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PHYSICO-CHEMICAL AND MICROBIOLOGICAL SAFETY OF WATER UTILIZED IN THE MEDICAL CELL, MBARARA CITY, SOUTHWESTERN UGANDA, A CROSS-SECTIONAL STUDY.

Christopher Okeny*, Wilson Kiduuma, Adrine Ikiiriza, Salim S Sonia, Sadrack Omara, Catherine N Abaasa

Department of Medical Laboratory Sciences, Mbarara University of Science and Technology, P.O Box 1410, Mbarara City Uganda, email @must.ac.ug.

Abstract

Introduction:

safe and readily available water is very vital for public health functions whether it is used for drinking, food production, washings, or recreation purposes. This study assessed the physical properties (temperature, total dissolved solids), chemical properties (pH, electrical conductivity), and microbiological properties (total coliform counts, Escherichia coli counts) of domestic water utilized in the medical cell, Mbarara city, Southwestern Uganda.

Method:

total coliform counts and total Escherichia coli counts were performed on Eosin Methylene blue medium and the organisms were subculture on MacConkey agar. This was followed by biochemical tests on individual bacterial colonies to identify the water contaminant.

Results:

the study revealed that temperature ranged from 21.30C to 25.50C, pH ranged from 5.23 to 7.91, electrical conductivity ranged from 110 to 189μS/cm, and total dissolved solids ranged from 54mg/dl to to112mg/dL. Microbiologically, 67.74% of the water samples had total coliform counts >10CFU/100mL and 6.45% had Escherichia coli counts > 0CFU/100mL of the water sample. 47.62% of the isolates were Enterobacter spp, 38.10% Citrobacter spp, 9.52% Escherichia coli, 9.52% Klebsiella spp, 7.14% Salmonella spp and 4.76% Pseudomonas spp.

Conclusion:

microbiologically, water supply had potential disease-causing pathogens which pose risks of waterborne disease outbreak to the population.

Recommendation:

The national water and sewerage corporation should inspect the water supply pipelines for leakages and repairs should be made where necessary.

Responsible stakeholders of the Medical cell, Mbarara City should ensure proper disposal of human wastes. Further studies can be done to genotype the microbial water contaminants and or determine their antimicrobial susceptibility patterns.

Keywords: Physical, Chemical, Microbiological, Safety, Domestic, Water, Submitted: 2023-08-10 Accepted: 2023-08-28

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1. Introduction

Water is an essential element of life on the planet Earth and about 75% of the Earth's surface is covered by water [1]. However, only 1% of the available water is drinkable [2], hence much of the world's population lacks access to sufficient and safe water supplies [3]. Globally, 2.1 billion people lack access to safe drinking water [4, 5]. Their water sources are fecally contaminated and can transmit waterborne and water-related diseases such as cholera, Shigellosis, Salmonellosis, and polio [6]. Each year contaminated drinking water is estimated to cause 502,000 diarrheal deaths [6]. In Sub-Saharan Africa, 42% of people are without basic water supply and 72% without basic sanitation [7]. Uganda has experienced two decades of economic growth, leading to a large population movement from rural areas to informal settlements around urban centers [8]. High population growth has stressed the water and sanitation services that are in existence [8]. Uganda has 45 million people, of which only 32% have access to basic water while 19% have access to basic sanitation, and 7 million Ugandans still practice open defecation [9].

Domestic water contamination has resulted in several waterborne and water-related infections worldwide [10]. Diarrheal diseases account for 1 in 9 infant mortality worldwide, making diarrhea the second leading cause of under 5 years mortality [11]. Diarrhea remains one of the leading infant and young children's morbidity in Uganda [12]. In 2016, the prevalence of diarrhea among children < 5 years In Uganda was 20% [13]. In 2017, diarrheal deaths reached 6.41% of total deaths, making the country to be ranked 27th worldwide [14]. Salmonellosis is one of the waterborne diseases and the global burden stands at 21%, of which 1% (222,000) succumb to the disease [15]. Salmonellosis remains an endemic disease in Uganda with a disease burden of 56,135 cases (144 cases in 100,000), 657 deaths, and 50,644 disability-adjusted life-years lost to typhoid by 2017 [16].

There is a need to have active monitoring and surveillance of water quality for domestic water through the enumeration of *Escherichia coli* in water samples [17]. However, this is not always done due to financial constraints. This study therefore assessed the physical, chemical, microbiological properties and microbiological contaminants of domestic water utilized in the Medical cell, Mbarara City, Southwestern Uganda

2. Materials and Methods

2.1. Study site and design.

This study was cross-sectional and involved quantitative methods of data collection. Domestic water points were randomly sampled. The study was conducted in the Medical cell, of Mbarara city located in South Western Uganda, 290 km from Kampala (The capital city of Uganda) on the Kampala-Kabale road from August –September 2022. Medical cell, Kamukuzi division, Mbarara city is located at the altitude 00 36'16" S of Equator and 30038'54" E of Greenwich meridian [18].

2.2. Inclusion criteria and exclusion criteria.

2.2.1. Inclusion criteria:

Water collection points with metallic taps that could withstand heat sterilization.

2.2.2. Exclusion criteria:

Water collection points with leakages and taps that could not withstand heat sterilization.

2.3. Sanitary Inspection of the Water Sources

A cross-sectional sanitary assessment was carried out in each of the selected domestic water points to identify the risks for contamination with fecal materials. The assessment adopted the standardized procedure which involved completing a nine-point standardized data form adopted from "The District Laboratory Practice in Tropical Countries Part 2" by Monica Cheesbrough,

* Corresponding author.
Email address: christopherokeny79@gmail.com (Christopher Okeny)

with a set of questions having "yes" and "no" options for designated risks [19]. For each "yes" answer (risk observed), a score of one point was awarded while zero point was given for each "no" answer (no risk observed). A final risk score was obtained by summing all "yes" scores thus giving the overall assessment of the risk profile of each water collection point. The total sanitary risk scores were converted to percentages. The aggregate risk scores were graded as very high (81-100%), high (51- 80%), medium (31- 50%), low (1- 30%) and nil (0%).

2.4. Sample collection and processing.

Water samples were collected after sterilization of the taps into sterile 300ml water collection bottles. Two water samples were collected per tap. One was for physicochemical measurements which were done on-site and the other sample was immediately placed in a cool box with ice packs, and transported to the Mbarara University of Science and Technology microbiology laboratory for microbiological analysis. Temperature, pH, electrical conductivity, and total dissolved solids of domestic water were measured on-site using the Hanna instrument HI98129. Microbiological properties were determined by performing total coliform count and Escherichia coli counts, in which, water samples were aseptically collected, transported, and analyzed. Water samples were serially diluted using normal saline and their aliquots were inoculated onto Eosin Methylene Blue (EMB) medium. The inoculated media were incubated at 37oC overnight and the culture plates read after 24 hours of incubation. Colony counts were done for plates with growth and purity plating of the isolates by subculture was done where there was mixed growth to obtain pure colonies on MacConkey medium. Escherichia coli colonies had a metallic golden sheen appearance on Eosin Methylene Blue medium [20]. Further biochemical tests for the identification of the isolates were performed and the biochemical tests were; Triple Sugar Iron (TSI) test, Sulphide Indole Motility (SIM) test, Citrate utilization test, and oxidase test for identification of the isolates to species level.

2.5. Biochemical tests.

These tests were used in combinations to deduce on the bacterial properties and hence their identifications based on observed properties.

Triple Sugar Iron (TSI) Agar, a composite medium was used to study different properties of bacteria (this case Gram-negative bacteria) for sugar fermentation, gas production, and hydrogen sulfide (H2S) production, phenol red and ferrous sulfate serves as indicators of acidification and H2S formation respectively [21]. The test was done by picking a well-isolated colony of the test organism with a sterile straight wire loop and inoculating the TSI medium by first stabbing through the center of the medium to the bottom of the tube and then streaking the surface of the agar slant and the tubes were incubated for 24 hours at 37°C in an incubator [22]. Alkaline slant / alkaline butt (K/K), indicated glucose, lactose, and sucrose non-utilizing bacteria like Pseudomonas spp (alkaline slant/alkaline butt), Acid slant/acid butt (A/A), with gas production indicated glucose, sucrose, and/or lactose fermenting bacteria like *Escherichia coli [22]*. Alkaline slant/acid butt (K/A), hydrogen sulfide gas production indicated glucose fermentation only by Salmonella typhi, the formation of carbon dioxide (CO2) gas was indicated by the presence of bubbles or cracks in the agar medium or its separation from the sides or bottom of the tube and the production of hydrogen sulfide gas required an acidic environment and was indicated by blackening of the butt of the medium in the tube [23].

The Sulphide Indole Motility (SIM) medium test determines the ability of the test organism to split the tryptophan molecule into Indole [24]. Indole is one of the metabolites obtained from the degradation of the amino acid tryptophan [25]. Bacteria that possess the enzyme tryptophanase are capable of hydrolyzing and deaminating tryptophan with subsequent production of Indole, pyruvic acid, and ammonia [26]. SIM medium was inoculated with the central stab of the test organism and incubated for 24 hours at 37°C. The test was interpreted by checking for bacterial motility depicted by hazy or feather-like growth away from the central stab line, observa-

tion for hydrogen sulfide production was depicted by blackening of the medium, and indole production checked by additions of ten (10) drops of freshly prepared Kovac's reagent down the inner wall of the tube where development of a bright red (Cherry red) color at the interface of Kovac's reagent and the broth within seconds showed positive indole production as the case with *Escherichia coli [27]*. The motility test was used to differentiate between the motile and non-motile bacteria species. A feather-like growth observed moving away from the stab line signified a motile bacterium and a positive test for bacteria such as *Escherichia coli and Salmonella typhi* [28]. The growth along the stab line indicated non-motile bacteria such as Shigella and Klebsiella spp [29].

Simon's Citrate Test, the ability of an organism to utilize citrate as its sole source of carbon and energy was determined using the Simon's citrate test [30]. The test was conducted by streaking the inoculum over the slant of Simon's citrate agar medium in a tube followed by 24 hours incubation period at 37°C. A positive test result for citrate utilization was shown by the presence of growth on the slant and bluish coloration of the medium as the case with *Klebsiella spp* while a greenish-colored medium depicted a negative test result [31].

Oxidase test, This test determines the presence of cytochrome oxidase enzyme among the Gram-negative bacteria. Oxidase-producing bacteria oxidize the reagent substrate tetramethyl - p- phenylenediamine for instance on a filter paper to indolphenol, a dark purple colored end product [32]. Oxidase papers were used where test colonies were smeared over a dry oxidase paper and observed for a deep purple color development within 10 seconds. Purple color development within 10 seconds was a positive test as the case with *Pseudomonas spp* and no color change meant a negative test [32].

2.6. Data analysis.

Data was analyzed using IBM SPSS 28 software, and the physico-chemical results were reported as percentages of each measured parameter. The microbiological properties were tabulated with the colony counts, Escherichia coli counts, and the isolates of each water sample. The percentage of water samples with colony counts greater than the WHO recommendations (i.e. >10CFU/ml of water sample) [6] was calculated from the total water sample analyzed and the results were analyzed as per the water samples.

2.7. Ethical consideration.

We were issued a clearance letter by the Faculty of Medicine Research Ethics Committee, Mbarara University of Science and Technology, and an introductory letter from the Medical Laboratory Science department which enabled us to get further clearance from the Department of Natural Resources and Environment, Mbarara City Council. We thereafter sought permission from the Local Council I, Medical Cell who introduced us to the community of Medical Cell, and we gained the consent of the residents of Medical Cell who consented that their water collection points be sampled before water collection.

3. Results and discussion

3.1. Sanitary inspections of the water collection points.

The sanitary environment of the water collection points was assessed using an adopted standardized sanitary inspection checklist from district laboratory practice in tropical countries by Monica Cheesbrough part 2, for establishing the likelihood of the possible sources of water contamination [33]. The highest risk of contamination was found at water points 1, 2, 3, 4, 7, and 9 with 37.5%, and no risk of contamination (0%) was found at collection points uniquely identified by the numerical numbers;13 to 20 consecutively. The results are shown in table 1.

These sanitary inspection grades of the water collection points showed a percentage likelihood of water point contamination. The grades show the risk factor(s) for contamination of a particular water collection point. 0 means no risk factor while 8 means 8 risk factors found at the water collection point that can potentially cause water

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contamination. The higher the grade percentage, the higher the risk of water contamination at a particular collection point and vice versa.

Table 1 showing sanitary inspection results for the water collection points.

Grade	Grade %	Water samples (PCMWA)
0	0	13, 14, 15, 16, 17, 18, 19, 20
1	12.5	5, 11, 12, 21 up to 62
2	25	6, 8, 10
3	37.5	1, 2, 3, 4, 7, 9
4	50	-
5	62.5	-
6	75	-
7	87.5	-
8	100	-

3.2. Physicochemical properties.

Water supplies were basically by the National Water and Sewerage Corporation (NWSC) and rain harvest. The pH of rain water ranged from 6.68 to 6.99 (WHO recommendations of 6.5 - 8.0). 48.08% of national water and sewerage corporation water samples had pH below 6.5. These water points deliver unsafe water for domestic use particularly for drinking. Temperature of all water collection points were >15°C, the temperature that makes drinking water palatable []. The electrical conductivity and total dissolved solids were all in agreement with the WHO recommendation of drinking water shown in the table 2.

Table 2: summary of the physicochemical properties of domestic water in reference to the WHO guidelines

Physico-chemical parameters	Water Source	Criteria	Number of sources deviation from WHO guideline	Sample ID (PCMWA)	% of source deviation from WHO guideline	Overall % Deviation from WHO guideline	WHO guidelines.
pH	NWSC	<6.5	25	1 to 5, 7, 36 to 49, 52, 53, 55, 58 and 59	48.08	40.32	6.5 - 8.0
	Rain harvest	<6.5	0	None	0	0	
EC (µS/cm)	NWSC	<1500	0	All (1 to 19, 30 to 62)	0	0	<1500
	Rain harvest	<1500	0	All (20-29)	0	0	
Temperature (°C)	NWSC	<15	52	1 to 19, 30 to 62	100	100	<15
	Rain harvest	<15	10	20 to 29	100	100	
TDS (mg/dl)	NWSC	<1000	0	All (1-19, 30-62)	0	0	<1000
	Rain harvest	<1000	0	All	0	0	

3.3. Microbiological properties.

Microbiologically, 50% of rainwater samples had total coliforms >10CFU/100ml and 0CFU/100ml Escherichia coli counts. 69.23% of the tap water samples were contaminated with coliforms and 7.69% Escherichia coli contamination. Escherichia coli is an indicator of recent faecal water contamination and hence this meant that tap water experienced recent faecal contaminations []. Other coliforms found in the water indicated long term water contamination and this suggested the proliferation of these organisms in the water storage tanks []. The overall result for all water samples indicated 67.74% contamination with total coliforms and 6.45% Escherichia coli contamination. These findings are summarized in the table 3.

Table 3 Microbiological properties of domestic water expressing the percentage contaminants and safety.

Water source	Bacterial Counts	Criteria	number of water points	Sample ID (PCMWA)	% composition of water points	WHO guidelines
Rain harvest	TCC (CFU/100ml)	<10	5	21, 25, 26, 27 and 28	50	<10
		>10	5	20, 22, 23, 24 and 29	50	
	E.coli (CFU/100ml)	0	10	All	100	0
NWSC	TCC (CFU/100ml)	<10	16	1, 3, 7, 11, 13, 16, 30, 32, 46, 47, 48, 53, 54, 55, 61 and 62	30.77	<10
		>10	36	2, 4, 5, 6, 8, 9, 10, 12, 14 to 19 31 33 to 45, 49 to 52, 56 to 60	69.23	
	E. coli (/100ml)	>0	4	8, 10, 14 and 17	7.69	0

3.4. Microbial water contaminants.

The Microbiological water contaminants of domestic water were Enterobacter spp, Citrobacter spp, Klebsiella spp, Escherichia coli , Pseudomonas spp and Salmonella spp and their percentages are shown in table 4.

Table 4: Microbial water contaminants of domestic water showing percentage composition of the contaminants.

Isolates	Number of samples	Samples PCMWA	Percentages (%)
Enterobacter spp	20	4, 5, 6, 9, 12, 13, 15, 17, 18, 19, 20, 22, 29, 31, 34, 35, 36, 37, 38 and 40	47.62
Citrobacter spp	16	33, 38, 41, 42, 43, 44, 45, 49, 50, 51, 52, 56, 57, 58, 59 and 60	38.1
Pseudomonas spp	2	2 and 23	4.76
Klebsiella spp	4	24, 33, 37 and 39	9.52
Escherichia coli spp	4	8, 10, 14 and 17	9.52
Salmonella spp	3	2, 14 and 23	7.14

Temperature generally influences water quality (physicochemical and biological characteristics) including the rate of chemical reactions in the water body, decrease in the solubility of gases, and improves the taste and color of water [18]. The temperature of water ranged from 21.20C to 25.50C and thus all the recorded water temperatures of the water collection points were above the WHO recommended value of <15°C [36, 37]. The high water temperature is attributed to global warming and as postulated by [38] the global land mean surface temperature increase was 0.85°C over the period from 1850 - 2012 [39]. Similarly, Uganda's National Adaptation Program of Action reported an average temperature increase of 0.28°C per decade between 1960 and 2010 and this further contributed to the rise in surface water temperature [40]. This result is in agreement with the study done by [41] in Jimma zone Southern Ethiopia which reported the water temperature range between 20.67 and 25.730C [41] and [18] in Mbarara municipality which also reported the water temperature range from 21.3 and 26.40C [18]. Total dissolved solids (TDS) including carbonate, bicarbonate, chloride, sulfate, phosphate, nitrate, calcium, magnesium, sodium, organic ions, and other ions determine the general nature of water quality [42]. They affect the taste of drinking water if found at concentrations above the WHO recommended value [6]. In this study, total dissolved solids were between 54 and 112mg/dl, and hence the total dissolved solids in all the water sources were within the WHO maximum allowable limit of <1000 mg/dl [43] hence making these water points suitable for domestic use. This was in line with other studies such as a study on the analysis of physiochemical parameters to evaluate the drinking water quality in the state of Perak, Malaysia [44] which reported that all drinking water points have TDS within the WHO-acceptable limit with the highest TDS value of 37mg/L and the lowest TDS values of 17.8mg/L and a study on physicochemical quality of selected drinking water sources in Mbarara Municipality, Uganda which also found that the TDS in all the water sources were below the WHO maximum allowable limit of 1000 mg/l hence making these water sources suitable for domestic use [18, 37].

Most biological activities take place only within a narrow pH range [45]. As a result, any pH variations beyond an acceptable limit can be fatal to cells and organisms [46]. The WHO recommended pH ranges between 6.5 and 8.0 [6]. However, this study recorded a pH range from 5.23 to 7.91 and found that 40.32% of the water collection points had a pH below the lower recommended limit of 6.5, and were slightly acidic. The slightly acidic nature of water is mainly attributed to sulfuric acid and nitric acid formed from increased sulfur dioxide gas and nitrous oxides emission into the atmosphere as a result of the combustion of fuels from vehicles engines which reacts with water and oxygen forming sulphuric acid and nitric acid

respectively that acidify rains with subsequent downpours to groundwater sources (R. Rwizi) [18, 47]. This result was in line with the study by [41] in the Jimma zone, Southern Ethiopia in which the overall mean pH value was 6.72 (ranged between 5.72 and 8.14), and only about half (52.3%) of the pH of water samples were within WHO standard (6.5–8.5) [41]. That study also found that except for tap water, the majority of other drinking water sources were slightly acidic (below a pH of 7). Electrical conductivity (EC) is the ability of any medium to carry an electric current [48]. Dissolved solids convey electric currents through water and among these solids include not only calcium, magnesium, and chlorides which exist as their respective ions in solutions [49]. This study recorded electrical conductivity ranging from 110 μS/cm to 189μS/cm. All the Electrical Conductivity values were within the WHO guidelines for drinking water quality of <1500 μS/cm [6].

Surveillance of water quality to ensure microbiological water safety is a vital public health function to prevent waterborne diseases [50]. Bacterial total coliform and *Escherichia coli* examinations are indicators of the hygienic condition of drinking water sources and major tools in the assessment of health risks borne by pathogens in water [51]. In this study, twenty water samples (32.26%) had bacterial total counts <10CFU/100ml of water and these were safe water collection points bacteriologically by the WHO guidelines for drinking water quality [6]. However, forty-two (67.74%) showed significant growth with bacterial total counts >10CFU/100ml of the water sample and these signified contamination of water delivered to end users at those water collection points. Four (6.45%) water samples from different collection points had *Escherichia coli* count > 0CFU/100ml of water sample and this indicated recent fecal contamination of those water collection points [34].

On identification of the isolates, *Enterobacter spp* and *Citrobacter spp* were the major contaminants with 47.62% and 38.10% respectively. *Klebsiella spp*, *Escherichia coli*, *Salmonella spp*, and *Pseudomonas spp* contaminated 9.52%, 9.52%, 7.14%, and 4.76% of the contaminated water samples respectively. The presence of bacterial pathogens in domestic water supply presents public health concerns with possible outbreaks of waterborne diseases [52]. Mbarara city experiences high population growth due to urbanization thus poor waste management is characterized by open defection and poor disposal of human and animal excreta coupled with leakages in the sewers system which contributes to contamination of domestic water supply [53]. In Mbarara City, it is estimated that 56% of human excreta is unsafely managed, 0.7% of the population practices open defecation according to the National Census and Population Report 2014 and the majority (61.9%) of pit latrine walls are unlined [53, 54]. Fecal materials are carried from uplands downstream by surface run-off, and unlined pit latrines further potentiate the risk of underground seepage of toilet water contents through the water cycle into the rivers (R. Rwizi), these contribute to the contamination of the rivers (River Rwizi), the primary source of water distributed in the area [51, 53]. Water contamination is also attributed to leakages in the pipeline distribution networks of water as well as the sewerage system within the area which in most cases results from urban developmental activities for instance road construction which causes damages to water pipes by the machines involved in the process [55]. This creates weak points through which the sewer flowing out of damaged pipelines mixes with the water system thus contamination of the water supplied upstream through the damaged pipes [53]. According to the sanitary inspection checklist data, there was no association between the risk of contamination of the water collection point and the actual contamination reflected by the microbiological analysis. This is because the water is supplied through underground pipes and the contamination was not due to the environmental sanitation around the collection point but rather the leakages in the distribution network as pointed out above.

Generalizability of the study results: This study was conducted in the medical cell, Mbarara City. However, the water sampled was represen-

tative of the water supply of Mbarara city during the period of August-September, 2022 since the water supply within this city was and is exclusively by the NWSC. The mismanagement of human and animal wastes as postulated by [53] and continuous damages to the sewers system within Mbarara city due to construction activities has culminated in domestic water contamination and hence the need for interventions to curb potential future waterborne and or water-related disease outbreak in the majority water consumers population.

4. Conclusion and recommendation.

The temperature of all water samples was above the WHO's recommendation for palatable/drinkable water. Microbiologically, the water supply had potential disease-causing pathogens which pose risks of a potential outbreak of waterborne diseases to the Medical cell and surrounding communities of Mbarara City. The presence of Escherichia coli in some water samples indicated recent fecal water contamination.

The national water and sewerage corporation should improve on the water treatment methods and also inspect the supply pipelines for leakages and repairs should be made to the pipelines where necessary. Water collection tanks and other storage facilities should be cleaned regularly. The responsible stakeholders should ensure the proper disposal of human waste.

Further studies can be done to genotype the water contaminants and or determine their antimicrobial susceptibility patterns.

5. Limitations.

Material and financial constraints limited the study to perform other tests like heavy metals analysis.

6. Data Availability.

The data sets generated and analyzed during the study are available from the corresponding author on request.

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9. List of abbreviations.

NWSC — National Water and Sewerage Corporation
WHO — World Health Organization
TCC – Total coliform counts
Coli – Escherichia coli
Spp – spices
PCMWA – Physico-chemical and microbiological water analysis
EC- Electrical conductivity.
PH – Potential hydrogen concentration.

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Author biography

Christopher Okeny ORCID 0009-0002-8865-4537, a practicing medical laboratory technician at Mbarara University of Science and Technology Clinical and Research Laboratory, 2023. He is Passionate about research, technical work and quality management of medical laboratories, teaching young and colleague professionals as well as offering career guidance to young people