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Study of some physicochemical and bacteriological characteristics of drinking water in three selected rural communities in Wamba, Nasarawa State, Nigeria.

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Abstract

Access to safe drinking water remains a challenge in rural Nigeria. This study was carried out to determine physicochemical and bacteriological characteristics of drinking water in three rural communities in Wamba, Nasarawa State. A cross-sectional survey was conducted, 5 water samples were in respective cases collected from stream, well and borehole sources. Standard techniques were used for physicochemical analysis. Total viable counts, Total Staphylococcal counts, Total coliforms & Feacal coliforms in the samples were obtained using pour plate method, growth on Mannitol salt agar, growth on MacConkey agar and Eosin Methylene Blue agar respectively. Stream Water had higher values for physicochemical parameters. Stream water exhibited the highest bacterial counts, followed by well and borehole water. The Bacteria isolated were *Escherichia coli, Staphylococcus aureus, Pseudomonas* spp, *Klebsiella* spp, *Proteus* spp and *Salmonella* spp. The acid lability attributes of the isolates were determined using Nutrient Agar buffered with (4-(2- Hydroxyethyl)-1- piperazineethanesulfonic acid) and Lipase activity was assessed using Trypticase soy agar supplemented with 1% Tween 80. Statistical analysis was performed using SPSS-PC statistical package, with a p-value of < 0.05 it was considered statistically significant. These findings indicate contamination of drinking water sources, posing health risk to consumers and it shows the need for water treatment, monitoring & public health education in rural communities.

Keywords: Physicochemical parameters; Bacteriological parameters; Drinking water; Rural communities

1. Introduction

Water is one of the basic requirements of human daily consumption, yet much of the world's population struggles to find consistent access to safe drinking water as recommended by the WHO drinking-water quality guidelines. Currently, about two billion people in the world live without access to safe drinking water (WHO, 2017). Bacteriological water quality is defined in terms of the absence or presence of indicator organisms. Drinking water does not cause an infectious disease if it is free from indicator organisms (Aderajew *et al*., 2019). More than half of the world's population lives in rural areas and most of them do not have access to safe drinking water supply. The neglect of rural areas in most developing countries in terms of pipe-borne water and sanitation facilities exposes the communities to a variety of water borne diseases. Bacterial and physicochemical contamination of water is most prevalent in Nigeria's rural areas where little or no attention is devoted to sources of potable water (Mutiat *et al*., 2023). Therefore it is necessary that the quality of drinking water should be evaluated at regular intervals, because continuous use of contaminated water causes the spread of varieties of water borne diseases (Patil *et al*., 2012).

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2. Material and methods

2.1. Study Area

Wamba Local Government Area is one of the 13 local Government Areas in Nasarawa state. It Coordinates 8˚56'0"N 8˚36'0"E. Wamba is renowned for the spectacular and beautiful Farin Ruwa Falls which is one of the longest cascades in Africa.

2.2. Sample Collection

A total number of 15 water samples were collected using 1 liter sterile bottles. 5 well water samples from Bambu community, 5 stream water samples from Kurize community and 5 borehole water samples from Gbude community. Samples from Bambu well were labeled BWW1 to BWW5 while those from Kurize stream were labeled KSW1 to KSW5 and those from Gbude borehole labeled GBW1 to GBW5. The water samples were collected on a weekly basis for 5 weeks.

2.3. Physicochemical Analysis

The pH of all water samples were checked using pH meter (Systronics 361, India). The electrical conductivity was measured using pre-calibrated conductivity TDS meter (Systronics 308, India). The total suspended solids (TSS) were measured using the portable TSS meter. Sulphate, Nitrates, Phosphates, Chloride, Dissolved oxygen, BOD and COD were analyzed using standard methods of American Public Health Association (APHA, 1992).

2.4. Bacteriological Analysis of Water Sample

Bacteriological analysis of each study sample was done to detect the presence of water borne pathogens such as *Salmonella, E. coli, Staphylococcus aureus, Klebsiella spp, Pseudomonas spp and Proteus spp*. Tenfold serial dilution was carried out using the water sample, 10^6 dilution factor was used, the samples were plated on different agar like Mannitol salt agar, Nutrient agar, Salmonella-Shigella Agar (SSA) and MacConkey agar and allowed for growth for 24hrs.

2.5. Most Probable Number (MPN)

The Most Probable Number or multiple tube fermentation technique was used for coliform enumeration. For the presumptive test for coliforms, three 10 ml, three 1ml, and three 0.1 ml volumes of the appropriate dilution of water samples were inoculated with the samples in respective nine fermentation tubes with inverted Durham tubes and then placed in lactose broth to detect gas production. The inoculated test tubes were incubated for 48 hours at 37°C, and those containing air bubbles were confirmed by plating on Eosin Methylene Blue agar (EMB) at 37°C for faecal coliforms (APHA 1992).

2.6. Total Viable Count

After serial dilutions of the water samples, the pour plate method was adopted. Molten agar was poured into sterile petri dishes aseptically. The diluted water samples were added to the molten nutrient agar and mixed gently. It was allowed to solidify for 5-10 minutes, the petri dishes were inverted and incubated at 37˚C for 24 hours. Plates containing between 30 and 300 colonies were selected and the colonies were counted using a colony counter and the number of colonies was recorded per plate. The numbers of colonies were multiplied by the final dilution factor to give the total viable cells/mL in the original samples.

2.7. Total Staphylococcal count

Mannitol salt agar was prepared according to manufacturer's instructions. The molten MSA was poured into sterile petri dishes aseptically; the diluted water samples were added to the agar and mixed gently. The agar was allowed to solidify for 5-10 minutes, the plates were inverted and incubated at 37˚C for 24 hours. The colonies with characteristic Staphylococcal morphology (round, convex, yellow pigmented) were counted. The total Staphylococcal count was calculated by multiplying the number of Staphylococcal colonies by the dilution factor.

2.8. Gram Staining

A thin smear of the bacterial colony was made on a clean grease free glass slide. The film was air dried and the smear was heat-fixed over a Bunsen burner flame. The slide was placed on a rack over a sink and the smear was covered with crystal violet reagent for one minute. The slide was rinsed with Gram's iodine for 30 seconds, the slide was rinsed slowly in water, after which 70% acetone was applied on the slide until no more dye ran off from the smear. The smear was

rinsed again and then covered with safranin for 30 seconds and then rinsed under slow running tap water, the slide was allowed to dry and observed using the oil immersion lens (x100).

2.9. Biochemical Analysis

The isolated organisms from the water samples were further confirmed by IMViC (Indole, Methyl Red, Vogues-Proskauer and Citrate utilization) tests (Cappucino and Sherman, 2002). Coagulase and catalase test was also carried out.

2.10. Determination of Acid Lability of the isolated Bacteria

From each agar plates, each bacterial isolate was suspended in sterile, dechlorinated water to produce a 1.0 McFarland turbidity equivalent. This suspension was kept at room temperature for 24h in order for bacteria to adapt to the aqueous environment. A previously titrated amount of acetic acid was mixed with 0.1 ml of this suspension to produce a final pH of 3.5. After rapid mixing, incubation was at room temperature for 10 min. 0.1 ml was sub-cultured onto a nutrient agar plate which was buffered with HEPES to produce a final pH of 7.5. This buffered agar plate neutralized the acetic acid while allowing the bacteria to grow. The number of survival was calculated based on the initial inoculum concentration obtained from quantitative plating of the bacterial suspension after 24hours at room temperature and the final number of CFU/ml that survived on the buffered agar plate.

2.11. Determination of Lipase activity of the isolated Bacteria

Trypticase soy agar plates supplemented with 1 per cent tween 80 (Polyoxyethylene Sorbitan Monooleate) served as a substrate. Colonies were inoculated onto the surface of the agar plate and allowed to incubate for 72 h at 37°C. The appearance of a turbid halo around the inoculum spot was taken as evidence of a positive test.

2.12. Statistical Analysis

Data was recorded, organized and summarized in sample descriptive statistics methods using SPSS-PC statistical package (SPSS 14 for windows version). These results were presented in correlations measures, ANOVAs, T-tests. Least Square of Differences (LSD) was applied to all physicochemical parameters and the bacterial counts to compare variation in water systems. The data was interpreted by their frequencies and magnitudes such as concentration of the organisms in a liter of water sample. P-value of less than 0.05 was considered statistically significant.

3. Results

3.1. Physicochemical parameter analysis

The pH of the water samples ranged from 6.5 to 7.2, the Turbidity ranged from 20.8 to 46.6 NTU. The electrical conductivity ranged from 144µs to 916µs. The chloride ranged from 36mg/L to 111mg/L, the Nitrate ranged from 42.1mg/L to 88.1mg/L. the Sulphate ranged from 101.1mg/l to 127.2mg/l, the Phosphate ranged from 3.2mg/l to 5.9mg/l, the dissolved oxygen ranged from 3.9mg/l to 15.0mg/l, the biochemical oxygen demand ranged from 120mg/l to 209mg/l, the chemical oxygen demand ranged from 117mg/l to 331mg/l and the total suspended solids ranged from 181mg/l to 401mg/l.

Table 1 Physicochemical Parameters of Water Samples from the three sources

Parameters	KSW	BWW	GBW
pH	7.2	6.8	6.5
Turbidity (NTU)	46.6	26.6	20.8
Conductivity (μS cm−1)	916	216	144
Chloride (mg/L)	111	36	59
Nitrate (mg/L)	88.1	42.1	45.4
Sulphate (mg/L)	127.2	114.2	101.1
Phosphate (mg/L)	4.9	5.9	3.2
DO (mg/L)	3.9	15.0	6.1
BOD (mg/L)	209	190	120
COD (mg/L)	331	117	142
TSS (mg/L)	401	204.2	181

DO= Dissolved oxygen, BOD= Biochemical oxygen demand, COD= Chemical oxygen demand, TSS= Total suspended solids, NTU= Nephelometric turbidity unit

All results are mean of quintuple values of the 5 samples KSW1-KSW5, BWW1-BWW5 and GBW1-GBW5 for the Stream, well and borehole water.

Table 2 Cultural, Morphological and Biochemical Characteristics of Bacterial isolated from different Water Sources

Cultural & Morphological	MP	GS	Biochemical Characteristics						Inference
			CAT	COA	IN	VP	MR	OX	
Golden Yellow on MSA	Cocci	+	+	+	-	-	-	-	*Staphylococcus aureus*
Greenish on EMB	Rod	-	+	-	+	-	+	-	*Escherichia coli*
Smooth whitish on NA, Black deposit on SSA	Rod	-	+	-	-	+	-	+	*Salmonella* spp
Smooth Greenish on NA and brown on MAC	Flat	-	-	+	-	-	-	+	*Pseudomonas* spp
Pinkish on MAC	Rod	-	+	+	-	-	+	+	*Klebsiella* spp
Smooth Colonies, moist and often have a characteristic fishy colour	Rod	-	+	-	-	-	+	-	*Proteus* spp

KEY: P=Pigment; MP=Morphology; GS=Gram Staining; CAT=Catalase, COA=Coagulase; IN=Indole; VP=Voges Proskauer; MR= Methyl Red; OX=Oxidase; CT= Citrate, + = Positive; - = Negative, MSA=Mannitol Salt Agar; EMB= Eosin Methylene Blue, MaC= MacConkey Agar, NA= Nutrient Agar, SSA= Salmonella Shigella Agar

The cultural, morphological and biochemical characteristics of Bacteria Isolates from different water sources is as shown in Table 2 above

Golden yellow colonies on MSA, cocci, Gram positive, catalase positive, coagulase positive, indole negative, Voges-Proskauer negative, methyl red negative and oxidase negative were suspected to be Staphylococcus aureus. Greenish metallic sheen on EMB, rod shaped, Gram negative, catalase positive, coagulase negative, oxidase negative, indole positive, Voges-Proskauer negative and methyl red positive were suspected to be Escherichia coli. Smooth whitish on NA, Black deposit on SSA, gram negative, catalase positive, oxidase positive, indole negative, Voges-Proskauer positive and methyl red negative were suspected to be Salmonella spp. Smooth greenish, none elevated on NA and brown on MAC, flat, gram negative, catalase negative, coagulase positive, oxidase positive, indole negative, Voges-Proskauer negative and methyl red negative positive were suspected to be Pseudomonas spp. Pinkish on MAC, rod shaped, gram negative, catalase positive, coagulase positive oxidase positive, indole negative, Voges-Proskauer negative and methyl red positive were suspected to be Klebsiella spp. Smooth cream colored colonies that were moist on MAC, rod shaped,

Gram negative, catalase positive, coagulase negative, oxidase negative, indole negative, Voges-Proskauer negative and methyl red positive were suspected to be Proteus spp.

Table 3 Total Bacteria Load Count from the three Different Water Sources

Water Sources	**Mean ± SD (CFU)**			
	Total Viable Count X10^6	**Total Coliform Count X10^6**	**Total Faecal Count X10^6**	**Total Staph Count X10^6**
KSW	29.03± 3.02	21.1±2.56	20.43±4.21	13.34±1.08
BWW	23.12±3.43	13.28±2.66	7.78±2.38	9.12±1.67
GBW	8.66±1.34	4.44±1.34	4.11±11.98	5.02±0.99

All results are mean of quintuple values of the 5 samples KSW1-KSW5, BWW1-BWW5 and GBW1-GBW5 for the Stream, well and borehole water respectively.

Table 3 above shows the total bacteria load count from different water sources. Stream Water had the highest counts amongst the water sources. Stream water had the highest total viable count at 29.03 ± 3.02 CFU x 10^6, followed by the well water at 23.12 ± 3.43 CFU x 10^6 and borehole water had the lowest at 8.66 ± 1.34 CFU x 10^6. This indicates that the stream water had the highest overall bacterial contamination. The bacterial load in the well water sample was lower than the stream water but still relatively high. The Total Fecal Count for well water was 7.78 ± 2.3 CFU x 10^6 which suggests less direct fecal contamination compared to that of stream. The bacterial load in the borehole water was the lowest among the three sources, indicating a better water quality.

3.2. Occurrence of Bacteria from the three different Water Sources

Table 4 below shows the percentage occurrence of bacteria among the different water sources. The isolates from well water had the highest from *Pseudomonas* spp 5(100%) followed by *Escherichia coli* 3(60%), *Staphylococcus aureus* 3(60%) and *Klebsiella* spp 3(60%). *Salmonella* spp and *Proteus* spp isolated from well both had the occurrence of 2 (40%). Bacteria isolated from stream water showed a high percentage occurrence of 4(80%) for *Escherichia coli and Pseudomonas spp, Staphylococcus aureus, Salmonella* spp*, Klebsiella* spp*, Salmonella* spp *and Proteus* spp had a percentage of occurrence of 3(60%) respectively. The bacterial isolates from borehole water had the least percentage of occurrences among all the water sources sampled. Only *Escherichia coli* and *Klebsiella* spp with a percentage of occurrence of 2 (60%) and Pseudomonas spp. with an occurrence of 1 (20%) was found in borehole water. All other isolates from the result of this had no occurrence as observed in borehole water.

Table 4 Occurrence of Bacterial isolates from the three Different Water Sources

Bacteria	**No. Isolated (%)**					
	No. Sample	**BWW**	**No. Sample**	**KSW**	**No. Sample**	**GBW**
E. coli	5	3 (60)	5	4 (80)	5	2 (40)
S. aureus	5	3 (60)	5	3 (60)	5	0 (0)
Salmonella spp	5	2 (40)	5	3 (60)	5	0 (0)
Pseudomonas spp	5	5 (100)	5	4 (80)	5	1 (20)
Klebsiella spp	5	3 (60)	5	3 (60)	5	2 (40)
Proteus spp	5	2 (40)	5	3 (60)	5	0 (0)

3.3. Acid lability of the isolated Bacteria

Table 5 below shows the percentage survival of isolated bacteria in acidic medium. *Pseudomonas* spp showed highest percentage survival in well water, stream water and borehole water. *Salmonella* spp and *Proteus* spp had least survival percentages across the 3 water sources.

Table 5 Acid Lability of Bacteria Isolated from Different Water Sources

Bacteria	No. isolated (%)					
	BWW	Survival	KSW	Survival	GBW	Survival
E. coli	3	2(66.6)	4	1(25.0)	2	0(0.0)
S. aureus	3	1(33.3)	3	2(66.6)	0	0(0.0)
Salmonella spp	2	0(0.0)	3	1(33.3)	0	0(0.0)
Pseudomonas spp	5	3(60.0)	4	2(50.0)	1	1(100)
Klebsiella spp	3	1(33.3)	3	0(0.0)	2	1(50.0)
Proteus spp	2	0(0.0)	3	1(33.3)	0	0(0.0)

3.4. Lipase activity of the Bacteria isolated from different water sources

Table 6 below shows the bacterial isolates that possessed the enzyme lipase to breakdown lipids. The percentage of bacteria that possessed the enzyme was generally higher in the isolates from stream water and lowest among the bacteria isolated from borehole water. Only *Klebsiella* spp isolated from the 3 water sources showed lipase activity.

Table 6 Lipase of Bacteria Isolated from Different Water Sources

Bacteria	No. isolated (%)					
	BWW	Lipase Positive	KSW	Lipase Positive	GBW	Lipase positive
E. coli	3	3(100)	4	2(50.0)	2	0(0.0)
S. aureus	3	0(0.0)	3	1(33.3)	0	0(0.0)
Salmonella spp	2	0(0.0)	3	2(66.6)	0	0(0.0)
Pseudomonas spp	5	2(40.0)	4	3(75.0)	1	0(0.0)
Klebsiella spp	3	2(66.6)	3	1(33.3)	2	1(50.0)
Proteus spp	2	1(50.0)	3	2(66.6)	0	0(0.0)

4. Discussion

This current study focused on the physicochemical and bacteriological analysis of drinking water samples collected from rural communities in Wamba, Nasarawa state, Nigeria. The slightly lower pH in the well and borehole water samples may be attributed to the presence of dissolved minerals and the influence of the surrounding geological formations (Egbueri, 2020). The turbidity levels were highest in the stream water at 46.6 NTU, followed by the well water at 26.6 NTU, and the borehole water at 20.8 NTU. The higher turbidity in the stream water is similar to a study by Akani et al., (2021) which attributed the high turbidity to the presence of suspended particles, organic matter, and sediments carried by surface runoff. The acid lability of Salmonella spp in stream water matches findings from a study by Wilkes et al. (2019), which reported that *Salmonella spp* isolated from agricultural runoff showed higher acid tolerance compared to those from more controlled environments like wells or municipal water systems. A study by Nandal et al., (2021) investigated the occurrence of lipase-producing bacteria in surface water and groundwater samples. A higher prevalence of lipase-producing bacteria in surface water samples was found compared to groundwater samples, which aligns with the results of this current study. These findings emphasize the importance of regular water quality monitoring, proper well construction and maintenance, and the implementation of effective water treatment and disinfection methods to ensure the provision of safe and potable water to the community. The results

obtained here can inform decision-making processes and guide the development of appropriate water management strategies to mitigate the risks associated with bacterial contamination in water sources.

5. Conclusion

This study concludes that the water samples are contaminated with pathogenic microorganisms and the water samples are unfit for consumption unless properly treated before consumption

Compliance with ethical standards

Disclosure of conflict of interest

No conflict of interest to be disclosed.

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