



Bacteriological examination of well water samples to confirm faecal contamination

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Abstract

A bacteriological analysis was conducted on well water samples in Enugu Metropolis to address the rising incidence of waterborne diseases. The study employed the Most Probable Number (MPN) technique at 37°C, utilizing MacConkey broth and Eosine methylene blue, to assess the microbial quality of the well water. Standard methods, including presumptive, confirmatory, and completed tests, were performed to detect *Escherichia coli*. The isolated *E.coli* was further characterized through gram staining and biochemical tests, and the Eijkman method was used to confirm its faecal origin. Wells with covered and unplastered interiors, particularly those near septic tanks and gutters, showed higher levels of faecal contamination. In areas like Abakpa, Maryland, Achara Layout, and Emene, well water is microbiologically unsafe for consumption or domestic use, requiring additional treatment measures such as boiling or disinfection. Proximity to pollution sources poses a significant risk of waterborne disease outbreaks, emphasizing the need for prevention and adherence to World Health Organization standards for untreated water.

Keywords: Bacteriological examination, faecal contamination, well water samples

Introduction

Groundwater samples have tested positive for microbiological and chemical contaminants [1]. Despite providing access to groundwater, traditional dug wells in developing areas face significant bacterial contamination risks resulting from poor waste disposal practices [2].

The consequences of groundwater contamination are dire for public health, particularly in vulnerable communities and developing countries reliant on groundwater for drinking [3]. World Health Organization [4] stipulates that safe drinking water must conform to established guidelines for physical, chemical, and bacteriological characteristics.

The presence of *E.coli* in drinking water is a known risk factor for gastrointestinal illness, characterized by diarrhea, nausea, and potentially life-threatening complications [5]. The combination of unsafe drinking water and inadequate sanitation results in an estimated 1.6 million deaths among children under five worldwide annually, with rural populations disproportionately affected [6].

The World Health Organization (WHO) reports that water contaminated with harmful microorganisms, causing illnesses like cholera, diarrhea, gastroenteritis, and typhoid, results in approximately 485,000 diarrhea-related deaths annually. Moreover, two billion people globally are exposed to water polluted with faecal matter [7]. *E.coli* is a ubiquitous commensal bacterium in the human gastrointestinal tract, but its pathogenic potential is realized in acute urinary tract infections and gastrointestinal tract infections, rendering it a reliable indicator organism for faecal [8].

The presence of fecal coliform bacteria in water is a reliable indicator of faecal contamination, implying that the water may be microbiologically unsafe for human consumption [9]. This research is to evaluate well water samples close to septic tanks and bacteriological parameters like *Escherichia coli* and total coliform are employed. Notably, *E.coli* is recognized as the most specific indicator of faecal contamination.

Materials and methods

Area of Study

This study took place in seven areas of Enugu State, Nigeria (Abakpa, Emene, New Haven, Uwani, Thinker's Corner, Maryland, and Achara Layout), located in the southeastern tropical rainforest zone.

Samples collection

Twenty-one well water samples were collected from seven areas in Enugu metropolis (Abakpa, Emene, New Haven, Uwani, Thinker's Corner, Maryland, and Achara Layout), with three samples per area. Using 25ml sterile bottles, samples were collected directly from wells without pre-treatment, ensuring no hand contact. The bottles were not filled to the brim. Within three hours, the samples were transported to the Department of Applied Microbiology laboratory for analysis.

Preparation of macconkey broth

A quantity 14.7g of MacConkey broth was weighed using weighing balance and mixed with 420ml of distilled water according to manufacturer guide. It was autoclaved at for 15 minutes. After 15 min, the conical flasks were removed vigorously before pouring into Petri dishes.

Preparation of eosine methylene blue agar

A quantity 15.79 powder eosine methylene was dissolve in 420 liter of water according to the manufacture's guide. Allow to soak for 10 minutes, swirl to mix and sterilize by autoclaving at 121°C for 15 distribution of the precipitate before pouring into sterile petridishes.

Presumptive test procedure

The coliform count was determined using the three-tube assay of the MPN technique, with the MacConkey broth used to carry out the test. Each sample underwent a serial dilution process, with three sets of lactose broth tubes prepared. These tubes were inoculated with 10 mL, 1.0 mL,

and 0.1 mL of the respective water samples and then incubated subsequently at 37°C for 48 hours. The presence of coliforms was inferred based on the observation of acid formation and a distinct color change occurring in any of the bijou bottles ^[10].

Confirmed test procedure

A loopful of inoculum from the positive presumptive tubes was transferred into bijou bottles containing brilliant green lactose bile broth to confirm the presence of coliforms. The tubes were incubated at 37°C for a duration on 24 to 48 hours. The definitive indication of the presence of coliforms was confirmed by a noticeable change in color within incubated media ^[10].

Completed test procedure

A loopful of broth from a positively confirmed test tube was carefully streaked onto an Eosin Methylene Blue agar plate to obtain pure colonies. These streaked agar plates were subsequently incubated at 37°C for duration of 24 to 48 hours. Further identification of the isolated colonies was carried out based on their morphological, cultural, and biochemical characteristics ^[11].

Gram staining

The gram staining was done according to the method as described by Tasié and George-Okafor ^[10]. A drop of physiological saline was added on a clean slide and a loop full of the organism transferred on it and spread evenly. The smear was hit fixed on a bunsen burner flame and allowed to air dry. The smear was stained with crystal violet for 1 minute and acetone alcohol was dropped onto the smear and allowed to run off and then washed with water, safranin was used as a counter stain for 1 min and finally washed off with water. The slides were allowed to air dry and examined under low power objective lens. The Gram-positive bacteria stained blue or purple while Gram-negative stained pink. Biochemical Characterization of the Bacterial Isolate

Catalase

Emulsify the test organism with 1 or 2 drops of hydrogen peroxide on a glass slide. An observed bubbling of air effect indicated oxygen gas production and such bacteria is said to be catalase positive ^[11].

Coagulase

Make a dense suspension of the organism using 1-2 drops of distilled water on a glass slide (Observe for auto agglutination). Mix with a loopful of EDTA plasma and observe for clumping or agglutination ^[11].

Citrate test

A loop full of the test organism was picked from the nutrient

agar plates, using a sterile glass rod and lightly inoculated on Simon citrate agar slants and incubated at 37°C for 24 hours. A positive test result was looked out for with the development of a blue colour in the medium ^[12].

Indole test

Inoculate a bijou bottle containing peptone water with the test organism. Incubate at 35-37°C for 24-48 hours. Then add 0.5mls of Kovac's reagent. Shake gently and examine for a red colour on the surface. The formation of pink to red ring colour indicates positive test while no colour change indicates negative test ^[12].

Oxidase test

Filter papers were soaked with tetramethyl-p-phenylenediaminedihydrochloride substrate. The filter papers were moistened with sterile distilled water. The isolates were collected with a wooden stick and smeared on the filter paper ^[13].

Methyl red test

A loop full of the test organism was inoculated into a tube containing 1% glucose phosphate was incubated at 37°C for 24h. A volume of 2.5ml of this suspension was transferred into a sterile tube and few drops of methyl red reagent were added. Absence of color change indicates negative result ^[14].

Voges proskauer test

Using a sterile inoculating loop, pick up well-isolated colonies of sample bacteria from 18 to 24 hours old culture and inoculate the broth. Incubate the tubes aerobically for 18 to 24 hours at 35±2°C. Following incubation, transfer 2 mL of broth to a clean test tube. Add 6 drops of Reagent A (5% α-naphthol solution) and mix properly by shaking. Add 2 drops of Reagent B (40% KOH solution) and mix properly by shaking. Observe for the formation of red-pink color at the surface of the medium within 30 minutes. Continuously shake the tube vigorously during the 30-minute waiting period. A positive result is indicated by the formation of a pink-red color over the surface of the medium. A negative result is indicated by a lack of pink-red color over the surface of the medium or the formation of the copper color ^[15].

Eijkman test

This test is very important in the confirmation of faecal *E. coli*. I.e. production of indole at 44°C or acid and gas at 44°C. Inoculate the suspected colonies into peptone water (as in indole test) and incubate at 44°C for 24 hours. Perform the indole test by adding Kovac's reagent and mix.

Results

Table 1: Random collection of well water samples in enugu metropolis

S/No	Location	Distance of Septic Tank and Well	Remarks
1	NO1. Street Ugbo-Oghe Abakpa	Near to septic tank and gutter	Well-constructed with cover and unplastered colorless and odorless
2	NO2. Street Abakpa	Near to septic tank and gutter	Well-constructed with cover and unplastered colorless and odorless
3	NO.30 Street Abakpa	Near to septic tank and gutter	Well-constructed with cover and unplastered colorless and odorless
4	Niger Steel Emene	None	Well-constructed with cover and unplastered colorless and

			odorless
5	No 22 Street Akpo-Oga Emene	Distance away	Well-constructed with cover and unplastered colorless and odorless
6	No 146 Line Emene	Distance away	Well-constructed with cover and unplastered colorless and odorless
7	No 22 Street New Haven	Distance away	Well-constructed with cover and unplastered colorless and odorless
8	No 3 Street New Haven	Distance away	Well-constructed with cover and unplastered colorless and odorless
9	No 6 Street New Haven	Distance away	Well-constructed with cover and unplastered colorless and odorless
10	No 3 Street Uwani	Distance away	Well-constructed with cover and unplastered colorless and odorless
11	No 22 Lane Uwani	Distance away	Well-constructed with cover and unplastered colorless and odorless
12	No 21 Street Uwani	Near to septic tank and gutter	Well-constructed with cover and unplastered colorless and odorless
13	No 10 Street Thinkers Corner	Distance away	Well-constructed with cover and unplastered colorless and odorless
14	No 7 Street Thinkers Corner	Distance away	Well-constructed with cover and unplastered colorless and odorless
15	No 126 Street Thinkers Corner	Distance away	Well-constructed with cover and unplastered colorless and odorless
16	No 2 Street Maryland	Near to septic tank and gutter	Well-constructed with cover and unplastered colorless and odorless
17	No 3 Street Maryland	Near to septic tank and gutter	Well-constructed with cover and unplastered colorless and odorless
18	No 4 Street Maryland	Near to septic tank and gutter	Well-constructed with cover and unplastered colorless and odorless
19	No 19 Street Achara Layout	Near to septic tank and gutter	Well-constructed with cover and unplastered colorless and odorless
20	No 40 Street Achara Layout	Near to septic tank and gutter	Well-constructed with cover and unplastered colorless and odorless
21	No 6 Street Achara Layout	Near to septic tank and gutter	Well-constructed with cover and unplastered colorless and odorless

N.B: the range between wells near to septic tank and gutters is between the averages of 10-25m

Table 2: Presumptive test (according to macrady table)

Quantity of water	50ml	10ml	1ml	MPN
NEW HAVEN	0	2	1	3
	0	1	2	3
	0	2	2	4
UWANI	1	2	3	12
	1	3	3	18
	1	4	3	30
Thinkers Corner	1	4	2	20
	1	5	1	35
	1	4	3	30
Emene	1	4	2	50
	1	5	1	50
	1	4	3	20
Maryland	1	5	4	160
	1	5	4	160
	1	5	3	90
Abakpa	1	5	4	160
	1	5	5	180
	1	5	3	90
Achara Layout	1	4	5	180
	1	5	5	40
	1	4	5	180

Table 3: Confirmatory test

S/No	Location	Metallic Green Sheen From Eosine Methylene Blue Plate	Remarks
01	NO1. Street Ugbo-Oghe Abakpa	+	<i>Escherichia coli</i> confirmed
02	NO2. Street Abakpa	+	<i>Escherichia coli</i> confirmed
03	NO.30 Street Abakpa	-	Absent
04	Niger Steel Emene	-	Absent
05	No 22 Street Akpo-Oga Emene	-	Absent
06	No 146 Line Emene	-	Absent
07	No 22 Street New Haven	-	Absent
08	No 3 Street New Haven	-	Absent
09	No 6 Street New Haven	-	Absent
10	No 3 Street Uwani	-	Absent
11	No 22 Lane Uwani	-	Absent
12	No 21 Street Uwani	-	Absent
13	No 10 Street Thinkers Corner	-	Absent
14	No 7 Street Thinkers Corner	-	Absent
15	No 126 Street Thinkers Corner	-	Absent
16	No 2 Street Maryland	+	<i>Escherichia coli</i> confirmed
17	No 3 Street Maryland	+	<i>Escherichia coli</i> confirmed
18	No 4 Street Maryland	-	Absent
19	No 19 Street Achara Layout	+	<i>Escherichia coli</i> confirmed
20	No 40 Street Achara Layout	-	Absent
21	No 6 Street Achara Layout	+	<i>Escherichia coli</i> confirmed

Key: +: Positive, -: Negative

Table 4: Result of biochemical test (completed test to confirm (*escherichia coli*))

S/N	Sample Source	Oxidase Test	Coagulase Test	Citrate Test	Indole Test	Methyl Red Test	Voges Proskauer Test	Organism
1	No1. Street Ugbo-Oghe Abakpa	-VE	+VE	-VE	+VE	+VE	--VE	<i>Escherichia coli</i> confirmed
2	No.22 Street Abakpa	-VE	+VE	-VE	+VE	+VE	--VE	<i>Escherichia coli</i> confirmed
3	No2 Street Maryland	-VE	+VE	-VE	+VE	+VE	-VE	<i>Escherichia coli</i> confirmed
4	No 4 Street Maryland	-VE	+VE	-VE	+VE	+VE	-VE	<i>Escherichia coli</i> confirmed
5	No. 19 Street Achara Layout	-VE	+VE	-VE	+VE	+VE	-VE	<i>Escherichia coli</i> confirmed
6	No. 6 Street Achara Layout	-VE	+VE	-VE	+VE	+VE	-VE	<i>Escherichia coli</i> confirmed

N.B: The Gram Reaction of *Escherichia coli* is Gram Negative Bacilli (Rod)

Table 5: Eijkman test result to confirm faecal *escherichia coli*

S/No	Location	Eijkman Test	Organism
1	No1. Street Ugbo-Oghe Abakpa	+VE	Faecal <i>Escherichia coli</i>
2	No.22 Street Abakpa	+VE	Faecal <i>Escherichia coli</i>
3	No2 Street Maryland	+VE	Faecal <i>Escherichia coli</i>
4	No 4 Street Maryland	+VE	Faecal <i>Escherichia coli</i>
5	No. 19 Street Achara Layout	+VE	Faecal <i>Escherichia coli</i>
6	No. 6 Street Achara Layout	+VE	Faecal <i>Escherichia coli</i>

Key: +: Positive

Discussion

Table 1 showed the streets, lanes and roads the well water samples were collected which also showed the distance between the septic tanks and well.

Table 2 showed an examination of well water samples from six Enugu locations (Abakpa, Emene, Uwani, Thinkers Corner, Maryland, and Achara Layout) revealed disparate levels of coliform bacterial contamination. Presumptive testing yielded positive results, characterized by acid and gas production in Durham tubes, in some samples, while others tested negative, indicating an absence of coliform bacteria.

Table 3 the confirmatory testing revealed the presence of coliform organisms, exhibiting green metallic sheen and pinkish coloration, on select incubated plates. The observed

green metallic sheen is consistent with *E. coli* a well-established indicator of faecal contamination.

Table 4 - Table 5 showed the gram stain, biochemical test were used to identification *E.coli* while the Eijkman test identified faecal *E.coli*. The findings align with Idowu et al. [16], who reported widespread bacterial contamination in wells in Sagamu, Nigeria, with prevalence rates of 95% for *Klebsiella spp*, 72% for *E.coli*, and 32.5% for *Salmonella typhi*. The research supports Atikul Islam et al. [17] findings, which uncovered substantial bacterial contamination in wells built by both government and non-government entities. Specifically, *E.coli* levels were higher during the wet season, with government-installed wells reaching 41 cfu/100 ml and non-government-installed wells at 21 cfu/100 ml. In contrast, the dry season saw significantly

lower levels, at 2 cfu/100 ml and less than 1 cfu/100 ml, respectively. Notably, 24% of government-installed and 31% of non-government-installed wells were deemed high-risk during the wet season, highlighting the potential health risks these wells pose to rural communities, especially during periods of heavy rainfall. The results are consistent with the study by Allamin et al. [18], which detected total coliforms in water samples ranging from 11.0 to 63.0 cfu/ml. Sabon Tasha recorded the highest levels, while Nasarawa had the lowest. Notably, only two sites - Sabon Tasha and Kakuri showed evidence of faecal contamination, with *E.coli* presence indicating recent faecal contamination in water samples from these locations. The findings align with Auta et al. [19], revealing alarming levels of bacterial contamination in well water samples from Dogon Dawa district. Using the Multiple Tube Fermentation technique, we detected excessively high total coliform and faecal coliform counts ($26.20-19.00 \times 10^2$ cfu/ml) in all 20 samples, far exceeding the recommended standard of zero coliforms per milliliter. This study underscores the severe contamination of well water by pathogenic microorganisms in the district. This research is in agreement with the research of Dawn et al. [20] that investigated bacterial contamination in Wukari well water, revealing high total viable counts ($0.86-3.04 \times 10^4$ cfu/ml) and total coliform counts ($0.24-1.84 \times 10^2$ cfu/ml). *Staphylococcus aureus* and *E.coli* were the most prevalent bacteria isolated.

Conclusion

Bacteriological examination of well water samples was carried out due to recent water borne disease. The examination showed some well water were contaminated with faecal materials due to its proximity to septic tank, gutters and covered and unplastered interior. *E.coli* an indicator organism was Isolated from some well water samples indicating that they are several organisms in such well water. Such water contaminated with faecal *E.coli* is unsafe for drinking and domestic use according to the World Health Organization standard for untreated water. Environmental protection agencies in Nigeria should ensure that well water should be several meters away from Septic tanks and gutters.

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