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ISOLATION AND CHARACTERIZATION OF RESISTANT BACTERIAL SPECIES ISOLATED FROM SHALLOW WELL WATER SITUATED CLOSE TO GRAVES AS A PUBLIC HEALTH MENACE IN OSOGBO, OSUN STATE

Ozabor P.T.[1,2]*, Oluwajide O.O.[1], Akeju A.O. [1], Onifade S.J.[1], Olaniyan S.O.[1,3], and Olaitan, J.O.[1]

[1]*Department of Microbiology, Osun State University, Osogbo*
[2]*Food Microbiology and Biotechnology Unit, Department of Microbiology, University of Ibadan*
[1,3]*Department of Biology, Baylor University, Texas, United States of America*

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This study was designed to evaluate the physicochemical and bacteriological qualities of well water situated close to graves. Total heterotrophic bacterial count (THBC), total coliform count (TCC) and fecal coliform count (FC) were done on nutrient agar, MacConkey and Eosine methylene blue agar, respectively, using spread plate method at incubation conditions of 37°C for 24 hours for THBC and TCC whereas 44.5°C for 24-48 hours for fecal coliforms. Membrane filtration was used for *Vibrio* spp. count on TCBS agar while conventional biochemical method was performed for bacterial identification. Isolated bacteria were subjected for antimicrobial resistant testing using 8 families of antibiotics. Bacteria with MAR index value of ≥75 were sent for genomic identification and sequencing. The average value of the ten water samples analyzed over the period of study had high total suspended solids (mg/l), phosphate (mg/l); magnesium ion (mg/l) contents and a very low dissolved oxygen mg/L content. The THB count was (5.0×10^7-1.35×10^8 CFU/ml), TCC count (3.55×10^5-1.04×10^6 CFU/ml), FC count (2.10×10^5-6.90×10^5 CFU/ml) while *Vibrio* spp. count was 45-144 CFU/100 ml and the MPN estimated reading for coliform was also high. The percentage occurrence and MAR index value of the bacteria isolated from the well water, respectively were *Vibrio* spp. 23%; 75, *Klebsiella* spp. 20%; 100, *Bacillus* spp. 14%; 100, *Staphylococcus* spp. 14%; 62.5, *E. coli* 12%; 62.5, *Pseudomonas* spp. 12%; 75, *Glycomyces* spp. 3%; 100 and *Proteus* spp. 3%; 50. The closeness of the well to the grave makes the decomposing leachates with high organic, inorganic, biological and poisonous metals sink into the underground aquifers.

Key words: Antibiotic resistant bacteria, physicochemical parameters, coliforms, most probable number, biological oxygen demand

INTRODUCTION

Water is one of the utmost indispensables as well as abundant compounds of the ecosystem. Planet earth have nearly 71 % of water needed by living organisms for subsistence and growth, of which 96.5% of the planet's water is found in seas and oceans, 1.7% in groundwater, 1.7% in the glaciers and the ice, a lesser fraction in other large water bodies, and 0.001% in the air as vapor, clouds and precipitation. 2.5% of the Earth's water is fresh water and 99% of that water is in ice and groundwater (1). A lesser amount of 0.3% of all freshwater is in rivers, lakes, and the atmosphere.

Nevertheless, increased human population and persistent man-made activities make water bodies highly contaminated with different detrimental pollutants. This therefore calls for an urgent necessity for safe drinking water to prevent the outbreak of various water borne diseases as well as improve quality of lives (2).

Groundwater is any water that lies beneath the earth's surface in pore spaces and within fractures of rock formations. Groundwater is usually regarded as great sources of water because it looks clear and clean (1). This is because it runs through so many layers of rocks and sediments which serve as a sort of natural filtration system.

The quality of ground water can be influenced by the non-presence and low concentration of various chemical constituents, heavy elements, metal ions and harmful microorganisms, physicochemical parameters such as: color, temperature, acidity, hardness, pH, sulphate, chlorides, DO, BOD, COD and alkalinity are important for testing of water quality. Heavy metals such as Pb, Cr, Fe, Hg are of special concern because they produce chronic poisons into the water bodies (3). Distances for setting well water to sources of contamination are not usually considered in the local communities of less-developed and developing countries. Grave involved numerous contaminating materials; buried body might seem harmless but start to decompose within the ground and releases potion water up to 30 to 40 liters characterized with high conductivity, pH and biochemical oxygen demand (BOD). Leaching of soils from necrosol (necroleachate) from burial and different constituents

***Corresponding Author**: Mailing address. Ozabor P.T., Department of Microbiology, Osun State University, Osogbo. E. mail: praise.ozabor@uniosun.edu.ng, Contact: +2347035235241, ORCID: 0000-0003-0047-8242.*

related with decomposition and burial processes such as embalming fluid which contains arsenic, mercury and formaldehyde (carcinogenic materials), artifacts; bones, varnishes, treated coffin, textile elements and jewelries, such increases the physical and chemical properties of cemetery soils with an increment in the of range of chemical, bacteria, and viruses that lead to changes in the soil profile and soil original constituents (4, 5). The necroleachate is high in pungent odor, ammoniac nitrogen, organic matter and releases salts into the surrounding soil and well water (6) leading to water contamination and lower water quality of wells situated around it (4, 5).

These 'necroleachates' also contain pathogenic bacteria (such as *E. coli, Enterobacter, Citrobacter, Klebsiella, Vibrio, Proteus, Salmonella, Staphylococcus,* etc.), fungi (such as *Aspergillus* spp., *Candida* spp., *Fusarium* spp.), parasites and viruses (7, 8). However, these pathogenic microorganisms associated with decomposition of interred bodies can cause dangerous infectious diseases such as tetanus, toxic contamination of food, paratyphoid fever, kidney inflammation and even death. Grave can then be considered landfills of sorts, as there is a higher than normal concentration of potentially contaminative materials located in one place (9, 10).

Considering all the factors stated above, the current study was undertaken to the investigate the bacteriological and physicochemical properties of shallow well water situated near graves as a public menace in selected sites in Osogbo, Osun State.

MATERIALS AND METHODS

Study Site. This study was carried out on ten (10) nucleated communities where ground wells are situated beside grave in communities within the Osogbo Local Government, Osogbo, Osun State, South-western Nigeria from the month of June to November 2020 as described in table 1. Osogbo is the capital of Osun State, located in Southwestern part of Nigeria which lies between longitude 7º46'N and latitude 4º34'E and share boundaries with Ikirun, Ilesa, Ede, Egbedore, Ogbomoso and Iragbiji and is easily accessible from any part of the state because of its central nature (11, 12). The city has a population of about 4,99,999 according to the 2006 census figures and an approximate land area of 2875 km^2 and 1,1050 ft elevation (13). The climate is characterized by wet season (warm, oppressive and overcast) followed by a dry season (hot, muggy and partly cloudy), with an annual average of temperature which ranges from 65ºF to 93ºF.

Collection of Water Samples. A total of 60 water samples were collected from ten (10) different well water sites situated close to graves at highly populated communities in Gbonmi, Odu, Oyekomi, Oke-baale, Sekoni, Alagbe and Balogun area located in Osogbo, Osun state, South West, Nigeria over the period on the same well at a sample in a month. The samples were collected using clean ethanol pre-sterilized drawer in to 1l pre-sterilized gallon sealed and labeled appropriately. The samples were immediately transported in a cooler with ice packs to the Microbiology laboratory of Osun State University for initial processing (14).

Physico-chemical Analysis of water samples. The physicochemical parameters such as: pH, temperature, electrical conductivity, and dissolved oxygen were measured in situ using standard instruments, total alkalinity, total hardness, calcium hardness, magnesium hardness, chloride test, bicarbonate test, Nitrate nitrite ion, flocculation test, chlorine residual test, total non-filterable solid test, carbonate test, bicarbonate test, presence of trace element, turbidity test, color test and biochemical oxygen demand were done with standard instruments and procedures at the point of collection and in the laboratory throughout the study period.

Isolation and enumeration of Bacteria

Spread plate method and Membrane filtration. A hundred microliter (100 µl) of each dilution factor 4 and 6 of the samples were inoculated into already sterile solidified nutrient agar, Eosine methylene blue agar and MacConkey agar plates. The inoculums were spread evenly with sterile spreader, left on the bench for 30 minutes to diffuse, inverted and incubated at 37°C for 24 hours and 42°C for 24-42 hours for fecal coliforms on MacConkey agar. 100 ml of each water sample were filtered through 0.45µm Millipore filter with the use of pre-sterilized membrane filtration unit machine, the filters were placed on Thiosulfate-citrate-bile salts-sucrose agar (TCBS) for *Vibrio* spp. count and MacConkey agar plates and incubated at 37°C for 24 hours for total coliform count (15, 16). Most probable number (MPN) method was employed using multiple fermentation tubes, presumptive isolation of coliform bacteria was made on MacConkey broth (17). After incubation at 37°C for 48 hours, the tubes with acid and gas were considered positive for coliforms. Positive tubes from MPN were determined following standard probability table as described by (18). In addition, the presence of *Escherichia coli* was confirmed by streaking a loopful of broth onto Eosine Methylene Blue (EMB) agar and evaluating for the formation of metallic green sheen color, a positive test for presence of *E. coli.*

Identification and characterization of bacterial isolates. Viable counts of the bacteria were determined after incubation, distinct colonies from each nutrient agar, Thiosulfate-citrate-bile salts-sucrose agar, Eosine methylene blue agar and MacConkey agar plates were counted and recorded in CFU/ml. Discrete colonies were picked for sub culturing onto prepared agar plates aseptically to get distinct colonies and stocked in agar slants for further experiments. Isolates were presumptively identified based on cultural (shape, color, opacity, elevation, colony size), morphological (Gram staining test) and biochemical tests (catalase, oxidase, citrate utilization test, indole, sugar fermentation and triple sugar iron tests) characteristics (15, 16, 19). *E. coli* was confirmed by the use of Eosine methylene blue agar which differentiated *E. coli* from other Gram-negative bacteria. The biochemical tests were interpreted to determine the presumptive nomenclature of the potential bacteria isolates through Bergey's Manual of Determinative Bacteriology and ABIS online (Advanced Bacterial Identification Software).

Antibiotic Susceptibility Testing of Bacteria Isolates. The antibiotic susceptibility testing was performed with the Kirby-Bauer agar disc diffusion method. Three (3) to five (5) colonies from 18 to 24-hour-old culture of each isolate were inoculated into 5 ml of sterile Ringer's solution to give a turbidity equivalent to 0.5 McFarland standards. A sterile cotton-tipped applicator was inserted into each inoculum tube, and swabbed onto the entire surface of the Mueller-Hinton agar plates, the antibiotics used in this study (Oxoid) includes: Oxacillin (1 mg), Cefepime (30 mg), Azithromycin (15 mg), Kanamycin (30 mg), Aztreonam (30 mg), Colistin sulfate (10 mg), Tigercycline (75 mg) and Doripenem (10 mg) were placed aseptically on the surface of the inoculated agar plates, using an 8-place disc dispenser on the plates which was then incubated at 37±2°C overnight (20). The diameters of the zone of inhibition on the plates were measured to the nearest millimeter and results interpreted as Sensitive (S) and Resistant (R) through a standard table EUCAST breakpoint tables version 11.0. Resistance to ≥1 antibacterial agent in ≥3 classes of antibiotics was used as an indicator of multidrug resistance (MDR).

RESULTS

Table 1 shows the description of the wells used in this study. Depth of the well water surveyed were very shallow, not more than 16 ft without cement rings inside, which makes percolation of organic and inorganic compounds in and out of the well and the surroundings easy, besides the distance of those well to aged grave and even public defecation and dump sites were as close as anything.

Physiochemical parameters and quantity of trace element of the well water survey were analyzed using standard procedures and compared with WHO and NIS-2015 standard (WHO, 2015). The average value of the ten-water sampled over the period of study were having high Total suspended solids (mg/l), Phosphate (mg/l); Magnesium ion (mg/l) content and a very low Dissolved oxygen (mg/l) content as depicted in Table 4.

The THBC ranged from 5.0×10^7-1.35×10^8 CFU/ml, TCC (3.55×10^5-1.04×10^6 CFU/ml), FC count (2.10×10^5-6.90×10^5 CFU/ml) while *Vibrio* spp. count was 45-144 CFU/100 ml and the MPN estimated reading for coliform was also high as shown in tables 2 and 3. All isolated bacteria were subjected for antimicrobial resistant testing using eight families of antibiotics. Bacteria with MAR index value of ≥75 were sent for genomic identification and sequencing. Figures 1 and 2 revealed the percentage occurrence and MAR index values of the bacteria isolated from the

well water respectively were: *Proteus* spp. 3%; 50, *Glycomyces* spp. 12%; 75, *Pseudomonas* spp. 12%; 75, *E. coli* 12%; 62.5, *Staphylococcus*sp 14%; 62.5, *Bacilllus* spp. 14%; 100, *Klebsiella* spp. 20%; 100 and *Vibrio* spp. 23%; 75.

Table 1: Description of the sampling sites.

Sampling Location	Sample Code	Latitude	Longitude	Well depth (ft)	Well Closeness to grave (m)
Obalende	OB	N7º 4620'	E4º3400.3288'	≤ 12ft	2
Alagbede	ALA	N7°4618.5916'	E 4°3402.5176'	≤ 10ft	2.5
Sekoni,	SK	N7°4615.3012'	E4° 3406.3588'	≤ 12ft	2
Biro	BR	N7°4615.1068'	E4° 3418.0912'	≤ 10ft	3
Ile – Oga	LG	N7°4616.5072'	E4° 3409.537'	≤ 14ft	2.5
Olu-Ajo	LA	N7º4616.4676'	E 4º3402.3052'	≤ 12ft	3
Odu	OD	N7º4614.3724'	E 4º3409.5772'	≤ 11ft	3
Oyekomi	OK	N7º4619.0272'	E4º3401.8228'	≤ 12ft	2
Gbonmi	GB	N7º4611.4924'	E 4º3415.1932'	≤ 16ft	3.5
Balogun	BG	N 7º4614.538'	E 4º3420.2008'	≤ 12ft	3

Table 2: Microbial growth count of targeted organisms in the well water samples.

Sample Code	THBC count (CFU/ml)	TCC count (CFU/ml)	FC count (CFU/ml)	*Vibrio* count (CFU/100 ml)
OB	1.15×10^{8}	8.3×10^{5}	5.6×10^{5}	6.6×10^{1}
ALA	1.05×10^{8}	1.04×10^{6}	6.2×10^{5}	1.28×10^{2}
SK	9.4×10^{7}	1.02×10^{6}	6.8×10^{5}	1.44×10^{2}
BR	7.9×10^{7}	9.3×10^{5}	5.8×10^{5}	1.01×10^{2}
LG	8.7×10^{7}	1.04×10^{6}	6.0×10^{5}	1.12×10^{2}
LA	1.22×10^{8}	6.5×10^{5}	4.1×10^{5}	9.5×10^{1}
OD	1.35×10^{8}	7.5×10^{5}	3.8×10^{5}	9.0×10^{1}
OK	8.0×10^{7}	1.05×10^{6}	6.9×10^{5}	5.5×10^{1}
GB	5.0×10^{7}	3.55×10^{5}	2.1×10^{5}	4.5×10^{1}
BG	6.0×10^{7}	6.0×10^{5}	3.4×10^{5}	5.5×10^{1}

Note: THB: Total heterotrophic bacteria; TC: Total coliform; FC: Fecal coliform; CFU/ml: colony forming unit per ml.

Table 3: Most Probable Number (MPN) analysis of the water samples.

Sample Code	Positive tubes out of five tubes in each inoculated			MPN/100 ml values
	10 ml	1 ml	0.1 ml	
OB	2	0	0	5
ALA	3	4	1	25
SK	3	2	0	14
BR	2	1	0	7
LG	3	2	0	14
LA	1	0	0	2
OD	1	0	0	2
OK	3	4	1	25
GB	0	2	0	4
BG	1	2	1	8

Note: Estimated number of coliform bacteria present in 100ml water sample collected with interpretation using BSI (1991) BS 5763.

Table 4: Summary of average value of physicochemical parameters and trace elements on water samples collected from the sampling sites and comparison with standard.

Parameters	**WHO standard**	**NIS 2015**	**Obalende (OB)**	**Alagbede (ALA)**	**Sekoni (SK)**	**Biro (BR)**	**Ile – Oga (LG)**	**Olu-Ajo (LA)**	**Odu (OD)**	**Oyekomi (OK)**	**Gbonmi (GB)**	**Balogun (BG)**
Color TCU		15										
Odor and taste		Unobjectionable						Unobjectionable				
Conductivity (µS/cm)	1000	1000	23.00	10.00	67.00	17.7	74.00	16.00	24.00	39.00	98.00	149.60
Temperature (C)	--	Ambient	30.9	30.6	31.4	31.2	30.9	30.6	31.4	31.7	30.9	31.60
Alkalinity (mg/l)	100		16.00	15.00	24.00	100.0	24.00	24.00	43.30	34.00	72.0	16.0
pH	6.5-8.5	6.50 - 8.50	7.0	7.4	7.5	7.0	7.2	7.0	7.1	7.1	7.2	7.3
Total hardness (mg/l)	500	150	90.00	78.00	89.00	115.00	95.00	80.00	90.21	78.00	105.10	73.00
Calcium hardness (mg/l)	75		24.00	23.00	26.00	80.00	62.00	23.00	35.00	53.71	54.0	24.00
Magnesium hardness (mg/l)	<50 – 150		37.00	35.00	38.00	36.00	28.00	35.00	38.00	36.00	50.00	48.00
Chloride (mg/l)	250	250	1.0	0.0	2.0	2.5	0.0	2.50	0.00	1.00	0.00	0.00
Total dissolved solids(mg/l)	500	500	-	-	-	-	-	-	-	-	-	-
Total suspended solids(mg/l)	40		457.9	453.4	458.1	669.2	512.9	374.3	393.5	411.2	574.8	434.4
Turbidity (NTU)	5	5	4.56	3.55	5.58	3.28	2.81	2.81	5.58	3.55	2.49	5.57
Iron (mg/l)	0.1	0.3	0.00	0.00	0.10	0.11	0.00	0.11	0.00	0.00	0.00	0.70
Phosphate (mg/l)	0.5	NA	8.70	6.00	12.00	0.33	0.13	6.70	8.50	4.60	4.40	1.60
Nitrate (mg/l)	50	50	0.04	0.03	0.06	0.025	0.00	0.06	0.02	0.31	0.00	0.05
Copper (mg/l)	1	1	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.10	0.01
Zinc (mg/l)	5	3		0.05	0.08	0.22	0.03	0.02	0.04	0.22	0.50	0.06
Chromium (mg/l)	NA	0.05	0.001	0.00	0.002	0.06	0.02	0.004	0.001	0.004	0.003	0.001
Lead		0.01	-	-	-	-	-	-	-	-	-	
Magnesium ion (mg/l)	-	0.200	10.50	9.22	12.00	9.0	7.0	12.00	8.00	10.00	11.00	12.00
Dissolved oxygen mg/l	≥ 6.5-8	≥ 6.5-8	4.3	3.8	5.6	3.50	3.80	4.2	3.4	5.3	3.8	4.3
Chemical oxidation (mg/l)			3.0	2.0	5.0	7.0	0.00	2.0	1.0	3.0	4.0	0.00
Biological Oxygen Demand (mg/l)		< 5	0.23	0.22	0.25	0.20	0.13	0.21	0.20	0.22	0.25	0.40
Manganese(mg/l)		0.2	0.001	0.000	0.002	0.002	0.00	0.001	0.003	0.002	0.002	0.10
Aluminum (mg/l)		0.2	0.01	0.00	0.02	0.01	0.00	0.10	0.20	0.10	0.20	0.01
Sulphate (mg/l)		100	8.00	7.00	10.00	10.00	0.00	10.0	7.00	5.00	4.00	0.00
Potassium (mg/l)			0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Flocculation (PPM)			26.00	25.00	28.00	20.00	25.00	25.00	27.00	26.00	21.00	20.00
Silica (mg/l)			0.05	0.04	0.09	0.38	0.09	0.09	0.42	0.06	0.04	0.10
Total coliform count cfu/ml		10	83×10^4	1.04×10^6	1.02×10^6	9.3×10^5	1.04×10^6	6.5×10^5	7.5×10^5	1.05×10^6	3.55×10^5	6.0×10^5
Fecal coliform count cfu/100 ml		0	5.6×10^4	6.2×10^5	6.8×10^5	5.8×10^5	6.0×10^5	4.1×10^5	3.8×10^5	6.9×10^5	2.1×10^5	3.4×10^5

Note: WHO: World Health Organization; NSDWQ: Nigerian Standard for Drinking Water Quality (NIS 554: 2015).

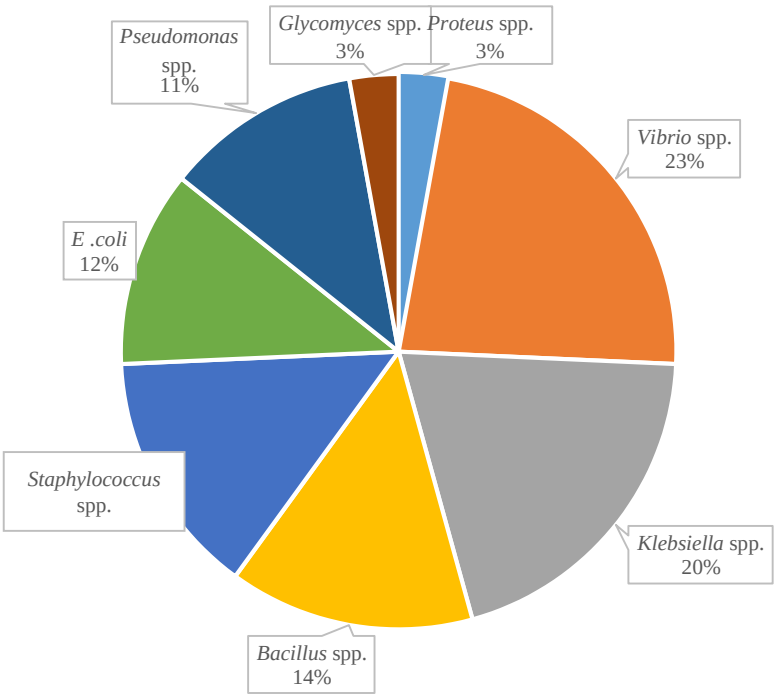

Figure 1: Percentage Occurrence of bacteria in surveyed well water samples.

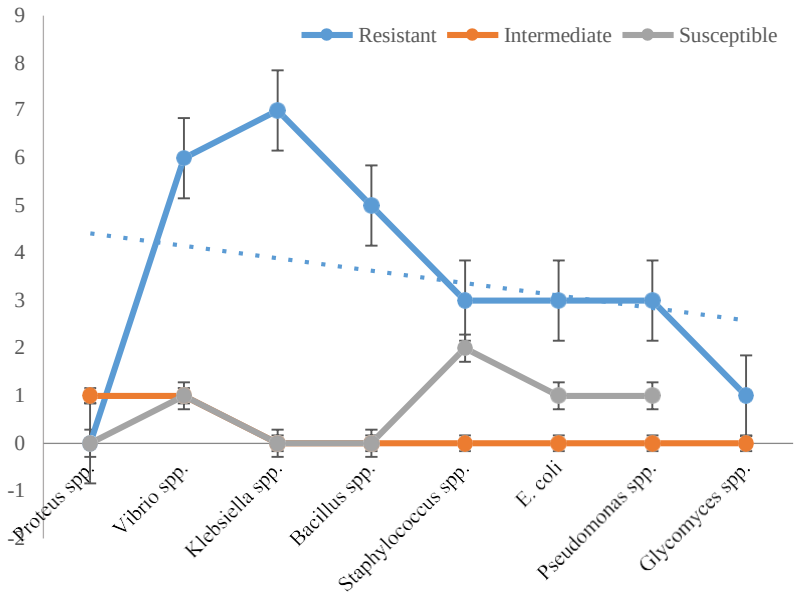

Figure 2: MAR index of the bacteria isolates from the well water surveyed.

DISCUSSION

According to the reports of Kumar and Kumar (2013), shallow groundwater has the tendency of contamination from the surroundings as they documented that nutrients and other constituents connected with burial decompositions affects nearby drinking water sources and ground water aquifers.

Eight (8) species of bacteria were isolated namely: *Proteus* spp., *Vibrio* spp., *Klebsiella* spp., *Bacillus* spp., *Staphylococcus* spp., *Escherichia coli*, *Pseudomonas* spp. and *Glycomyces* spp. (22, 23) had earlier documented the species of bacteria and fungi isolated from necrosols collected from urban cemeteries in Poland to be: *B. cereus, B. megaterium, Enterococcus faecalis, Escherichia coli, Serratia megaterium, Staphylococcus epidermidis* and coagulase negative staphylococci (CNS). This is in line with some of the bacterial organisms isolated in this work. Previous reports also documented the isolation of similar organisms from water sources situated close to grave sites (24, 25). Rodrigues and Pacheco (2010) and Kutlu *et al.* (2015) also documented the isolation of *E. coli*, *Enterococcus* spp., *Staphylococcus* spp., and *Glycomyces* spp. from funeral decomposed artifacts such as: jewelry, metal dental fillings and caskets. In addition, Adesakin *et al*. (2020) predominantly identified *Enterobacteriaceae* such as: *Pseudomonas* spp., *E. coli*, *Enterococcus* spp., *Vibrio* spp. and *Bacillus* spp. in domestic water samples situated close to burial grounds. This is in line with some of the organism isolated from this study. These pathogenic organisms are associated with the gut and skin microbiome which can consequently be released into the environment through decomposition of man or animal.

From the results of the total heterotrophic bacteria count (THBC), total coliform count (TCC), fecal coliform (FC) and the twenty-seven (27) different physicochemical parameters conducted in this study, namely: color, taste, odor, conductivity (10.00-149.60 µs/cm), temperature (30.6-31.7℃), alkalinity (15.00-100.00 mg/l), pH (7.0-7.5), total hardness (78.00-115.00 mg/l), calcium hardness (23.00-80.00 mg/l), magnesium hardness (28.00-48.00 mg/l) , chloride (0.00-2.50 mg/l), total dissolved solids (nil), total suspended solids (374.3-572.8 mg/l), turbidity (2.81-.5.58 NTU), iron (0.00-0.70 mg/l), phosphate (0.13-12.00 mg/l), nitrate (0.00-0.06 mg/l), copper (0.00-0.10 mg/l), zinc (0.02-0.22 mg/l), chromium (0.00-0.06 mg/l), lead (nil), magnesium (7.0-12.00 mg/l), dissolved oxygen (3.50-5.6 mg/l), chemical oxidation (0.00-7.00 mg/l), biological oxygen demand (0.13-0.25 mg/l), manganese (0.00-0.03 mg/l), aluminum (0.00-0.20 mg/l), sulphate (0.00-10.00 mg/l), potassium (nil), flocculation (20.00-28.00 PPM) and silica (0.04-0.42 mg/l). It could be deduced that the water samples were high in total suspended solids, phosphate and magnesium while the total dissolved oxygen was observed to be low. However, the reports of (28, 24) can be compared to the results obtained in this work. They reported the following physicochemical parameters from shallow wells around cement factories which are also vital points of water contamination in the society: turbidity (17.5-51.5 NTU), pH (4.10-5.04), total dissolved solids (270-959 mg/l), total suspended solids (53.0-70.0 mg/l), conductivity (4.30-893 µS/cm), iron (0.25-1.16 mg/l), manganese (0.33-0.92 mg/l), zinc (0.075-0.127 mg/l) while no activity was recorded for lead (29, 30) also documented a similar report for total hardness (32.0-114 mg/l), calcium hardness (18.0-59.0 mg/l), magnesium hardness (18.0-57.0 mg/l), chloride (32.0-48.0 mg/l), total dissolved solids (60.0-130), alkalinity (17.9-59.0 mg/l), acidity (3.80-10.20 mg/l), total suspended solids (0.20-0.25 mg/l), nitrates (0.15-0.30 mg/l), phosphates (0.15-0.35 mg/l), pH (5.15-7.23), temperature (26.2-29.2℃), turbidity (4.00-7.00 NTU) and conductivity (38.7-84.5 µS/cm) from wells situated in Ile-Oluji, popularly known for cocoa plantation.

The THBC, TCC and FC obtained from this work ranged from: 5.0×10^7-1.35×10^8 CFU/ml; 3.55×10^5-1.04×10^6 CFU/ml and 2.10×10^5-6.90×10^5 CFU/ml, respectively. This count is higher than the one documented by other researchers where isolation of Enterobacteriaceae was reported from well water situated around Ile-Oluji, Ondo State, Nigeria (popularly known for cocoa plantation) ranged from 2.79×10^8-9.66×10^8 CFU/ml (29, 31).

The bacteria isolated from this study were subjected to eight (8) panels of antibiotics namely: oxacillin (1 mg), cefepime (30 mg), azithromycin (15 mg), kanamycin (30 mg), aztreonam (30 mg), colistin sulphate (10 mg), tigercycline (75 mg) and doripenem (10 mg). The percentage (%) of multiple antibiotic resistance (MAR) index for *Virbrio* sp., *Klebsiella* sp., *Bacillus* sp., *Staphylococcus* sp., *Escherichia coli*, *Pseudomonas* sp., *Glycomyces* sp., and *Proteus* sp. are: 75%, 100%, 100%, 62.5%, 62.5%, 75%, 100% and 50%, respectively. The fact that all the 8 isolated bacterial species have MAR index of 50% and above is noteworthy. This implies that the well waters are potential threats to the consumers.

RECOMMENDATION

It is highly recommended that domestic well water samples should not in any way be situated close to grave sites. Pathogenic organisms and chemical leachates from the decomposing bodies can sink into the ground water aquifers and cause harmful effect when consumed

and/or used for other domestic purposes.

CONCLUSION

The current study has discovered that approximately all the bacteriological data and physico-chemical parameters of the well water samples had values beyond the maximum tolerable limits which could be as a result of the grave leachates sinking into the shallow groundwater aquifers. Nevertheless, further research is necessary to determine the extent of effect sitting a grave close to well water bodies can cause. This will be useful for decision making with respect to local, state, regional, or national policy decision making as par sitting drinking water channels, changes in cemetery management and infrastructures.

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