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BACTERIOLOGICAL ASSESSMENT AND ANTIMICROBIAL PROFILE OF COLIFORM ISOLATES FROM GROUNDWATER NEAR SEPTIC TANKS IN AGO-IWOYE TOWN, NIGERIA

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ABSTRACT

Groundwater sources generally regarded as safe source of drinking water, 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 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, Groundwater, Septic, 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 and 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 sp* and *Shigella sp* 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.

MATERIAL AND METHODS

Study Area

Ago-Iwoye is a rural community in Ijebu North local government area of Ogun State. Geographically, it lies between latitude 6056'N and 7000'N and longitude 3.054'E and 4.000'E, with neighboring town such as Oru, Awa, Ijebu Igbo and Ijebu Ode.

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 (table 1). The distance between the septic tank and the well ranged between 10 metres – 15metres. The sterile sample 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.

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

Key: F- Figbigbade, K- Koroko, E- Elemele, A- Ayegbami, I- Imere.

Laboratory examination and procedure

Using a modified version of the procedure described by Egberongbe et al. (2012). 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 sub-cultured onto MacConkey and Eosin Methylene Blue agar, the resultant pure isolates were then identified and characterized using cultural and biochemical characteristics.

Data Analysis

Data obtained was entered and analyzed using Microsoft Excel 2013. Data obtained were adequately represented as tables in appropriate sections.

RESULTS AND DISCUSSION

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 were analyzed. The temperature of all samples was within the range of 29°C and 30°C which were in conformity with WHO prescribed limit for drinking water (table 2). 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°C and 27.7°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 (table 2). The 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 un-objectionable 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.

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

SAMPLE	TEMPERATURE(⁰C)	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

Key: F- Figbigbade, K- Koroko, E- Elemele, A- Ayegbami, I- Imere.

Bacteriological analysis of water samples

Result analysis showed that 90% of the well water samples collected was unsafe for consumption due to the high total viable count observed in this study (Table 3). 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 these 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%) (Table 4). 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 sp* were also reported. Presence of fecal contaminants makes water unsuitable for human consumption.

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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

Key: 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

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) (Table 5). Majority of the isolates were susceptible to the antibiotics 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.

Table 5: Antimicrobial susceptibility testing of the bacteria isolates from 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

Key: CPR: Ciprofloxacin, GEN: Gentamicin, AUG: Augmentin, CXM: Cefixime, CAZ: Ceftacidime, OFL: Ofloxacin, NIT: Nitrofurantion, CRX: Cefuroxime, R: Resistant

CONCLUSION

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.

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