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Impact of the Proximity of Septic Tanks on the Bacteriological Quality of Well Water from Private House-holds in Ado Ekiti, Nigeria

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Authors' contributions

This work was carried out in collaboration between all authors. Authors KA and OAO designed the study, performed the statistical analysis, wrote the protocol. Author EIO wrote the first draft of the manuscript. Authors EIO and OAO managed the analyses of the study. Author KA managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

This study evaluated the effects of the proximity of septic tank on the bacteriological quality of domestic well water of some private house-holds in "Aba Erinfun", Ado Ekiti, Ekiti State, Nigeria. Twenty (20) samples of private house-holds well water at different locations in the community were analysed for total bacteria count, total coliform count and characterization of isolates. The distance between the wells and the nearest septic tanks ranged from 2.74 m - 42.90 m. Total viable count ranged from 2.4 - 25.4 $\times 10^5$ cfu/ml while that of coliform count was 0 - 12.0 $\times$ 10^5 cfu/ml. The Isolates characterized and identified in the water samples include *Escherichia coli, Bacillus* spp, *Staphylococcus* spp and *Pseudomonas* spp.

These results showed that the distance between septic tanks location and water wells has significance in terms of bacterial pollution of the well water. However, other factors like population density, environmental hygiene and ringing/non ringing of wells may also affect the quality of well water. Irrespective of the distance of the water wells from the nearest septic tank, the presence of coliform organism was noticed.

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1. INTRODUCTION

Water is essential for life sustenance. A satisfactory supply must be made available to consumers [1] But despite the fact that water is perhaps the most abundant substance in nature, one-sixth of the world population still does not have access to safe drinking water [1,2]. Water has its source from rivers, lakes, oceans, rainfall and underground water.

Water is examined microbiologically to determine its sanitary quality and its suitability for general use. The aim being that it will be accepted for internal consumption and other uses in contact with man [2,3]. Water that contains poisonous chemical substances, pathogenic organisms (infective and parasitic agents), Industrial or other waste or sewage is referred to as being contaminated or polluted [4]. The growing imbalance between water supply and demand has led to chronic water shortage and to the use of water collected from spring, wells, rivers and even rain. Apart from the quantitative shortages, the quality of drinking water is becoming a serious public health issue for the past few years [2,3,5,6].

Water that is wholesome and fit for drinking rapidly deteriorates because of inefficient management of the piped water distribution system and largely because of the direct discharge of untreated sewage into the water sources [7]. The source of water contamination responsible for the spread of infectious disease is almost invariably faeces. Faecal contamination of water is established by the isolation of an organism that occurs only in faeces, never free-living in nature. The most important pathogens transmitted through the water route are *Salmonella typhi, Escherichia coli, Campylobacter, Shigella* and the other organism causing diarrhoea [8]. Ideally drinking water should not contain any microorganism such as faecal pollutants or any bacteria indicative of faecal pollution, since the presence of these micro organisms has been seen as an indicator for faecal contamination of water. Microbial tests are useful for monitoring the microbial quality of water used for human consumption. Therefore, evaluation of microbial quality of water is an important weapon to the achievement of potable water for daily consumption [9].

According to the environmental campaign organization WWF, pollution from toxic chemicals threatens life on this planet. Every ocean and every continent from the tropics to the once-pristine polar regions is contaminated [4,10].

The main objective of this study is to examine the effect of the proximity of septic tanks on the microbiological quality of domestic house-holds well water. This is with a view to ascertaining the microbial properties of such well water in that location in order to safeguard public health.

2. MATERIALS AND METHODS

2.1 Materials

Glass sampling bottles were obtained from BISO Lab in Ado-Ekiti, Ekiti State, the fetchers (plastic buckets) and the rope (Thin trawling ropes) were bought from “Oja Oba” market in Ado-Ekiti. The measuring tape used for the measurement of the distance from the wells to the septic tanks was obtained from the Department of Quantity Surveying, Federal Polytechnic, Ado-Ekiti, Ekiti State.

The sampling bottles were coded, wrapped with aluminum foil alongside the lids and sterilized in an autoclave at 121°C temperature for 15 minutes, the fetchers and ropes also were sterilized for 10 minutes at a temperature of 100°C and oven dried for 30 minutes at a temperature of 60°C. The bottles, the lids and the fetchers were coded according to the location/site.

2.2 Collection of Water Samples

The water samples were collected using the plastic fetchers between 14th and 15th of July 2016 aseptically in sterilized sampling bottles and taken to the microbiology laboratory of the research laboratory, School of Science & Computer Studies, Federal Polytechnic, Ado-Ekiti for analysis. Microbial analysis was done within 3 hours after sample collection. Samples were collected in duplicates at each of the identified house-holds in accordance with the coded sampling bottles. The distance from the septic tank to the wells were measured and noted in meters, the topography of the wells to the septic tanks was also noted. The samples were collected from (20) twenty different private house-holds water wells located within “Aba Erinfun” in Ado-Ekiti. One sterilized fetcher was used for all the wells (See Table 1). Preventive measures were taken to avoid contamination of the water samples by any form.

2.3 Serial Dilution

The samples were vigorously shaken and serially diluted. The process was repeated up to 10^{-3} from the serially diluted water samples. 1ml each was pour plated in nutrient agar for total bacterial enumeration. Eosin methylene blue agar was used for *E. coli* count. Purified cultures were characterized biochemically and physiological by criteria [11,12].

2.4 Determination of Total Bacterial Count

Determination of bacterial load in the water samples were done in duplicates. Bacterial plate count was carried out using the pour plate method with nutrient agar [12]. This method was based on the serial dilution of water samples which was then pipetted into each sterile petri dish. About 20 ml of molten nutrient agar was cooled to 45°C and poured into each petri dish containing 1ml of the water sample. Plates were allowed to cool and set after which they were incubated in inverted position at 37°C. After 24 hours of incubation, the plates were counted using a colony counter.

2.5 Determination of Total Coliform Count

Determination of total coliform load in the water samples were done in duplicates. Coliform count was carried out using the pour plate method with eosin methylene blue (EMB) agar [11-13]. About 20 ml of molten EMB agar was cooled to 45°C and poured into each petri dishes containing 1ml of the water samples. Plates were allowed to cool and set after which they were incubated in inverted position at 37°C. After 24 hours of incubation the plates were counted using colony counter.

2.6 Characterization and Identification of Isolates

The isolates were classified on the basis of biochemical and physiological appearance.

Positive tubes of the presumptive test were sub-cultured on eosin methylene blue (EMB) agar for the enumeration of *Escherichia coli* and other enteric coliforms. All the inoculated media were incubated aerobically at 37°C for 24-48 hours after which the isolates were further characterized by a combination of colonial and morphological characteristics on solid media, both biochemical test [11,13].

Table 1. Sample coding

S/N	Sample/Code	Distance (m)	Location/Name
1.	MOZ	42.90	Star FM
2.	LDP	24.82	Diamond
3.	ABB	5.64	Christ Villa
4.	DPP	35.05	Prime
5.	LZL	32.21	Bugar 1
6.	SOP	35.71	Bugar 2
7.	DOZ	6.40	House of Rep.
8	NOL	9.83	Red gate
9.	POP	35.89	Hollysam
10.	SRZ	2.74	Halleluyah
11.	MLO	34.59	ATM
12.	AOB	37.67	Divine favour
13.	SOZ	18.29	FCT 1
14.	DKO	21.95	FCT 2
15.	LBB	12.80	Lekki phase 1
16.	ZOP	24.08	Matured student 1
17.	LMK	4.75	Matured student 2
18.	BOA	6.38	Virgin
19.	DOL	31.80	House of senate
20.	ZLM	30.53	Americana

2.7 Gram Staining

A sterilised inoculating loop was used to take a sample of the innoculum from the agar and spread on the microscopic slide and heat fixed by passing through a bunsen burner flame.

A smear of the organisms from a 24 hours old culture was dried in cool air, heated till fixed, covered with crystal violet and left for 2 minutes. The stain was then washed off in tap water. Lugol's iodine was poured on the smear, left for 30 seconds. Absolute alcohol was dropped over the slanted slide and washed. This smear was then counterstained with 0.5% safranin for 30 seconds, washed with water and dried between filter paper, prior to examination under the microscope using oil immersion [13].

2.8 Data Analysis

All experimental analysis was done in duplicates. The average of each duplicate determination was taken as the representative results.

3. RESULTS AND DISCUSSION

3.1 Total Viable Counts of Water Samples from Private House-holds Well at Aba Erinfun, Ado Ekiti

The total viable count of analysed well water samples obtained from private house hold wells at Aba Erinfun is shown in Table 2. The values ranged from 2.4 - 25.4×10^5 cfu/ml implying the water samples have a high bacterial load, potentially making these water samples a threat to public health.

The spread of diseases through pollution and faecal contamination of water particularly in developing and underdeveloped countries is common and well reported [1,9,10,14,15,16,17]. It was observed that all the water samples collected were contaminated with high total viable counts. Sample LDP had the highest total viable count (25.4×10^5 cfu/ml) with a distance of 4.82 m from the nearest septic tank which is within the minimum allowable distance of septic tanks to water wells. The well was covered but un-ringed, this may perhaps be responsible for the high total viable count, un-ringed water wells may be polluted due to percolation of run-off water from the environment. The high microbial count may also be due to fetching water from the well with contaminated fetcher or poor sanitation as observed around the well site. The implication of high total viable count in water samples is risk of diarrhoea, dysentery and typhoid fever arising from consumption of polluted water [1,18-20].

Well MOZ (42.90 m) had the longest distance between the wells and the nearest septic tank. Despite this considerable long distance of the water well from a septic tank, it had a high viable count (4.2×10^5 cfu/ml). According to Inspect Amedia [21], the minimum distance of 15.24 m away from the nearest septic tank will ensure that well water is free from coliform contamination. The well was covered but un-ringed and this may possibly account for its high total viable count. Similarly, wells DPP and MLO with a good distance from the nearest septic tank of 35.05 m and 34.59 m had high total viable count of 3.8×10^5 cfu/ml and 3.2×10^5 cfu/ml respectively, probably because they are both without covers and unringed, this perhaps may account for the contamination of MLO water samples. They also contain *Escherichia coli.*

Table 2. Total viable count (cfu/ml) of private households well water from Aba Erinfun, Ado Ekiti

S/N	Samples	No of colonies × 10^5 cfu/ml
1.	MOZ	4.2
2.	LDP	25.4
3.	ABB	5.8
4.	DPP	3.8
5.	LZL	6.8
6.	SOP	5.8
7.	DOZ	6.0
8.	NOL	15.2
9.	POP	4.8
10.	SRZ	4.4
11.	MLO	5.0
12.	AOB	6.0
13.	SOZ	7.0
14.	DKO	2.4
15.	LBB	4.0
16.	ZOP	6.0
17.	LMK	3.6
18.	BOA	7.0
19.	DOL	9.0
20.	ZLM	5.0

Table 3. Total coliform count of private household well water samples from Aba Erinfun

S/N	Samples	No of colonies × 10^5 cfu/ml
1.	MOZ	0
2.	LDP	0
3.	ABB	0
4.	DPP	2.5
5.	LZL	0
6.	SOP	12.0
7.	DOZ	7.1
8.	NOL	0
9.	POP	8.4
10.	SRZ	6.0
11.	MLO	3.2
12.	AOB	10.4
13.	SOZ	9.0
14.	DKO	2.0
15.	LBB	1.5
16.	ZOP	1.0
17.	LMK	0
18.	BOA	3.0
19.	DOL	0
20.	ZLM	1.6

3.2 Coliform Count of Private Households Well Water from Aba Erinfun, Ado Ekiti

The value for the total coliform count as shown in Table 3 ranges from 0 – 12.0×10^5 cfu/ml. The highest total coliform count was in sample SOP (12.0×10^5 cfu/ml) with a distance of 35.71m from the nearest septic tank. This high incidence of coliform in sample SOP may likely be due to the fact that the well is un-ringed and partly, the considerable large number of students living in the area. In a previous study [22], it was suggested that if the number of people accessing a particular well is high, there may be a high degree of contamination. Coliform bacteria are pathogenic organism mainly of faecal origin, therefore any water source used for drinking or cleaning purpose should not contain any organism of faecal origin [6,23,24]. Therefore, water from well SOP is unfit for human consumption. Several other factors apart from distance of the wells to the nearest septic tanks may promote bacterial contamination of the water wells. Some wells may be covered, but if they are located in a dirty environment and highly populated house, the microbial load may be high. Likewise, uncovered wells located in dirty environment and densely populated house will have higher microbial loads [2,6,16,25]. Wells POP, SOZ and DKO were located well above the minimum allowable septic tank location distance, but also had high coliform bacteria and *Escherichia coli* counts. This may probably be due to the large number of people accessing the well water. Samples MOZ, LDP, ABB, LZL, NOL, LMK and DOL were all free of coliform organism, interestingly among these wells, some had their distance from the nearest septic tank well below

Table 4. Physiological characteristics of organisms in the water samples (appearance)

S/N	Samples	Colour	Shape	Surface (appearance)
1.	MOZ	Cream	Regular	Smooth
2.	LDP	Yellow, cream	Regular, Irregular	Smooth
3.	ABB	Cream	Regular, Irregular	Smooth
4.	DPP	Yellow, cream	Regular, Irregular	Smooth, Rough
5.	LZL	Yellow, cream, Green	Regular, Irregular	Smooth
6.	SOP	Cream	Regular	Rough
7.	DOZ	Cream	Regular, Irregular	Smooth
8.	NOL	Cream	Regular, Irregular	Smooth
9.	POP	Green, cream	Regular, Irregular	Smooth
10.	SRZ	Green, cream	Regular, Irregular	Smooth
11.	MLO	Yellow, cream	Regular	Smooth
12.	AOB	Green, cream	Irregular	Smooth, Rough
13.	SOZ	Green, cream	Irregular	Smooth, Rough
14.	DKO	Green, cream	Regular, Irregular	Smooth, Rough
15.	LBB	Cream	Regular, Irregular	Smooth, Rough
16.	ZOP	Cream	Irregular	Smooth, Rough
17.	LMK	Yellow, Cream, Green	Regular, Irregular	Smooth
18.	BOA	Yellow, Green	Irregular	Smooth, Rough
19.	DOL	Yellow, Cream	Regular, Irregular	Smooth, Rough
20.	ZLM	Green, Cream	Regular	Smooth,

Table 5. Microorganisms Isolated from different water sources

Sample	Microorganism isolated	Biochemical test					
		Nitrate reduction	Catalase	Coagulase	Indole	Oxidase	Gram staining
MOZ	*Pseudomonas*	-	-	+	-	+	-
LDP	*Bacillus*	-	+	-	-	-	+
ABB	*Staphylococcus*	-	+	+	_	_	+
DPP	*Staphylococcus*	-	+	+	-	-	+
LZE	*Staphylococcus*	-	+	+	_	_	+
SOP	*Escherichia coli*	-	-	-	+	_	-
DOZ	*Staphylococcus*	-	+	+	_	_	+
NOL	*Pseudomonas*	-	_	+	_	+	-
POP	*Escherichia coli*	-	-	-	-	+	-
SRZ	*Escherichia coli*	-	_	_	+	_	-
MLO	*Escherichia coli*	-	-	-	+	-	-
AOB	*Bacillus*	-	+	_	_	_	+
SOZ	*Escherichia coli*	-	_	_	+	_	-
DKO	*Escherichia coli*	-	_	_	+	_	-
LBB	*Staphylococcus*	-	+	+	_	_	+
ZOP	*Pseudomonas*	-	_	+	_	+	-
LMK	*Staphylococcus*	-	+	+	_	_	+
BOA	*Staphylococcus*	-	+	+	_	_	+
DOL	*Pseudomonas*	-	_	+	-	+	-
ZLM	*Bacillus*	-	+	-_	-_	_-	+

Key: + = Positive, - = Negative

the standard of 15.24 m, i.e 50 feet, while others were within the acceptable distance. Although wells ABB, NOL and LMK were within the minimum septic tank distance, a lesser number of people access these wells and therefore, the wells may be less prone to gross contamination from other sources. Well SRZ has the shortest distance (2.74 m) from a septic tank, and although the well has a cover, it is un-ringed. The very high total coliform count of 6.0×10^5 cfu/ml coupled with the fact that *Escherichia coli* was also isolated from it is an indication of gross pollution implying that the water is unsafe for human consumption [10,22]. This attests to the fact that excreta might have an easy entrance into water from the nearest septic tank, which can decrease with increasing distance from pollution and defaecation sites [22].

Other species like S*taphylococcus* spp*, Bacillus* spp *and Pseudomonas* spp found in the water samples possibly got into the water through handlers skin, hand, saliva when talking, sneezing and coughing when fetching the water which produces droplets*. Pseudomonas* spp found in the water samples possibly got into the water from droplets that could settle on the water.

4. CONCLUSION

It can be concluded from this study that the minimum standard distance of 15.24 m of wells from the nearest septic tank might not be enough to guarantee the safety of domestic well water. Findings from this study clearly highlight the non-conformity of most of the well water samples with the WHO standard recommendation for potable water quality. The water well with the least distance of 2.74 m was found to contain high coliform count and *Escherichia coli*, despite the fact that it is always covered, hence, the closeness of a well to septic tank may imply a higher tendency for contamination, therefore a considerable distance well above 15.24 m amongst other factors, will be essential for standard quality domestic well water.

This study has shown that there is a high incidence of well water contamination by pathogenic organisms due to inadequate spacing from the septic tanks and the water wells. To reduce the spread of these incidences, water wells must be maintained in good hygienic sanitary conditions, in and around the wells. There must be thorough cleaning of water storage equipment and public health can be safeguarded by carrying out routine water treatment with appropriate chemicals.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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