococcus aureus* were isolated from320 rural drinking water samples. Consequently, in this study, the temporary/makeshift homes have been associated with increased microbial population due to lack of hand washing as elicited from the questionnaire respondents. However, the least prevalence microbe in this study, *Candida* spp may have occurred least due to it being a predominantly isolated fungal contaminant of domestic tap water as reported by Ayanbimpe *et al.,* (2012). The total coliform counts that were significantly different and higher in temporary/makeshift homes strongly indicated poor hygiene and sanitary practices (Costerton *et al.,* 1999). WHO (2017) certify that total coliform should be accepted in a potable water if only the

counts lies bellow two (2) coliform per unit. Total coliform counts obtained, from water sample in the homes under study did not meet the WHO (2017) standards. However, fecal coliforms were not significantly different at a probability level greater than 0.05 between the homes despite the temporary homes having higher loads of fecal coliform. Consequently, the counts obtained from both homes; do not satisfy the permissible counts of fecal coliform in drinking water according to World Health Organization report (WHO 2017). WHO (2017) reports that potable water should be devoid of fecal coliform. The high fecal colifom loads seen in the temporary/makeshift home water samples may be due to the practice of open defecation as seen from the questionnaire survey report. Thus, this high presence may be associated with diseases. Generally, the high or increased loads of microbes in stored drinking water samples from temporary/makeshift homes over that of the permanent homes as strongly indicated from the survey report suggests that the size of the households of the temporary homes is implicated in microbial increase, especially, as 5-7 years old persons constitute more than 50% of the household size.

V. CONCLUSION AND RECOMMENDATIONS

Most stored drinking water samples from temporary or makeshift homes are laden with high bacterial contaminants than the stored drinking water samples obtained from the permanent homes. The microbial water quality assessment revealed also that the water samples had heterotrophic bacteria and coliform bacterial counts higher than the World Health Organization (WHO) satisfactory limit of microbes in drinking water. The stored drinking water from both the permanent and temporary or makeshift homes had potent pathogens like *Vibrio cholerae*, *Escherichia coli*, *Staphylococcus aureus*, *Salmonella* and *Klebsiella*. Inhabitance of homes, especially the temporary/makeshift homes must wake up in their hygiene and sanitary practices during sourcing and storing of home drinking water towards ensuring clean safe and wholesome water for their drinking. It is also recommended that inhabitants must practice the habit of regular hand washing with soap and water before refilling water storage containers or vessels kept in their homes.

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