

TES Trends in Environmental Sciences

Bacteriological Quality of Well Water in Ekpoma, Edo State, Nigeria

Iyevhobu Kenneth Oshiokhayamhe^{1,2,3}, Ken-Iyevhobu Benedicta Agumeile^{3,4}, Asibor Ernest^{2,5}, Obohwemu Kennedy Oberhiri^{6,7}, Oseni David Idahosa^{3,8}, Momodu Kawthar Osilama^{1,3} and Nnamudi Promise^{1,3}

¹Department of Medical Microbiology, Faculty of Medical Laboratory Science, Ambrose Alli University, Ekpoma, Edo State, Nigeria

²Department of Medical Laboratory Science, Faculty of Applied Health Science, Edo State University, Iyamho, Edo State, Nigeria ³Saint Kenny Diagnostic and Research Centre, Ujoelen, Ekpoma, Edo State, Nigeria

⁴Department of Microbiology, Ambrose Alli University, Ekpoma, Edo State, Nigeria

⁵Department of Histopathology and Cytopathology, Faculty of Medical Laboratory Science, Ambrose Alli University, Ekpoma, Edo State, Nigeria

⁶Faculty of Health, Wellbeing and Social Care, Global Banking School, Oxford Brookes University, Birmingham, United Kingdom ⁷PENKUP Research Institute, Birmingham, United Kingdom

⁸Department of Chemical Pathology, Faculty of Medical Laboratory Science, Ambrose Alli University, Ekpoma, Edo State, Nigeria

ABSTRACT

Background and Objective: Water is the most common liquid on our planet, vital to all life forms. It is a chemical substance with the chemical formula H_2O . It is a molecule containing one oxygen and two hydrogen atoms combined by covalent bonds. This study aims to assess the bacteriological quality of well water in Ekpoma, Edo State, Nigeria, to determine its suitability for consumption and potential health risks. Materials and Methods: Four different wall water samples were collected from four different wells in some quarters at Ekpoma, Esan West Local Government Area, Edo State, Nigeria. The different well water samples collected were taken to the Microbiology Laboratory of Saint Kenny Diagnostic and Research Center for analysis. The microbiological quality of well water in Ekpoma was investigated using standard bacteriological methods. Microbiological tests were conducted on the sample to detect the types and number of heterotrophic and coliform bacteria in it. The coliform count of the well water samples was determined using the multiple tube techniques and the Eijkman test for differentiation of Escherichia coli. **Results:** The mean total viable count/heterotrophic plate count of the water samples ranged from 3.35×10^{6} - 5.30×10^{8} (cfu/mL), and the total coliform count is within the range of <2-9/100 mL of the water samples. The bacteria isolated include Escherichia coli, Klebsiella pneumoniae, Proteus mirabilis, and Salmonella sp., Shigella sp., Staphylococcus aureus, Streptococcus faecalis, Vibrio cholerae, and Vibrio parahaemolyticus. The presence of these microorganisms (bacteria) in well water samples is of public health importance. Conclusion: The number of coliform bacteria recorded in the study is higher and falls short of the WHO standard. It implies that the well water did not meet the World Health Organization standard for drinking water, hence the water needs to be boiled or filtered before drinking to avoid waterborne diseases in the Ekpoma community.

KEYWORDS

Bacteriology, well water, coliform, drinking water, public health

Copyright © 2025 Oshiokhayamhe et al. This is an open-access article distributed under the Creative Commons Attribution License, which permits unrestricted use, distribution and reproduction in any medium, provided the original work is properly cited.



INTRODUCTION

Water is the most common liquid on our planet, vital to all life forms. It is a chemical substance with the chemical formula H_2O . It is a molecule containing one oxygen and two hydrogen atoms combined by covalent bonds. It is a liquid at ambient conditions, but it often co-exists with its solid state, ice, and gaseous state, water vapor or steam. Water covers 70.9% of the Earth's surface and is vital for all known forms of life^{1,2}. On Earth, 96.5% of the planet's water is found in oceans, 1.7% in groundwater, 1.7% in glaciers and the ice caps of Antarctica and Green land, a small fraction in other large bodies, and 0.001% in the air as vapor, clouds (formed of solid and liquid water particles suspended in air), and precipitation. Only 2.5% of the Earth's water is in fresh water, and 98.8% of that water is ice water and ground water. Less than 0.3% of all freshwater is in lakes and the atmosphere, and an even smaller amount of the Earth's freshwater (0.003%) is contained within biological bodies and manufactured products³.

The hydrological cycle of evaporation, transpiration (evapotranspiration), condensation, precipitation, and runoff, which typically leads to the sea, is how water on Earth moves continuously. Overland precipitation is influenced by transpiration and evaporation. Additionally, it can be found in a liquid crystal condition close to a hydrophilic surface^{4.5}. Nearly every region of the World has seen improvements in safe drinking water throughout the past ten years. Still, more than 2.5 billion people lack proper sanitation, and billions more lack access to safe water. Access to clean water and GDP per capita are correlated. However, according to some analysts, over half of the World's population would be vulnerable due to water by 2025. According to a November, 2009 estimate, water demand would be 50% higher than supply in some emerging nations by 2030. Water is essential to the global economy because it can be used as a solvent for a wide range of chemicals and makes transportation and industrial cooling easier. Water resources can be rain, river, well, or groundwater water and it is one of the major components of environmental resources that can cause pollution to humans during human activities on the Earth's surface⁶. Poor water quality can pose a health problem enough to threaten health before consumption cannot be overemphasized, but irregularity or acute shortage of potable water to the populace has led to people drinking water from hand-dug wells and other sources, including streams, ponds, rivers, and lakes^{7,8}. This water could be polluted with agricultural, industrial, and domestic wastes. Industrial pollution may involve seepage of used water containing chemicals such as metals and radioactive compounds or contaminated water from damaged pipelines that infiltrate into the groundwater and can be in hand-dug wells⁹. Also, domestic pollution may involve seepage from septic tanks, pit latrine cesspools, and privies. In addition, agricultural pollution is also from irrigation and runoff water carrying fertilizers, pesticides, herbicides, and faecal matter to a water source. Polluted or contaminated water could serve as a source of water to another community, unknowingly to the consumer, that the water has been contaminated in its source by seawater intrusion into the coastal aquifer. However, the World Health Organization¹⁰ recommends that wells should be located at least 30 m away from latrines and 17 m from septic tanks. The presence of faecal coliforms or *Escherichia coli* is an indicator for the presence of waterborne pathogens. The consumption of contaminated water could lead to various waterborne diseases, which include typhoid fever, diarrhea, cholera, and dysentery. Diarrhea is the most common water-related disease, and it is most prevalent in children¹¹⁻¹⁴. Every 20 sec, a child dies from water-related disease¹³. Diarrhea remains the second leading cause of death among children under five globally.

Water can dissolve many substances, giving it a varying taste and odour. Humans and other animals have developed senses that enable them to evaluate the potability of water. However, pure water is tasteless, odourless, colourless, and free from faecal contamination and toxic chemicals¹³. Bacteriological examination of water is, therefore, a powerful tool to prevent the presence of microorganisms that might constitute a health hazard. Water from streams, wells, rivers, ponds, and lakes usually contains large numbers and varieties of microorganisms because they are exposed to direct pollution and usually do not meet the standard qualities of potable water. This work aims to investigate the bacteriological quality of well water in some quarters in Ekpoma, Edo State, Nigeria.

MATERIALS AND METHODS

Collection of samples: Four different wall water samples were collected from four different wells in some quarters at Ekpoma, Esan West Local Government Area, Edo State, Nigeria, from August, 2024 to December, 2024. The different well water samples collected were taken to the Microbiology Laboratory of Saint Kenny Diagnostic and Research Center for analysis.

Media used: The media used in this project work include the following: Nutrient agar, MacConkey agar, *Salmonella Shigella* agar (SSA), Thiosulphate Citrate Bile Salt Sucrose (TCBS) agar, Eosin Methylene blue (EMB) agar, and peptone water.

Sterilization of materials: All glassware was washed in foaming detergent water and later rinsed properly in warm water. After which the materials were sterilized using a hot air oven at 160°C for 1 hr. Media were sterilized in an autoclave at 121°C for 15 min at 15 psi pressure and were allowed to cool to about 45°C. Wire loops were stylized by passing them through a Bunsen burner flame until red hot before use. The incubator, hot air oven, and other equipment used were also sterilized thoroughly to achieve maximum sterilization.

Bacteriological analysis: Microbiological tests were conducted on the sample to detect the types and number of heterotrophic and coliform bacteria in it. The coliform count of the well water samples was determined using the multiple tube techniques and the Eijkman test for differentiation of *Escherichia coli*¹³.

Multiple tube techniques: This technique is used for the detection and enumeration of coliform bacteria and *Escherichia coli*. It is divided into three stages: Presumptive test, Confirmatory test & Completed test¹³.

Presumptive test: In this test, undiluted samples of water were used. On each well water sample, five bijou bottles containing 10 mL of sterile double-strength MacConkey broth were inoculated with 10 mL of the water samples. Five bijou bottles containing 5 mL of sterile single MacConkey broth were inoculated with 1 mL of the water samples, and another five bijou bottles containing 5 mL of sterile single MacConkey broth were inoculated with 0.1 mL of the water samples. A Durham tube was filled and inverted in each of the bijou and was labeled with the samples' code numbers. They were incubated at 37°C for 24 hrs. Gas productions at the end of incubation were indicated by the presence of air space at the top of the Durham's tubes, while the change in color of the medium from red to yellow indicates lactose utilization. The number of positive reactions from each set was recorded and compared with the most probable number (MPN) table for the readings of the MPN of each water sample.

Confirmatory test/Ejikman test for *E. coli***:** The bottles from the multiple tube technique showing positive reaction from each sample were subcultured into peptone water, and it was divided into two sets. A set was incubated at 37°C for 24 hrs, while the other set was incubated in a water bath at 44°C for 24 hrs. After incubation, 1 or 2 drops of Kovac's reagent were added to the culture at 44°C. A red ring coloration on the top of the culture (indole positive), confirms the presence of *E. coli*.

Completed test: This test for the presence of other coliforms and *E. coli* as well. From the positive tubes of the confirmatory test, a loopful of the sample was inoculated on MacConkey agar and incubated at 37°C for 24-48 hrs. The morphology of the various colonies observed was recorded.

Faecal streptococcal count: A heavy inoculum from the multiple tubes showing positive reactions was subcultured into glucose azide broth and incubated at 44°C for 24 hrs. The cultures showing positive results indicated by a change of color from red to yellow in the medium were again subcultured onto MacConkey agar and incubated at 37°C for 24 hrs. After incubation, a tiny pink or red colony was formed. This confirms *Streptococcus faecalis* because it is only *Streptococcus faecalis* that grows on MacConkey agar with such colonies.

Total viable count (Aerobic heterotrophic plate count): The upper plate method was used to estimate the number of heterotrophic bacteria. A tenfold serial dilution was made. One milliliter (1 mL) from the 10^{-4} and 10^{-6} dilutions was aseptically transferred with the aid of disposable sterile syringes into a sterile Petri dish, and molten Nutrient agar (NA) was poured over the inoculum. Each of the Petri dishes was gently rocked to ensure uniform distribution of the water sample within the molten agar in the Petri dishes. The agar was allowed to solidify, and the plates were incubated at 30°C for 24 hrs. At the end of the incubation, the total heterotrophic plate count (cfu/mL) of the water samples was calculated using the number of viable organisms per ml of water = number of colonies per plate×dilution factor.

Isolation of other bacteria from the water samples: Inoculum from the nutrient agar plate used for total viable counts was subcultured into MacConkey agar and incubated at 37°C for 24 hrs. To isolate bacteria and other coliforms that are present in the water samples. Characterization of the bacteria present in the water sample was based on each isolate's cultural, morphological, and biochemical reactions. The characteristic growth and colonial appearance of the organisms on the growth media, Nutrient agar (NA), MacConkey agar (MAC), Eosin methylene blue (EMB) Agar, *Salmonella Shigella* Agar (SSA), and Thiosulfate-Citrate Bile Salts Sucrose (TCBS) agar were observed and noted.

Bacteriological characterization: Isolated colonies from the agar medium were subjected to staining using the Gram staining techniques as described by Christian Gram in 1884¹³. Gram staining procedure; a thin smear of the bacterial colony was made on a grease-free microscope slide with a sterilized wire loop. The smear was heat-fixed by passing it over a Bunsen burner flame four times. The smear was covered with crystal violet and allowed to stay for 60 sec. The stain was flooded off with clean water; the smear was flooded with Lugol's iodine (mordant) and flooded off with water after 30 sec. The smear was flooded with acetone to decolorize the primary stain and flooded off immediately. The smear was counterstained with safranine and allowed to stay for 60 sec, and flooded off with water. The back of the slide was wiped with clean cotton wool and placed on a draining rack for the smear to air-dry. The stained smear was examined under the microscope using the x100 oil immersion objective lens.

Biochemical test: Various biochemical tests were carried out for the identification and confirmation of isolates¹³.

Catalase test: This test was done to identify organisms that can produce the enzyme catalase. A drop of distilled water was placed on a clean glass slide, and inoculum suspected to be catalase positive was emulsified on it, and a few drops of Hydrogen Peroxide (H_2O_2) were added to the suspension of the organisms. The production bubbles indicate a positive test

Oxidase test: This test was used for those bacteria that have the ability to produce the enzyme cytochrome oxidase, which is capable of catalyzing the transfer of electrons (e) from an electron donor in the bacteria and a redox dye. This test was performed by soaking a small section of filter paper in 1% aqueous Tetramethyl-paraphenylene-diamine-dihydrochloride (oxidase reagent). Some fresh culture was scraped with a sterile L-bent glass rod and impregnated on the soaked filter paper and observed for color change. A positive reaction was recorded when a dark purple coloration appeared within 5-10 sec of impregnation.

Indole test: This test demonstrates the ability of certain bacteria to decompose the amino acid tryptophan to indole, which accumulates in the medium. This test was carried out by inoculating the test organism(s) in bijou bottles containing sterile peptone water and incubating at 37°C for 24 hrs. About 0.02-0.03 mL of Kovac's reagent (P-methyl amino benzaldehyde) was added to the culture after incubation. The bottles were allowed to stand, and a reddish ring superimposed on the culture medium was indicative of indole production.

Motility test: This was used to differentiate those bacteria that are motile from the non-motile organisms. The agar stab method was used, and Nutrient agar of reduced concentration was also used. With the aid of a sterile straight wire, an inoculum was obtained and stabbed on a sterile semi-solid agar medium or nutrient gelatin contained in the bottle two-thirds (2/3) of its depth and incubated at 37°C for 14 hrs. Motility was observed when spikes or cloudiness appeared around the stab lines (or point of inoculation) due to the migration of motile bacteria from the point of inoculation.

Citrate utilization test: This test determines the ability of some organisms to utilize citrate as their sole source of carbon and ammonia as their only source of energy for growth. The medium contains sodium citrate and ammonium salt, and the indicator bromothymol blue. Colonies of suspected bacteria were emulsified into citrate medium and incubated at 37°C for 24 hrs. Citrate utilization was indicated by turbidity and a change in color from light green to blue.

Urease test: This tests the ability of certain organisms to produce the enzyme urease, which decomposes urea into ammonia, and carbon (IV) dioxide.

$$NH_2$$
-CO- NH_2 - H_2O ---> $2NH_3$ + O_2

This test was carried out by inoculating the test organism(s) in a bijou bottle containing urea to give ammonia and carbon (IV) dioxide (CO_2). With the release of this ammonia, the medium becomes alkaline, as shown by a change in the color of the indicator to pink.

Sugar fermentation test: The sugars used were glucose, maltose, mannitol, lactose, and sucrose. The bacteria isolated were inoculated into 5 mL of peptone water in bijou bottles and incubated at 37°C for 24 hrs. A drop of the peptone water containing test bacteria was inoculated into the bottles containing the sugar solution with an indicator (phenol red). The bijou bottles were incubated at 37°C for 24 hrs to check for acid and gas production. A color change from red to yellow appearance is an indication of acid production, while a displacement of the solution in the Durham tubes indicates gas production.

RESULTS

The results from the various well water samples revealed the presence of some pathogenic bacteria. Table 1 shows the mean total viable count (cfu/mL) for bacteria, the lowest viable count is 3.33×10^6 , which is present in sample B, while the highest total viable count was observed in D, which is 5.30×10^8 .

The most probable number (MPN) of total coliform cells/100 mL of the water samples is shown in Table 2. The least total coliform count is 33/100 mL in sample C, while samples A and B had the highest count \geq 2400/100 mL.

Table 3 shows the *E. coli* count using the Eijkman test. Among the samples analyzed, sample D had the highest *E. coli* count, followed by sample B, C, and the lowest sample, A, which accounted for < 2/100 mL.

Table 4 shows the *Streptococcus faecalis* count cells/100 mL of the water samples. Among the samples analyzed, *Streptococcus faecalis* was detected only in sample D as 7/100 mL.

Table 5 shows the presence (+) or absence (-) of bacterial isolates across four samples (A, B, C, and D). *Shigella sp., Klebsiella pneumoniae, Vibrio parahaemolyticus,* and *Vibrio cholerae* were detected in all samples. *Escherichia coli, Salmonella sp., Proteus mirabilis, Staphylococcus aureus,* and *Streptococcus faecalis* showed varying distributions, with some isolates absent in specific samples. This variation highlights differences in bacterial occurrence across the tested samples.

Sample				Bacteria
ł				3.10x10 ⁷
3				3.35x10 ⁶
				3.62x10 ⁸
0				5.30x10 ⁸
Table 2 [.] Most probable n	umber (MPN) of colifor	m cells/100 mL of water sample	PC	
Sample	5×10 mL	5×1 mL	5×0.1 mL	MPN index
A	5	5	5	≥2400
3	5	5	5	≥2400
	4	3	1	33
)	5	4	4	350
Table 3: Escherichia coli co	ount/100 mL Eijkman te	est of the water samples		
	Number	of bottles showing positive cha	ange	
Sample	 10 mL	 1 mL	0.1 mL	MPN/100 ml
λ	0	0	0	<2
5	2	1	0	7
_	2	1	0	7
-				
)	2	1 well water samples using gluc	1 ose azide broth at 44°C for 24	9 4 hrs
C D Fable 4: Faecal streptocod	2 cci count/100 mL of the Number	1 well water samples using gluce of bottles showing positive ch	ose azide broth at 44°C for 24 ange	
D Fable 4: Faecal streptocod	2 cci count/100 mL of the Number	well water samples using gluc of bottles showing positive ch	ose azide broth at 44°C for 24 ange	
) Table 4: Faecal streptocod Gample	2 cci count/100 mL of the Number	well water samples using gluc of bottles showing positive ch	ose azide broth at 44°C for 24 ange	4 hrs
D Fable 4: Faecal streptocod Sample	2 cci count/100 mL of the Number 	well water samples using gluc of bottles showing positive ch 1 mL	ose azide broth at 44°C for 24 ange 0.1 mL	4 hrs MPN/100 ml
) Fable 4: Faecal streptocod Sample A 3	2 <u>cci count/100 mL of the</u> Number <u>10 mL</u> 0	well water samples using gluce of bottles showing positive ch <u>1 mL</u> 0	ose azide broth at 44°C for 24 ange 0.1 mL 0	4 hrs MPN/100 ml <2
D Table 4: Faecal streptocod Sample A 3 C	2 <u>cci count/100 mL of the</u> Number <u>10 mL</u> 0 0	well water samples using gluce of bottles showing positive ch <u>1 mL</u> 0 0	ose azide broth at 44°C for 24 ange 0.1 mL 0 0	4 hrs MPN/100 ml <2 <2 <2
D Table 4: Faecal streptocod Sample A S C D	2 <u>cci count/100 mL of the</u> Number 10 mL 0 0 0 2	well water samples using gluc of bottles showing positive ch 1 mL 0 0 0 0 0 0	ose azide broth at 44°C for 24 ange 0.1 mL 0 0 0 0	4 hrs MPN/100 ml <2 <2 <2 <2 <2
D Table 4: Faecal streptocod Sample A S C D	2 <u>cci count/100 mL of the</u> Number 10 mL 0 0 0 2	well water samples using gluce of bottles showing positive ch <u>1 mL</u> 0 0 0 0 0 0 0 0	ose azide broth at 44°C for 24 ange 0.1 mL 0 0 0 0	4 hrs MPN/100 ml <2 <2 <2 <2 <2
D Fable 4: Faecal streptocod Sample A 3 5 5 5 5 5 5 5 5 5 5 5 5 5 5 5 5 5 5	2 cci count/100 mL of the Number 10 mL 0 0 0 2 rganisms isolated from	well water samples using gluce of bottles showing positive ch 1 mL 0 0 0 0 0 the well water sample S	ose azide broth at 44°C for 24 ange 0.1 mL 0 0 0 1 1 amples	4 hrs MPN/100 ml <2 <2 <2 <2 <2
o Table 4: Faecal streptocod Sample A S Table 5: Distribution of or Solates	2 <u>cci count/100 mL of the</u> Number 10 mL 0 0 0 2	well water samples using gluce of bottles showing positive ch <u>1 mL</u> 0 0 0 0 0 0 0 0	ose azide broth at 44°C for 24 ange 0.1 mL 0 0 0 0 1	4 hrs MPN/100 ml <2 <2 <2 <2 7
able 4: Faecal streptocod ample able 5: Distribution of or able 5: Distribution of or solates scherichia coli	2 cci count/100 mL of the Number 10 mL 0 0 0 2 rganisms isolated from	well water samples using gluce of bottles showing positive ch 1 mL 0 0 0 0 0 the well water sample S B	ose azide broth at 44°C for 24 ange 0.1 mL 0 0 0 1 1 amples	4 hrs MPN/100 ml <2 <2 <2 <2 7 D
able 4: Faecal streptocod	2 cci count/100 mL of the Number 10 mL 0 0 0 2 rganisms isolated from - A 	well water samples using gluce of bottles showing positive ch 1 mL 0 0 0 0 0 the well water sample S B	ose azide broth at 44°C for 24 ange 0.1 mL 0 0 0 1 1 amples 	4 hrs MPN/100 ml <2 <2 <2 <2 7 D
able 4: Faecal streptocod	2 cci count/100 mL of the Number 10 mL 0 0 0 2 rganisms isolated from - A 	well water samples using gluce of bottles showing positive ch 1 mL 0 0 0 0 0 the well water sample S B	ose azide broth at 44°C for 24 ange 0.1 mL 0 0 0 1 1 amples 	4 hrs MPN/100 m <2 <2 <2 <2 7 D
Sample Sample Sample Sample Solates	2 cci count/100 mL of the Number 10 mL 0 0 0 2 rganisms isolated from - A 	well water samples using gluce of bottles showing positive ch 1 mL 0 0 0 0 0 the well water sample S B	ose azide broth at 44°C for 24 ange 0.1 mL 0 0 0 1 1 amples 	4 hrs MPN/100 m <2 <2 <2 <2 7 D
Sample Sample	2 cci count/100 mL of the Number 10 mL 0 0 0 2 rganisms isolated from - A 	well water samples using gluce of bottles showing positive ch 1 mL 0 0 0 0 0 the well water sample S B	ose azide broth at 44°C for 24 ange 0.1 mL 0 0 0 1 1 amples 	4 hrs MPN/100 m <2 <2 <2 <2 7 D
Sample Sample Sample Sample Salates Solates Solates Scherichia coli Shigella sp. Salmonella sp. Klebsiella pneumoniae Vibrio parahaemolyticus Proteus mirabilis	2 cci count/100 mL of the Number 10 mL 0 0 0 2 rganisms isolated from - A 	well water samples using gluce of bottles showing positive ch 1 mL 0 0 0 0 0 the well water sample S B	ose azide broth at 44°C for 24 ange 0.1 mL 0 0 0 1 1 amples 	4 hrs MPN/100 m <2 <2 <2 <2 7 D
Sample Sample A Sample A Solates	2 cci count/100 mL of the Number 10 mL 0 0 0 2 rganisms isolated from - A 	well water samples using gluce of bottles showing positive ch 1 mL 0 0 0 0 0 the well water sample S B	ose azide broth at 44°C for 24 ange 0.1 mL 0 0 0 1 1 amples 	4 hrs MPN/100 m <2 <2 <2 <2 7 D

Table 1⁻ Mean total viable count (cfu/mL) of bacteria in the water samples

+: Present and -: Absent

Table 6 shows the cultural, morphological characteristics isolated from the various well water samples. Escherichia coli was present in samples B, C, D, and absent in sample A. Klebsiella pneumoniae, Shigella sp., Vibrio parahaemolyticus, and Vibrio cholerae were present in all the samples.

DISCUSSION

The result of the bacteriological analysis of the well water samples collected from some quarters in Ekpoma, Edo State, revealed that the coliform bacteria of all the water samples exceeded the WHO guidelines for potable water⁹. Most of the communities in Ekpoma depend on well water as their major source of water supply for consumption. These well waters are highly contaminated due to improper construction of wells, human and animal activities around the well, leaks from waste water disposal, and a low water table, which makes it vulnerable to contamination. Consumption of this well water directly by humans may lead to various health issues. Hence, to avoid or reduce the risk of contamination, wells should be dug a long distance away from where human and animal activities are carried out¹⁵.

Morphology (gram reaction and shape) Gram-ve rod Slender Gram-ve Cocco bacilli Gram-ve rod Gram-ve cocco bacilli Gram-ve comma-shape	TOM + ' +	Ą					1				
reaction and shape) Gram-ve rod Slender Gram-ve Cocco bacilli Gram-ve rod Gram-ve cocco bacilli Gram-ve comma-shape											
	+ , +		CAT OXI	URE	CIT	GLU	MAL	SUC	LAC	MAN	ldentified organism
	, +		'	·	·	A/G	A/G	A	A/G	A/G	Escherichia coli
	· ·										
	+	'	'	ı	'	۷	ı	·	ı		Shigella sp.
	+										
		ı	ı	ı	'	A/G		ı	·	A/G	Salmonella sp.
		+	'	+	+	A/G	٩	A	۷	A	Klebsiella pneumoniae
	+	I	+	ı	+	۷	٩	۷	۷	A	Vibrio parahaemolyticus
Pale colored colonies swarming over Gram-ve rod	+	ı	ı	+	+	A/G	٩	۷	·		Proteus mirabilis
the agar surface on MCA											
Smooth pale and glistering Gram+ve cocci in cluster		+	·	ı	+	A/G		ı	·	A/G	Staphylococcus aureus
colonies on MCA											
Smooth yellow colonies on TCBS Gram-ve comma-shape	+	1	+	ı	+	A	٩	٩	٩	A	Vibrio cholerae
Small pale yellow colonies on Gram+ve cocci in chains		ı	ı	ı	'	۷	٩	۷	۷	A	Streptococcus faecalis
SSA, tiny colonies on MCA											

Table 6: Cultural: morphological, biochemical and sugar fermentation characteristics of bacteria isolated from the well water samples

OXI: Oxidase test, EMB: Eosin methylene blue, URE: Urease test, SSA: Satmonella shigella agar, CIT: Citrate utilization test, MAC: MacConkey agar and TCBS: Thiosulphate citrate bile salt sucrose agar

Trends Environ. Sci., 1 (2): 174-183, 2025

The various organisms isolated include *Escherichia coli*, *Shigella* sp., *Salmonella* sp., *Klebsiella pneumoniae*, *Vibrio parahaemolyticus*, *Proteus mirabilis*, *Staphylococcus aureus*, *Vibrio cholerae*, and *Streptococcus faecalis*. These isolated bacteria are of public health importance. The presence of these bacteria indicates the contamination of the water in the well. The consumption of such water by individuals is a risk to human health⁸. The total coliform count cells/100 mL of water sample shown in Table 2 is a clear indication that the water samples are not safe for immediate consumption due to the high coliform count. Potable water is a transparent liquid without color, odor, or taste, but when infected with pathogenic organisms, these qualities are lost, and such water becomes harmful to both human and animal populations.

The isolation and identification of *E. coli* and *Streptococcus faecalis* are exclusively of faecal origin. Their presence in water is evidence of faecal pollution of human and animal origin. Their absence, however, does not mean that the water is safe because other organisms may be present. *Escherichia coli* produces enterotoxins (heat-labile and heat-stable), which cause diarrhea in humans. It is equally the most common cause of urinary tract infection in young women¹⁶. *Streptococcus faecalis* also produces exotoxins and causes a wide range of infections, such as sore throat, sepsis, scarlet fever, and bacteremia, among many others.

Salmonella and *Shigella* sp. are highly pathogenic to humans. *Salmonella typhi* causes enteric fever and Salmonellosis. It is the major cause of typhoid fever. *Shigella* sp. is known to be a major cause of bacillary dysentery and shigellosis. The presence of *Salmonella* and *Shigella* sp. in well water indicates a gross contamination of the well water with faecal origin. The presence of *Klebsiella pneumoniae*, which is, bacteria of medical importance and an enteric microorganism, cause a small proportion (about 1%) of infection in immune-compromised patients¹⁶. *Staphylococcus aureus* is a microflora of the skin, mouth, nose, and gastrointestinal tract. It is responsible for most food poisoning, septicemia, otitis media, and boils due to its ability to produce enterotoxin and enzymes that are harmful to humans.

Infections caused by *Vibrio parahaemolyticus* usually present in one of three major clinical syndromes. 60-80% of infections are caused by gastroenteritis, and 50% by septicemia¹⁷. The most common presentation of gastroenteritis, with symptoms including diarrhea (sometimes watery and bloody) with abdominal cramps, nausea, vomiting, headache, chills, and low-grade fever¹⁸. Infection is usually self-limiting and of moderate severity, lasting approximately 3 days in an immunocompetent patient, and can be treated with oral rehydration alone¹⁹. Fatal cases of septicemia may occur in immunocompromised patients or those with pre-existing medical conditions (such as liver disease, cancer, heart disease, or diabetes). Since the number of coliform bacteria recorded in the study is higher and falls short of the WHO standard, it is suggested that well water could be potable source of microbial infection which posing a potential health hazard to users. It is obvious from the result obtained that well water in Ekpoma does not meet the WHO¹⁹ standard for drinking water. Effective management can control potential adverse health consequences that could be associated with unsafe well water usage. Thus, in such areas, water treatment is necessary for the water in the well to be safe for consumption.

CONCLUSION

The bacteriological quality of well water in Ekpoma was not within the acceptable standard due to inadequate maintenance and infrequent washing. The bacteriological analysis of well water is a step in inspecting the sanitary condition and quality of water. The potability of water for human consumption is determined bacteriologically by its coliform- *Escherichia coli* load rather than the presence of other bacteria. From the samples analyzed, the high *E. coli* and total coliform cells/100 mL of water observed reflected the poor quality of the well water, despite the clarity. Consequent upon this, it is recommended that the people should be enlightened on the need to follow the appropriate standard for siting wells to

avoid faecal contamination. To avoid bacterial contamination of well water, good sanitary measures and the treatment of well water before consumption in the area should be embraced by all. Also, given the seeming inability of the government to provide pipe-borne water in recent times, deep wells (bore holes) that are less prone to contamination from surface run-off should be constructed to replace hand-dug wells. Also, regulatory agencies should intensify their efforts towards providing clean and potable water to the public and encourage in campaign for the cleanliness of the environment and good refuse and sewage disposal systems.

SIGNIFICANCE STATEMENT

This study discovered a high level of bacteriological contamination in well water in Ekpoma, Edo State, which can be beneficial for public health authorities and local communities in identifying and mitigating sources of waterborne diseases. The findings highlight the urgent need for proper well maintenance, strategic siting, and routine treatment to ensure water safety for domestic use. This study emphasizes the importance of replacing shallow hand-dug wells with deeper boreholes, which are less susceptible to surface contamination. Moreover, it underscores the necessity of awareness campaigns on sanitary practices in water handling. This study will help the researchers to uncover the critical areas of rural water safety management and microbial pollution that many researchers were not able to explore. Thus, a new theory on decentralized water safety strategies may be arrived at.

ACKNOWLEDGMENT

The authors would like to acknowledge the management, Laboratory and technical staff of Saint Kenny Diagnostic and Research Centre, Ujoelen, Ekpoma, Edo State, Nigeria for their excellent assistance and for providing medical writing support/editorial support in accordance with Good Publication Practice (GPP3) guidelines.

REFERENCES

- 1. Kılıç, Z., 2020. The importance of water and conscious use of water. Int. J. Hydrol., 4: 239-241.
- 2. Musie, W. and G. Gonfa, 2023. Fresh water resource, scarcity, water salinity challenges and possible remedies: A review. Heliyon, Vol. 9. 10.1016/j.heliyon.2023.e18685.
- 3. Lauro, S.E., E. Pettinelli, G. Caprarelli, L. Guallini and A.P. Rossi *et al.*, 2021. Multiple subglacial water bodies below the south pole of Mars unveiled by new MARSIS data. Nat. Astron., 5: 63-70.
- 4. Al Hallak, M., T. Verdier, A. Bertron, C. Roques and J.D. Bailly, 2023. Fungal contamination of building materials and the aerosolization of particles and toxins in indoor air and their associated risks to health: A review. Toxins, Vol. 15. 10.3390/toxins15030175.
- 5. Sajini, F.I., 2021. Human population growth and the socioeconomic effects in Warri Metropolitan City Delta State Nigeria. Ling. Cult. Rev., 5: 878-889.
- 6. Grigg, N.S., 2023. Water Resources Management: Principles, Methods, and Tools. 1st Edn., John Wiley & Sons, Inc., New Jersey, USA, ISBN: 978-1119885962, Pages: 416.
- 7. Oshiokhayamhe, I.K., K.I.B. Agumeile, O.L. Eromosele, A.C. Agbomehe, L.N. Ozurumba-Dwight and O.D. Idahosa, 2023. Bacteria load assessment from hands of students in a Tertiary University in South-South Nigeria. J. Biotechnol. Res., 9: 44-49.
- 8. Ibo, E.M., O.R. Umeh, B.O. Uba and P.I. Egwuatu, 2020. Bacteriological assessment of some borehole water samples in Mile 50, Abakaliki, Ebonyi State, Nigeria. Arch. Agric. Environ. Sci., 5: 179-189.
- 9. Wen, X., F. Chen, Y. Lin, H. Zhu and F. Yuan *et al.*, 2020. Microbial indicators and their use for monitoring drinking water quality-A review. Sustainability, Vol. 12. 10.3390/su12062249.
- Farkas, K., D.I. Walker, E.M. Adriaenssens, J.E. McDonald, L.S. Hillary, S.K. Malham and D.L. Jones, 2020. Viral indicators for tracking domestic wastewater contamination in the aquatic environment. Water Res., Vol. 181. 10.1016/j.watres.2020.115926.

- 11. Saingam, P., B. Li and T. Yan, 2020. Fecal indicator bacteria, direct pathogen detection, and microbial community analysis provide different microbiological water quality assessment of a tropical urban marine estuary. Water Res., Vol. 185. 10.1016/j.watres.2020.116280.
- 12. de Kraker, M.E.A., E. Tartari, S. Tomczyk, A. Twyman and L.C. Francioli *et al.*, 2022. Implementation of hand hygiene in health-care facilities: Results from the WHO Hand Hygiene Self-Assessment Framework Global Survey 2019. Lancet Infect. Dis., 22: 835-844.
- 13. Vika, V., S. Ndhleve, N. Mbandzi and M.D.V. Nakin, 2024. Assessment of physico-chemical and microbiological parameters of Mthatha River in Eastern Cape, South Africa. Environ. Forensics, 25: 417-430.
- 14. WHO, 2022. Guidelines for Drinking-Water Quality: Fourth Edition Incorporating the First and Second Addenda. 4th Edn., WHO, Switzerland, ISBN: 978-92-4-004506-4, Pages: 614.
- 15. Sousa, V.S. and M.R. Teixeira, 2020. Metal-based engineered nanoparticles in the drinking water treatment systems: A critical review. Sci. Total Environ., Vol. 707. 10.1016/j.scitotenv.2019.136077.
- 16. Ali, A., A. Parisi, M.C. Conversano, A. Iannacci, F. D'Emilio, V. Mercurio and G. Normanno, 2020. Food-borne bacteria associated with seafoods: A brief review. J. Food Qual. Hazards Control, 7: 4-10.
- Narayanan, S.V., T.C. Joseph, S. Peeralil, M.P. Mothadaka and K.V. Lalitha, 2020. Prevalence, virulence characterization, AMR pattern and genetic relatedness of *Vibrio parahaemolyticus* isolates from retail seafood of Kerala, India. Front. Microbiol., Vol. 11. 10.3389/fmicb.2020.00592.
- Gavilan, R.G., J. Caro-Castro, C.J. Blondel and J. Martinez-Urtaza, 2023. *Vibrio parahaemolyticus* Epidemiology and Pathogenesis: Novel Insights on an Emerging Foodborne Pathogen. In: *Vibrio* spp. Infections, Almagro-Moreno, S. and S. Pukatzki (Eds.), Springer, Cham, Switzerland, ISBN: 978-3-031-22997-8, pp: 233-251.
- 19. Slavik, I., K.R. Oliveira, P.B. Cheung and W. Uhl, 2020. Water quality aspects related to domestic drinking water storage tanks and consideration in current standards and guidelines throughout the world-A review. J. Water Health, 18: 439-463.