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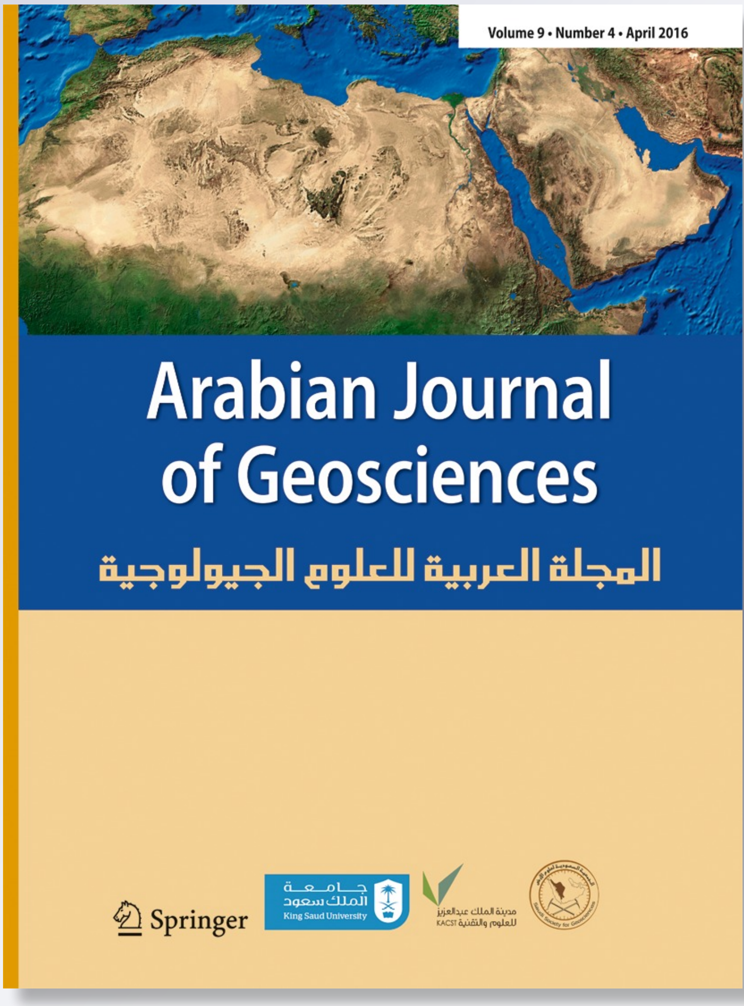

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ORIGINAL PAPER

Physicochemical assessment and bacteriological studies of hand-dug wells of major markets in south western, Nigeria

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Abstract Rapid population in developing nations has imposed stress on groundwater resources, thus the need to assess physicochemical and bacteriological impact of microbes on hand-dug wells along some major markets in Ibadan Southwestern Nigeria. Water samples from hand-dug wells were measured sequentially, and total dissolved solute (TDS), pH, electrical conductivity (EC), salinity, and temperature were measured in situ. Water samples were analyzed at a Microbiology Laboratory. Most probable techniques used for micro-organism analysis were in three stages: presumptive test used for confirmation of *Escherichia coli*, confirmed test for total viable bacteria count (TVBC), and complete test to reconfirm the presence of coliform. Presumptive test showed high rate of *E. coli* in most of the hand-dug wells with (37.5 %). Confirmed test revealed *Staphylococcus aureus* to be 25 %, followed by *Proteus vulgaris* (14.6 %), *Bacillus species* (12.5 %), *Pseudomonas aeruginosa* (8.3 %), and *Klebsiella* spp. (2.1 %) respectively. Total viable bacteria counts are 500 to 192,000. Physicochemical results (total dissolved solute (TDS), pH, electrical conductivity (EC), salinity, temperature) when compared with WHO (2006) and SON (2007) revealed all the parameters to be within the permissible limits except pH (5.8 to 9.56), and high values of the parameters were caused by organic matter. High *E. coli* in the study area revealed influence of human and animal fecal that could lead to urinary tract infection; *Staphylococcus aureus* that comes from sewage could lead to diarrhea, gastrointestinal disease, and multiplication in tissues. High value of pH and *E. coli* above the permissible limits showed contamination of hand-dug wells in the study area.

Keywords Pathogens · Bacteriological · Coliform · Disease · Epidemic

Introduction

Rapid growth in population, which enhances increase in water demand for domestic, market, agricultural, and industrial, has imposed stress on groundwater resources, USAID (2005), by increasing contamination of the surface and groundwater in developing countries which is the most economic and valuable natural resource for various human activities especially those tapping water from shallow unconfined aquifers (Amadi 2011; Olasehinde and Amadi 2009). The amount and type of dissolved ions in groundwater are a composition of the local geology, geochemical characteristics, prevailing human activities, and application (Ezeigbo 1989). The deterioration of groundwater in terms of quality through anthropogenic interference could lead to waterborne disease. Generally, waterborne diseases are transmitted through the ingestion of water and food that are contaminated by fecal materials that carry infective dose of various microbial pathogens. Waterborne diseases remain a major cause of death and illness in various developing countries. The global spatial distribution of these diseases showed that Africa and Asia accounts for a large percentage of such that includes cholera, typhoid fever, paratyphoid, bacillary dysentery, amoebic dysentery, gastroenteritis, and infective hepatitis (Lucas and Gilles 1973; Foster and Chilton 2003).

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In drinking water, microbes pathogens are the contaminants with the greatest chance of reaching levels high enough to cause acute health effect; such microbes are *Escherichia coli*, *Staphylococus aureus*, *Proteus vulgaris*, and *Baccillus* spp. (Jesperson 2004; Chinedu et al. 2011; EPA 2011; IDLO 2006). Pathogens are disease-causing micro-organisms that include Bacterial pathogens (*Acinetobacter*, *Aeromonas*, *Bacillus*, *Campylobacter*, *Enterobacter sakazakii*, *E. coli*, *Klebsiella*, *Legionella*, *Pseudomonas aeruginosa*, *Salmonella*, *Shigella*, *Staphylococcus aureus*, *Vibrio*, and a typical mycobacteria that can grow in water and soil (WHO 1997): Viral pathogens (*adenoviruses*, *astroviruses*, *caliciviruses*, *enteroviruses*, *hepatitis A virus*, *hepatitis E virus*, *rotaviruses*, and *orthoreoviruses* (Grabow et al. 2001): Protozoan pathogens (*Acanthamoeba*, *Balantidium coli*, *Blastocystis*, *Cryptosporidium*, *Cyclospora cayetanensis*, *Entamoeba histolytica*, *Giardia intestinalis*, *Isospora belli*, *Microsporidia*, *Naegleria fowleri*, *Toxoplasma gondii* (WHO 2003)): Helminth pathogens (dracunculiasis and fascioliasis (Cairncross et al. 2002)) which require intermediate hosts to complete their life cycles but are transmitted through drinking water by different mechanisms. Coliforms could travel through a distance of 70.7 m (232 ft) from sewage trenches intersecting groundwater, while traveling through sand and pea gravel aquifer in 35 h will cover 30.5 m (100 ft) from primary sewage-injected subsurface (Crane and Moore 1984). Presence of septic systems and well depth was found to be related to detection of coliforms in groundwater, although these relationships were not statistically significant (Francy et al. 2000). Effect of distance from pollution sources was more pronounced on fecal and total coliform counts, which decreased with increasing distance from waste dumps and defecation sites (Adekunle et al. 2007). Also, other factors such as the environment where the well was sited and the level of hygiene of the well, in terms of the use of drawers and the population of people the well is serving and its surroundings could be considered as other possible sources of contamination aside the septic tanks.

Statistics revealed that inhabitants of Ibadan Metropolis in Nigeria suffer mainly from diarrhea, gastroenteritis, malaria, measles, tuberculosis, cholera, and typhoid fever (Iyun 1994). This had led to divers' investigation on the quality of water samples drawn from private wells and springs in various southwestern regions Adekunle (2008); Shimizu et al. (1980); Snow (1894); Swerdlow (1992); Swerdlow et al. 1992. The study area could easily breed microorganisms due to diver's reasons such as dirty environment, poor town planning, dilapidated infrastructure, and indiscriminate sitting of wells and boreholes (Valenzuela et al. 2009) since coliform bacteria are widely found in nature (Binnie et al. 2002). The study therefore examined various pattern of physicochemical and bacteriological impact of microbes on hand-dug wells along some major markets.

Materials and method

The study area

Ibadan, the study area known as the city at the edge of savannah, the third largest metropolitan area, by population, in Nigeria, after Lagos and Kano with a population of 282,585 people (139,515 male and 143,070 female) in 2006, has a relief pattern that can be described as undulating with an elevation varying between 150 and 235 m above the sea level and longitude 07° 23′ 18.2″ N with latitude 03° 54′ 27.4″ E and a dendritic type of drain located within the basement complex of south-western Nigeria, underlain by a migmatite-gneiss complex which consist of banded gneiss with associated quartzite, hornblende biotite granite gneiss, quartzite schist, mica schist, and amphibolite layers, quartzites form prominent topographic features in the study area. The area has a well-accessible road network with minor paths which provides real accessibility to the wells. The two main problems man contends with in the study area are the quantity (source and amount) and quality of water (Fig. 1). The type of wastes which constitute environmental pollution in the area is domestic refuse consisting of degradable food wastes, leaves, dead animals, corpse, and non-degradable ones such as plastics, bottles, nylon, medical, and hospital wastes, generated in households, hospitals, industries, and commercial centers (Omoleke 2004).

Data collection

Sampling was carried out during the dry season, and a total of 22 water samples were collected in hand-dug wells in major markets around Oje, Bere, Idi-Arere, Mapo, Oja-Oba, Bode, and Molete Market respectively. The procedure of collection was divided into three:

1. Procedure done before the field sampling
2. Field sampling
3. Laboratory methods

Procedure done before the field sampling involved using clean porcelain polyethylene plastic bottles to collect the water samples before the field procedure, and bottles were autoclaved at 121 °C for 15 min and were not opened until the point of collection.

For the field sampling, total dissolved solvent (TDS), salinity, temperature, and pH were measured in situ with a pH meter instrument before the water samples were collected. Samples were collected into sterile bottles and sealed with paper tape after disinfection with spirit lamp of dispensing point with flame. The samples were then placed in a container filled with iced block to maintain the microbial population and process microbial presence.

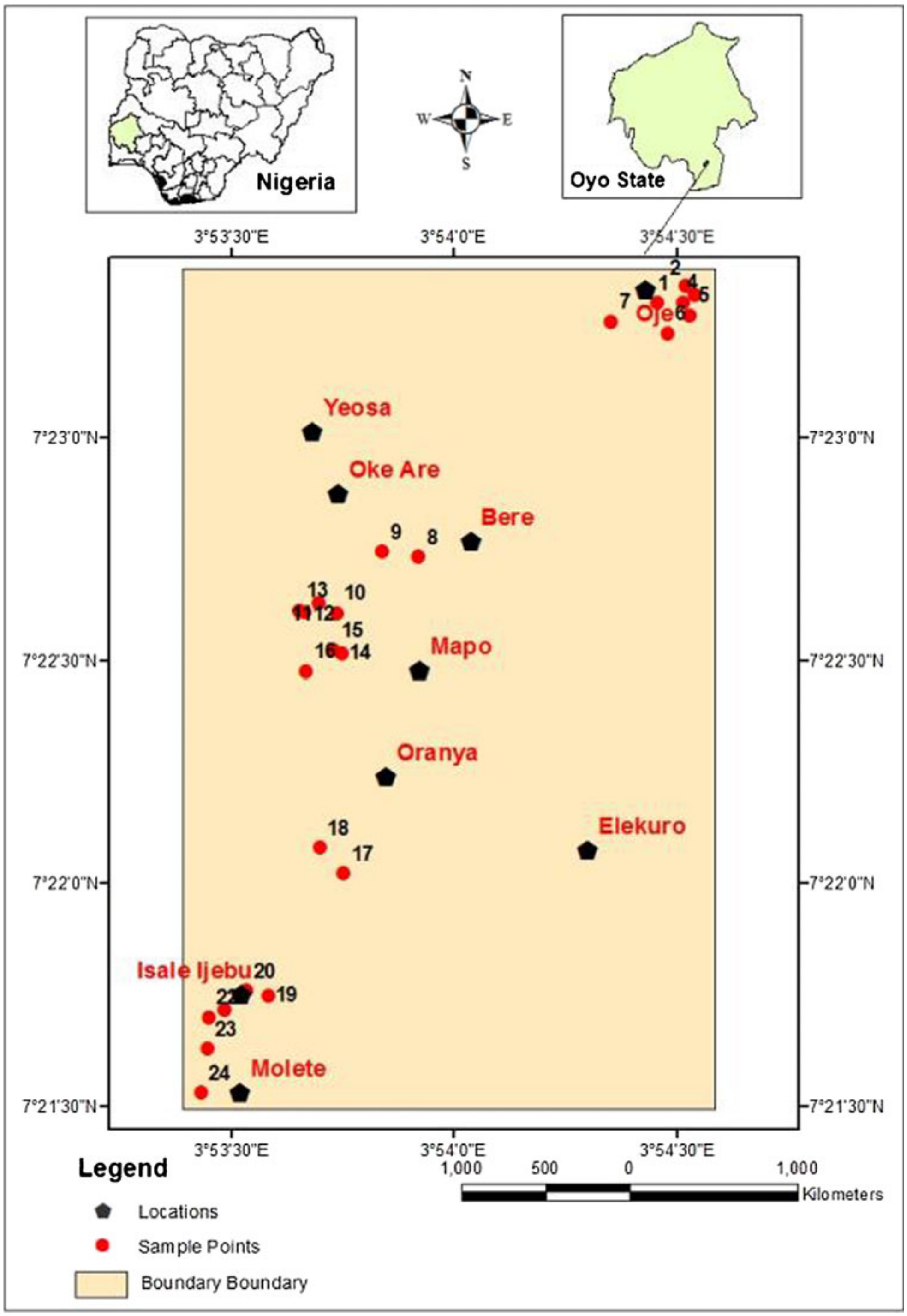

Fig. 1 Location map of the study area with sampling points

The laboratory method includes presumptive test, confirmation test, and complete test. The presumptive test was used to detect and enumerate fecal coliform in the underground water; nine separate bottles were randomly picked and labeled 1–9 to be a representative sample for the whole study to detect the coliform level. One hundred milliliters of double strength of lactose broth was added to bottles 1–3, 100 ml of single strength of lactose broth was added to bottles 4–9, 10 ml of the water sample was then added to bottles 1–3, 1 ml of water sample was to bottles 4–6, and 0.1 ml of water samples were added to bottles 7–9. Durham tube was then inverted over each bottle before sterilization after which all the bottles with lactose broth the water samples were later incubated at 37 °C for 24 h. Evidence of coliform gas observed from the Durham tube with bubbles coming at the top of the tube indicates the presence of coliform, while the absence of Durham tube coliform could be detected through the change in color of the original media from red to yellow, and the absence of gas indicates absence of coliform.

The confirmed test involves serial dilution, streaking method, and pour plate method. Serial dilution is done to reduce the microbial load present in water samples; before plating, each of the positive and negative of the presumptive test was diluted to tenfold in peptone water (0.1 % nutrient broth) before been plated on the appropriate media. In the streaking method, 36.4 g of MacConkey agar was diluted to 700 ml of water and sterilized at 121 °C for 15 min, the mixture was then poured into petri-dish and was allowed to cool and solidify before streaking, organisms were thereafter incubated inversely at 37 °C for 24 h, and results were observed after the 24th hour by identifying different organisms on each plate which includes gram-positive and gram-negative. In Pour plate method, 1 ml of water was added to sterile nutrient broth on two different bottles each with Petri-dish plate and allowed to cool at 45 °C or chick tolerance, and then, 1 mL of water was added to 9 ml of nutrient blood which was shacked thoroughly after which was poured immediately into the Petri-dish plate which was allowed to solidified and incubated inversely at 37 °C for 72 h. Total viable bacteria count (TVBC) began by dividing the plates into four segments with permanent marker for an easy counting of microorganism, counting and recording each of the four segments separately, summing up the whole count, and converting the value to the colony-forming unit (CFU/ML) by using formula number of organisms × $\left[\frac{1}{10}\right]^{-3}$.

The Indole test is the final analysis of the water sample. Subculture organisms' colonies were taken from the plate with inoculating loop into the sterile peptone water after which it was incubated for 72 h, and then a drop of shelling reagent was added to peptone water, for the observation of the color to ascertain the presences of coliform or gram-staining organism. After the few minutes, there was a presence of yellow color which indicates the presence of *E. coli*.

Results and interpretation

Physiochemical results

Total dissolved solid (TDS), pH, temperature, biochemical oxygen demand (BOD), electrical conductivity (EC), and salinity (Table 1) of the different markets were compared with SON (2007), and WHO (2006) results revealed all parameters to be within the permissible standard with the exception of pH in some of the markets due to high rate of indiscriminate disposal of refuse and uncontrolled drain. The mean values for the parameters are 438.50 ± 267.17, 6.59 ± 0.940, 27.10 ± 0.778 °C, 5.60 ± 1.264, 525.00 ± 218.25, and 0.04 ± 0.039, respectively.

Table 1 Mean ± SD concentration (mg/l) of physiochemical parameters in the various water sources in the study area

Physical parameters	Oje market	Bere market	Mapo market	Oja-oba market	Idi-arere market	Bode market	Molete market	SON limit (2007)
pH	8.20 ± 0.917	6.59 ± 0.141	6.86 ± 0.940	6.76 ± 0.530	6.90 ± 0.336	8.00 ± 0.183	8.29 ± 0.212	6.5–8.5
Temp. (°C)	29.10 ± 0.504	30.35 ± 0.778	27.10 ± 0.552	26.80 ± 0.505	29.20 ± 0.781	28.50 ± 0.387	30.20 ± 0.566	–
BOD (mg/l)	6.00 ± 0.405	5.61 ± 0.535	6.00 ± 0.910	6.80 ± 1.264	8.03 ± 0.132	5.60 ± 0.441	5.40 ± 0.387	–
TDS (mg/l)	535.00 ± 176.230	498.50 ± 70.003	827.30 ± 173.900	654.30 ± 267.170	690.00 ± 9.899	539.00 ± 61.230	438.50 ± 4.950	1500
Electrical conductivity (µS/cm)	668.30 ± 202.130	705.00 ± 74.450	966.60 ± 138.684	766.60 ± 218.250	905.00 ± 63.640	705.00 ± 70.470	525.00 ± 7.070	1000
Salinity (mg/l)	0.05 ± 0.011	0.05 ± 0.007	0.07 ± 0.039	0.05 ± 0.002	0.06 ± 0.007	0.05 ± 0.000	0.04 ± 0.000	–

Table 2 Occurrence of isolated organisms from the different locations

Locations	ST	EC	BS	PA	PV	KS
1	+	+	−	−	−	−
2	−	+	−	−	+	−
3	−	+	+	+	−	−
4	+	+	−	−	−	−
5	+	+	−	−	−	−
6	+	+	−	−	+	−
7	+	−	−	−	−	−
8	+	+	−	−	+	−
9	+	−	−	−	+	−
10	+	−	−	−	−	−
11	+	+	−	+	+	−
12	+	+	−	−	−	−
13	−	+	+	−	−	−
14	−	+	+	+	−	−
15	−	+	+	−	−	−
16	−	+	−	−	+	−
17	−	+	−	−	−	−
18	+	+	−	−	−	+
19	+	−	−	−	−	−
20	−	+	+	+	−	−
21	−	+	+	−	−	−
22	−	+	−	−	+	−

Keys: *ST Staphylococcus aureus*, *PV Proteus vulgaris*, *EC Escherichia coli*, *KS Klebsiella species*, *BS Bacillus species*, + present, *PA Pseudomonas aeruginosa*, − absent

Bacteriological results

Microbiological quality assessment (Table 2) of hand-dug wells was conducted and was observed that none of the water samples were found to be bacteria free. The 18.2 % of the four water samples are suitable for drinking after the coliform test, while the remaining 81.8 % contains high coliform level thus importable for drinking. Total viable bacteria count was observed to also be high, while prevalence of bacteria pathogens of a total of six bacteria species (two gram positive and four gram negative) was recovered from the water samples circulating at the study area. *E. coli* (Fig. 2) were found to be the most predominant organism with prevalence rate of 18 (37.5 %) and closely followed by the prevalence rate of 12 (12.5 %) *Staphylococcus aureus*, then the prevalence rate of 7 (14.6 %) of *Proteus vulgaris*, and the prevalence rate of 6 (12.5 %) of *Baccillus species* and prevalence rate of 4 (8.3 %) of *Pseudomonas aeruginosa*, respectively, while the least isolated organism in the study area was *Klebsiella species*. The overall 48 strains of bacteria isolated belonging to six species were recovered. Mapo market was found to be most contaminated, and Bode market was least contaminated with bacterial (Fig. 3).

Fig. 2 Result showing *Escherichia coli* on petri-dish

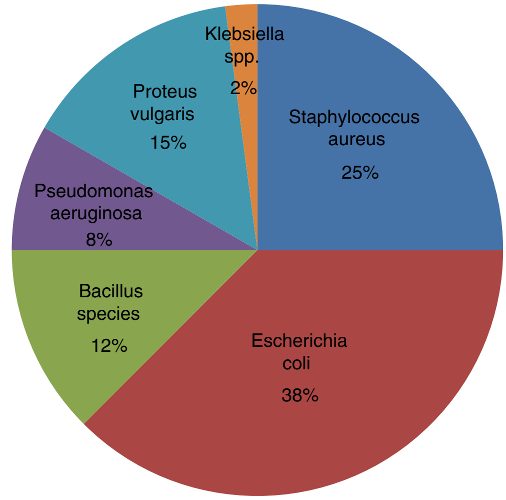

Fig. 3 Percentage of isolated organisms in the study area

Discussion

Growth and survival of micro-organisms (Thomas et al. 2012) in drinking water are of public health (CFSPH 2009), because the presence of such in water is a risk factor to developing country infections. Eighteen (81.8 %) of the examined well water were found not to be acceptable for drinking. This observation might have adduced that the examined well water is not properly managed in terms of hygiene and possibly environmental cleanliness. The fact that *E. coli* was the most prevalent in this study further stressed that the examined well water was contaminated with fecal samples (Oshoma et al. 2009). *E. coli* are well-known for causing serious gastroenteritis disorder such as watery diarrhea, hemorrhagic colitis, and/or uremic syndrome (CFSPH 2009). *Staphylococcus aureus* which was the second most prevalent in the study has been linked with various hospital acquired infection (Duquette and Nuttall 2004). Some strains of these organisms especially the meticillin-resistant strains are even more virulent due to their multidrug-resistant attributes (CFSPH 2009), and they have also been implicated as the etiology of many community

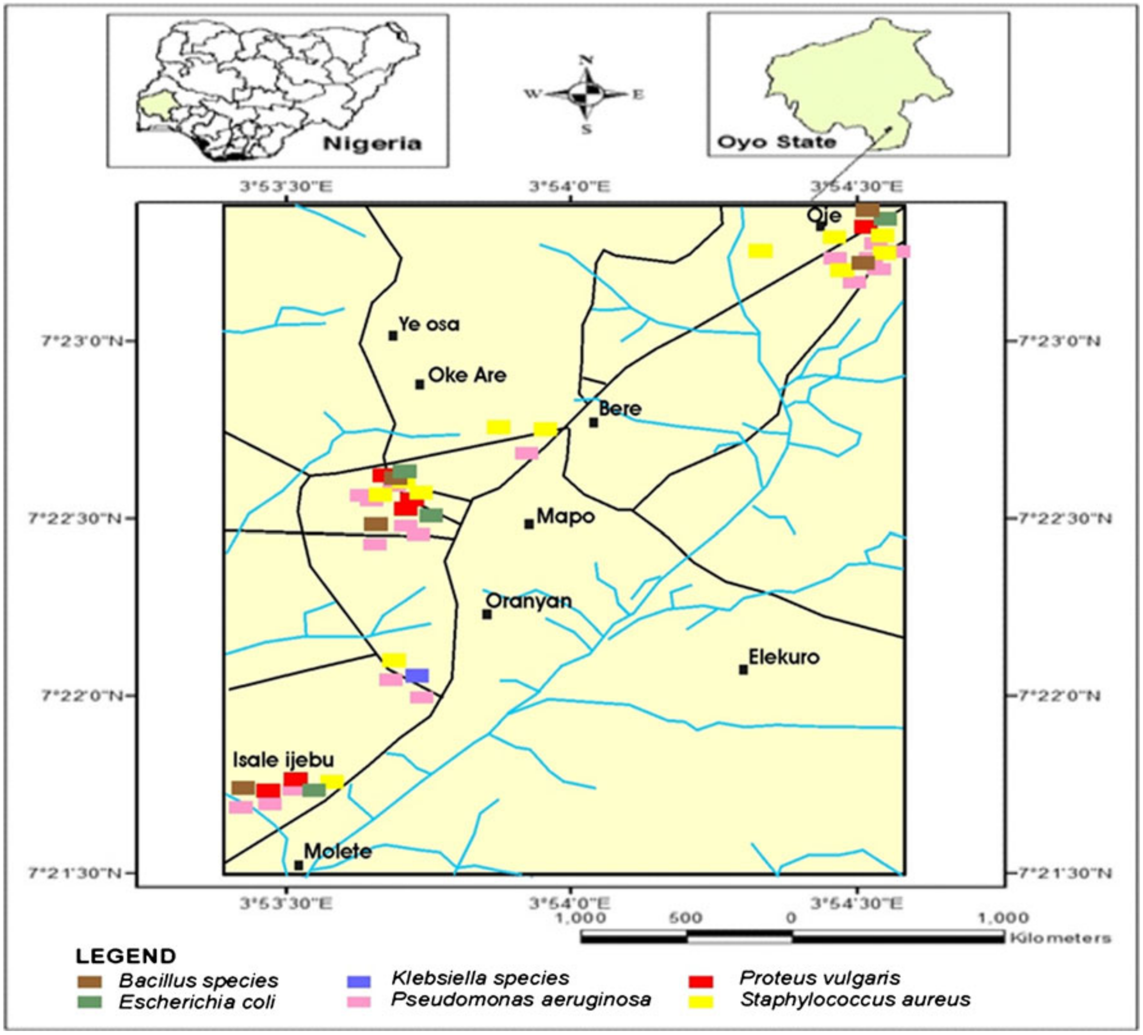

Fig. 4 Distribution of *Bacillus species*, *Escherichia coli*, *Klebsiella species*, *Pseudomonas aeruginosa*, *Proteus vulgaris*, and *Staphylococcus aureus* found in the well locations

associated and nosocomial infection (Duquette and Nuttall 2004). Until recently, these strains were susceptible; however, resistance seems to be increasing, and multiple antibiotic resistant strains have started to emerge. *Proteus vulgaris*, one of the isolated organisms, are known as common inhabitants of the human guts and frequently implicated in urinary tract infections (Senior 1979). *Bacillus species*, known as opportunist pathogens, since the late nineteenth century and reports of cases, have appeared at the rate of about 6 years for the past decade (Logan 1988). Also, some species of this organism especially the species subtilis are known to produce bacitracin, a toxic antibiotic to human tissue in addition to their antagonistic effect against other pathogenic bacteria (Brooks et al. 2005). *Pseudomonas aeruginosa*, another major nosocomial pathogens recovered in the study, was found to be associated with catheter-associated urinary tract infection which cannot be overemphasized (Fluit et al. 2001). The virullency of this bacterial isolate has been linked to their cell associated factors like alginate, lipopolysaccharide (LPS), flagellum, pilus, and non-pilusadhesms as well as with exoenzymes or secretory virulence factors like protease, elastase, phospholipase, pyocyanin, exotoxin A, exoenzyme S, hemolysins (rhamnolipids), and siderophores (Matheson et al. 2006). Another common human intestinal floral which was isolated from some of the well waters was *Klebsiella* sp. This organism in frequently associated with hospital acquired infection (Sung-sheng and Tsai 2009). According to some authors, certain underlying diseases such as malignancy, cirrhosis, biliary tract, disorders, diabetes mellitus (DM), and alcoholism may impair an individual's defenses and increase the risk of *Klebsiella pneumoniae* infection (Tsay et al. 2002). These bacteria may also be implicated in bacteremia thereby causing significant morbidity and mortality in general population (Tsay et al. 2002). Metastatic infections such as pyogenic brain abscess, meningitis, and endophthalmitis are the most common and/or important characteristics of *Klebsiella* infections. Higher counts of total viable bacteria were observed in the study area compared with coliform counts. These high counts were due to the poor level of hygiene and sanitation observed, since hand-dug wells in the environ are constantly exposed to contamination from human activities and unplanned layout of houses that led to minute distances between wells, septic tanks, and refuse dumps (Fig. 4).

Conclusions

The type of bacteria isolated from the examined well water samples is similar to that reported by Shittu et al. (2008). This observation might have adduced that the source of such water contamination may be similar. Such source may include improper disposal of refuse, contamination of water by sewage, and surface runoff. Heterotrophic count (HPC) measures a range of bacteria that are naturally present in the environment (EPA 2002). The total bacteria counts for all the water samples were generally high exceeding the limit of 1.0×10^2 cfu/ml which is the standard limit of heterotrophic count for drinking water (EPA 2002). The high total heterotrophic count is indicative of the presence of high organic and dissolved salts in the water. The primary source of bacterial contamination includes surface runoff, pasture, and other land area where animal waste is deposited (Shittu et al. 2008). Additional sources include seepage or discharge septic tanks, sewage treatment facilities, and nature soil/plant bacteria (EPA 2002). Thus, from the bacteriological results, the well waters are highly contaminated and are not good for human consumption, agriculture, industrial, municipal uses, among others. It is therefore recommended that wells should be properly located, human activities should be controlled to prevent water body, and diverse educational talks should be organized for the people in the environment.

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