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RESEARCH PAPER

Bacteriological assessment of pipe-borne, borehole, and well water sources available to students in Nasarawa State University Keffi, Nasarawa State, Nigeria

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Abstract. The provision of clean water, microorganisms-free water is crucial in preventing the transmission of waterborne diseases. This study was conducted with the objective of evaluating the bacteriological quality of water sources accessible to students within Nasarawa State University Keffi. Sixteen samples, comprising ten from piped source, three from boreholes, and three from wells, were subjected to bacteriological quality assessment. The total bacterial count was analyzed using the pour plate technique, while the total coliform count and bacteriological index were assessed through the most probable number technique. Among the various water sources examined, pipe-borne water exhibited the lower bacterial contamination with a mean of 0.6×10^6 CFU/100 mL in contrast to borehole and well water sources, which recorded mean of 1.6×10^6 and 3.2×10^6 CFU/100 mL, respectively. Additionally, pipe-borne water demonstrated the lowest mean total coliform count, registering 22 MPN/100 mL. Notably, the water samples were found to harbor bacteria such as *Escherichia coli*, *Klebsiella pneumoniae*, *Enterobacter aerogenes*, and *Citrobacter* sp. Alarming findings from this study highlight the unsuitability of most water samples for human consumption, as they fail to meet the quality standards established by the World Health Organization. Consequently, it is necessary to implement measures aimed at safeguarding water sources from contamination and curbing the proliferation of diseases. Furthermore, it is pertinent to prioritize adequate treatment of domestic water sources prior to consumption to ensure public health and well-being.

Keywords: pipe-borne water; well water; borehole water; Nasarawa State; Nigeria; bacteriological assessment

1. Introduction

Access to clean water is a fundamental human right and an essential resource for the existence of man and other living things (Joanne, 2000). Even, a minor loss of one per cent of body fluids can lead to dehydration in humans, while fluid loss reaching ten percent poses high risk of mortality (Onyeze et al., 2013). Scientifically, water is defined as a chemical compound with the

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formula H₂O ([Bramer, 2011](#)). A significant portion of the biochemical reactions that take place during the metabolism and growth of living cells relies on water and an aqueous environment.

Conventional water sources encompass streams, springs, rivers, lakes, reservoirs, wells, and boreholes. According to [Okafor \(1985\)](#), rivers and lakes hold a more 0.33 per cent of freshwater at any given time, whereas the atmosphere contains only 0.035 percent of freshwater. Given the scarcity of freshwater, many countries prioritize its treatment and recycling ([Okafor, 2011](#)).

Groundwater, in conjunction with surface water from streams and lakes, represents an essential supply of drinking water and stands as the largest freshwater reserve, accounting for 0.76 percent of such reserves after glaciers ([Plummer et al., 2013](#)). However, these water sources are susceptible to contamination stemming from both point and non-point sources. Common issues such as subpar plumbing systems, pipe leaks, the proximity of water supply pipelines to pollution sources, buried underground pipes in disrepair, and inadequate facilities for human waste disposal render the water distribution system susceptible to microbial contamination ([Chukwurah, 2001](#)).

Contaminated water supplies contribute to the proliferation of water-borne diseases. The quality of water quality inevitably deteriorates within distribution networks, mainly due to pipe leaks and infiltration issues ([Cheesbrough, 2000](#)). Several studies ([Adedeji et al., 2017](#); [Kamal & Hashm, 2021](#); [Nwandkor & Ifeany, 2015](#)) have reported unsatisfactory bacterial qualities of pipe-borne water, groundwater, and other natural water sources, with coliform counts significantly exceeding the recommended levels set by the World Health Organization (WHO). In a study conducted by [Okoko and Idise \(2014\)](#), *Micrococcus sp.*, *Streptococcus sp.*, and *Pseudomonas sp.* were isolated from pipe-borne water sources. The predominant water-borne diseases include typhoid and paratyphoid diseases (Salmonellosis), cholera, dysentery, and schistosomiasis ([Udoessien, 2003](#); [Ukpong & Okon, 2013](#)).

Providing safe drinking water to both urban and rural populations is essential for preventing water-borne diseases ([Okorafor et al., 2012](#)). According to [World Health Organization \(2022\)](#), water supplies contaminated with microorganisms, which can lead to illnesses such as cholera, diarrhea, gastroenteritis, and typhoid, are projected to cause 485,000 diarrheal deaths annually. Shockingly, at least two billion people worldwide are exposed to water contaminated with fecal matter ([WHO, 2022](#)).

In Nasarawa State, the lack of safe drinking water and poor sanitation has resulted in several reported cholera incidences over the past decade ([Odama, 2021](#); [Ojeme, 2014](#)). Additionally, there were reported cholera outbreak cases in which more than thirty Nasarawa State University students hospitalized at the South Atlantic Petroleum Medical Clinic due to the consumption of contaminated water ([Linus, 2018](#)). Furthermore, there were additional instances of cholera outbreaks that went unreported as they were managed by private clinics and hospitals located off the school campus.

Due to the risk of fecal contamination, microbial contamination of drinking water presents the most significant threat to the safety of potable water supplies ([WHO, 2022](#)). Considering the vital role of water in our daily lives, conducting microbiological examinations of water is imperative ([Shittu et al., 2008](#)). Therefore, it was crucial to assess the quality of the water supplied to the Institution from the Mada waterworks and other water sources available to students, as well as investigate the effectiveness of the decontamination processes. The primary objective of this study was to conduct a comprehensive bacteriological analysis of pipe-borne, borehole, and well water sources accessible to students within Nasarawa State University, Keffi, (NSUK), located on main campus in Nasarawa State, Nigeria.

2. Materials and method

2.1. Study area

This study was carried out at Nasarawa State University, Main Campus, located in the Keffi local government area of Nasarawa state from January to July 2015. The study area's geographical

location is illustrated in Figure 1, situated at latitude 8°51' N and longitude 7°54' E. The campus is approximately 68 kilometres from Abuja, the Federal Capital Territory, and 128 kilometres from Lafia, the capital of Nasarawa State (Akwa et al., 2007). It shares borders to the north by Kaduna State, to the west with the Abuja Federal Capital Territory, to the south with Kogi and Benue states, and to the east with Taraba and Plateau states. The inhabitants of this area primarily comprise students, academic and non-academic staff, as well as the local residents.

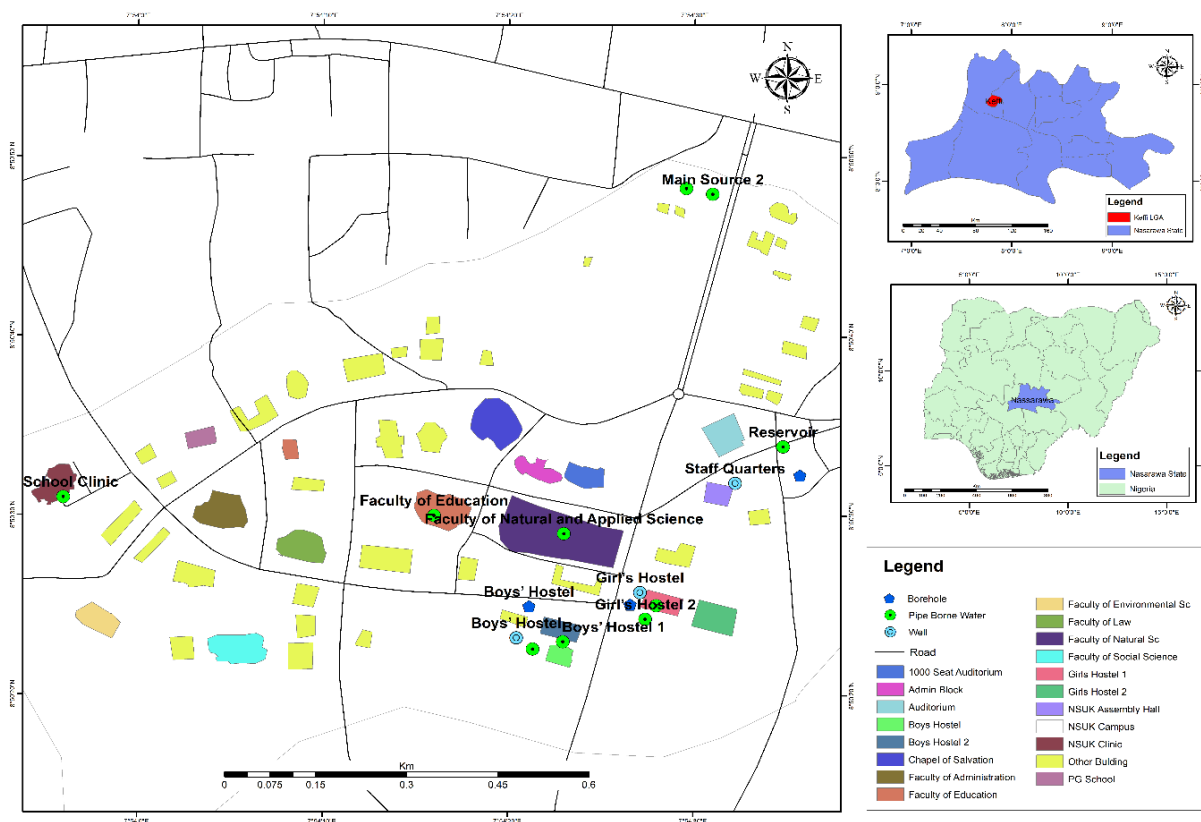


Figure 1. Map of Nasarawa State University, Keffi adapted from the Administrative Map of Nasarawa State

The communities in the Keffi local government of Nasarawa state primarily rely on pipe-borne water, which is managed by the state government through the Mada waterworks. There are two primary sources that directly supply pipe-borne water to the distribution from the water board’s distribution line. Additionally, the campus features a large capacity storage tank that serves as a water reservoir, providing alternatives when the pipe-borne water supply falls short. Borehole and well water sources are also available as alternatives in case of insufficient pipe-borne water supply in the study area.

2.2. Sample collection

Sixteen water samples were collected aseptically using sterile 100 mL sample bottles at various distinct points within Nasarawa State University Campus, Keffi, Nasarawa state, encompassing different water sources (i.e., pipe-borne, borehole, and well water), as shown in Table 1. Specifically, two samples were collected from two primary pipe-borne water sources, one from the water reservoir, and seven samples from various location across the Institution’s premises, including the boys’ and girls’ hostels. Furthermore, three samples were procured from the both borehole and well water sources. These collected samples were then promptly

transported by laboratory, securely stored within ice-containing cellophane bag, ensuring their arrival within two hours for subsequent analysis.

Table 1. Description of sample locations

S/N	Water Samples	Description
1	Pipe borne RSV	Reservoir
2	Pipe borne MS1	Main Source 1
3	Pipe borne MS2	Main Source 2
4	Pipe borne BH1	Boys' Hostel 1
5	Pipe borne BH2	Boys' Hostel 2
6	Pipe borne GH1	Girl's Hostel 1
7	Pipe borne GH2	Girl's Hostel 2
8	Pipe borne FAE	Faculty of Education
9	Pipe borne CLN	School Clinic
10	Pipe borne FANP	Faculty of Natural and Applied Science
1	Borehole GH	Girl's Hostel
2	Borehole BH	Boys' Hostel
3	Borehole STQ	Staff Quarters
1	Well GH	Girl's Hostel
2	Well BH	Boys' Hostel
3	Well STQ	Staff Quarters

2.3. Sterilization and media preparation

The materials employed in this study were subjected to meticulous sterilization procedure to eliminate any potential microbial contamination. Following a thorough cleansing, all glassware and bottles were sterilized in a hot-air oven at 160°C for a duration of two hours. Additionally, the media utilized underwent sterilization at 121°C for 15 minutes within an autoclave. The inoculating wire loop was effectively sterilized through exposure to a Bunsen burner flame until it reached a red-hot state. Furthermore, the work surfaces were disinfected using appropriate disinfectants or antiseptic solutions, specifically 95% ethanol.

The preparation of the media was carried out in strict accordance with the manufacturer's instructions. The selected media included nutrient agar (NA), MacConkey broth, brilliant green lactose bile broth (BGLB), and Eosin Methylene Blue (EMB) agar. These components were proportioned accordingly, dissolved in an appropriate amount of water, and subjected to homogenization for several minutes. Subsequently, constant agitation was maintained to ensure complete dissolution of the media. Cotton wool was employed to seal the containers, which were then covered with aluminum foil before undergoing the autoclaving process.

2.4. Analysis of samples

The bacteriological analysis of the samples was carried out using a combination of total bacterial count (TBC) and most probable number (MPN) technique. In water and food Microbiology research, bacteriological analysis is commonly employed to offer a whole evaluation of the number and viability of bacteria within a sample (Food and Drug Administration, 1992). It includes a qualitative investigation to isolate and identify the primary germs present in the water sample, as well as a quantitative analysis to assess the concentration of bacterial bio-indicators.

TBC entails counting the number of bacterial cells in a sample using a variety of techniques, including microscopy and colony counting, which may count both viable and nonviable cells (Jay, 2004). Each technique is recognized for its speed, simplicity, and widespread acceptance in

measuring bacterial contamination in water. To determine TBC, we utilized the pour plate method. The water samples were serially diluted into sevenfold (10^{-1} to 10^{-7}) using distilled water. Subsequently, 1 mL of the diluted water sample (10^{-7}) was carefully dispensed into a petri dish containing 19 mL of molten nutrient agar. These prepared plates were then incubated at 37 °C for a duration of 48 hours. Following incubation, the former colonies were observed, manually counted, and the results were calculated and recorded.

The Most Probable Number (MPN) approach, also known as the dilution method, is a technique for determining microbial population density by examining the presence or absence of microorganisms in several aliquots of subsequent dilutions, eliminating the requirement for individual cell or colony counts ([Alexander, 1965](#)). This cost-effective method which is also known for its ease of implementation. The MPN technique as described by [Bartram and Ballance \(1996\)](#), was employed to determine both the total coliform count and the bacteriological index (most probable number) of the water samples. This technique was conducted through three successive stages; the presumptive, confirmed, and completed tests, respectively.

2.4.1. The presumptive test

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.

2.4.2. The confirmed test

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.

2.4.3. The completed test

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.

2.5. Identification of isolated organisms

The isolates were identification using the Gram staining technique, primarily based on their morphological characteristics, including color, shape, and texture. To achieve species-level identification, a battery of biochemical tests was conducted, following the methods described by [Baron and Finegold \(1990\)](#). These tests included Indole, Methyl red, Voges-Proskauer, Citrate utilization, and Sugar Fermentation tests. The obtained results were subsequently cross-referenced with Bergy's manual of determinative bacteriology for confirmation ([Buchanan & Gibbons, 1974](#)).

3. Results and Discussion

3.1. Results

The Table 2 provides an overview of the total viable bacterial counts, with pipe-borne water exhibiting the lowest mean total bacterial count, followed by borehole and well water sources. Pipi-born water showed a total bacterial count ranging from 0 to 1.5×10^6 CFU/100 mL, with a

mean value of 0.6×10^6 CFU/100 mL. Borehole water exhibited a range of 0.8×10^6 to 2.5×10^6 CFU/100 mL, with a mean of 1.6×10^6 CFU/100 mL. Well water had a range of 2.4×10^6 to 4.5×10^6 CFU/100 mL, with an average value of 3.2×10^6 CFU/100 mL.

Table 2. Total viable bacterial count at 37°C and Total Coliform Count

Water Samples	Total viable bacterial count at 37°C			Total Coliform Count (MPN/100 mL)		
	Pipe-borne (CFU/100 mL)	Borehole (CFU/100 mL)	Well (CFU/100 mL)	Pipe-borne	Borehole	Well
1	0.3×10^6	1.5×10^6	2.7×10^6	28	210	150
2	0.2×10^6	0.8×10^6	2.4×10^6	20	20	28
3	0.0×10^6	2.5×10^6	4.5×10^6	21	75	460
4	1.0×10^6			11		
5	0.7×10^6			20		
6	1.1×10^6			75		
7	1.5×10^6			14		
8	0.2×10^6			15		
9	0.4×10^6			11		
10	0.5×10^6			7		
Mean	0.6×10^6	1.6×10^6	3.2×10^6	22	102	331

The MPN technique revealed that pipe-borne water had a mean total coliform count of 22 MPN/100 mL, ranging from 7 to 75 MPN/100 mL (Table 2). Well water samples exhibited a mean total coliform count of 331 MPN/100 mL, with a range of 28-460 MPN/100 mL. Borehole water samples had an average total coliform count of 102 MPN/100 mL, with a range of 20-210 MPN/100 mL. These findings highlight that well water had the highest bacteriological index, emphasizing its potential as a source of water-borne diseases due to contamination from external sources.

Among the various water sources, pipe-borne water exhibited the lowest total coliform count and bacteriological index, whereas the other sources displayed higher values. As depicted in Table 3, the identified organisms included *Escherichia coli*, *Klebsiella Pneumonia*, *Enterobacter aerogens*, and *Citrobacter sp.* Notably, well water showed the highest frequency of isolated organisms, while pipe-borne water had the lowest occurrence.

Table 3. Morphological characteristics, gram reaction, and biochemical tests for isolated bacteria

Morphological characteristics	Gram Staining	Indole	Methyl red	Voges-Proskauer	Citrate	Sugar fermentation		Likely isolated organism
						Glucose	Lactose	
Greenish-metallic sheen	GNR	+	+	-	-	+	-	<i>Escherichia coli</i>
Brown dark-centered, mucoid colonies	GNR	-	-	+	+	-	-	<i>Klebsiella pneumonia</i>
Dark-centered, mucoid colonies	GNR	-	-	+	+	+	+	<i>Enterobacter aerogens</i>
Small, circular convex purple colonies	GNR	-	+	-	+	+	+	<i>Citrobacter sp.</i>

Key: GNR = Gram Negative rods, + = positive, - = negative

The organisms listed in Table 3 were identified through isolation on E.M.B. agar, relying on their distinctive morphological characteristics, gram staining reactions, and biochemical reactions. Gram staining consistently revealed that all the isolated bacteria belong to the gram-negative category. The distribution of these isolates across the various samples is detailed in the preceding table.

Table 4. Bacterial isolates from the water sources

Isolates	Various Water Sources		
	Pipe-borne	Borehole	Well
<i>Escherichia coli</i>	+	++	+++
<i>Klebsiella pneumoniae</i>	+	+	++
<i>Enterobacter aerogens</i>	0	+	++
<i>Citrobacter sp.</i>	0	+	+

Key: 0 = no growth, + = light growth, ++ = medium growth, +++ = heavy growth

As shown in Table 4, *Escherichia coli* was the predominant bacterial isolate, followed by *Klebsiella pneumoniae*. Among the water sources examined, pipe-borne water exhibited the lowest contamination, with borehole water being the next least contaminated. In contrast, well water was found to be the most contaminated, hosting a higher frequency of bacterial isolates compared to the other water sources. Notably, *Citrobacter sp.* was the least frequently isolated organism, with a lower occurrence rate than *E. coli*, *K. pneumoniae*, and *E. aerogens*.

3.2. Discussion

As presented in Table 2, pipe-borne water emerged as the least contaminated water source. Regrettably, only one out of the nine pipe-borne water sources, with CFU/mL of 0.0×10^6 , met the stringent requirements stipulated by World Health Organization (WHO). These findings align with prior studies ([Jamalludeen, 2019](#); [Kamal & Hashm, 2021](#); [Okoko & Idise, 2014](#)) where the mean coliform counts in pipe-borne water sources exceeded the predetermined limit of 0 CFU/100 mL.

On the contrary, well water samples exhibited the highest degree of contamination, boasted total bacterial count of 4.5×10^6 CFU/100 mL, a result consistent with the previous study conducted by [Adedeji et al. \(2017\)](#). Borehole water sources also fell short of meet the stipulated requirements for total bacterial count, echoing the findings of [Bashir et al. \(2018\)](#), who observed the presence of high fecal coliforms. This outcome was attributed to the proximity of the latrine system to the borehole water source, which was 30 meters below the recommended depth by the WHO. The water sources failed to comply with WHO's Coliform Forming Unit requirements. Furthermore, the type and quantity of organisms present in the water samples will determine the health risk poses to consumers upon ingestion.

Pipe-borne water sources displayed the lowest total coliform count, with values ranging from 7 to 75 MPN/100 mL. This finding diverged from the reports by [Ohanu et al. \(2012\)](#) who recorded coliform counts in tap water ranging from 188 to 1000 coliform aerobic isolates. Notably, the range of total coliform count for pipe-borne water did not align with the parameters outlined by the [WHO \(2008\)](#). Nevertheless, the difference was relatively minor when compared to other water sources.

In contrast, well and borehole water sources exhibited higher total coliform counts, ranging from 28 to 460 MPN/100 mL and 20 to 210 MPN/100 mL, respectively. These results were consistent with previous studies ([Adedeji et al., 2017](#); [Anake et al., 2013](#); [Bashir et al., 2018](#); [Sawyer et al., 2019](#)) which reported similar elevated counts. Unfortunately, both well and borehole water sources failed to meet the permissible range stipulated by regulatory standards.

The contamination of these water sources could be attributed to their proximity of the latrine systems and sewage dumps, which were located approximately 50 meters from water sources. Additionally, some of the well water sources lacked adequate covering, and unsanitary conditions

prevailed in their vicinity. The presence of coliforms in water serves as an indicator of potential enteropathogens that pose health risk to human ([Cheesbrough, 2000](#)). Addressing the issue of these indicator organisms in water sources is imperative to safeguard human health. Furthermore, it is essential to consider that pipe-borne water may also be susceptible to contamination due to faulty pipes or insufficient plumbing systems ([Onyeze et al., 2013](#)).

The morphological characteristics, gram reaction, and biochemical tests consistently pointed to the presence of gram-negative enteric rods; *Escherichia coli*, *Klebsiella pneumoniae*, *Citrobacter sp*, and *Enterobacter aerogens* among the probable organisms isolated. The finding was in line with previous studies carried out on pipe-borne water, where enteric gram-negative rods were the predominant organisms identified ([Adedeji et al., 2017](#); [Afzal et al., 2021](#); [Bashir et al., 2018](#); [Ibrahim et al., 2013](#); [Jamalludeen, 2019](#); [Kamal & Hashm, 2021](#); [Okoko & Idise, 2014](#); [Shakya et al., 2021](#)). The presence of *E. coli*, in particular, raised concerns as it is an indicator of potential enteropathogens in water sources, signifying their unsuitability for consumption (WHO, 2008).

Nevertheless, microbial water quality can exhibit substantial and rapid variations. Periodic spikes in pathogen concentrations can considerably heighten disease risks and contribute to water-borne diseases outbreaks. The gravest microbiological concerns are linked to water sources contaminated with human or animal feces (WHO, 2008). Such contamination may result from factors like inadequate public waste disposal facilities, insufficient waste collection and treatment systems, and on-site sanitation facilities discharging directly into water bodies. Additionally, bacteriological analysis may reveal contamination levels that particularly concerning during epidemics such as cholera or typhoid ([WHO-OECD, 2003](#)). Biofilms within the water distribution system can contribute to the presence of potential pathogens in drinking water pipes. Issues like corroded pipelines, faulty joints, back siphonage, crossing over the sewage pipes, and excessive pressures in the pipelines can all contribute to water contamination.

Furthermore, the shortage of potable drinking water is pervasive issue across Nasarawa state. Consequently, the collection and storage of water under unhygienic conditions may lead to contamination. Safeguarding water supplies is this a significant public health responsibility, with the WHO emphasizing the microbiological quality of drinking water. Outbreak of water-borne diseases continue to pose a tremendous burden on society, emphasizing the importance of ensuring that water is free from microbial and physical impurities and aesthetically acceptable to consumers. Therefore, physical and or chemical treatment of water before use for domestic purposes is imperative.

These water sources within the Keffi community in Nasarawa State are, however, potential hazards. They serve as potential route for transmission of water-borne diseases that are frequently reported in that area. In addition to consumption, the use of contaminated water for domestic and agricultural purposes can also contribute to the spread of water-borne diseases.

4. Conclusion and recommendations

This investigation unequivocally concludes that the quality of the water samples falls far below acceptable standards. It is evident that these water sources are highly susceptible to contamination by water-borne pathogens, highlighting the paramount importance of ensuring safe drinking water sources for the well-being of the populace.

Preserving water quality throughout the entire distribution network, whether through chemical or physical treatments, is of utmost significance in preventing water-related illnesses that can gravely impact public health. To achieve this, it is imperative for the state government to task its water agencies with the responsibility of effectively treating the water and ensuring the utmost protection of the piped distribution system to prevent contamination. Routine water analysis within the distribution system by Mada waterworks is equally essential to maintain consistency and compliance with permissible limits.

Moreover, addressing the specific contamination points for each of these water sources is crucial. Boreholes and wells must be securely covered and located at a safe distance from sewage

tanks and latrines. The proximity of animals to the well must be managed as they can contribute to the contamination. Additionally, a thorough inspection and potential reconstruction of pipes used for conveying water sources should be undertaken to ensure their integrity.

To ensure drinking water safety and quality while safeguarding public health, the implementation of comprehensive water safety framework and the execution of well-planned water supply strategies should be a top priority.

Eliminating the prevalence of contaminated water consumption and water-borne diseases necessitates a collaborative effort involving the state government, its agencies, and other stakeholders such as academic institutions and healthcare organizations. These entities must work collectively with a shared goal of progressively eradicating water-borne diseases. Institutional bodies like Nasarawa State University and Federal Medical Centre should rigorously study the trends in water-borne disease and generate accurate data.

In conclusion, the responsibility for ensuring the safety of our water sources for consumption lies with each and every one of us. It is a collective obligation to protect public health by addressing water quality issues comprehensively.

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