Isolation of *Escherichia coli* and *Staphylococcus aureus* in Surface Water Sources in Katabi Subcounty, Wakiso District.

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Abstract

Background:[a]

Unsafe water, inadequate sanitation, and insufficient hygiene account for an estimated 9.1 percent of the global burden of disease and 6.3 percent of all deaths. This study aimed to isolate *Escherichia coli* and *Staphylococcus aureus* in surface water sources in Katabi Subcounty, Wakiso District. The specific objectives were to isolate *E.coli* and *Staphylococcus aureus* in water sources in the Katabi sub-county and to determine the physico-chemical parameters of the water sources.

Methodology:

A cross-sectional and snowball sampling method was applied while collecting water samples from the wells, boreholes, and other groundwater in the selected areas of Wakiso District (Katabi division, and Kajjasi division).

Results:

A total of 34 (n=34) water samples were obtained including borehole water 61.8% (n1=21/34), spring water 11.8% (n2=4/34) and open well water 26.4% (n3=9/34). Freshwater samples were directly analyzed from the water source for pH, temperature, and dissolved oxygen, then different means of physic-chemical parameters were recorded, Mean temperature for open well water was 23.5°C±1.092, pH=5.21±0.432, and dissolved oxygen was 4.075±1. 555). The mean temperature for spring water was 22.98°C±0.907, pH=5.7± 0.781, and dissolved oxygen was 4.075±1.555. For borehole water, the mean temperature was 22.9°C±1.339, pH=5.7±1.441, and dissolved oxygen was 4.9±1.549). A total of 10 samples fermented MSA after overnight incubation at 37°C changing the media color from pink to yellow, borehole water samples were 60% (6), spring water samples were 20% (2) and open well water samples were 20% (2). Also, *S. saprophyticus* 76.9% (10) and *S.epidermidis* 23.1% (3) were identified.

Conclusion and recommendation:

All the samples analyzed did not show growth of *E.coli* and *S.aureus* but had other organisms including *S.saprophyticus* and *S.epidermidis* which could be harmful to human health when consumed.

Further investigation of possible pathogenic organisms that are present in drinking water under the acidic pH should be done.

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1 Background

Access to safe drinking water and sanitation is a human right as declared by the UN General Assembly in 2010 (Meier *et al.,* 2014). Safe drinking water is important as a health and development issue at national, regional, and local levels. It is of economical and public health concern to access safe drinking water.

Worldwide, over one billion people lack access to an adequate water supply while more than twice as many lack basic sanitation (UNICEF *et al.,* 2019). Unsafe water, inadequate sanitation, and insufficient hygiene account for an estimated 9.1 percent of the global burden of disease and 6.3 percent of all deaths (CDC, 2016). This burden is disproportionately borne by children in developing countries, with water-related factors causing more than 20 percent of deaths of people under the age of 14. Nearly half of all people in developing countries have infections or diseases associated with an inadequate water supply and sanitation (Bartram *et al.,* 2005). Waterborne diseases and death continue to be a worldwide burden in both developed and developing countries, the heaviest being diarrhoeal diseases. In Uganda, waterborne diseases are among the major public health problems (Nafi'u *et al.,* 2016). The prevalence of waterborne diseases in Uganda is also attributed to poor hygiene and environmental sanitation including the inadequate supply of safe water (WHO, 2012). Only 33% of the urban population in Uganda had access to adequate sanitation in 2012, a 1% rise since 1990 while 2% still practiced open defecation (WHO, 2014).

The majority of the rural populations in Uganda get their water supplies from unprotected water sources, underground water, streams, spring wells, ponds, and lakes (UN-Water World Water Assessment Programme, 2006). Water supply in the urban areas of Wakiso District is provided by NWSC while the rural areas access water from hand-dug wells, deep boreholes, shallow wells, and protected springs. Safe water coverage in Wakiso District stands at 64 percent for the rural areas and 20 percent for the urban areas. The functionality of water sources stands at 81 percent while the safe sanitation coverage (latrine per household) stands at 92 percent. However, access to water poses a serious issue and was reported as low as 25% in Nabweru sub-county (s/c). The quality of the water such as the content, color, smell, and taste was also cited as problematic by citizens in Mende s/c, Entebbe 'B', Kasanje s/c, and Kira Town Council, among others. (Namara, 2015).

Despite the many benefits of improved sources of potable water for human development, many developing countries including Uganda seem to allocate insufficient resources to meet the sustainable development goal (SDG) target for sanitation and potable water. There are also great inequalities in access to clean water and sanitation. As a result of the insufficient supply of water, most households depend on hand-dug wells and boreholes (groundwater) for drinking water.

Despite the endeavor of the Ministry of Health (MOH) in Uganda to sensitize the public about water-borne diseases due to water contamination and improper waste disposal, there have been increasing cases of water-borne diseases. It is therefore very important to analyze the quality of surface water to ascertain whether they are safe for drinking and advise the population accordingly.

2 MATERIALS, AND METHODS

Study design.

The study was cross-sectional and the snowball sampling method was applied while collecting water samples from the wells, boreholes, and other groundwater in the selected areas of Wakiso District (Katabi division, and Kajjasi division).

Study area.

The study was conducted in Wakiso district located in the Central region of Uganda 0.0630° N, 32.4467° E. Wakiso district is boarded by Luwero district to the North, Mukono district to the East, Kampala district to the Southeast, Lake Victoria district to the South, Mityana district to the West, Nakaseke to the Northeast, and Mpigi district to southwest. Wakiso has a population of 2007700 people and Entebbe municipality has a population of 69958 people (UBOS, 2014). The District Headquarters at Wakiso town council are located 16kilometres, by road, northwest of Kampala, Uganda's capital and largest city. The total land area of Wakiso district is 2807.75 sqkms, with a land area under water being 65.8sqkm, the area under open water is 53.3sqkms, area under permeable wetland is 12.5sqkms, and district perimeter is 154kms and finally, the percentage covered by international boundary being 0.32% (District planning unit, 2009)

Inclusion and exclusion criteria.

Inclusion criteria

Only water from boreholes, springs, and open wells will be used in this research.

Exclusion criteria

All tap water from the national water and Sewerage Corporation was not used in this research.

Sample size.

The sample size was 4 springs, 9 open wells 21 boreholes making a total of 34 water sources in the katabi sub-county and namulanda village. 120mls of water were collected from each water source and physical-chemical parameters were taken from the site or directly at the water source.

Sampling sites

The different sites include open wells, springs, and boreholes used by people in the community of Katabi division and Namulanda village in Kajjasi town council wakiso district were sampled.

Water sampling

The glass bottles used for water sampling had a capacity of 120 ml. They were fitted with screw caps. The cap and neck of the bottle were protected from contamination by a suitable thin aluminum foil that will withstand repeated sterilization at 180ºC. Sterilized bottles were opened just before sample collection. Each water sample was given a code number identifying the source as an open well, spring, or borehole. Date of collection, name of the water source, GPS of the location of the water source were also be recorded.

Physico-chemical parameters like pH, temperature and dissolved oxygen, weather condition of the day the water sample is collected were recorded. Samples collected near swaps and homesteads were also noted.

Sterile bottles were held by one hand and the other hand was used to remove the stopper which will be retained in the hand while the bottle is filled with water and then recap the bottle with the stopper. The water sample bottle was labeled with the sample code number and placed in a cool box to be transported to the laboratory for analysis.

Data collection

The physico-chemical parameters such as temperature, dissolved oxygen, and pH were taken directly from the water source and recorded in the data collection tool and while the bacteriological analysis, water samples were filtered and cultured on Nutrient Agar, any growth on the plate was recorded on the data collection tool, then differential media were used such MSA and Larly trypton mannitol broth and any growth on this media were also recorded in the data collection tool and finally, Gram stain was also done and the results were also recorded on the data collection tool.

Data management and analysis.

The collected data was then entered into a Microsoft excel dataset; data validation was also done by the principal investigators to ensure the correctness of entry. The dataset from a data spreadsheet was imported into Stat version 13 software for analysis. Baseline characteristics were summarized with frequency tables, pie charts, and bar graphs

Laboratory procedures.

The collected water samples were filtered using a membrane filter technique. The membrane filter was transferred to nutrient agar and incubated at 37°C overnight, if any growth was seen on the nutrient agar, then the colony was picked and subcultured on the MSA for identification as *Staphylococcus aureus* and laryle tryptone manital broth for *E.coli.* Any growth on differential media was subjected to Gram staining.

Laboratory Procedure

Bacteriological water analysis

The water was poured into the top of the filtration unit and a hand pump was manually operated. The membrane filter was picked using sterile forceps and transferred to media plates using a rolling motion to avoid entrapment of air. A small amount of sample water was then poured into the petri dish on top of the membrane filter. The sample water prevents the bacteria on the filter from going into shock.

The lid was placed back onto the petri dish. The dish was sealed by placing two pieces of tape around the dish to prevent airflow into the dish that would kill the bacteria. Each dish was inverted and placed inside an incubator at 37°C for 24 hrs. This allowed the bacteria captured by the filter to grow and form a visible colony. This procedure was repeated for each of the three samples from the same source was filtered.

Ethical consideration.

Approval from IRB was not required since the study didn't involve the use of human subjects but the permission was though from the chairperson of the village or any other elderly person in the community near the source of water in question.

Quality assurance and Quality control.

The meter for measuring pH, temperature, and dissolved oxygen was always calibrated as per the manufacturer's instructions. All the containers that were used to collect water samples were sterilized before they were used, each water sample was labeled with water sample code for open well, spring, or borehole and was accurately recorded in the worksheet which had the name of the water source, code, GPS, date of collection, physico-chemical parameters, location, and elevation. The data were double recorded both manually and electronically on the computer. During analysis, each water sample was filtered using separate membrane filter paper, and each sample of the bacteriological filtering unit was disinfected using 70% alcohol. Culture plates and tubes were sterilized in the hot air oven and culture media and the broth were sterilized in the autoclave. After preparation, the media were incubated overnight at 37°C. This was to observe any growth and media that had growth was not used. All the results were recorded in the worksheet. All cultures were autoclaved before discarding.

3 RESULTS

A total of 34 (n=34) water samples were obtained, borehole water samples were 61.8% (n1=21/34), spring water samples were 11.8% (n2=4/34), and open well water samples were 26.4% (n3=9/34). Freshwater samples were directly analyzed from the water source for physical-chemical parameters such as pH, temperature, and dissolved oxygen, then the water samples were collected and transported under a cold chain to the University of Kisubi laboratory for analysis.

3.1 Physico-Chemical Parameters

The physic-chemical parameters included pH, temperature and dissolved oxygen were taken at the site of water sample collection of different types of water like boreholes, springs and open wells and the minimum, maximum and the mean were as seen in the Table 4.2.

Mean temperature for open well water was 23.5°C±1.092, pH=5.21±0.432, and dissolved oxygen was 4.075±1. 555). Mean temperature for spring water was 22.98°C±0.907, pH=5.7± 0.781, and dissolved oxygen was 4.075±1.555. For borehole water, the mean temperature was 22.9°C±1.339, pH=5.7±1.441, and dissolved oxygen was 4.9±1.549)

The Table 3 shows the variation in pH, temperature and dissolved oxygen in open wells

Nine (9) open wells were sampled where the highest pH was 5.59 and the lowest pH was 4.13. The highest temperature was 25.1°C while the lowest was 22.1°C. Dissolved oxygen was recorded at its highest at 7.6mg/l and the lowest reading was 2.0mg/l (Table 4.3). Among the water samples collected from springs, the pH range was 3.57 to 5.57. The temperature range was 21.8 - 24.3°C. Dissolved oxygen ranged from 2.3 - 5.2mg/l (Table 4.4).

A total of twenty one (21) borehole water samples were analyzed, the highest pH was 7.72 and the lowest was 1.81, the highest temperature was 26.3°C and the lowest was 21.3°C, and the dissolved oxygen was in the range of 2.6mg/l -7.4mg/l (Table 4.5).

3.2 Growth on the Nutrient media

All the samples had growth on nutrient agar after overnight incubation at 37°C under aerobic conditions.

3.3 Sub-culturing On Lauryl Tryptose Mannitol Broth

There was no bubble formation in Lauryl Tryptose Mannitol broth which was indicative that *E.coli* was not present in all the samples. However, there was turbidity that indicated the growth of other coliforms but not *E.coli*.

3.4 Sub-Culturing On Mannitol Salt Agar (MSA)

Pink colonies were formed on MSA from which, 11 were of borehole water samples and 2 were of open well water sample, yellow colonies were formed on MSA from which, 2 were of open well water samples, 2 of spring water samples and 6 were of borehole water samples.

Two borehole water samples had *S.epidermidis* and one open well water sample had *S.epidermidis* while 6 borehole water samples had *S. saprophyticus,* 2 open well water samples had *S.saprophyticus* and 2 spring water samples also had *S.saprophyticus*

3.5 Fermentation MSA after an overnight incubation at 37°C by different water sources

A total of 10 samples which fermented MSA after overnight incubation at 37°C changing the media

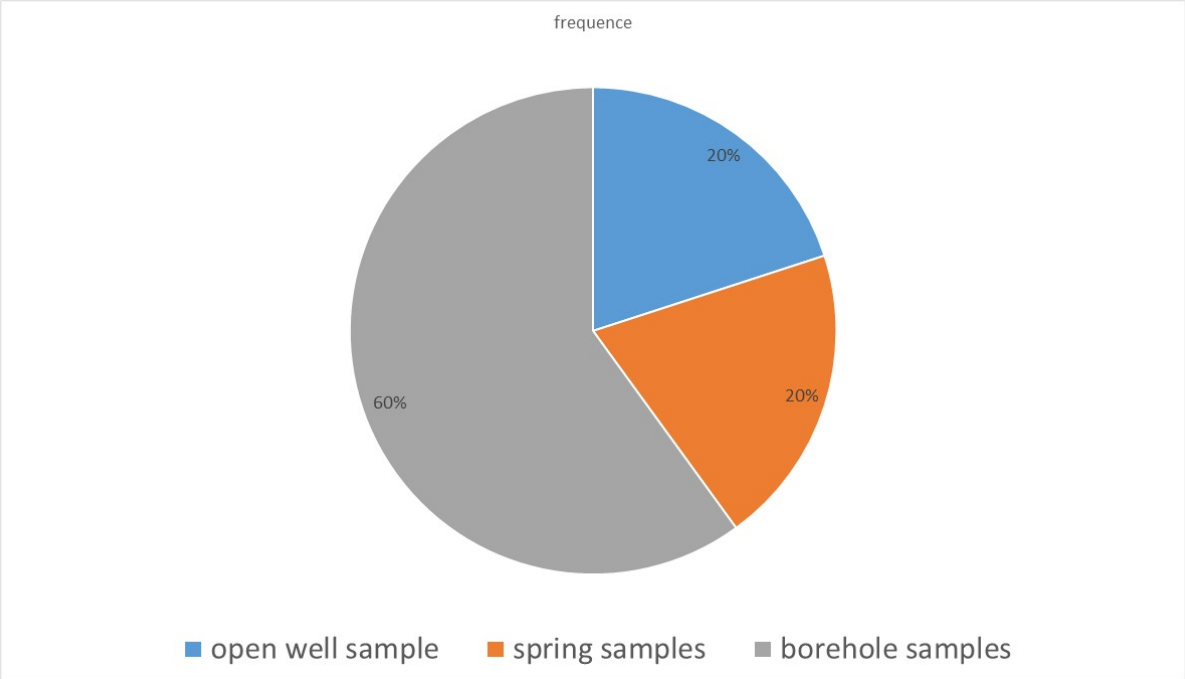

Chart 1. Show plates with pink colonies

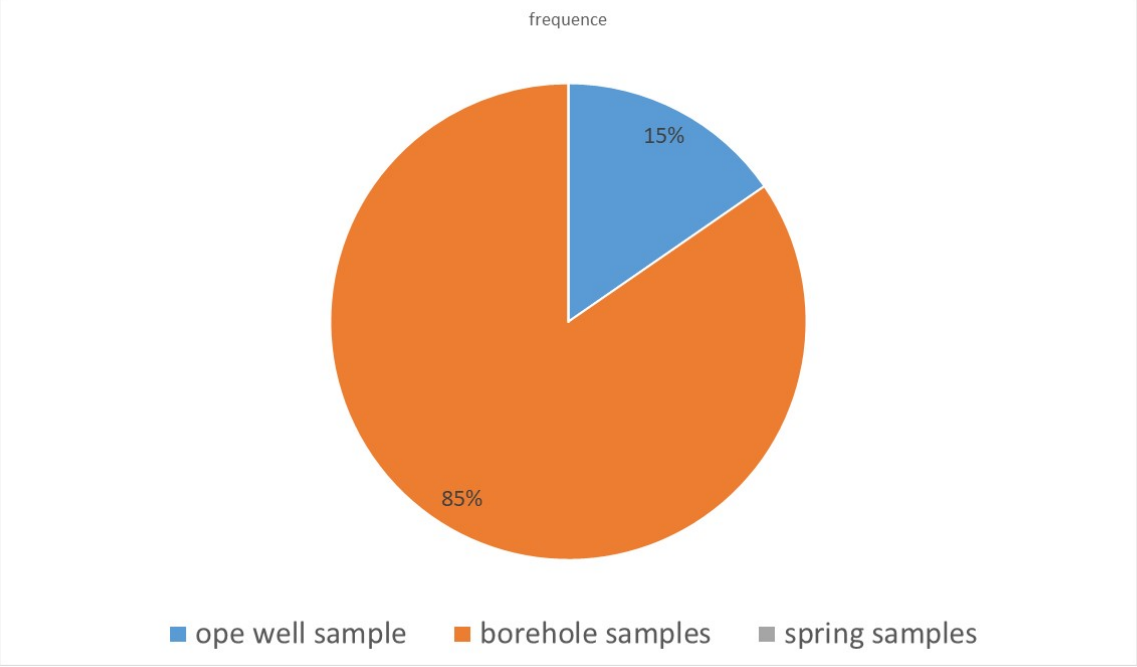

Chart 2. show plates with yellow colonies

Table 1. Shows **the type of water samples collected**

Type of water sample	No of samples	Percentage
Borehole water sample	21	61.80%
Spring water sample	4	11.80%
Open well water sample	9	26.40%
Total water sample collected	34	100%

Table 2. s**hows the physico-chemical parameters**

TYPE OF WATER	MEAN TEMP°C	SD OF TEMP +/-	MEAN pH	SD pH +/-	MEAN DO	SD DO +/-
Open well	23.5	1.092	5.21	0.432	4.075	1.555
spring	22.975	0.907	4.83	0.781	3.75	1.124
Borehole	22.98	1.339	5.7	1.441	4.9	1.549

Table 3. shows the variation in pH, temperature and dissolved oxygen in open wells

OPEN WELLS	TEMPERETURE (°C)	p H	DISSOLVED OXYGEN(mg/l)
W001	22.1	5.42	2.7
W002	22.1	5.47	2
W003	23.9	5.23	7.6
W004	25.1	5.52	5
W005	24.4	4.87	3.8
W006	23.1	5.43	4.9
W007	22.7	5.23	3.4
W008	24.4	4.13	3.2
W009	22.1	5.59	3.5

Table 4. shows **the variation in pH, temperature and dissolved oxygen in springs**

SPRINGS	pH	TEMPERETURE(°C)	DISOLVED OXYGEN(mg/l)
S001	5.57	24.2	5.2
S002	5.38	21.8	3.1
S003	4.8	23.4	4.4
S004	3.57	22.5	2.3

colour from pink to yellow, borehole water samples were 60% (6), spring water samples were 20% (2), and open well water samples were 20% (2) (Table 4.6).

3.6 Gram staining

S.aureus as the organism of interest was not identified from all water samples. However, *S. saprophyticus* was identified from ten samples, contributing 76.9% growth of the isolates. *S.epidermidis* contributed 23.1% (3) of the total number of organisms seen after Gram stain (Table 7).

4 DISCUSSION, CONCLUSION, AND RECOMMENDATIONS

Temperature and pH

There was no growth of *Escherichia coli* on the media during this study. *S.aureus* was not also isolated. The pH of the water was generally acidic. The pH and temperature determine bacterial growth. This is supported by findings of Wahyuni *et al.*, (2015) that Most bacteria survive at an alkaline pH than an acidic PH. The optimal pH for bacterial growth is between 7-7.8 for aerobic and 6.8-7.2 for anaerobic organisms (Shcherbakova *et al.,* 2005). The pH

Table 5. shows the variation in pH, temperature and dissolved oxygen in boreholes

BOREHOLE	pH	TEMPERATURE	DISOLVED OXYGEN
B001	5.64	23.2	6
B002	5.45	23.8	3.4
B003	7.17	22	3.2
B004	6.88	22.2	6.8
B005	6.78	21.7	3
B006	6.84	22.9	5.2
B007	4.8	26.3	4.2
B008	3.02	25.2	4.8
B009	3.93	25.1	3.4
B010	6.54	23.9	5.6
B011	6.97	22.7	4.6
B012	7.72	21.6	3.2
B013	6.31	21.5	7
B014	6.11	21.3	3.2
B015	6.17	22	6.9
B016	6.08	21.9	4.5
B017	6.06	22	3
B018	5.75	22.3	7.2
B019	3.74	23.5	7.4
B020	1.81	23.3	5.5
B021	5.6	24.2	2.6

Table 6. shows Fermentation MSA after an overnight incubation at 37°C by different water sources

Reading of MSA	Frequency	Percentage
Borehole water sample	6	60%
spring water sample	2	20%
Open well water sample	2	20%
Total	10	100%

Table 7. hows Gram staining results

Organism	Frequency	Percentage
S. saprophyticus	10	76.9%
S. epidermidis	3	23.1%
Total No of organism	13	100%

differs in different seasons, where the pH may be below 6.0 and in the dry season it's between 6.5-8.5 (Ojok, 2017).

Dissolved oxygen

Staphylococcus aureus and *E. coli* are affected by a decrease in dissolved oxygen because they utilize a lot of oxygen (Kiamco *et al.,* 2017). In this study, the levels of dissolved oxygen were 7mg/l and below while the reference range is 7-14mg/l. Probably this affected the survival of *S.aureus* and *E.coli* in the water samples analyzed.

Dug outwells

In this study, *S.aureus* and *E.coli* were not identified from any water sample but *S.epidermidis* and *S.saprophyticus* were identified. This differed from a study by Oyedum *et al.,* (2016), that reported that the well water contained *Salmonella spp, Shigella spp, E. coli, Staphylococcus spp,* and *Streptococcus*

spp. These results differ from the findings of this study in Wakiso, Uganda.

Borehole and spring water

Obioma *et al.*, (2017) observed that borehole water usually has less microbial load than any other surface water. However, in this study borehole water was more contaminated than any other water source like open wells and springs. In other study by An *et al.,* (2005), *E. coli* and *S.aureus* were found in spring water.

5 Conclusion

All the samples analyzed did not show the growth of *E.coli* and *S.aureus.* However, *S. saprophyticus* and *S.epidermidis* were identified and these can be harmful to human beings when they are consumed in water.

Recommendation

Further investigation of the possible pathogenic organisms that are present in drinking water under acidic water conditions should be done.

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List of Abbreviations

BBLT Bachelor of Biomedical Laboratory Technology
CO_2 Carbon dioxide
Coli Escherichia coli
EPA Environmental Protection Agency
MSA Mannitol Salt Agar
MoH Ministry of health
NWSC National Water and Sewage Corporation.
PTU Power Transfer Unit
SDG Sustainable development goal
UBOS Uganda Bureau of Statistics.
UCG Uganda Clinical Guideline
UN United Nations
UNICEF United Nations Initiative Children Emergency Fund.
UNIK University of Kisubi
VHI Village Healthy Inspector
WHO World Health Organisation.
WSP Water Safety Plan.

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