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Assessment of bacteriological quality of water sources from an agrarian settlement in South-East Nigeria

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This study assessed the bacteriological quality of groundwater, rainwater and surface water sourced from an agrarian settlement in South-eastern Nigeria. Agamede, Amede, Ihenyi, Mgbuji and Umuhu communities in Eha-Amufu served as sampling locations. Samples were sourced from River Ebenyi, hand-dug wells and rooftop harvested rainwater. Bacterial isolation, identification and enumeration followed standard methods. Coliform counts were above WHO permissible standard except groundwater sourced at Mgbuji. Whereas a significant difference occurred in the means of coliform counts of lactose-fermenters (LF) of groundwater and rainwater, no significant difference occurred among non-lactose fermenters (NLF) across all locations. There was no significant difference between the mean coliform colony count of LF and NLF across surface water in different locations. Bayesian Paired Samples T-Test and Post-Hoc showed no significant difference in the total colony count of LF and NLF coliforms and means of total colony count between and within water sources across stations. However, the mean total coliform count in rainwater was the highest. Our results demonstrate that water sources from the agrarian settlements were contaminated by coliforms whilst settlers and others that consume them via drinking and domestic purposes are vulnerable, especially the aged, children and immunocompromised.

Key words: Water quality, bacteriology, agrarian settlement, coliform.

INTRODUCTION

Comparatively, escalating demands for potable drinking water exist in urban than rural settlements because most people in rural settlements (especially agrarian settlements) consume poor quality and contaminated water out of ignorance of the huge public health implications. Quality water is cardinal to the survival,

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livelihood and well-being of humans yet it is scare. Only 2.7% of the earth's water is freshwater (in lakes, rivers and ground) out of which only 1% is accessible (WHO, 2012). The inaccessible 1.7% is hidden in deep aquifers and glaciers. Consequently, humans suffer due do water scarcity. Water scarcity affects four out of every 10 people (WHO, 2012). This is exacerbated in regions with increased microbial contamination or pollution.

Beside cases of scarcity, anthropological activities such as: inadequate urban management, industrial pollution, over-fertilization, use of pesticides and agricultural wastewater (especially from animal husbandry) result in contamination of drinkable water sources to millions of people. Contaminated water transmits waterborne diseases such as cholera, diarrhoea, dysentery, hepatitis A, typhoid, and polio. By 2025, half of the world's population will be living in water-stressed areas (UNWWAP, 2014). It has been recorded that at least 2 billion people use drinking water source contaminated with faeces. Pathetically, majority of people without access to safe water are from Africa (UNWWAP, 2014; Martins et al., 2016; Nwabor et al., 2016). Contaminated drinking water is estimated to cause 485,000 annual diarrhoeal deaths, killing about 1.8 million children yearly and over 220 million people required preventative treatment for schistosomiasis in 2017 (WHO, 2012). In least developed countries, 22% of health care facilities have no water service. More so, approximately 3.1% of the global annual death (1.7 million) and 3.7% of the annual burden (disability) (54.2 million) are caused by the use of unsafe water (WHO, 2012).

In Nigeria, diarrhoea leads to over 70,000 annual deaths of children under five. More than 45 million people practice open defecation in Nigeria (FMWR, 2015) consequently contaminating natural water sources. Only 26.5% of the population use improved drinking water sources. Most people residing sideways of water bodies (especially in rural and agrarian settlements) still source and drink untreated water from lakes, rivers, springs, streams, wells and other water bodies regardless of the state of these water bodies with (FMWR, 2015; Martins et al., 2016; Nwabor et al., 2016). Although the practise of indiscriminate dumping and dispersal of wastes from home, hospitals, markets, factories and industries to water bodies is not recent, the continual adoption of this method of waste dispersal, especially during rainy season, will continue to be drawbacks in surface water backdrop. There is need for consistent monitoring of the rate and impact of microbial contamination of water bodies especially in rural and semi-urban areas where high dependence on surface and rain water is rife.

Objectives

This study was designed to assess the total colony count of different water sources from different communities in Eha-Amufu. Hence, the objectives include:

(1) Examining the distribution of total lactose-fermenting (LF) and non-lactose-fermenting (NLF) coliforms from different water sources and locations; to draw inference on the substrate and pollution source responsible for microbial presence and growth.
(2) To evaluate, within each water source, the variation of microbial load across different locations in Eha-Amufu.
(3) Compare total coliform count from different sources of water to communities in different locations in order to assess the source with higher health and environmental concern.

MATERIALS AND METHODS

Study area

Eha-Amufu is a town in South-eastern Nigeria. Coordinates – Universal Transverse Mercator (UTM): LN63; geographical coordinates in decimal degrees (WGS84): latitude: 6.667, longitude: 7.767; geographical coordinates in degrees minutes seconds (WGS84): latitude: 6 40' 00", longitude: 7 46 ' 00". Daylight: sunrise: 06:17:35; sunset: 18:08:08. It is located at latitude: 6° 39' 32.94" N; longitude: 7°45 ' 34.60" E. It is situated at elevation of 109 meters above sea level (http://www.tageo.com/index-e-ni-v-00-d-m2792190.htm). Eha-Amufu has an average temperature of 27.0°C and average precipitation of 1669 mm (CLIMATE-DATA.ORG). It is traversed by the Ebenyi River (ER) and its tributaries. The residents are dominantly of the Igbo speaking tribe and mainly rural farmers, fishermen, artisans and traders. Two distinct seasons occur in the area: the dry and wet seasons. The dry season stretches from November to April or May while the rainy season begins around May and ends in October (Nnamonu et al., 2018).

Sample collection

Surface water samples were sourced from three sampling points along each of these Ebenyi Rivers: Ebenyi Agamede, Ebenyi Amede, Ebenyi Ihenyi, Ebenyi Mgbuji and Ebenyi Umuhu using 200 ml sterilized borosilicate glass bottles. Ground water and rain water were as well collected from three randomly selected locations in each of these communities: Agamede, Amede, Ihenyi, Mgbuji and Umuhu. Ground water was sourced from hand-dug wells while rain water was harvested directly from rooftop at three randomly selected locations in each of these communities: Agamede, Amede, Ihenyi, Mgbuji and Umuhu. All samples were collected using 200 ml sterilized borosilicate glass bottles. All the 200 ml sterilized borosilicate glass bottles were immediately labelled and transported in ice-pack to the laboratory for bacteriological analysis within 6 h of sample collections.

Bacteriological analyses

Bacterial isolation, identification and enumeration

Isolation and identification of microorganisms were carried out with the following media: nutrient agar, Simon's citrate agar and urea agar base (manufactured by Hardy Diagnostics; 1430 West McCoy Lane, Santa Maria, CA 93455, USA); MacConkey agar, peptone water, kligler iron agar and urea medium (manufactured by Central Drug House Limited, NewDelhi, India).

In the laboratory, 10 µl of each water sample was inoculated onto a MacConkey agar plate, and evenly spread using sterile glass spreader. Each sample was duplicated. The MacConkey plates

were incubated at 37°C for 24 h. The plates were examined and isolates were enumerated based on grown colonial morphology, colour and texture. Different colonies were subcultured to obtain distinct colonies. Distinct colonies from the agar were examined for ability to ferment lactose. Non-lactose fermenting colonies were further inoculated onto deoxycholate citrate agar (DCA) and incubated overnight at 37°C. A representative of each distinct colony type was Gram stained and subjected to biochemical tests according to the WHO Manual for the Laboratory Identification and Antimicrobial Susceptibility Testing of Bacterial Pathogens of Public Health Importance in the Developing World (WHO/CDS/CSR/RMD/2003.6).

Growth on Kligler iron agar

Presumptive identification was based on the reaction of the isolate on Kligler iron agar (KIA). Discrete colonies were carefully picked from the agar media using pointed inoculating wire loop. Tubes of KIA were inoculated by stabbing the butt and streaking the surface of the slant. The caps of the tubes were loosened before incubation. After incubation for 24 h at 37°C, the KIA slants were observed for reactions and results recorded. Yellowing of the butt indicated glucose fermentation; yellowing of slant indicated lactose fermentation; while reddening of the slant indicated inability of the organism to ferment lactose. Gas production was indicated by air bubbles, cracks or displacement of the medium. Hydrogen sulphide production was indicated by blackening of the medium

Sulfide-indole-motility (SIM) screening test

Sulfide-indole-motility medium (SIM) is a commercially available combination medium that combines three tests in a single tube: hydrogen sulfide (H_2S) production, indole production, and motility. The indole reaction is not useful for screening suspected *Shigella* isolates because strains vary in their reactions in this test. SIM medium was prepared from the dehydrated medium according to manufacturer's instruction. *Escherichia coli,* which is indole positive, H_2S negative and motility positive, was used as control. The SIM media were inoculated with a straight inoculating wire, by making a single stab about 1-2 cm down into the medium and was incubated overnight at 37°C. The surface of the motility agar was dry when used, since moisture is known to cause a non-motile organism to grow down the sides of the agar, creating a haze of growth and appearing to be motile. The motility reaction in SIM was indicated by the presence of diffused growth (appearing as clouding of the medium) away from the line of inoculation. The organisms that did not grow out from the line of inoculation were recorded as non-motile. As in Kligler iron agar, H_2S production was indicated by blackening of the medium. Indole production was tested by adding 0.5 ml of Kovac's reagent to the tube. Indole positive organisms produced red colour at the top of the test tube.

Viable cell count

Number of colonies was determined by converting the number of colonies per 10 µl to colony forming unit per 1000 µl (CFU/ml).

Site comparison and data analyses

Since we were mainly interested in the coliform count in different water sources across different locations within the study area, we compared the coliform counts using Bayesian statistical analysis due to its robustness which considers the dynamics and variations (prior probabilities) across sampling time and flow gradient (such as upstream to downstream) of water sources. One-way analysis of variance (ANOVA) was conducted using JASP software (version 0.9.2). Our hypotheses were that substrate type and water source have effect in coliform counts across different locations. Also, we hypothesised that a significant difference in coliform count exists within each water source across different locations. Post-Hoc Test was used to compare the difference between the mean of coliform loads within sample groups. Bayes Factor (BF_{10}) of the Bayesian statistic is similar to the *p*-value of classical frequentist. Results of the Bayesian analyses were appropriately interpreted (Kass and Raftery, 1995). BF_{10} value up to three (3) was considered significant and showed evidence supporting the alternative hypothesis.

RESULTS

Table 1 and Figure 1 display Bayesian ANOVA comparisons showing BF_{10} and Post-Hoc test of compared samples and subsamples. Bayesian ANOVA was conducted to test the effect of different locations, substrate and water sources on the occurrence of coliform count. Colony counts were stated as dependent variable while location, substrate type (LF and NLF) and water source were stated as fixed factors where relevant.

There was a significant difference in the means of coliform counts in groundwater (GW) and rainwater (RW). Within the ground water samples with lactose fermenters (Figure 1a), Agamede had mean count of 2.667×10^2 and standard deviation (SD) of 2.517 while Amede had mean count of 2.333×10^2 and SD of 0.577. Ihenyi had the highest mean count of 3.0×10^2; however the SD was 0.0 because the values were essentially constant across the sample size (N). Other locations (Mgbuji and Umuhu) have zero mean count and zero SD (Figure 1a). There was no significant difference between the mean coliform colony count of non-lactose fermenters across ground water in different locations (Figure 1b). Agamede had mean count of 28.333×10^2 and SD of 12.50 while Amede had mean count of 14.67×10^2 and SD of 12.86. Umuhu had mean count of 11.67 and SD of 10.12. Ihenyi had the lowest mean count of 4.67×10^2 and SD of 4.16. Mg had zero mean count and zero SD (Figure 1b).

Similar to the GW, there was a significant difference between the coliform colony count of lactose-fermenters (LF) in rainwater (RW) across different locations (Figure 1e). Umuhu had the highest mean count of 54×10^2 and SD 10.82 followed by Agamede with mean count of 45×10^2 and SD 10.44 whilst Amede, Ihenyi and Mgbuji had comparatively low mean count of 19.33, 11.67 and 9.67 respectively. The SD was 16.17, 6.81 and 4.04. The main effect of the difference lies within the Mgbuji and Umuhu locations as showed by the Post-Hoc test (Table 1). There was no significant difference between the mean coliform colony count of non-lactose fermenters across rainwater in different locations. Although a significant BF_{10} 12.29 was detected as an analysis of effect, no significant difference in colony count was observed across variations of location. Mgbuji had the highest mean colony count of 250.33×10^2 and SD 146.28. Mean

Table 1. Bayesian ANOVA comparisons showing BF_{10} and Post-Hoc test of compared samples and subsamples.

Sample	Compared subsamples	Bayes factor (BF_{10})	Post-Hoc test ($BF_{10.\,C}$)
GW_LF	Locations (Ag, Am[a], Ih, Mg[b], Um[b])	3.897	4.288
GW_NLF	Locations (Ag, Am, Ih, Mg, Um)	2.622	Very low
Locations	LF_C and NLF_C	1.418	Very low
RW_LF	Locations (Ag, Am, Ih, Mg[a], Um[b])	31.59	3.778
RW_NLF	Locations (Ag, Am, Ih, Mg, Um)	12.29	Very low
SW_LF	Locations (Ag, Am, Ih, Mg, Um)	0.28	Very low
SW_NLF	Locations (Ag, Am, Ih, Mg, Um)	1.99	Very low
Total C_LF	Locations (Ag, Am, Ih, Mg, Um)	0.352	Insignificant
Total C_NLF	Locations (Ag, Am, Ih, Mg, Um)	0.457	Insignificant
Water source	RW, SW; GW	0.993	
	RW; SW		0.458
	RW; GW		1.527
	SW; GW		0.302

$BF_{10.\,c}$, Posterior odds or Corrected Bayes Factor; BF_{10} from the value of 3 and above shows a significant difference in relationship. GW, groundwater; RW, rainwater; LF, lactose-fermenter; NLF, non-lactose fermenter, C, colony count; Ag, Agamede; Am, Amede; Ihenyi, Ih; Mg, Mgbuji; Um, Umuhu. [a,b] Locations with different superscript differ significantly and those with no superscript do not differ significantly or cannot be compared.

colony counts for Amede, Ihenyi, Agamede, and Umuhu were 23, 11.67, 0.67 and 0.67 with respective SD of 18.36, 12.50, 0.58 and 1.16 (Figure 1f).

There was no significant difference between the mean coliform colony count of lactose and non-lactose fermenters across surface water in different locations (Table 1). There were low BF_{10} and lack of significance in variations of locations. Umuhu and Ihenyi had high mean colony count of 20 and 18.89 while Amede, Agamede and Mgbuji had low mean count of 4.22, 3.67 and 1.67 (Figure 1g).

There was weak BF across all models. There was no significant difference in the colony count across compared locations. The means of the locations were 93.11, 32.11, 19.11, 6.0 and 5.56 for Amede, Agamede, Ihenyi, Mgbuji and Umuhu separately (Figure 1i). Bayesian Paired Samples T-Test was carried out to test if there is a significant difference in the total colony count of LF and NLF coliforms. With BF_{10} of 1.418 (Table 1), there was no significant difference across the priors tested. There is evidence in support of the null hypothesis that colony count between LF and NLF was evenly distributed across the locations. LF_C had a mean count of 11.72×10^2 with 95% Credible Interval (CI) 6.154 to 17.29. Colony of Non-lactose fermenter (NLF_C) had a mean count of 32.53×10^2 with 95% CI 15.28 to 49.79 (Figure 2).

The credible interval (CI) is overlapping at some extent, making the analysis between lactose fermenting and non-lactose fermenting insignificantly different from one another although both CI look different. Colony count of non-lactose-fermenters (NLF_C) was higher than colony of Lactose-fermenters (LF_C). This may suggest that the study area has multiple alternative substrates sustaining the proliferation of varieties of coliforms from many sources across all tested locations (Figure 2). There was no significant difference between the mean of total coliform colony count of lactose and non-lactose fermenters across all water sources in different locations (Table 1). Figure 1c had weak BF across all models. No significant difference in the colony count across compared locations. The means of the locations were 22.80, 14.27, 11.73, 6.87 and 2.93 for Umuhu, Ihenyi, Agamede, Amede and Mgbuji separately. Figure 1d had weak BF across all models. No significant difference in the colony count across compared locations. The means of the locations were 63.40, 53.67, 25.07, 14.73 and 5.80 for Amede, Mgbuji, Agamede, Ihenyi and Umuhu independently.

Bayesian Paired Samples T-Test and Post-Hoc comparison were used to compare the means of total colony count between and within water sources. There was no significant difference in the total coliform count of different sources of water to the communities in different locations. This statistically suggests that all water sources (GW, SW and RW) have similar microbial load. However, the mean total coliform count in RW was the highest while GW had the least coliform count (Figure 3).

DISCUSSION

The observed alarming level of ignorance with regards to the grave side effects of consuming contaminated water among people in rural settlements (especially agrarian settlements) motivated the present study. This study specifically evaluated the bacteriological quality of ground, rain and surface water sourced from an agrarian settlement.

The bacteriological analysis of ground water (hand-dug well) showed that lactose fermenter (*E. coli*) was isolated from water sourced from wells in three communities

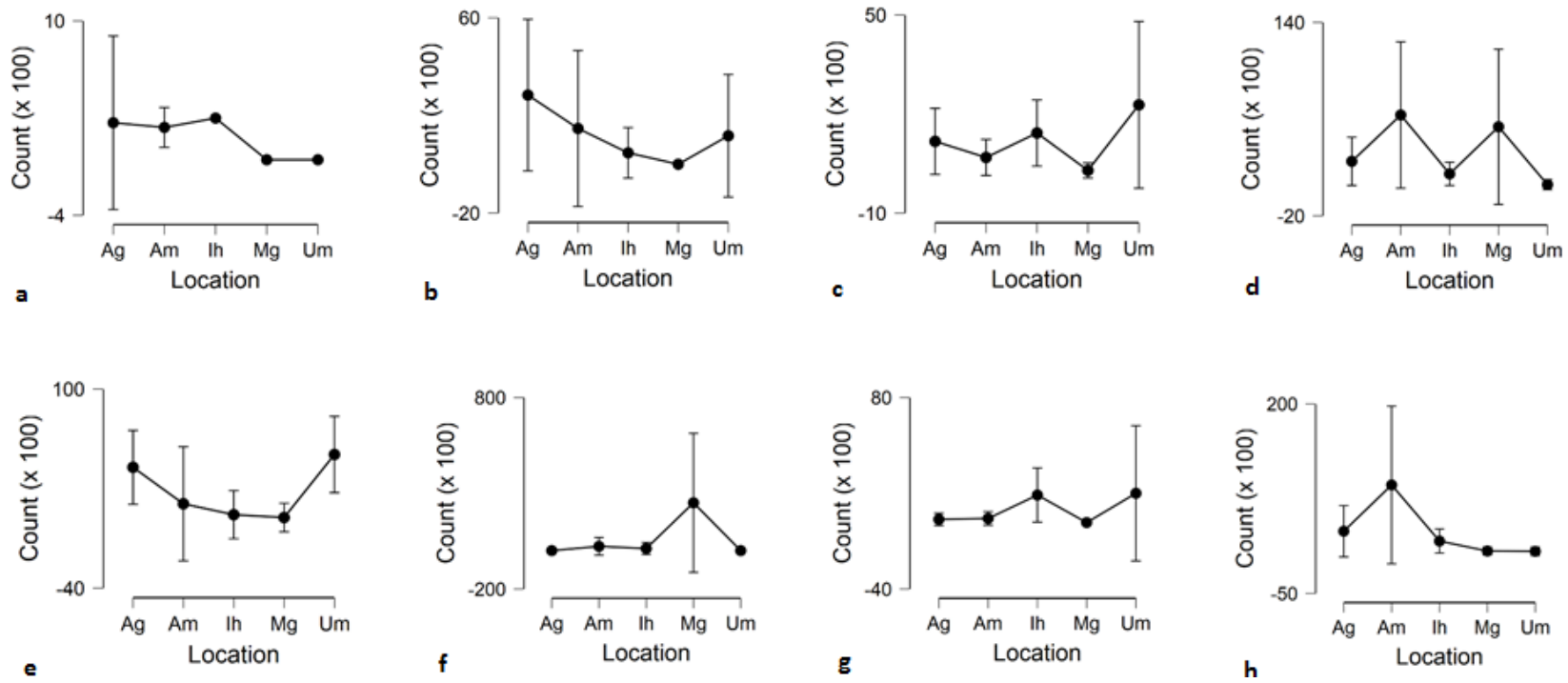

Figure 1. Comparison of lactose and non-lactose fermenting bacteria count in water samples (ground, rain and surface) sourced from Agamede, Amede, Ihenyi, Mgbuji and Umuhu in Eha-Amufu. **(a)** counts of lactose fermenters in groundwater **(b)** counts of non-lactose fermenters in groundwater **(c)** total colony count of lactose fermenters **(d)** total colony count of non-lactose fermenters **(e)** counts of lactose fermenters in rainwater **(f)** counts of non-lactose fermenters in rainwater **(g)** counts of lactose fermenters in surface water **(h)** counts of non-lactose fermenters in surface water. Ag, Agamede; Am, Amede; Ihenyi, Ih; Mg, Mgbuji; Um, Umuhu.

(Ihenyi, Agamede and Amede) while two communities (Mgbuji and Umuhu) recorded zero level of *E. coli*. These results consonant with many studies (Anyanwu and Okoli, 2012; Nwabor et al., 2016; Bolaji et al., 2017; Onuigbo et al., 2017; Obeta and Mamah, 2018). Specifically, the significant difference within this group was between the mean colony count of Amede and Mgbuji or Umuhu. This suggests that Mgbuji and Umuhu have lower level of faecal pollution compared to Amede. Contrary to the believe of some of the natives in the rural settlements that nothing in water kills, *E. coli* and other feacal and total coliforms are infectious organisms of public health importance. The presence of these organisms indicates that there is animal or human waste contaminant in water. They cause intestinal infections, dysentery, hepatitis, typhoid fever, cholera, gastroenteritis which sometimes lead to other complications and death as well (Nwabor et al., 2016). Consequently, WHO has recommended that any water that contains these organisms should not be used for drinking, cooking nor processing of food, and similar domestic purposes including brushing/ washing of mouth without disinfecting it first (WHO, 2011; Martins et al., 2016; Nwabor et al., 2016). We have observed in our study area that most hand-dug wells were not properly covered. When covers are available, they are not elevated, and this allows run-off to have its way into the wells during rainy season. Similarly, in some residence toilets and suck-away pits are not distanced / well positioned away from the wells (Nwabor et al., 2016). Pathetically, open filled / bush defecation is still practiced by some persons in the area.

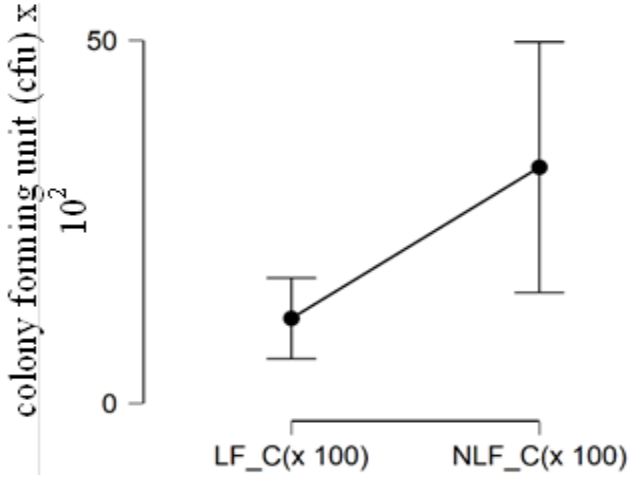

Figure 2. Comparative 95% credible interval plot of mean colony count between lactose fermenting (LF) and non-lactose-fermenting (NLF) coliforms, colony count (C) (x 10^2); Y-axis is colony forming unit (cfu) x 10^2 or Count (x 100).

Additionally, there is serious problem of poor sanitation. For instance, the kind / nature of buckets and other containers used in drawing out water from the hand-dug wells is not in any way a cause of concern for most persons in the area.

There was no significant difference between the mean coliform colony count of non-lactose fermenters across ground water in different locations. Whereas non-lactose fermenters count in ground water sourced from four communities (Agamede, Amede, Umuhu and Ihenyi) were above WHO permissible standard, ground water sourced from Mgbuji recorded zero count. This is in agreement with Anyanwu and Okoli (2012) and Obeta and Mamah (2018). We therefore tend to report that during the period of this study, ground water sourced from Mgbuji met the WHO bacteriological standard for drinking water. This may also imply that there is better sanitation and waste management practices at Mgbuji compared with other communities studied.

Similar to the groundwater, there was a significant difference between the coliform colony count of lactose-fermenters (LF) in rainwater (RW) across different locations (Figure 1e). The main effect of the difference lies within the Mgbuji and Umuhu locations as showed by the Post-Hoc test (Table 1). There was no significant difference between the mean coliform colony count of non-lactose fermenters across rainwater in different locations (Figure 1f). Although a significant BF_{10} 12.29 was detected as an analysis of effect, no significant difference in colony count was observed across variations of location. The coliform colony count of both lactose-fermenters and non-lactose fermenters at each location showed coliform counts were above WHO permissible standard. Residents in the study areas were observed to use this rainwater as drinking water without further treatment. They also used same water for food processing and cooking. Preparation of uncooked foods like 'Abacha', washing of fruits without addition of salt, rinsing of plates and pots, teeth brushing, and bathing were other activities involving the use of the contaminated rainwater.

The source of contamination of the rainwater could be as a result of poor hygienically way of harvesting the rain water, deposition of animal dungs on the rooftops (animals such as birds, squirrels, rodents and other small mammals). High settling rate of microbes on rooftops of some houses, lack of and derelict management of toilets in the area especially open field defecation may have led to rampant defecation within the environment which could be contributory. There is need for continual reorientation of rural settlers, especially farmers, on the need to treat rain water harvested from rooftops and other sources before drinking and utilisation. There was a significant difference in the means of coliform counts in groundwater and rainwater. Rainwater recorded more coliforms that groundwater. This could be due to the hygiene level applied while harvesting the rainwater and suggests that rainwater in the area has higher risk of waterborne diseases than groundwater. There was no significant difference between the mean coliform colony count of lactose and non-lactose fermenters across surface water in different locations. However, coliform counts at individual locations showed values above WHO permissible standard. These results agree with Kolawo et al. (2011), Ihuma et al. (2016), Martins et al. (2016) and Nwabor et al. (2016). The commonest sources of these organisms in water are animal and human wastes (EPA, 2002). Candidly, open defecation system is still common in rural settlements and this serves as the major means of surface water contamination. Some nomadic

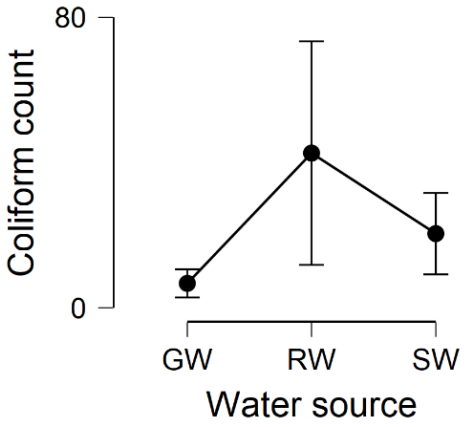

Figure 3. Comparison of mean coliform count of different water sources across all sampling sites. GW, groundwater; RW, rainwater; SW, surface water.

herdsmen often take their animals to available surface water for rehydration and these animals urinate, defecate, and transfer other bodily microbes into same water that rural settlers source water for drinking and other domestic purposes. Some abattoirs in rural settlements are sighted very close to rivers and streams for easy access to water. Rotten meats, animal dungs, blood and skeletal parts are often washed into the water bodies. These heavily contribute to microbial load of surface water. Open field waste disposal is also practiced in the studied rural settlements.

Bayesian Paired Samples T-Test was carried out to test if there is a significant difference in the total colony count of LF and NLF coliforms showed that there was no significant difference across the priors tested. This supports the null hypothesis that colony counts between LF and NLF were evenly distributed across the locations (Figure 2). This may suggest that the study area has multiple alternative substrates sustaining the proliferation of varieties of coliforms from many sources across all tested locations. This suggests that issues of hygiene and environmental sanitation be considered seriously in rural settlements.

Bayesian Paired Samples T-Test and Post-Hoc comparison showed no significant difference in the total coliform count of different sources of water to the communities in different locations suggesting that all water sources (GW, SW and RW) have similar microbial load. However, the mean total coliform count in RW was the highest while GW had the least coliform count (Figure 3). This depicts that rooftops and entire environment where the rain water is harvested are contaminated by coliforms. This also suggests high load of coliforms in the environment of the study area and a need for awareness and intervention on the locations with high dependence on RW and SW since considerably higher public health impact is more imminent than areas with available or high dependence on GW (Figure 3).

Conclusions

This study has demonstrated that ground, rain and surface water sourced from rural agrarian settlements are vulnerable to coliform contamination. They also have poor bacteriological quality and are detrimental to health of those that drink and use them for other domestic purposes with regards to WHO recommendation. The state of this water should be of public health concern.

RECOMMENDATION

Further studies should adopt test methods describing sensitive and differential membrane filter (MF) medium; MI agar or MI broth should be used for the simultaneous detection and enumeration of both total coliforms (TC) and *E. coli* in water samples within 24 h or less based on their specific enzyme activities.

Limitations

The impact of meteorological variables like wind speed and direction in transferring coliforms from a source to location(s) was not studied. Also, the effect of environmental variables like humidity and temperature of the study site was not considered. The strain and pathogenicity of the isolates were not identified.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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