ORIGINAL PAPER

TITLE: Bacteriological Assessment and Antimicrobial Profile of Coliform Isolates from Groundwater near Septic Tanks in Ago-Iwoye Town, Nigeria

RUNNING TITLE: Bacteriological Assessment of Coliform Isolates

ABSTRACT

Groundwater sources generally regarded as safe source of drinking water for majority of the population in the developing world are vulnerable to fecal contamination as a result of proximity to septic tanks. Hence, this study investigated the physicochemical, bacteriological qualities and antimicrobial susceptibility profiles of coliforms in from selected ground water sources located near septic tanks in Ago-Iwoye town. Physicochemical and bacteriological assessment were carried out according to standard methods as described by Cheesbrough and disc diffusion method was used for antibiotic susceptibility test. Results from this study showed that 90% of samples were heavily contaminated with coliforms (*Escherichia coli* (30%), *Klebsiella sp* (30%), *Salmonella sp* (15%), *Enterobacter sp* (10%), *Shigella sp* (10%) and *Proteus sp* (5%)) with total viable counts ranging from 2 cfu/ml to 200 cfu/ml. Majority of the isolates were susceptible to antibiotics used in this study but resistance to augmentin was largely observed. All well water samples were non turbid, odourless and colourless with temperature ranging from 29^0C-30^0C, pH ranging from 6.5 -7.3, which was all in compliance with the WHO standard. This concludes that most well water samples in close proximity with septic tanks are unsafe for drinking or other domestic activities. The presence of antibiotic resistant bacteria can lead to severe health issues.

Key words: Coliforms, Well water, Septic tanks, Bacteriological

INTRODUCTION

Groundwater as source of drinking water should be free from pathogenic microbes (Dayanti *et al.*, 2018). In the developing world, the majority of the human population depends on ground water as a source of drinking water (Bogena, 2015) Water from dug wells are often believed to be clean and heathy, thereby assuring better quality than the surface open waters (Akaniro, *et al*., 2019). However, ground water are vulnerable microbial contaminants which causes water borne such as dysentery, diarrhea and cholera (Takal & Quaye-Ballard, 2018).

Septic tanks are widely used to treat small volumes of wastewater in rural and suburban areas (Yang, *et al*., 2017). During installation of the septic tanks, site specific conditions and local ground water flow are often ignored (Farouq, *et al*., 2018). Septic tank effluents are discharged into drain fields where it penetrates in to the soil (Yang, *et al*., 2017). These effluents contain high amount of bacteria which may seep into groundwater used for consumption particularly in areas with close proximity with septic tanks and this poses health risk to the human population in the environment (Phillips, *et al*., 2015). Numerous diseases affecting the population of the developing world are attributed to contamination of groundwater (Bouderbala, 2019). Sewage has been reported to serve as the primary source of pathogenic microbes contaminating ground water (Farouq, *et al*., 2018). However, the situation is worsening due to shallow water table and unsafe method of sewage system construction (Farouq, *et al*., 2018).

Coliform bacteria have been reported to majorly constitute microbial contaminants of ground water, could also be used as an indicator in areas susceptible to water pollution (Sriyono, *et al*., 2019). Detection of fecal coliforms belonging to the enteric bacteria family in ground water have been used as an indicator of sewage contamination (Farouq, *et al*., 2018). Presence of enteric bacteria such as *Eschericha coli*, *Salmonella sps and Shigella sps* in groundwater makes ground water unsafe for consumption due to their ability to cause disease conditions in humans (Oluwasola, *et al*., 2017).

The study aimed at examining the presence of coliform bacteria in well waters located in close proximity with septic tanks at Ago-Iwoye.

2. MATERIAL AND METHODS

2.1 Study area

The samples were collected from five different well in Ago-Iwoye, a rural community in Ijebu North local government area of Ogun State. Geographically, it lies between latitude 6056'N and 7000'N and longitude 3054'E and 4000'E, with neighbouring town such as Oru, Awa, Ijebu Igbo and Ijebu Ode.

2.2 Sample Collection

Five (5) samples of water were collected by standard method (Cheesbrough, 2000) using the plastic fetchers consisting of two hundred (200ml) were collected aseptically in sterile sampling bottles from 5 different private wells in Ago-Iwoye, the bottles were labeled with full details of: source, date and numbers and taken to the Microbiology laboratory of Olabisi Onabanjo University, Ago-Iwoye, Ogun State for further analysis within 24 hours.

2.3 Laboratory examination and procedure

Immediately after sample collection, analysis for temperature, pH and turbidity was carried out on all samples. The total viable count was also determined and was expressed as colony forming unit per ml (cfu/ml). The pour plate method was employed and samples were initially cultured onto nutrient and MacConkey agar. Isolates from primary culture were then subcultured onto

MacConkey and Eosin Methylene Blue agar, the resultant pure isolates were then identified and characterized using cultural and biochemical characteristics.

3. RESULTS AND DISCUSSION

3.1 Physical and chemical analysis of water samples

The results of the physical and chemical analysis of well water samples collected from five different areas which include Koroko, Imere, Figbigbade, Elemele and Ayegbami in Ago Iwoye township is shown in table 2. Temperature, pH and turbidity of the water samples was analysed. The temperature of all samples were within the range of 29^{o}C and 30^{o}C which were in conformity with WHO prescribed limit for drinking water. However, the temperature observed were a little bit higher when compared with studies carried out by Olubanjo *et al*, 2019 and Oyem *et al* 2014, which recorded mean temperature of 24.05^{o}C and 27.7^{o}C respectively. This might be due to difference in climatic conditions and location of sample collection used in the study.

The result of the analysis also showed that the pH values from samples collected were between 6.5 and 7.3 indicating water samples was slightly acidic to neutral. pH of samples was within the WHO and NIS permissible pH limits of 6.5 – 8.5 and 6.5 -7.5 respectively. The result obtained in this study was similar to the study carried out by of Saana *et al*, 2016 but disagrees with studies carried out by Jamuna, 2018 that reported a higher pH range between 7.6 – 8.4 and Dirisu *et al*, 2016 which recorded a lower pH range of 4.74 – 5.1. This might due to difference in geographic locations where the study is carried out, metal leaching and dissolution of acidic gases could have an effect on the water pH level.

All water samples obtained in this study were non-turbid, odour was unobjectionable and colour was within recommended safe limit for drinking water that is in conformity with WHO standard but this finding does not agree with the study carried out by Taiwo *et al*, 2015 where samples were slightly turbid and coloured, these characteristics reveals pollution of the groundwater.

3.2 Bacteriological analysis of water samples

Result analysis showed that 90% of the well water samples collected were unsafe for consumption due to the high total viable count observed in this study. This could be due to the presence of high amount of organic and dissolved salts in the well water. Presence of coliforms such as such as *Escherichia coli, Klebsiella sp, Salmonella sp, Enterobacter sp, Shigella sp* and *Proteus sp* indicates fecal contamination of well water sample, the proximity of the wells to septic tanks might have played a significant role in this findings. Also unsterile permanent water drawers could also aid microbial contamination of the well water during handling. The findings of this study was similar to that Mukhopadhay *et al*, 2012 where coliforms were found in well water from rural and urban households.

Escherichia coli and *Klebsiella sp* had the highest frequency of occurrence (30%) followed by *Salmonella sp* (15%), *Shigella sp (10%), Enterobacter sp* (10%) and *Proteus sp* (5%). Surface runoff, land use and animal waste deposited, also seepage from septic tanks contribute to the high frequency of the *Escherichia coli* and *Klebsiella sp.* this finding is similar with studies carried out by Abolude *et al*, 2016 in Zaria (Nigeria), Dayanti, *et al*, 2018 in West Jakarta (Indonesia), and Onyango, *et al*, 2018 in Isiolo County (Kenya); where high frequency of Eschericha coli and Klebsiella spp were also reported. Presence of fecal contaminants makes water unsuitable for human consumption.

3.3 Antimicrobial resistance

Antimicrobial susceptibility testing of isolates was carried out using disc diffusion techniques. Broad and narrow spectrum antibiotics which includes Ceptazidine(30mg), Cefuroxime(30mg), Gentamicin(10mg), Cefixime(5mg), Ofloxacin(5mg), Augmentin(30mg), Nitrofurantoin(300mg) and Ciprofloxacin(5mg). majority of the isolates were susceptible to the antibotics used. However high resistance to augmentin was observed in almost all the isolates. the findings of this study were similar to the study carried out by Akaniro *et al*, 2019 where augmentin resistance was also observed among bacteria isolates from groundwater.

4.0 CONCLUSION

The physicochemical properties of the well water samples were within the safe limits of the WHO standard of drinking water. However, all samples had the presence of coliform bacteria with high bacteria counts exceeding the limits of compliance of the EPA (2003) standard of zero total coliform per 100ml of water. Presence of fecal contaminants in all samples makes water unsafe for drinking and also indicates proximity to a common source of contamination which could be investigated further. Majority of the pathogenic bacteria isolates observed were susceptible to antimicrobials used. Yet, Augmentin resistance was observed in almost all the isolates. well water obtained for drinking from this area must be properly purified and treated before it can be deemed safe for drinking or any other domestic activities. Proximity to septic tanks, unsterile drawers, well construction and human activities could play significant roles in contamination of the well water. Therefore, a further investigation on these factors as well as role of soil porosity and extensive analysis of water samples should be carried out.

TABLES

Table 1: Area of sample collection in Ago-Iwoye

S/N	SAMPLE AREA	SAMPLE CODE
1	Koroko,	K
2	Imere	I
3	Figbigbade	F
4	Elemele	E
5	Ayegbami	A

Table 2: Physical and chemical analysis of the various well water samples.

SAMPLE	TEMPERATURE(^{0}C)	PH	TURBIDITY	COLOUR	ODOUR
F	30	7.1	Non-turbid	Colourless	Odourless
K	29	6.7	Non-turbid	Colourless	Odourless
E	30	6.5	Non-turbid	Colourless	Odourless
A	29	7.3	Non-turbid	Colourless	Odourless
I	29	6.8	Non-turbid	Colourless	Odourless

Keyword: F-(Figbigbade) K-(Koroko) E-(Elemele) A-(Ayegbami) I-(Imere)

Table 3: Total viable count of the bacteria isolates from the various well water samples.

SAMPLE CODE	TOTAL VIABLE COUNT(cfu/ml)
Fww1	80
Fww2	2
Fww3	23
Fww4	7
Kww1	72
Kww2	17
Kww3	38
Kww4	46
Eww1	188
Eww2	70
Eww3	200
Eww4	45
Aww1	57
Aww2	12
Aww3	140
Aww4	57
Iww1	152
Iww2	19
Iww3	56
Iww4	16

Keyword: Fww-(Figbigbade well water) Kww-(Koroko well water) Eww-(Elemele well water) Aww-(Ayegbami well water) Iww-(Imere well water)

Table 4: Percentage occurrence of the isolates from the well water samples

Organism	**Frequency**	**Percentage Frequency(%)**
Escherichia coli	6	30
Klebsiella sp	6	30
Salmonella sp	3	15
Shigella sp	2	10
Enterobacter sp	2	10
Proteus sp	1	5
Total	20	100

Table 5: Antimicrobial susceptibility testing of the bacteria isolate from the various water samples.

Isolated organism	CPR	GEN	AUG	CXM	CAZ	OFL	NIT	CRX
Escherichia coli	24	17	R	13	20	25	20	15
Klebsiella sp	R	18	15	R	20	15	17	10
Salmonella sp	20	15	R	15	25	20	R	R
Enterobacter sp	15	20	15	20	R	R	10	15
Shigella sp	25	22	R	R	15	30	15	R
Proteus sp	15	30	R	15	10	10	20	15

KEYWORD: CPR: Ciprofloxacin GEN: Gentamicin AUG: Augmentin CXM: Cefixime CAZ: Ceftacidime OFL: Ofloxacin NIT: Nitrofurantion CRX: Cefuroxime R(Resistance)

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