Investigation of *Escherichia coli* Distribution in Drinking Water Wells Close to Septic Tanks in Densely Populated Areas of Osogbo, Osun State, Nigeria

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Abstract: Groundwater, which serves as the major drinking water source to densely populated areas in Osogbo metropolis, are usually in close proximity to defective septic tanks. Faecal contamination of such water system is inevitable. This work investigated the faecal contaminants in eighty wells from five locations. Total coliform count (TCC) was determined using Most Probable Number (MPN) and membrane filtration; *Escherichia coli* was recovered using chromogenic media. Antibiotic susceptibility profile was obtained for *E. coli* isolates. All wells were > 15.24m from nearest septic tank; averaging 78m. Depth to water surface ranged between 9ft and 48ft with a mean of 23.8ft while 16.2% were deeper than 30ft to water surface. All samples (100%) had coliforms. TCC ranged between 1.0 x 10^2 and 4.0 x 10^5 cfu/ml (except Kasmo); with a mean 3.8 x 10^4 cfu/ml. Mean TCC for wells was highest in Igbona area and lowest for Oke-baale. *Escherichia coli* was isolated from 48.8% of samples. Five samples had two strains of *E. coli* as revealed on chromogenic media. The study recorded 100% resistance to ticarcillin and meropenem; 52.3% to tigecycline. Aztreonam and Colistin inhibited 92.6% and 91.0% of isolates respectively. Multidrug resistance was evident in 79.5% of isolates. The well water samples analyzed were neither safe for drinking nor put into such uses that may facilitate ingestion by humans. *E. coli* was most susceptible *in-vitro* to aztreonam and colistin, thus suggesting their use in the treatment of gastrointestinal syndromes resulting from consumption of contaminated groundwater.
Key words: Indicator organism, public health, septic tank, well water contamination,

INTRODUCTION

Water has always been a necessity to humans and is essential to the survival of all organisms. It is a crucial component of metabolic processes and serves as a solvent in body solutions (Alison, 2001). Water is essential for growing food, household uses including drinking, cooling, sanitation, as a critical input into industry, for tourism and cultural purposes, and for its role in sustaining the earth's ecosystem (Mark *et al.,* 2002). In addition to its use for direct human consumption, it is also integrally linked to the provision and quality of ecosystems service. Water quality is a key environmental issue involving natural water resources and local rural communities. Groundwater is the largest reserve of potable water in regions where humans live. Major environmental pressures have an impact on the quantity and quality of groundwater resources (Danielopol *et al*., 2003) which are generally perceived as being less vulnerable to contamination than surface water (Pearce, 2006) given the natural filtering ability of the subsurface. Although groundwater is believed to be free of pathogens, many well water systems are unprotected and contamination could occur because drinking water wells are rarely if ever monitored (Valenzuela *et al.,* 2009). Seepage from leaking septic tanks could pose a serious source of fecal contamination to poorly monitored wells. Therefore, it is important to detect fecal contamination in well water, especially if there are no pre-consumption water treatment systems (Atherholt *et al*., 2003) in order to reduce waterborne diseases which is a major public health goal in developing countries (Alison, 2001).

Globally, the most common contamination of raw water sources is from sewage, particularly fecal pathogens and parasites. Access to safe drinking water and sanitation is critical in terms of health. Unsafe drinking water contributes to numerous health problems in developing countries.

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Waterborne diseases account for 4.1% of the global disease burden and account for 1.8 million deaths every year while about 88% is ascribed to unsafe water supply, sanitation and poor personal hygiene (World Health Organization, 2014). The World Health Organization (WHO) reports that 884 million people lack access to even basic drinking water service, 159 million of which are dependent on surface water and 423 million people on unprotected springs and wells (WHO, 2017; Abdulkadir *et al.,* 2018). Detection of total coliform and *Escherichia coli* are considered the best way to determine if a water supply is protected from microbial contamination (Vendrell and Atiles, 2003). *E. coli* originates in the gut of a warm-blooded animal, and its presence in water indicates contamination from human and animal feces (USEPA, 2009) and high likelihood of presence of disease-causing organisms as well (Vendrell and Atiles, 2003). The United States Environmental Protection Agency requires that septic tanks be installed at least 15.24m (50 feet) from a well that is used for drinking water (InspectAPedia, 2010). Although an existing septic system closer to a well may be safe, it is important to maintain these systems properly.

In Osogbo, southwest Nigeria, hand dug wells are present in virtually every home as homeowners are required to provide a source of potable water for drinking and for other domestic or household uses. These wells are dug many times without taking into consideration the proximity of septic tanks and wastewater systems in the home, as well as, in neighboring compounds. Homeowners are also obliged to put in place soak away systems to manage household wastewater as there is no provision for treatment of wastewater in the town. These installations become necessary as Government-owned water distribution facilities are unable to meet the water requirement of the served community due to lack of maintenance, or increased population. This scarcity of potable water has made people find alternative sources of water, and well water has been a ready source (Adelekan, 2010) since it is convenient and available all year round. In light of the above, the main objective of this study is to assess the contamination of hand dug wells by fecal matter - taking into consideration the EPA's recommendation for acceptable distance between a water well and a septic tank, and using it as a yardstick to evaluate the influence that proximity from a septic tank has on well water quality in selected areas in Osogbo, Osun State Nigeria.

MATERIALS AND METHODS

Study Area:

This study was carried out in five different locations with Osogbo having three sampling locations and one location each from Olorunda and Ede North local government areas.

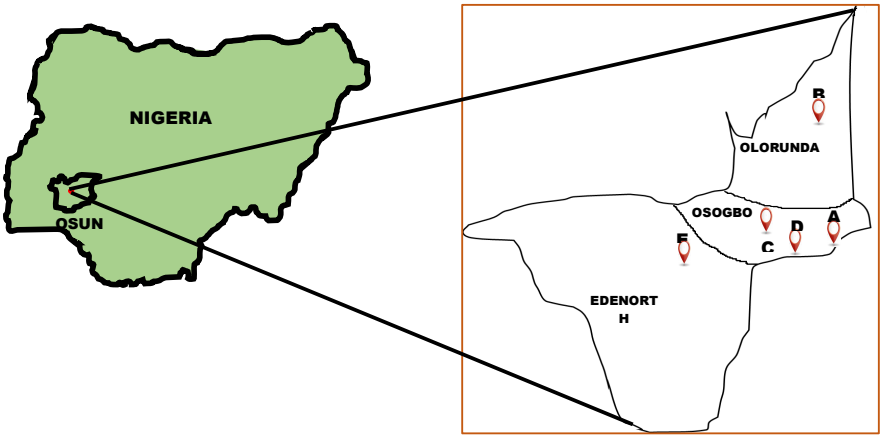

Figure 1: Map of the study areas, indicating the sampling locations

Determination of proximity to septic tank and depth of well:

The criterion for sampling a well was ≥ 15.24m from the nearest septic tank, which is the minimum distance between a well and a septic tank as stipulated by the Environmental Protection Agency (InspectAPedia, 2010). Measurements of distance between the wells and septic tanks was done using a tape rule. All the 80 wells selected were between distances of more than 15.24m (50ft) from the nearest septic tank. Again, the depth of well to water table was determined by measuring the length of the drawer used to draw samples using a tape rule.

Collection of well water samples:

A total of 80 wells were chosen for the study, 20 around Kasmo area while 15 wells were chosen from the other four locations. The samples were collected at random from the different areas. Water samples were collected from hand dug wells using manual drawers that had been previously sterilized with an autoclave, 1 litre of which was then transferred into the properly labeled sterilized glass; stored in ice-cold containers for transport to the laboratory and analyzed within 6 hours of collection. All the samples were collected in duplicates between 6 a.m. and 10 a.m. in the morning.

Membrane Filtration Method:

The total coliform count was determined using the Membrane filtration technique. Each well water sample (500ml) was serially diluted with ringer solution to thin out the bacterial load. A minimum volume of 100ml of the diluted water sample was then introduced aseptically into a sterile membrane filtration assembly containing a sterile membrane filter (filter pore size was 0.45µm). A membrane filtration pump was used to apply vacuum and the sample was drawn through the membrane filter. All organisms were retained on or within the filter which was then transferred onto sterilized Membrane Lauryl Sulphate agar in a petri dish and incubated at 44^{o}C. The number and different colors of colonies which grew were observed and recorded. The total coliform was computed and reported per /100 mL of the sample using the equation below:

$$\text{Total coliform colonies/100mL} = \frac{\text{colonies counted x reciprocal of dilution factor}}{\text{volume of sample filtered}}$$

Most Probable Number (MPN) Assay:

MacConkey purple broth (single and double strength) was prepared in sterilized McCartney bottles with inverted sterilized Durham tubes to detect CO_2 gas production. For each sample, three bottles each were prepared in three rows and clearly labeled to identify the well water sample and volume (10ml, 1ml, 0.1ml) to be inoculated. The bottles were aseptically inoculated and then incubated at 37^0C ± 0.5^0C. Un-inoculated bottles were also incubated as controls. After 24 ± 3 hours, each tube was gently swirled and examined for growth indicated by a color change of broth from purple to yellow as well as CO_2 gas production. In cases where no gas had formed, bottles were re-incubated for an additional 24 ± 3 hours and reassessed. For tubes with growth, the presence of gas in inverted Durham tubes within 48 ± 3 hours signified a positive presumptive reaction, indicating presence of coliforms. Positive and negative results were recorded and MPN of coliforms/ml calculated from the number of positive McCartney bottles. For tubes with a positive presumptive reaction, the confirmative test was done by streaking out the positive presumptive test onto sterile Eosin Methylene Blue (EMB) agar and incubated at 44^0C for 48 hours.

Isolation of *Escherichia coli*:

The distinct colonies with metallic green sheen on EMB agar were picked and confirmed by streaking onto HiChrome agar. Colonies with a blue/violet appearance were selected and analyzed further by gram staining and biochemical tests.

Antibiotic Susceptibility Testing –

All the recovered isolates were screened against commonly prescribed antibiotics using the Kirby-Bauer disc diffusion method.

Antibiotic discs used (Oxoid, UK) included aztreonam (30µg), colistin (10µg), Fosfomycin(50µg), gentamicin (high level) (120µg), levofloxacin (5µg), meropenem (10µg), nalidixic acid (30µg), nitrofurantoin (300µg), streptomycin (10µg), sulphonamides (300µg), ticarcillin (75µg), tigecycline (15µg) and trimethoprim/sulfamethoxazole (1.25/23.75µg). *Escherichia coli* ATCC 25922 (Rockville, USA) was used as control. Resistance to ≥ 1 agent in ≥ 3 antibiotic classes was indicative of multidrug resistance (MDR) among the isolates. Multiple Antibiotic Resistance (MAR) index for each test isolate was also calculated as proposed by Krumperman (1983) and Adeleke *et al*. (2014). The MAR index was calculated using the formula:

$$MAR\ index = \frac{\text{No of antibiotics against which isolate is resistant}}{\text{Total no of antibiotics against which isolate was tested}}$$

RESULTS

Determination of proximity to septic tank and depth of well:

All the wells (100%) studied were more than 50 ft (15.24m) from the nearest septic tank. One of the wells (in Kasmo area) was situated in between two septic tanks (sample W1-A on Table 1), while yet another (in Oke-Baale) was located very close to both a septic tank and a public latrine. The depth to water table for each of the wells were also assessed. Of all the 80 wells analyzed, the depth to water table was lesser or equal to 30ft in 83.8% of the wells while 16.2% were deeper than 30ft to the water surface. 58.8% of the well had covers fitted and were reported by users to be always properly covered. A high percentage of the wells also had no pumping system fitted; only 12 (15%) had functional pumping systems, and as such, water had to be drawn manually from them with the aid of manual drawers. The proximity of the 80 wells to septic tanks averages 78m, while the depth to water surface for all the wells ranged between 9ft and 48 ft with a mean of 23.8ft (Table 2).

Membrane Filtration Method / Most Probable Number (MPN) Assay:

All the water samples analyzed revealed the presence of coliforms. The details of the results for each sample is given on table 4. The total coliform count for all samples (except samples from Kasmo area which was not determined with membrane filtration method) ranged between 1.0 x 10^2 and 4.0 x 10^5cfu/ml; with a mean of 3.8 x 10^4cfu/ml (Table 4). The mean total coliform count was highest for wells in the Igbona area while it was lowest for wells in Oke-baale (Table 3). The mean coliform count was higher for wells with water table deeper than 30ft into the ground; as well as open wells than in covered wells and much lower in wells that had pumping machines than those in which water was obtained with manual drawers (Table 4).

Isolation of *Escherichia coli*:

Escherichia coli was recovered from quite a high number of the samples – seventeen out of the 20 samples (85.0%) from Kasmo area, while 66.7%, 33.3%, 26.7% and 20.0% were recovered in samples from Oke-Baale, Igbona, Owode Ede and Ota Efun respectively. Nearly half of the samples revealed the presence of *E. coli* as the organism was isolated from 48.8% (39 out of 80) of the samples. Five of the samples (3 from Oke-Baale and 2 from Owode Ede) revealed the presence of two different strains of *E. coli* as revealed on chromogenic media. However, two of these had relatively low coliform counts with the MPN method (240 and 460 respectively).

Antibiotic Susceptibility Testing/MAR indexing:

The antibiotic resistance pattern of the recovered isolates revealed that all the isolates were resistant to ticarcillin and meropenem while 52.3% were resistant to tigecycline. Aztreonam appeared to be the most effective of the antibiotics, inhibiting 25 out of 27 (92.6%) isolates, closely followed by Colistin at 9.0% resistance (Table 5).

Multidrug resistance to three or more classes of antibiotics was evident in 79.5% of the isolates (35 out of 44). The multiple antibiotic resistance (MAR) indices calculated for the isolates equally shows that only 2 out of the 44 *E. coli* isolates tested had Multiple antibiotic resistance (MAR) indices of < 0.2 (Table 6 and Figure 1). 95.5% of them had MAR indices ≥ 0.2.

Table 1: Details of well water samples from various locations across the study area

KASMO AREA (A) (7.7600^{O} N; 4.6064^{O} E)			OTA EFUN (B) (7.8187^{O} N; 4.5875^{O} E)			IGBONA (C) (7.4736^{O} N; 3.7613^{O} E)			OKE BAALE (D) (7.7685^{O} N; 4.5716^{O} E)			OWODE EDE (E) (7.7127^{O} N; 4.4954^{O} E)		
ID	**Distance from Septic Tank (m)**	**Depth to water table (ft)**	**ID**	**Distance from Septic Tank (m)**	**Depth to water table (ft)**	**ID**	**Distance from Septic Tank (m)**	**Depth to water table (ft)**	**ID**	**Distance from Septic Tank (m)**	**Depth to water table (ft)**	**ID**	**Distance from Septic Tank (m)**	**Depth to water table (ft)**
W1-A*	a.16 b.22	24	W2-A	60	9	W3-A	70	45	W4-A	30	9	W5-A	100	45
W1-B	21	18	W2-B	100	9	W3-B	50	30	W4-B*	60	12	W5-B	120	25
W1-C	43	15	W2-C*	120	30	W3-C	110	45	W4-C*	150	9	W5-C	90	9
W1-D*	22	18	W2-D	80	45	W3-D	100	27	W4-D	100	18	W5-D	60	15
W1-E	41	18	W2-E	90	18	W3-E*	110	30	W4-E	80	25	W5-E	105	30
W1-F*	39	33	W2-F	120	15	W3-F	75	15	W4-F*	60	36	W5-F	110	40
W1-G*	32	15	W2-G*	70	12	W3-G	65	12	W4-G*	70	30	W5-G**	125	30
W1-H*	37	33	W2-H*	80	27	W3-H	120	12	W4-H	90	45	W5-H**	80	20
W1-I*	40	27	W2-I	100	36	W3-I	150	9	W4-I*	100	24	W5-I*	70	15
W1-J*	36	30	W2-J	85	30	W3-J*	70	18	W4-J**	70	36	W5-J	90	18
W1-K*	30	48	W2-K	105	9	W3-K	80	12	W4-K	120	21	W5-K	100	20
W1-L*	43	36	W2-L	125	12	W3-L*	95	45	W4-L**	80	15	W5-L	60	15
W1-M*	22	18	W2-M	110	18	W3-M	115	45	W4-M*	50	24	W5-M	90	25
W1-N*	37	33	W2-N	90	24	W3-N*	150	27	W4-N*	90	30	W5-N*	110	18
W1-O*	40	27	W2-O	60	9	W3-O*	60	24	W4-O**	60	30	W5-O	70	9
W1-P*	91	18	--	--	--	--	--	--	--	--	--	--	--	--
W1-Q*	89	24	--	--	--	--	--	--	--	--	--	--	--	--
W1-R*	84	21	--	--	--	--	--	--	--	--	--	--	--	--
W1-S*	85	21	--	--	--	--	--	--	--	--	--	--	--	--
W1-T*	53	30	--	--	--	--	--	--	--	--	--	--	--	--

Legend: ID = Sample code; * = Samples from which *E. coli* isolates were recovered; **= Samples from which two different strains of E. *coli* were recovered.

Table 2: MPN Decimal Dilution for the Well Water Samples

Broth Medium (MacConkey Broth)	Qty of Broth Medium	Volume of Original Sample	Qty of Inoculum	No. of Culture Bottles
Double strength	10ml	10ml	10ml	3
Single strength	9ml	1ml	1ml	3
Single strength	9ml	0.1ml	1ml	3

Table 3: MPN and Total Coliform Counts of well water samples from various locations across the study area

KASMO AREA (A) (7.7600^{O} N; 4.6064^{O} E)			OTA EFUN (B) (7.8187^{O} N; 4.5875^{O} E)			IGBONA (C) (7.4736^{O} N; 3.7613^{O} E)			OKE BAALE (D) (7.7685^{O} N; 4.5716^{O} E)			OWODE EDE (E) (7.7127^{O} N; 4.4954^{O} E)		
Sample ID	**MPN index /100ml**	**Total coliform count (cfu/ml)**	**Sample ID**	**MPN index /100ml**	**Total coliform count (cfu/ml)**	**Sample ID**	**MPN index /100ml**	**Total coliform count (cfu/ml)**	**Sample ID**	**MPN index /100ml**	**Total coliform count (cfu/ml)**	**Sample ID**	**MPN index / 100ml**	**Total coliform count (cfu/ml)**
W1-A*	>1100	ND	W2-A	>1100	8.0×10^3	W3-A	>1100	1.8×10^4	W4-A	>1100	2.0×10^3	W5-A	240	1.6×10^3
W1-B	>1100	ND	W2-B	>1100	2.8×10^4	W3-B	>1100	7.0×10^4	W4-B*	>1100	5.0×10^3	W5-B	>1100	2.3×10^3
W1-C	75	ND	W2-C*	>1100	4.0×10^2	W3-C	>1100	2.0×10^5	W4-C*	460	8.0×10^3	W5-C	>1100	1.4×10^4
W1-D*	>1100	ND	W2-D	>1100	6.3×10^4	W3-D	460	1.0×10^5	W4-D	1100	1.2×10^3	W5-D	>1100	5.0×10^2
W1-E	240	ND	W2-E	>1100	4.0×10^2	W3-E*	240	2.8×10^4	W4-E	>1100	2.0×10^3	W5-E	>1100	2.0×10^2
W1-F*	>1100	ND	W2-F	460	2.5×10^4	W3-F	1100	6.0×10^4	W4-F*	>1100	1.0×10^3	W5-F	>1100	8.0×10^2
W1-G*	34	ND	W2-G*	1100	5.5×10^4	W3-G	460	3.0×10^4	W4-G*	>1100	1.7×10^3	W5-G**	>1100	1.8×10^4
W1-H*	>1100	ND	W2-H*	>1100	1.0×10^3	W3-H	>1100	2.0×10^5	W4-H	>1100	2.5×10^4	W5-H**	>1100	1.3×10^4
W1-I*	290	ND	W2-I	>1100	1.0×10^2	W3-I	>1100	5.3×10^4	W4-I*	>1100	7.0×10^3	W5-I*	460	6.0×10^3
W1-J*	>1100	ND	W2-J	>1100	1.0×10^2	W3-J*	1100	6.6×10^4	W4-J**	240	5.5×10^3	W5-J	>1100	4.0×10^4
W1-K*	>1100	ND	W2-K	>1100	3.2×10^4	W3-K	460	1.5×10^4	W4-K	1100	1.6×10^4	W5-K	460	6.0×10^3
W1-L*	1100	ND	W2-L	>1100	2.0×10^3	W3-L*	>1100	5.0×10^4	W4-L**	460	6.0×10^3	W5-L	>1100	3.5×10^4
W1-M*	>1100	ND	W2-M	>1100	2.0×10^5	W3-M	>1100	4.0×10^5	W4-M*	240	4.0×10^2	W5-M	240	1.0×10^4
W1-N*	>1100	ND	W2-N	>1100	2.0×10^5	W3-N*	>1100	6.0×10^4	W4-N*	>1100	5.0×10^4	W5-N*	460	6.0×10^2
W1-O*	>1100	ND	W2-O	>1100	6.0×10^2	W3-O*	1100	2.0×10^4	W4-O**	>1100	4.0×10^3	W5-O	460	7.0×10^2
W1-P*	150	ND	--	--	--	--	--	--	--	--	--	--	--	--
W1-Q*	1100	ND	--	--	--	--	--	--	--	--	--	--	--	--
W1-R*	460	ND	--	--	--	--	--	--	--	--	--	--	--	--
W1-S*	460	ND	--	--	--	--	--	--	--	--	--	--	--	--
W1-T*	290	ND	--	--	--	--	--	--	--	--	--	--	--	--
MCC	**810**	**ND**		**1144**	**4.1×10^4**		**968**	**9.1×10^4**		**960**	**8.9×10^3**		**874**	**9.9×10^3**

Legend: ID = Sample code; ND = Not determined; MCC = Mean Coliform Count; * = Samples from which *E. coli* isolates were recovered; ** = Samples from which two different strains of E. *coli* were recovered.

Table 4: Distribution of samples on the basis of proximity to septic tanks and depth to water table

		MEAN COLIFORM COUNT		KASMO AREA	OTA EFUN	IGBONA	OKE BAALE	OWODE EDE	TOTAL (%)
		MPN /100ml	Membrane filtration (cfu/ml)						
Distance from Septic Tank (m)	≤ 15.24m	0	0	0	0	0	0	0	0 (0.0)
	> 15.24m	943	3.8×10^4	20	15	15	15	15	80 (100.0)
	TOTAL	**943**	**3.8×10^4**	**20**	**15**	**15**	**15**	**15**	**80**
Depth to water table (ft)	≤ 30 ft	910	3.0×10^4	15	13	11	12	13	64 (80.0)
	> 30 ft	1074	6.9×10^4	5	2	4	3	2	16 (20.0)
	TOTAL	**943**	**3.8×10^4**	**20**	**15**	**15**	**15**	**15**	**80**
Protective Cover	COVERED	919	3.2×10^4	16	7	6	7	11	47 (58.8)
	OPEN	977	4.3×10^4	4	8	9	8	4	33 (41.2)
	TOTAL	**943**	**3.8×10^4**	**20**	**15**	**15**	**15**	**15**	**80**
Pumping facility	PUMP	704	1.7×10^4	7	1	1	1	2	12 (15.0)
	NO PUMP	985	3.9×10^4	13	14	14	14	13	68 (85.0)
	TOTAL	**943**	**3.8×10^4**	**20**	**15**	**15**	**15**	**15**	**80**

Table 5: Antibiotic Resistance profile of bacterial isolates obtained from well water samples in Osogbo, Southwestern Nigeria.

		Resistant Isolates; n (%)												
Location	**Total[a]**	**ATM**	**COL**	**FOS**	**CN**	**LEV**	**MEM**	**NAL**	**NIT**	**STR**	**S3**	**TIC**	**TGC**	**SXT**
KASMO	17 (38.6)	**ND**	4 (23.5)	**ND**	7 (41.2)	**ND**	**ND**	1 (5.9)	8 (47.0)	3 (17.6)	**ND**	17 (100)	12 (70.6)	6 (35.3)
OTA EFUN	3 (6.8)	1 (33.3)	0 (0.0)	0 (0.0)	0 (0.0)	1 (33.3)	3 (100)	**ND**	0 (0.0)	**ND**	3 (100)	3 (100)	0 (0.0)	**ND**
IGBONA	5 (11.4)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	5 (100)	**ND**	0 (0.0)	**ND**	5 (100)	5 (100)	4 (80.0)	**ND**
OKE BAALE	13 (29.6)	1 (7.7)	0 (0.0)	7 (53.8)	0 (0.0)	2 (15.4)	13 (100)	**ND**	1 (7.7)	**ND**	3 (23.1)	13 (100)	5 (38.5)	**ND**
OWODE EDE	6 (13.6)	0 (0.0)	0 (0.0)	1 (16.7)	0 (0.0)	1 (16.7)	6 (100)	**ND**	1 (16.7)	**ND**	0 (0.0)	6 (100)	2 (33.3)	**ND**
TOTAL	**44 (100)**	**2 (7.4)**	**4 (9.0)**	**8 (29.6)**	**7 (15.9)**	**4 (14.8)**	**27 (100)**	**1 (5.8)**	**10 (22.7)**	**3 (17.6)**	**11 (40.7)**	**44 (100)**	**23 (52.3)**	**6 (35.2)**

LEGEND: ATM, aztreonam;COL, colistin; FOS, fosfomycin; CN, gentamicin; LEV,levofloxacin; MEM, meropenem; NAL, nalidixic acid; NIT,nitrofurantoin; STR, streptomycin; S3, sulphonamides; TIC, ticarcillin; TGC, tigecycline; SXT, trimethoprim/sulfamethoxazole.**Total[a]=** Total number of isolates tested

Table 6: Multiple Antibiotic Resistance (MAR) Indices of bacterial isolates

MAR index	KASMO AREA (n = 17)	OTA EFUN (n = 3)	IGBONA (n = 5)	OKE BAALE (n = 13)	OWODE EDE (n = 6)	TOTAL (n = 44)
0	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
0.1	2 (11.8)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	2 (4.5)
0.2	0 (0.0)	0 (0.0)	0 (0.0)	1 (7.7)	3 (50.0)	4 (9.0)
0.3	3 (17.6)	1 (33.3)	1 (20.0)	6 (46.1)	2 (33.3)	13 (29.5)
0.4	3 (17.6)	2 (66.7)	4 (80.0)	5 (38.5)	0 (0.0)	14 (31.8)
0.5	5 (29.4)	0 (0.0)	0 (0.0)	1 (7.7)	1 (16.7)	7 (15.9)
0.6	3 (17.6)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	3 (6.1)
0.7	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
0.8	1 (5.9)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	1 (2.3)
0.9	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
1.0	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)

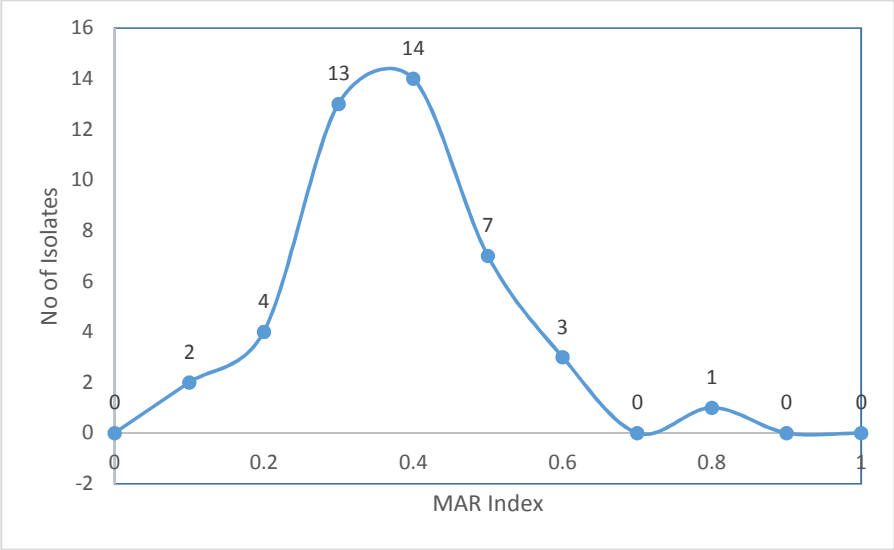

Figure 1: The Frequency of Multiple Antibiotic Resistance Indices of bacterial isolates

DISCUSSION

Ground water particularly hand-dug wells supply drinking water for more than half of the Nigerian population, and there is hardly any home in Osogbo which does not have a minimum of one well for domestic use. In highly congested areas, these wells are easily inundated by surface run-off and flood, thus increasing the chances of contamination with faecal coliform bacteria. The objectives of the present study aimed at detecting the faecal contamination of well water by septic tanks in selected locations in Osogbo, and the results revealed a high rate of contamination as all the wells analyzed were contaminated with coliforms. Total coliform counts were very high with *Escherichia coli* present in 48.8% of the wells. About 57 (71.3%) of the wells had coliform counts of 1100/100ml and above across the five locations (Table 3) even though all the wells were at distances above the standard of 15.24m from the nearest septic tank, as stipulated by the Environmental Protection Agency (InspectAPedia, 2010). Many authors who have studied the effects of septic tank proximity to drinking water wells have reported similarly high values of total coliform in different groundwater samples in their studies conducted within the country (Ifabiyi, 2008; Adetunji and Odetokun, 2011; Akinbile and Yusoff, 2011; Fubara-Manuel and Jumbo, 2014; Oluwasola *et al.*, 2017) and outside the borders of Nigeria (Arwenyo *et al.,* 2017; Rohmah *et al*., 2018). The Environmental Protection Agency (EPA) considers Total Coliform as an indicator of other pathogens in drinking water, and it is used to determine satisfactory water treatment and integrity in the distribution system (USEPA, 2013). Therefore, there should be no such organisms in any drinking water source as the maximum limit of contaminant of pathogenic bacteria such as Total coliform and *Escherichia coli* is 0 for drinking water (Rohmah *et al.,* 2018). The presence of extremely large amounts of coliforms in this study clearly violated the guidelines for drinking water quality (WHO, 2010; USEPA, 2013). Of all the eighty (80) samples, only two (both in Kasmo area) had coliform counts less than 100 in 100ml of sample, and even one of these harbored one strain of *Escherichia coli*. This high coliform count, indicative of faecal contamination of water, has been associated with the development of several water borne gastrointestinal illnesses (Mackenzie *et al.,* 1995; Kaper *et al.,* 2004; Maier, 2009). This portends serious health risks for infants, young children, and people with impaired immune systems (USEPA, 2013; Rohmah *et al.,* 2018). Houses in Osogbo are built very close to each other, and many of the houses especially in the selected areas are not properly laid out because of the relatively small sizes and disjointed nature of private land ownership. House owners in many instances, do not pay cognizance to location of septic tanks in neighboring houses such that distances between wells and septic tanks in nearby compounds may be below the recommended distance. The selected areas are densely populated and as such, a high population of users of these wells could also contribute to the high coliform counts in all the wells. This is in line with previous studies in different areas of the country (Shekwolo and Brisbe, 1999; Shimizu *et al.*, 2009; Adetunji and Odetokun, 2011) and beyond (Obiri-Danso *et al.,* 2009).

In this study, it was observed that the coliform counts were higher than the mean coliform count in wells deeper than 30ft to the water table using both the MPN and the membrane filtration methods, as the values were much higher than the values obtained for wells at 30 ft or lesser to the water surface (Table 4). The reasons for this were not quite clear but could be due to leaching of contaminating microorganisms through the soil from the septic tanks due to the soil type, topography as well as groundwater flow (Rohmah *et al.,* 2018).

Previous studies have identified leachate from septic tanks as a major potential source of groundwater contamination from pathogens (Gerba and James, 2005; Fong *et al.,* 2007; Adetunji and Odetokun, 2011).

The covered wells and those fitted with pumping facilities had lower coliform count. This is understandable as these properties result in limited exposure of the wells to environmental contaminants and, probably better management practices by users.

The antibiotic resistance pattern of the recovered isolates revealed that all the isolates were resistant to ticarcillin and meropenem, which are both beta lactam antibiotics although in different classes - beta lactam and carbapenems respectively. This pattern of resistance suggests that the isolates were most likely to be extended spectrum beta lactamase producers and /or carbapenemase producers (Frédéric *et al.,* 2002). About 52.3% were resistant to tigecycline. Aztreonam appeared to be the most effective of the antibiotics as it was unable to inhibit only 2 out of 27 (7.4%) isolates tested against it. This was closely followed by Colistin as only 4 out of 44 isolates (9.0%) were resistant to it (Table 5). Aztreonam and Colistin have been reported as active *in-vitro* against Enterobacteriaceae (Ku *et al.,*2015).

A total of 35 isolates (79.5%) exhibited multidrug resistance to three or more classes of antibiotics. The high level of multi resistance in isolates from wells supplying water for domestic use and possible ingestion by humans in this study is worrisome and underscores the severity of carriage of resistant organisms in this environment. The multiple antibiotic resistance (MAR) indices calculated for the isolates equally reveal a trend which heralds serious public health implications. Only 2 out of the 44 *E. coli* isolates tested had Multiple antibiotic resistance (MAR) indices of < 0.2 (Figure 1). 95.5% of them had MAR indices ≥ 0.2. This high value indicates that these organisms are originating from an environment that is already pressurized with the indiscriminate use of several antibiotics. The implications of these high MAR indices are far reaching and suggests a need for the constant surveillance of bacteria antibiotic susceptibility patterns from groundwater samples in different environments and strict government policies and regulations regarding antibiotic prescription to ensure the decline in the spread of resistant bacteria strains.

The result of this study suggests proof of well water contamination by fecal coliform especially in this study where the EPA's recommendations for sighting of a well is attained. Since all the well water analyzed in this study were found to be contaminated by pathogens of fecal origin, they are consequently, neither fit to be drunk nor safe to be put into such uses that can facilitate their ingestion by humans. In light of this, recommendations for minimum proximity of septic tanks from wells should be reviewed and updated by government policies; regular monitoring of wells used for domestic purposes should be also be carried out prevent the consumption of contaminated water and its accompanying health effects.

Conflict of Interests

The authors have not declared any conflict of interests.

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