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Antibiotic susceptibility of bacterial isolates and water quality index of water sourced from closed ground water and open hand dug well in Koko Community, Delta State, Nigeria

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SUMMARY. Water samples were collected from a semi urban community in Nigeria with the aim of investigating the water quality index and antibiotic profile of bacterial isolates of closed ground water and open hand dug wells. Physicochemical and microbiological analyses were carried out using standard analytical methods. pH of groundwater and hand dug well ranged from 4.16 to 5.74 and 4.83 to 5.22 respectively. The total suspended solid of water samples for hand dug well ranged from 1.2-9.2mg/l. Also iron concentrations for groundwater and hand dug well water samples ranged from 0.15-0.54 mg/l and 0.62-1.12 mg/l respectively. Microbial analysis of the water samples revealed the presence of bacteria such as *S. aureus, Klebsiella* sp., *E. coli, B. subtilis, Pseudomonas, Aeromonas hydrophila* and *Enterobacter aerogenes* and fungi such as *Aspergillus niger, Penicillium notatum, Aspergillus flavus, Rhizopus stolonifer* and *Saccharomyces cerevisiae.* The total heterotrophic bacterial count of water samples for groundwater and hand dug well samples indicated that values ranged from 2.9 - 4.4 $\times 10^3$ cfu/ml and 5.4 – 8.6 $\times 10^3$ cfu/ml respectively. Total coliform of water samples for groundwater and hand dug well samples indicated that values ranged from 5-8 MPN/100ml and 10-20 MPN/100ml respectively. *E. coli* count of water samples for groundwater and hand dug well samples indicated that values ranged from 0.0 MPN/100ml and 4-8 MPN/100ml respectively while total fungal count of groundwater and hand dug well samples indicated that values ranged from 0.0 - 6.0×10^2 cfu/ml and 3.5×10^2-17.0×10^3 cfu/ml respectively. Variable antibiotic susceptibility patterns were observed in antibiotic inhibitory zone (mm) among the tested bacterial isolates. Evaluation of Water Quality Index indicated values of 34.4 for groundwater source indicating good water quality and 67.31 for open hand dug well

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indicating water was of poor quality. Findings from this study revealed that groundwater sources had better and acceptable quality compared to those of open hand dug wells, hence it is recommended that critical measures be put in place to ensure the safety of both sources of water in Koko community.

Keywords: antibiotic susceptibility, ground water, hand dug wells, microbiological analysis, water quality

Introduction

Research findings have revealed that a considerable percentage of all diseases which cause mortality in the developing countries are directly and indirectly related to poor drinking water quality (Jeffre, 2008), and over 20,000 children die per day (approximately six million annually) due to water borne diseases resulting from availability of safe drinking water (TWAS, 2002). The geological constraints limit accessibility of many human communities to water that is adequate in terms of quantity, quality and sustainability (Tchobanoglous *et al.,* 2003) and lack of adequate supply of potable water is a critical challenge in Nigeria and other developing countries.

In these parts of the world, the usual sources of drinking water are streams, rivers, wells and boreholes which are mostly untreated and associated with various health risks (Agbarie and Obi, 2009). Paucity of infrastructure for effective treatment and distribution of water accounts for the incidence of high morbidity and mortality rate associated with water borne diseases in developing countries (Muhammed *et al.,* 2007). The quality of water influences the health status of any population, hence, analysis of water for physical, biological and chemical properties including trace element contents are very important for public health studies (Orewole *et al.*, 2007).

A reliable supply of clean wholesome water is highly essential in a bid to promote healthy living amongst the inhabitants of any defined geological region (Ndinwa *et al.,* 2012). In advance industrialized world, delivery of safe drinking water and sanitation technologies are, however, not affordable (Ashaye *et al*., 2001; Adekunle *et al*., 2004). Consequently, given the renewed global commitment towards the millennium development goals (MDG) marked for 2015, the importance and contribution of locally sourced, low cost alternative drinking water schemes to sustainable access in rural, sub-urban and urban settings of developing countries cannot be over emphasized (UNDESA, 2004).

The objective of this study was to evaluate the antibiotic susceptibility profile of bacterial isolates and water quality index of closed groundwater and open hand dug wells water sourced from Koko community located in Delta State, Nigeria.

Materials and methods

Sample collection

Water samples were collected from closed ground water wells (GW) and open hand dug wells (HDW) at different locations covering the geographic land mass of Koko community, from November to December 2016 and January to March 2017. The water samples were collected using sterile sampling bottles and transported to the laboratory for microbiological and physico-chemical analysis.

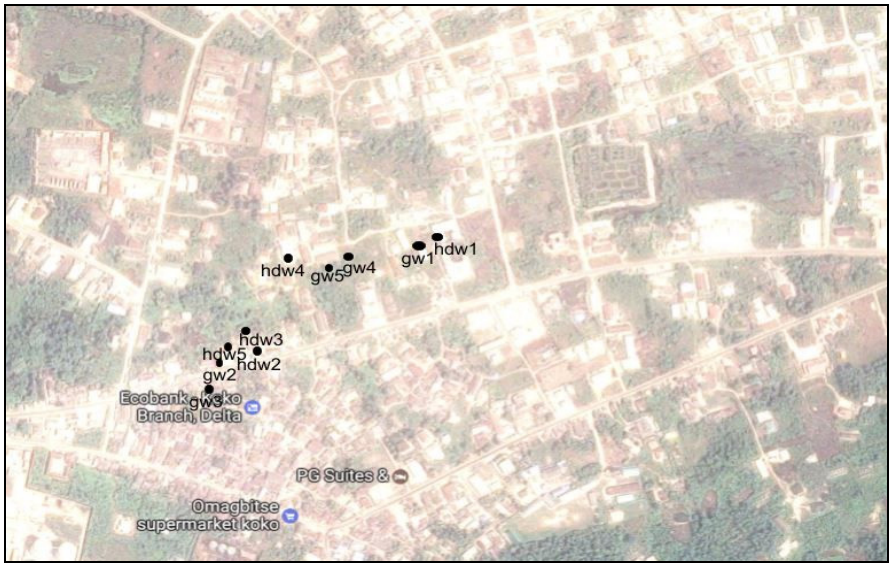

Figure 1. Google earth map indicating the respective sampled codified locations in Koko community, Delta State, Nigeria

Determination of heterotrophic bacterial and fungal counts:

Enumeration of the total viable bacterial and fungal counts were determined using serial dilution and pour plate methods as described by Harley and Prescott (2002). Media use was sterile Peptone Water as diluent, Nutrient Agar (for bacterial count) and Potato Dextrose Agar (for fungal count). Plating was done in duplicates and the Nutrient Agar plates were incubated at 35°C for 48h in an incubator while the Potato Dextrose Agar plates were incubated at room temperature for 5 days. Sub-culturing of representatives of the various colonies onto agar plates of the same media were made for purification. The bacterial isolates were Gram-stained (Cheesbrough, 2000). Phenotypic profiling of both Gram-positive and Gram-negative bacteria was

undertaken using API 50CHB and API 20E strips (BioMerieux, Marsielle, France). Additional tests of spore stain and oxidase were also performed. Observable colonial characteristics of the fungal isolates which were noted, microscopic observation of portions of their mycelia and spores using wet mount technique were used in identifying the fungal isolates. Observed fungal spores and mycelial fragment were compared to illustrations contained in Barnett and Hunter (1972).

Determination of coliform and *E. coli* counts:

These tests were carried out according to methods stated by Cheesebrough (2006) and were conducted in three stages: presumptive stage, confirmatory stage and completed stage.

Determination of antibiotic susceptibility profile of the bacterial isolates:

The antibiogram of the isolates was determined using the disc diffusion method (Harley and Prescott, 2002). The test bacterial isolates were inoculated unto Muller-Hinton Agar and followed by application of the discs (Oxoid) impregnated with different antibiotics. Antibiotic disc contained the following antibiotics: Ciprofloxacin (CPX, 10 µg), Chloramphenicol (CH, 30 µg), Sparfloxacin (SP, 10 µg), Amoxacillin (AM, 30 µg), Augmentin (AU, 30 µg), Gentamicin (CN, 30 µg), Pefloxacin (PEF, 10 µg), Streptomycin (S, 30 µg), Erythromycin (E, 10 µg), Ampiclox (APX, 30 µg), Zinnacef (Z, 20 µg), Ofloxacin (OFX, 5 µg) and Recephin (R, 25 µg). After 24 hours of incubation at 30 ^{o}C, the diameter of the zone of inhibition of each antibiotic disc was measured using a ruler and recorded.

Physicochemical analyses:

pH, electrical conductivity, turbidity, total suspended solids (TSS), total dissolved solids (TDS), dissolved oxygen (DO), biochemical oxygen demand (BOD_5) were analyzed according to standard analytical procedure (APHA, 1993, Ademoroti, 1996).

Evaluation of heavy metals:

The levels of iron (Fe), manganese (Mn), zinc (Zn), copper (Cu), chromium (Cr) and lead (Pb) were analysed using the atomic absorption spectrophotometer (AAS) (Buck Scientific, Model 210 VGP).

Evaluation of the water quality index of the sampled waters:

The WQI scores were generated using the Weighted Arithmetic Index method as described by Chatterjee and Raziuddin (2002).

$$WQi = \{[(Vactual - Videal) / (Vstandard - Videal)] \times 100\}$$

Table 1.

Water Quality Index (WQI) rankings

Range	Ranking/Status
0-25	Excellent
26-50	Good
51-75	Poor
76-100	Very poor
Above 100	Unsuitable for drinking

Source: Chatterjee and Raziuddin (2002)

Statistical analysis

The non-parametric analogue of the unpaired student T test was utilized to determine if the differences between the respective microbial and physicochemical data recorded for the ground water and hand dug well water samples was statistically significant ($p<0.05$).

The results of mean heterotrophic bacterial and fungal counts were in the order 10^2 to10^3 cfu/ml respectively. Generally, water samples from open hand dug wells had higher counts (5.4 x 10^3 to 8.6 x 10^3 cfu/ml) than ground water (table 2). Total microbial counts are important parameters which for indicative of the hygienic and portability properties of drinking water.

Table 2.

The heterotrophic microbial and coliform counts of the water samples

Sampling month	Water type	Mean HBC ($\times10^3$cfu/ml)	Mean HFC ($\times10^2$cfu/ml)	TCC (MPN/100ml)	*E. coli* count (MPN/100ml)
November 2016	GW	4.4	0.0	5	0.0l
	HDW	6.3	5.5	10	5
December 2016	GW	3.6	0.0	5	0.0
	HDW	5.4	3.5	10	4
January 2017	GW	4.2	4.0	7	0.0
	HDW	6.5	7.5	10	5
February 2017	GW	2.9	4.0	5	0.0
	HDW	8.0	9.0	10	4
March 2017	GW	3.7	6.0	8	0.0
	HDW	8.6	17.0	20	8
p-values	-	0.022	0.000	0.015	0.000

KEY: GW = Closed groundwater sample, HDW = hand dug well, HBC = Heterotrophic bacterial count, HFC = Heterotrophic fungal count, TCC = Total coliform count

The results showed that the greatest frequency was observed for *Bacillus* spp. (30.1 %) and the least predominant among the bacterial isolates was *Escherichia coli* (6.0 %). Regarding the fungal isolates *Aspergillus flavus* was the most predominant (26.5 %) and *Rhizopus stolonifer* (11.8 %) was the least predominant (table 3). Bacterial isolates identified in the water samples include: *Micrococcus* sp.*, Klebsiella* sp., *Escherichia coli, Bacillus* sp.*, Pseudomonas aeruginosa* and *Aeromonas hydrophila*. Fungal isolates identified in the water samples collected include *Aspergillus niger, Penicillium notatum, Aspergillus flavus, Rhizopus stolonifer* and *Saccharomyces cerevisiae.* These microbial organisms are of public health importance. The high microbial load and variety of microorganisms detected and isolated from these water samples may be attributed to the poor sanitary and water handling practices.

Table 3.

Percentage frequency of occurrence of bacterial and fungal isolates from the respective water samples

Bacterial isolate	**No. of bacterial isolates**	**% frequency of occurrence**	**Fungal isolate**	**No. of fungal isolates**	**% frequency of occurrence**
Micrococcus spp.	18	21.7	*Aspergillus niger*	8	23.5
Klebsiella spp.	7	8.4	*Penicillium notatum*	8	23.5
Escherichia coli	5	6.0	*Aspergillus flavus*	9	26.5
Bacillus spp.	25	30.1	*Rhizopus stolonifera*	4	11.8
Pseudomonas aeruginosa	8	9.6	*Saccharomyces cerevisiae*	5	14.7
Aeromonas hydrophila	20	24.1			
	n =83	**100**		n =34	**100**

Variable antibiotic susceptibility patterns were observed in the antibiotic inhibitory zones (mm) among the tested bacterial isolates (Table 4). Findings showed that seven (7) antibiotics did not exhibit inhibition zones against tested Gram negative bacterial isolates (*Klebsiella* sp., *Aeromonas hydrophila, E.coli* and *Pseudomonas aeruginosa*) where as five (5) antibiotics showed no inhibition against Gram positive bacterial isolates (*Micrococcus* and *Bacillus*). The widest zone of inhibition was observed for ofloxacin (30.0 mm) against *Klebsiella* sp., which indicates susceptibility of the bacterial isolate. This observation could be ascribed to the fact that many bacteria isolated from the surface water possess an important ecological quality, namely that of resistance plasmids, which can be picked up in the course of selective processes. Researches had reported the increased incidences of bacterial resistance to antibiotics which contribute to the prevailing trend of antibiotic abuse and misuse by a larger human population most especially in the developing world (Omogbai and Ikenebomeh, 2013, Imarhiagbe *et al*., 2016). Spanggard *et al.* (1993) had earlier observed the possibility of members of bacterial groups residing in a common environment to express a similar antibiotics pattern if they share in a common pool of R-factor plasmids.

Table 4.

Zone(s) of inhibition (mm) representing antibiotic sensitivity/resistance patterns elicited by the bacterial isolates against the test drugs

Gram +ve	PEF (10 µg)	CN (30 µg)	APX (30 µg)	Z (20 µg)	AM (30 µg)	R (25 µg)	CPX (10 µg)	S (30 µg)	SXT (30 µg)	E (10 µg)
(1)	0.0	13.0	0.0	17.0	0.0	20.0	24.0	0.0	0.0	11.0
(2)	19.0	25.0	0.0	20.0	12.0	28.0	30.0	14.0	0.0	18.0
Gram -ve	**SXT(30 µg)**	**CH (30 µg)**	**SP (10 µg)**	**CPX (10 µg)**	**AM (30 µg)**	**AU (30 µg)**	**CN (30 µg)**	**PEF (10 µg)**	**OFX (5 µg)**	**S (30 µg)**
(3)	0.0	08.0	0.0	18.0	0.0	14.0	20.0	0.0	27.0	15.0
(4)	0.0	0.0	0.0	15.0	0.0	0.0	16.0	0.0	20.0	0.0
(5)	0.0	0.0	0.0	20.0	0.0	0.0	18.0	0.0	25.0	0.0
(6)	0.0	13.0	15.0	22.0	0.0	12.0	20.0	10.0	30.0	10.0

KEY: (1) *Micrococcus* sp., (2) *Bacillus* sp., (3) *Aeromonas hydrophila*, (4) *Pseudomonas aeruginosa*, (5) *E. col, (6) Klebsiell*a sp. Ciprofloxacin (CPX), Chloramphenicol (CH), Sparfloxacin (SP), Amoxacillin (AM), Augmentin (AU), Gentamicin (CN), Pefloxacin (PEF), Streptomycin (S), Erythromycin (E), Ampiclox (APX), Zinnacef (Z), Ofloxacin (OFX) and Recephin (R) **S**= Streptomycin.

Table 5 revealed the result of the physicochemical analysis of the water samples from closed groundwater and open hand dug well from Koko community. pH value is significant determinant for the suitability of water for several purposes. pH of the groundwater samples and hand dug well water samples ranged from 4.40-5.741 and 4.83-5.54 respectively. The findings show that water from this location is slightly acidic; this observation may be due to the geological conditions (King and Ekeh, 1990). Electrical conductivity for groundwater samples and hand dug well water samples ranged from 52.7-86.2 µS/cm and 145.3-330 µS/cm respectively. Measurement of turbidity has been described as a very important parameter when evaluating the quality of a drinking water. Findings from this study revealed that water sourced from closed ground water and open hand dug wells had a mean 0.0 and 2.82 NTU respectively. The highest values of TDS and TSS were observed in open hand dug wells (4.54 mg/L and 98.10 mg/L respectively). Dissolved oxygen for both groundwater samples and hand dug well water samples ranged from 5.5-7.1 mg/L and 4.5-6.6 mg/L respectively. BOD_5 for groundwater samples and hand dug well water samples ranged from 0.4-1.1mg/L and 1.9-4.7mg/L. Also heavy metal analysis of water samples from closed groundwater and open hand dug wells is indicated in Table 5.

Table 5.

Physicochemical analysis of water samples from closed Groundwater (GW) and open Hand Dug (HDW) wells from Koko community

Parameter	Water type	Month (A)	(B)	(C)	(D)	(E)	(F)	(G)
pH	GW	5.19	5.74	4.54	4.40	4.16	4.81±0.65	6.5-8.5
	HDW	5.54	4.83	5.12	4.92	5.22	5.13±0.28	
EC (µS/cm)	GW	60	57.8	63.8	52.7	86.2	64.1±12.99	NA
	HDW	330	152.6	174.1	145.3	193.1	199.02±75.6	
Turbidity (NTU)	GW	0.0	0.0	0.0	0.0	0.0	0.00±0.00	5.0
	HDW	0.0	2.7	8.8	0.0	2.6	2.82±3.59	
TSS (mg/l)	GW	0.0	0.0	0.0	0.0	0.0	0.00±0.00	500
	HDW	1.2	5.1	9.2	2.1	5.1	4.54±3.14	
TDS (mg/l)	GW	31	28.9	31.7	28.1	42.9	32.52±5.99	500
	HDW	160	76.1	86.3	72.3	95.8	98.10±35.79	
DO (mg/l)	GW	5.9	6.3	5.5	6.8	7.1	6.32±0.65	NA
	HDW	6.1	4.5	5.2	5.9	6.6	5.66±0.82	
BOD_5 (mg/l)	GW	0.4	0.0	0.0	1.5	1.1	0.60±0.67	NA
	HDW	4.7	2.3	1.9	3.7	4.8	3.48±1.34	
p-values	-	0.000	0.000	0.000	0.000	0.000	0.000	-

(A) November 2016, (B) December, 2016, (C) January, 2017, (D) February 2017, (E) March 2017, (F) Mean ± S.D, (G) WHO limit, (NA) Not available

Table 6.

Water Quality Index of closed groundwater and open hand dug sources

	Closed ground water (GW)					Open hand dug wells (HDW)				
Parameter	V_i	S_i	W_i	q_i	W_iq_i	V_i	S_i	W_i	q_i	W_iq_i
pH	4.81	6.5-8.5	0.22	0	0	5.13	6.5-8.5	0.22	0	0
EC (µS/cm)	64.1	250	0.37	25.6	9.51	199.02	250	0.37	79.61	29.54
TDS (mg/L)	32.5	500	0.004	6.50	0.02	98.10	500	0.004	19.62	0.07
TSS (mg/L)	0.00	500	0.003	0	0	4.54	500	0.004	0.91	0.003
DO (mg/L)	6.32	5	0.37	86.3	32.11	5.66	5	0.37	93.13	34.67
BOD_5 (mg/L)	0.60	5	0.37	12.0	4.47	3.48	5	0.37	69.60	25.91
			ΣW_i= 1.34		ΣW_iq_i= 46.1			ΣW_i= 1.34		ΣW_iq_i = 90.2
WQI	$(\Sigma W_iq_i / \Sigma W_i) = 46.1/1.34 =$ **34.4**					$(\Sigma W_iq_i / \Sigma W_i) = 90.2/1.34 =$ **67.31**				
Ranking	**Good**					**Poor**				

Observed (Vi), Standard values (Si), Unit weights (qi), and quality rating (qi)

The observed water quality was supported by the findings from the analytical physico-chemical parameters (Table 6). Water quality index (WQI) value obtained closed groundwater sources and open hand dug wells was 34.4 and 67.31 respectively. The derived water quality index of the respective water

samples revealed that the overall water quality of the samples sourced from open hand dug wells was poor as compared with closed ground water. Based on the WQI values of the samples, it can be inferred that the water samples from open hand dug wells are unfit for direct consumption by the inhabitants of this community.

Table 7.

Levels of heavy metals (mg/l) in water samples collected from closed groundwater and open hand dug wells sited in Koko town, Delta State

Parameter	Water type	Month						
		(A)	(B)	(C)	(D)	(E)	(F)	(G)
Fe	GW	0.28	0.15	0.54	0.26	0.17	0.28±0.156	0.30
	HDW	0.62	0.79	1.12	0.84	0.62	0.80±0.205	
Mn	GW	0.01	0.01	ND	0.01	ND	0.004±0.005	0.1
	HDW	0.05	0.01	0.14	0.09	0.055	0.07±0.049	
Zn	GW	0.08	0.03	0.18	0.07	0.09	0.09±0.055	5.00
	HDW	0.17	0.24	0.62	0.45	0.13	0.32±0.207	
Cu	GW	ND	0.01	ND	0.004	ND	0.0028±0.004	1.0
	HDW	0.02	0.02	0.04	0.061	0.027	0.03±0.017	
Cr	GW	ND	ND	ND	ND	ND	0.00±0.00	0.050
	HDW	0.003	ND	0.05	0.032	0.019	0.02±0.021	
Pb	GW	ND	ND	ND	ND	ND	0.00±0.00	0.01
	HDW	ND	ND	0.02	0.005	ND	0.005±0.009	
p-values	-	0.001	0.001	0.001	0.005	0.001	0.001	

Key: (A) November 2016, (B) December, 2016, (C) January, 2017, (D) February 2017, (E) March 2017, (F) Mean ± S.D, (G) WHO limit, iron (Fe), manganese (Mn), zinc (Zn), copper (Cu), chromium (Cr) and lead (Pb)

Generally, heavy metal levels were below recommended permissible levels by World Health Organisation for drinking water. Iron concentration in both groundwater samples and hand dug well water samples ranged from 0.15-0.28mg/L and 0.62-1.12 mg/L (Table 7).

Conclusions

The results obtained from this research indicate that the water samples collected from this community had poor microbiological and overall Water Quality indices and therefore is unsuitable for direct human consumption. Several measures which include boiling, filtration and addition of flocculants such as aluminium salts would invariably enhance the potability of the water and reduce the risk of developing

water borne gastroenteritis which could arise from the direct consumption of the contaminated water. The entitled authorities ought to ensure proper awareness for treatment of the water sources to safeguard the public health of the population.

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