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Microbiological quality of water samples obtained from water sources in Ishaka, Uganda

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Abstract

Objectives: This study aimed to evaluate the microbiological quality of water sources in Ishaka division, Bushenyi district.
Methods: Water from taps, wells and springs were sampled for the cross-sectional investigation. The enumeration and identification of microbes (*Escherichia coli, Salmonella, Shigella, Proteus, Staphylococcus aureus* and total coliforms) in water samples were carried out using a variety of methods. *Escherichia coli* was enumerated using the membrane filtration method; *Salmonella, Shigella* and *Proteus* using a two-step enrichment method; *Staphylococcus aureus* using the surface spread method and total coliforms using the most probable number technique. Mannitol salt agar was used for enumeration of *Staphylococcus Aureus* and violet red bile agar was used for enumeration of total coliforms and *Escherichia coli*; xylose lysine deoxycholate agar was used for both *Salmonella* spp. and *Shigella* spp. API-20E was used to phenotypically identify the Enterobacteriaceae contaminants in water. These included *Escherichia coli, Proteus mirabilis, Proteus vulgaris, Salmonella* spp. and *Staphylococcus aureus*.
Results: *Escherichia coli* counts in the water from springs and wells ranged from 0 to 314 cfu/mL ($p = 0.173$) and 0 to 3 cfu/mL ($p = 0.269$), respectively, while tap water had no incidence of *Escherichia coli*. Highest level of bacterial contamination in water sources, beyond acceptable WHO (0 cfu/100 mL) limits for drinking water, was reported: *Proteus* spp., 34 (54.8%), followed by total coliforms, 24 (38.7%), *Shigella* spp., 22 (35.5%) and least were *Salmonella* spp. (8.1%) and *Staphylococcus aureus* spp. (8.1%).
Conclusion: It is therefore concluded that spring and well community water sources in Ishaka division, Uganda, are significantly contaminated with pathogenic bacteria and thus unsafe for drinking without adequate water treatment (disinfection and filtration).

Keywords

Escherichia coli, *Salmonella*, *Staphylococcus aureus*, *Shigella*, *Proteus*, total coliforms, drinking water sources

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Introduction

The quality of water is an important aspect of public health and environmental sustainability. Pathogenic bacteria present in sources of water is a significant threat to human wellbeing. Some of the most reported bacteria in sources of water are *Escherichia coli, Salmonella* spp., *Shigella* spp. and *Staphylococcus aureus*. They have been associated with outbreaks of water-borne ailment. Recently, the occurrence of antibiotic-resistance microorganisms/antimicrobial resistance (AMR) in water sources has increased concern.[1] It is important to monitor and

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investigate microbial quality of water to effectively manage water quality and ensure public health.

Indicator microorganisms have improved the ability to identify the presence of pathogenic microorganisms in water sources.[2] Various studies have shown the presence of *Salmonella* spp., *Shigella* spp. and *Yersinia* spp. in sources of water and their impact on human health.[3] The occurrence of AMR bacteria in drinking water systems and the risks posed to human health have also been identified.[4] A study in Kenya summarises the value of evaluating the physicochemical and bacteriological quality of water sources in rural settings.[5] It is imperative to improve microbial assessment in a bid to ensure the safety and suitability of drinking water especially in developing countries because presence of these will reduce quality of water and lead to diarrhoea, stomach cramps, urethritis, cystitis and abscess when consumed.[6] In Lesotho and Ethiopia, contamination of water sources by *E. coli* spp. has been identified in both protected and unprotected water sources (up to 4800 cfu/100 mL and 43,500,000 cfu/mL, respectively, for Lesotho) resulting from animal faeces and leakage of human waste as a result of unhygienic sanitation practices.[7,8] In Pakistan, *Shigella* spp. was found in drinking water, cow milk and vegetables; while in Brazil, *S. aureus* spp. was identified in small number of water fountain samples.[9,10]

The presence of pathogenic bacteria in water sources is an important public health concern and essential management of the quality of water is key to environmental sustainability and public health. This study aimed to evaluate the microbiological quality of water sources in western Uganda. Specifically, testing for *E. coli* spp., *Salmonella* spp., *S. aureus* spp., *Shigella* spp., *Proteus* spp. and total coliforms in water sources within Ishaka division, Bushenyi district, Uganda.

Methodology

The study aimed at studying the microbial water quality in 10 villages in Ishaka,[10] Bushenyi using a cross-sectional and descriptive study between January and December 2022. The target microbes *E. coli* spp., *Salmonella* spp., *S. aureus* spp., *Shigella* spp., *Proteus* spp. and total coliforms were investigated.

Study site

Ishaka division of Bushenyi-Ishaka municipality belongs to a sub-county located within the Bushenyi district, western Uganda. The division is bordered by Rubirizi, Sheema, Mitoma and Rukungiri to its northwest, east, south and west, respectively. It lies between 00 32 S and 30 11 E with an approximate population of 250,400, averaging 4.5 persons per household.[11] This study was undertaken in Ishaka division which consisting of five parishes – town ward, ward IV, ward III, Kasenyi and Buramba.[12] Approval for research was obtained from the Department of Public Health, School of Allied Health Sciences; the Directorate of Higher Degrees and Research and the Kampala International University Research and Ethics Committee (Ethics approval number: KIU-2022-115). Afterwards, the research was logged with the Uganda National Council for Science and Technology. The Ishaka division office also permitted field data collection.

Sample location and size

Two villages each were randomly selected from each of the five wards. Thirty-one points (sampled in duplicates making a total of 62 samples) were systematically selected and tested from the water sources in each of the 10 villages. The water sources included wells, piped water and springs. The sample points are shown in Figure 1.

Sampling procedure

For each community, sterile, impermeable and acid-washed containers were used to collect water samples (200 mL) in duplicates using standard sampling methods[14] from protected springs, dug wells and public taps. Samples were stored in a temperature-controlled water cooler and transferred to the laboratory within an hour of collection.

Laboratory methods

E. coli was enumerated using the membrane filtration method. The water sample was filtered through a 0.45 µm membrane and the colonies that grew on MacConkey agar were presumptive *E. coli*. These colonies were then sub-cultured onto eosin methylene blue agar to confirm the identification.[15–17] *Salmonella, Shigella* and *Proteus* species were enumerated using a two-step enrichment method. First, the water sample was incubated in sterile peptone water overnight. Then, 1 mL of the mixture was enriched in Rappaport-Vassiliadis enrichment broth for 24 h. The enriched sample was then streaked onto xylose lysine deoxycholate (XLD) agar, and the presumptive colonies were further confirmed using Analytical Profile Index (API)-20E.[15,17] *S. aureus* was enumerated using the surface spread method. The water sample was serially diluted, and 0.1 mL of each dilution was spread onto Mannitol salt agar plates. The plates were incubated at 37°C for 48 h, and the characteristic golden yellow colonies were further sub-cultured onto Baird-Parker agar. The identification was confirmed using gram staining, coagulase test and API 20 Staph.[16–18] Total coliforms were enumerated using the most probable number (MPN) technique. A three-tube assay was set up, with each tube containing 10 mL of lactose broth. The water sample was added to the tubes in increasing dilutions, and the tubes were incubated at

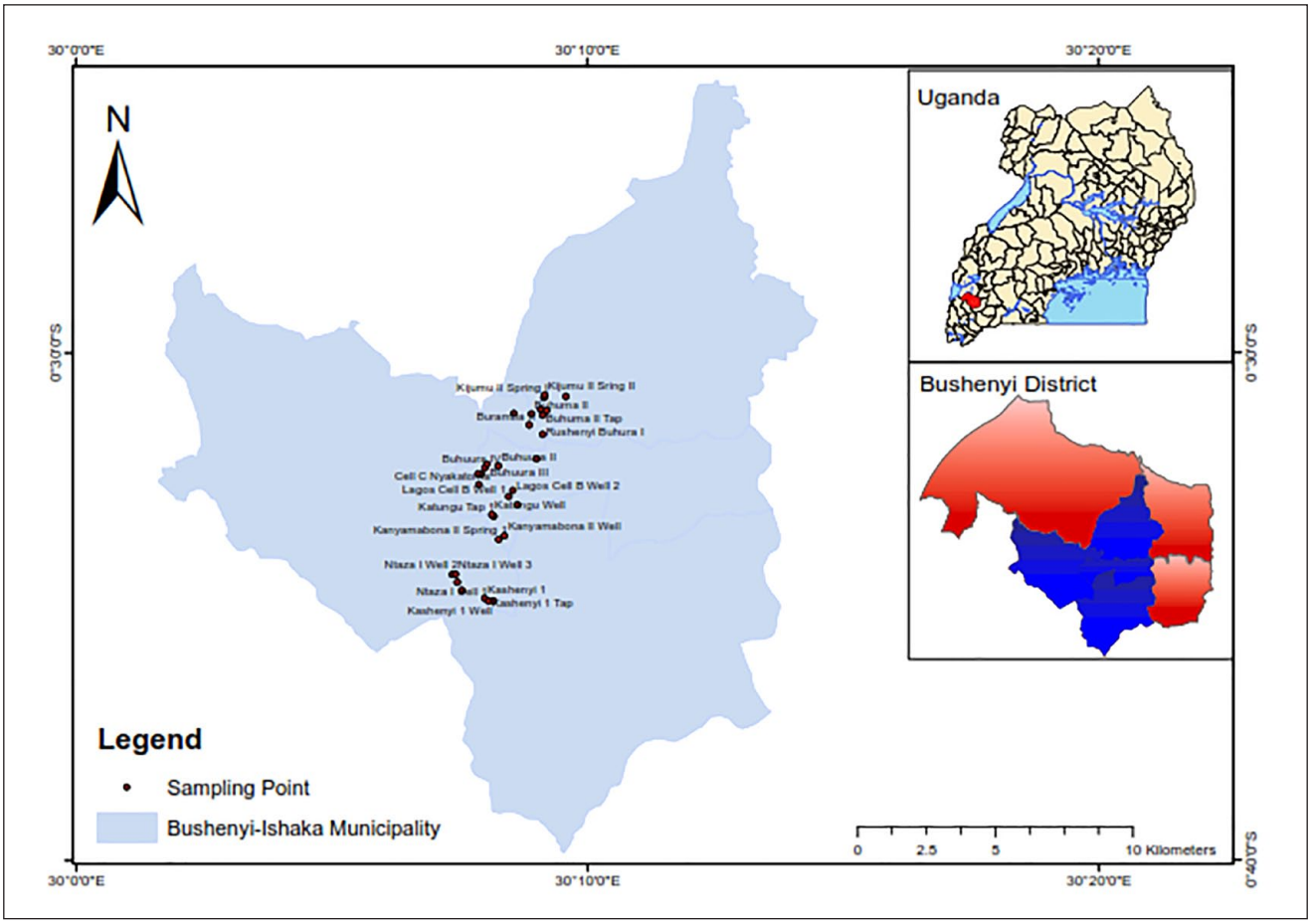

Figure 1. Map showing location of sample points in Ishaka division, generated using ArcMap 10.6.[13]

37°C for 24–48 h. Then they were examined for acid and gas production, and the total coliform counts were estimated using the MPN table.[16–18]

Colonies of the spread plate cultures were directly counted (only plates having 30–300 colonies) and recorded as a function of colony forming units per milliliter (cfu/mL) using the following formula (equation (1))[19]:

$$\text{cfu / mL} = \frac{\text{colonies counted}}{\text{volume plated}} \times \text{dilution factor} \quad (1)$$

The water quality was assessed by comparing the obtained microbial loads against the WHO acceptable limits for drinking water, that is 100 cfu/mL for *S. aureus*, 0 cfu/mL for *E. coli, Salmonella* spp. and *Shigella* spp.[20]

Purification of bacterial isolates. Purification of *S. aureus* was done on Mannitol salt agar with golden yellow colonies, *E. coli* on eosin methylene blue (EMB) agar with metallic green sheen while *Salmonella* spp. and *Shigella* spp. were purified on XLD forming red colonies with black centres and pink/red colonies, respectively.[21]

Biochemical analysis of the isolated enteric microbes. This was performed using *API-20E test*, to distinguish among members of the Enterobacteriaceae family.[22]

Statistical analysis and software. For data analysis and plotting graphs, the data collected from the study were entered using Microsoft Excel[23] and imported to GraphPad Prism (version 10)[24]. Location coordinates of the sample points were imported to ArcMap 10.1[13] and were used to plot the map of the area.

Results

All tap water samples had no *E. coli* contamination that was not significantly different from the 0 cfu/mL limits ($p = 1.000$). However, the springs and wells were most contaminated with *E. coli* counts ranging from 0 to 314 cfu/mL and 0 to 3 cfu/mL, respectively. The mean *E. coli* counts from both the springs ($p = 0.269$) and wells ($p = 0.173$), as shown in Table 1, were not significantly different from the 0 cfu/mL limits. *S. aureus* was identified in the growth on

Table 1. Mean bacterial counts in different water sources.

Bacteria	Range (cfu/mL)	Mean ± SD	One sample *T*-test *p*-value
E. coli			
Taps	0	0	1.000
Springs	0–314	10.38 ± 53.0	0.269
Wells	0–3	0.31 ± 0.87	0.173
S. aureus			
Taps	0–4500	625.0 ± 1494	0.249
Springs	0–300	24.09 ± 62.65	⩽0.0001
Wells	0–7800	497.25 ± 1947.56	0.427
Coliform bacteria			
Taps	0	0	1.000
Springs	0–467	30.65 ± 108.09	0.108
Wells	0.0–95	18.25 ± 27.68	0.019

Table 2. Number of water samples contaminated with pathogens beyond 0 cfu/mL limits.

Bacterial contaminants	Number of samples with unacceptable limits of bacteria, *n* (%)			Total (*N* = 62)
	Taps (*n* = 12)	Springs (*n* = 34)	Wells (*n* = 16)	
E. coli (cfu/mL)	0	4 (11.8)	2 (12.5)	6 (9.7)
Total coliforms (cfu/mL)	0	10 (29.4)	14 (87.5)	24 (38.7)
S. aureus (cfu/mL)	2 (16.7)	2 (5.9)	1 (6.25)	5 (8.1)
Salmonella species	0	1 (2.9)	4 (25.0)	5 (8.1)
Shigella species	2 (16.7)	16 (47.1)	4 (25.0)	22 (35.5)
Proteus species	5 (41.7)	17 (50.0)	12 (75.0)	34 (54.8)

culture plates. The wells and taps were highly contaminated with *S. aureus* that ranged from 0 to 7800 cfu/mL and 0 to 4500 cfu/mL, respectively. However, their mean counts were not significantly different from the 0 cfu/mL limits. Notably, spring water had significantly ($p \leqslant 0.0001$) lower mean counts of *S. aureus* (24.09 ± 62.65 cfu/mL) compared to the 0 cfu/mL limits. A *p*-value of ⩽0.0001 (Table 1) means that there is less than a 0.0001% chance of obtaining a mean of *S. aureus* that is at least as far from the population mean as the observed sample mean. This is a very low probability; we conclude that there is a significant difference between the *S. aureus* mean and the population mean. All tap water was safe from total coliforms followed by majority of spring water with exception of one sample with high coliform count of 467 cfu/mL. However, most of the well water had significantly higher ($p = 0.019$) total coliforms as shown in Table 1. However, the *p*-value for coliform bacteria is still close to the significance level of 0.05, so the results can be considered marginally significant.

Comparative analysis of the E. coli *contamination from the different water sources*

Springs were found to have the highest number of occurrence for *E. coli* with a mean value ± standard deviation of 10.38 ± 53.80 counts (cfu/mL) as seen in Table 2 and Figure 2. There was no *E. coli* in all water sampled from taps; mean ± standard deviation for wells was 0.31 ± 0.87 (Figure 2, Table 2).

S. aureus *contamination in water samples*

Though the research did not set out to identify *S. aureus* in the water samples, during the investigation *S. aureus* was identified by gram staining in the growth on culture plates. *S. aureus* contaminants occurred most in taps (625.0 ± 1494 cfu/mL) and wells (497.25 ± 1947.56 cfu/mL) while springs showed least occurrences (24.09 ± 62.65 cfu/mL) (from Table 2). However, the *S. aureus* counts were not significantly different from all water sources as shown in Figure 3.

Total coliform contamination in water samples

Springs had the highest counts of total coliforms with a range of 0–467 cfu/mL and mean of 30.65 ± 108.09 cfu/mL. This was followed by wells with mean of 18.25 ± 27.68 cfu/mL while none of tap water was contaminated with total coliform as shown in Figure 4.

Percentage of water samples with unacceptable microbial loads

Majority of the analysed samples were highly contaminated beyond acceptable WHO limits for drinking water with

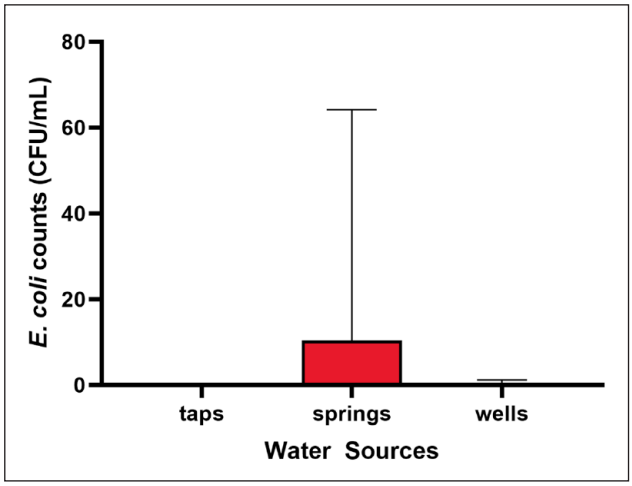

Figure 2. *E. coli* contamination in water sources.

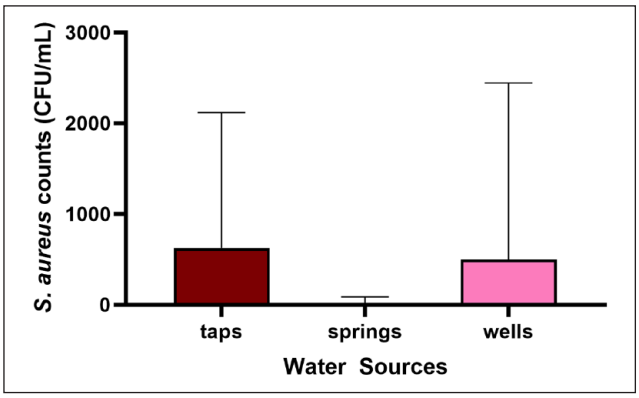

Figure 3. *S. aureus* contamination in water sources.

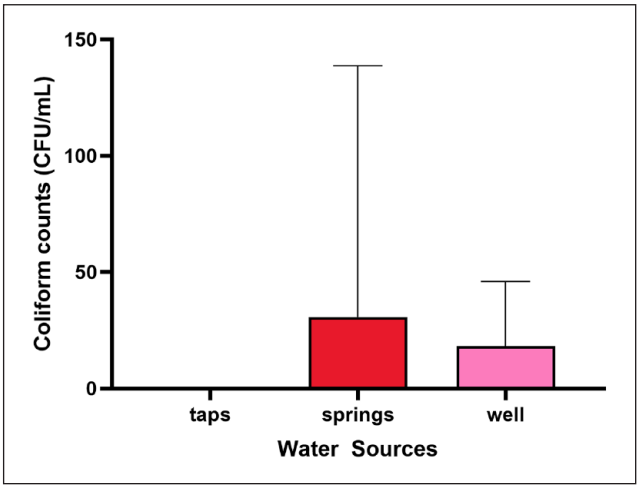

Figure 4. Total coliform contamination in water sources.

Proteus spp. 34 (54.8%), followed by total coliforms, 24 (38.7%), and least were *Salmonella* spp. (8.1%) and *S. aureus* (8.1%). High rates of *Salmonella* spp. were reported

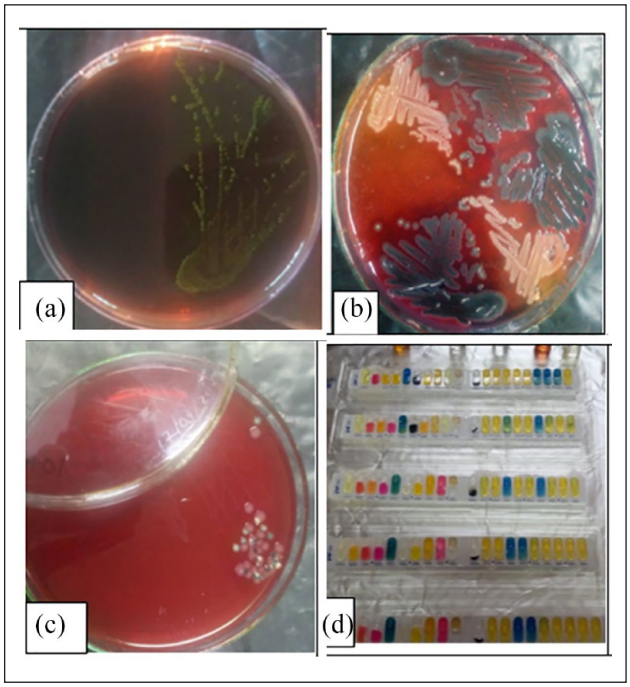

Figure 5. Identification of bacterial species (a, *E. coli* on EMB; b, *Salmonella* and *Proteus* subcultures; c, mixed culture of *Shigella* and *Salmonella*; d, selected results of API 20E).

in wells (25.0%) and none of the tap water. *Proteus* spp. contamination was highest in well water (75.0%), followed by spring water (50.0%) and then tap water (41.7%). *Shigella* spp. contamination is mostly in spring water (47.1%) and least in tap water (16.7%). Tap water had highest number of samples with *S. aureus*, beyond acceptable limits (16.7%) while none had *E. coli* contamination. Moreover, 12.5% and 11.8% of the analysed well and spring water, respectively, were highly contaminated with *E. coli* beyond acceptable limits, as shown in Table 2.

Phenotypic identification of bacterial species

The phenotypic identification of the contaminating bacterial species was done using API-20E; *E. coli* and *Shigella flexneri* were the only species detected in the analysed samples. Two species of *Salmonella* spp. were also identified: *Salmonella typhi* and *Salmonella paratyphi* (shown in Figure 5).

Discussion

E. coli, Salmonella *and* Shigella *from the water sources in Ishaka division*

In a similar study,[25] mean ± standard deviation of *E. coli* for spring, trough and tap water sources in Kenya was found to be 35.92 ± 8.89, 0.0 ± 0.0 and 6.0 ± 0.54, respectively. While in Ghana,[26] about 79.5% of the 122 samples (from dams, boreholes, streams, had dug wells, rivers and canals)

tested for *E. coli* as against 0.8% and 4.9% (of 115 hand pumps and 185 tap water) sampled in Pakistan.[27] The absence of *E. coli* in taps sampled from this study compared to that of Onyango et al.[25] It can be attributed to the successful use of residual chlorine and absence of leakages (which can be sources of contamination) in the water supply lines. However, in this study, the presence of *E. coli* in the wells can be attributed to their easy access by animals which increases the chances of faecal contamination. This is also the reason for the microbe's presence in springs. *E. coli* in drinking water causes diarrhoea, urinary tract infection and meningitis.[20,28] Possible remedies of *E. coli* contamination include locating sanitation facilities away from water sources, lining the water sources and disinfecting drinking water by boiling, flocculating or chlorine tablets, filtration.[20]

One of the most common causes of intestinal illness worldwide and the etiological agent of more serious systemic illnesses like typhoid and paratyphoid fevers is salmonella.[29] Out of 448 water samples, four (0.89%) were found positive with *Salmonella* in Iran. While in Burkina Faso, 476 samples of water from taps, wells, reservoir and water channels had 0%, 2%, 35% and 31% *Salmonella* spp., respectively.[30,31] High rates of *Salmonella* spp. were reported in wells (25.0%); they were not found in any water samples taken from taps. The primary factor driving the load of *Salmonella* in surface waters was determined to be surface runoff.[29]

Shigellosis is largely a food- and water-borne illness in both underdeveloped and industrialised nations. In some parts of the world, distinct *Shigella* species are more common than others, and shigellosis is more prevalent. In Pakistan, in a study with 1020 water samples, 4.41% of the samples were positive with *Shigella* spp.[10] In this study, *Shigella* spp. contamination is mostly in spring water (47.1%) and least in tap water (16.7%). The occurrence of the microorganisms may be attributed to faecal contamination resulting from runoff/wash-off from unlined human latrines and animal faeces (from grazing areas around water sources) to water sources.

Infectious dose, host defenses and immunity

Outbreak investigations suggest that the infectious dose may be less than 1000 organisms. Factors including serotype, level of host defenses against infection, method of transmission and immunological status can affect the infectious dosage.[32] Only 10–100 organisms are required to produce disease in the *Shigella* species, and the bacteria are more resistant to stomach acid and can easily pass through the gastric acid barrier.[33] Severe gastroenteritis and bacteraemia are risk factors for salmonellosis, shigellosis and *E. coli* infections for patients who have chronic underlying conditions, such as cancer and diabetes, as well as immune suppression, such as HIV/AIDS.[32–34] If there is recent stomach surgery, the use of acid-reducing drugs, or the use of antacids diminish gastric acid production, the infectious dose is decreased and the risk of infection is increased. The infectious dose is reduced for several weeks after taking antibiotics that momentarily change the normal intestinal microbiota, which also serves as a barrier to infection.[32,34]

Limitations of study

The study was limited by geographical location and did not investigate the effects of environmental factors which could be significant to how the microbes will develop and cause sicknesses.

Recommendation

Community education and health promotion of appropriate water safety plans can reduce the current practices of use of untreated water (from unprotected wells and protected springs) thereby reducing the risk of exposure to contaminated water and increase the use of treated water, which in this study was found to be free from *E. coli, Salmonella* and *Shigella*. In future construction, lining of sanitation facility can be encouraged to ensure that sewage does not become a threat to water sources. Further studies should investigate the effects of environmental factors, such as temperature and pH, on the growth and survival of microbes in water samples.

Conclusion

Public health initiatives are prompted by the current study's finding that there are bacteria in the drinking water source used by the people of Ishaka division, as these bacteria can cause severe illnesses in humans. In the study area, *E. coli* spp., *Salmonella* spp., *S. aureus* spp., *Shigella* spp., *Proteus* spp. and total coliforms were found. They may be the etiologic factor for water-borne illnesses such as typhoid, diarrhoea and dysentery. It can be concluded that microbiological water quality is related to the source of water. This is because most of the pathogenic microorganisms (*E. coli, Salmonella* and *Shigella*) were found in springs and wells possibly due to their lack of undergoing any treatment.

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Author contributions

Jemimah Fwangmun Wamyil: Conceptualisation, methodology, formal analysis, resources, data curation, writing – original draft preparation, writing – review and editing, visualisation. Ogbuagu Chukwuanugo Nkemakonam: Methodology, writing – review and editing, supervision. Oyebadejo Samson Adewale: methodology, writing – review and editing, supervision. Fwangmun Benard Wamyil: Methodology, data curation, writing – original draft preparation, writing – review and editing. Jackim Nabona: Data

curation, writing – review and editing. Ibrahim Ntulume: Writing – review and editing, visualisation. All authors have read and agreed to the published version of the manuscript.

Declaration of conflicting interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Ethics approval

Ethical approval for this study was obtained from Kampala International University Research and Ethics Committee (KIU-REC) (approval number/ID KIU-2022-115).

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Informed consent

Not applicable.

Trial registration

Not applicable.

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