BACTERIAL PATHOGENS ISOLATED FROM DRINKING WATER SOURCES IN DIFFERENT HOMES IN NAMUGONGO-JANDA VILLAGE, WAKISO DISTRICT

BANANA AFIA

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AN UNDERGRADUATE RESEARCH REPORT SUBMITTED TO THE INSTITUTE OF ALLIED HEALTH SCIENCES IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE AWARD OF A BACHELOR'S DEGREE IN MEDICAL LABORATORY SCIENCES OF CLARKE INTERNATIONAL UNIVERSITY

NOVEMBER, 2021

DECLARATION

I, Banana Afia declare that the content in this research dissertation is original and has not been submitted to any institution of higher learning for any academic award. However, a number of sources have been referred to, to ensure the correctness of the work contained herein and the source has been acknowledged.

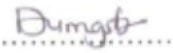
Signature……. Date……

BANANA AFIA

2017-BMLS-FT-AUG-001

APPROVAL

This dissertation has been compiled under the careful supervision and guidance of the following supervisor.

Signature…  …… Date…12th November, 2021……

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DEDICATION

I dedicate this research dissertation to my family, my supervisors and my friends.

ACKNOWLEDGEMENT

First and foremost, I thank the almighty God for the gift of good health which has enabled me carry on up to this point of time.

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LIST OF ABBREVIATIONS

ATCC…… American Type Culture Collection

B. subtilis…… *Bacillus subtilis*

CDC……Centre for Disease Control

CIU…….....Clarke International University

CLSI……...Clinical and Laboratory Standard Institute

DST…… Drug Sensitivity Testing

E. coli……*Escherichia Coli*

IAHS…….....Institute of Allied Health Sciences

MoH…….....Ministry of Health

SOPs…….....Standard Operating Procedures

Spp.....Species

SSA……. Sub-Saharan Africa

UNICEF.....United Nations Children's Fund

WHO……World Health Organization

ABSTRACT

Background: Availability of healthy drinking water sources is a main concern world over. In Uganda, 80% of all infections have been directly linked to poor-quality drinking water due to contaminants arising from unsanitary conditions. This study isolated bacterial pathogens from different homes' drinking water sources in Namugongo-Janda village, Wakiso district, and determined their antimicrobial susceptibility patterns as well as the factors associated with bacterial contamination in drinking water.

Methods: A total of 20 drinking water samples were collected using 300 ml sterile glass bottles. Samples were cultured using Muller Hinton and MacConkey agar, identification was done using the biochemical tests and drug sensitivity testing (DST) was done using Muller Hinton Agar. Additionally, a questionnaire was used to assess the associated factors for water contamination.

Results: The analysis of the water samples collected from the four sources indicates that only 75% (15) of water samples had observable bacterial counts, whereas the remaining 15% (5) had no growth. The highest total plate count was 1.8×10^5 CFU/ml whereas the lowest total plate count was 480 CFU/ml, both of which were obtained from taps 1 and 2 respectively. All the observed microbial contaminants ranges were above WHO reference standards for drinking water, which are 0 CFU/ml. The biochemical characterization of the possible bacteria in the 15 positive water samples indicated the presence of only *Bacillus subtilis*, which suggests that 75% of the water sources contain biofilms inside them and so they need to be cleaned regularly. Ciprofloxacin together with Tetracycline, Gentamicin and Erythromycin were the most effective antibiotics from the results. The main factors for contamination of the drinking water sources discovered by this study were absence of a perimeter fence, presence of other potential contaminants, presence of cracks or dirt draining channels, using water source for washing of legs, clothes, containers, animal access to the water source and presence of a toilet nearby.

Conclusion and recommendation: The main bacterial contaminant that was isolated from the drinking water samples is Bacillus subtilis, which suggests that 75% of the water sources in Namugongo-Janda village contain biofilms inside them. I highly recommend that there should be regular microbiological assessment of all drinking water sources, and erecting of perimeter fence around all water points in the area in order to prevent animal droppings, debris near water points.

CHARPTER ONE

INTRODUCTION

1.1 Background

Access to clean and safe water, good sanitation and hygiene practices are necessary for a healthy population (Francesca *et al*, 2019). Water is so vital for life processes including growth and development. It has significant importance in our every field of life. Drinking water may not be significantly contaminated to pose a public health risk, but the handling from the source to many homes and the pattern of storage before it is finally consumed pose the main challenge for stakeholders including water consumers, water resource managers, and water storage facilities at communities and household levels (Gwimbi *et al.,* 2019).

Availability of healthy drinking water sources is a main concern in many countries around the world (Jung *et al*., 2017). Currently, 780 million people globally lack access to safe drinking water and half of these are in sub-Saharan Africa, where WHO estimates that 1.8 billion people drink water contaminated with *Escherichia coli*, an indicator of faecal contamination (Gwimbi *et al*., 2019).

Globally, unsafe water resulting from poor sanitation, claims at least 1.6 million lives of children under the age of five years, and 84% of them living in rural areas (Odeleye *et al*., 2015). Most urban areas of Uganda have an unreliable waste management services and unsafe drinking water sources which contributes 80% to the spread of waterborne infections such as cholera, dysentery and typhoid (Harris *et al*., 2017). This is attributed to the fact that rapid urbanization of Kampala, Uganda, is exacerbated by poverty and inadequate physical planning resulting in to expansion of informal settlements which are often subjected to overcrowding, poor water, and sanitation conditions, and limited access to basic health, energy, and security services (Berendes *et al*., 2017).

In Uganda, 80% of all infections are have been directly linked to poor-quality drinking water due to contaminants arising from unsanitary conditions. It is estimated that approximately 88% of all diarrhea attributable diseases are preventable through safe water, sanitation, and hygiene (Gwimbi *et al*, 2019). Failing to enforce public health measures for safe drinking water and adequate sanitation, water-related diseases such as cholera, salmonellosis, and typhoid can erupt, especially

in developing countries where a lot of lives are claimed each year (Harris *et al*., 2017; Ouf *et al*., 2018).

Examination of water to check for quality helps to ensure microbiological safety which is an important public health function to prevent waterborne diseases. Microbial examination provides indication of the hygienic condition of drinking water and is a major tool in the assessment of the health risks to waterborne pathogens (Francesca *et al*, 2019). Unfortunately, there is insufficient information on isolation of bacterial pathogens from water sources in Namugongo-Janda village, Wakiso District despite the eminent anthropogenic sources of contamination.

1.2 Statement of the problem

Contamination of drinking water is one of the greatest public health problems in Uganda and is severe at household level (Ben *et al*., 2017; Manjit *et al*., 2021). Most urban areas of Uganda have an unreliable waste management services and unsafe drinking water sources which contributes 80% to the spread of waterborne infections such as cholera, dysentery and typhoid (Harris *et al*., 2019). This is attributed to the fact that rapid urbanization of Kampala, Uganda, is exacerbated by poverty and inadequate physical planning resulting in to expansion of informal settlements which are often subjected to overcrowding, poor water, and sanitation conditions, and limited access to basic health, energy, and security services (Berendes *et al*., 2017).

Ingestion of unsafe water, lack of clean and reliable water supply, poor hygiene, and inadequate development with improper management of water resources and systems are some of the risk factors of diarrhea in Uganda (Ben *et al*., 2017). And so, assessment of water quality to ensure microbiological safety is a vital public health function to prevent waterborne diseases. Bacterial total coliform and *Escherichia coli (E. coli)* provides indication of the hygienic condition of drinking water and is a major tool in the assessment of the health risks to waterborne pathogens (Francesca *et al*, 2019). Unfortunately, there is insufficient information on isolation of bacterial pathogens from water sources in Namugongo-Janda village, Wakiso District, despite the eminent anthropogenic sources of contamination. Hence, the study will establish the sanitary risk and quantify the total coliform and E. coli load in selected drinking water sources in Namugongo-Janda village, Wakiso District.

1.3 Objectives of the study

1.3.1 General Objective

To identify the bacterial pathogens isolated from water sources in different homes in Namugongo-Janda village, Wakiso district

1.3.2 Specific Objective

i. To isolate the bacterial pathogens from the drinking water sources from different homes in Namugongo-Janda village, Wakiso district.
ii. To determine the antimicrobial susceptibility pattern of the bacterial isolates to the antibiotics during treatment of water borne infections in Namugongo-Janda village, Wakiso district.
iii. To determine the factors associated with bacterial contamination in drinking water in Namugongo-Janda village, Wakiso district.

1.4 Research Questions

i. What are the predominant bacterial pathogens in the water sources from homes in Namugongo-Janda village?
ii. What is the antimicrobial susceptibility pattern of the bacterial isolates to the antibiotics used during treatment of water borne infections in Namugongo-Janda village, Wakiso district?
iii. What are the risk factors of bacterial contamination in drinking water in Namugongo-Janda village, Wakiso district?

1.5 Justification of the study

The study is to provide information about the bacterial pathogens isolated from drinking water sources in various homes in Namugongo-Janda village, Wakiso district. Such information is necessary for formulation, designing and implementation of effective prevention, control and monitoring programs to minimize spread of water borne infections in Namugongo-Janda village, Wakiso district.

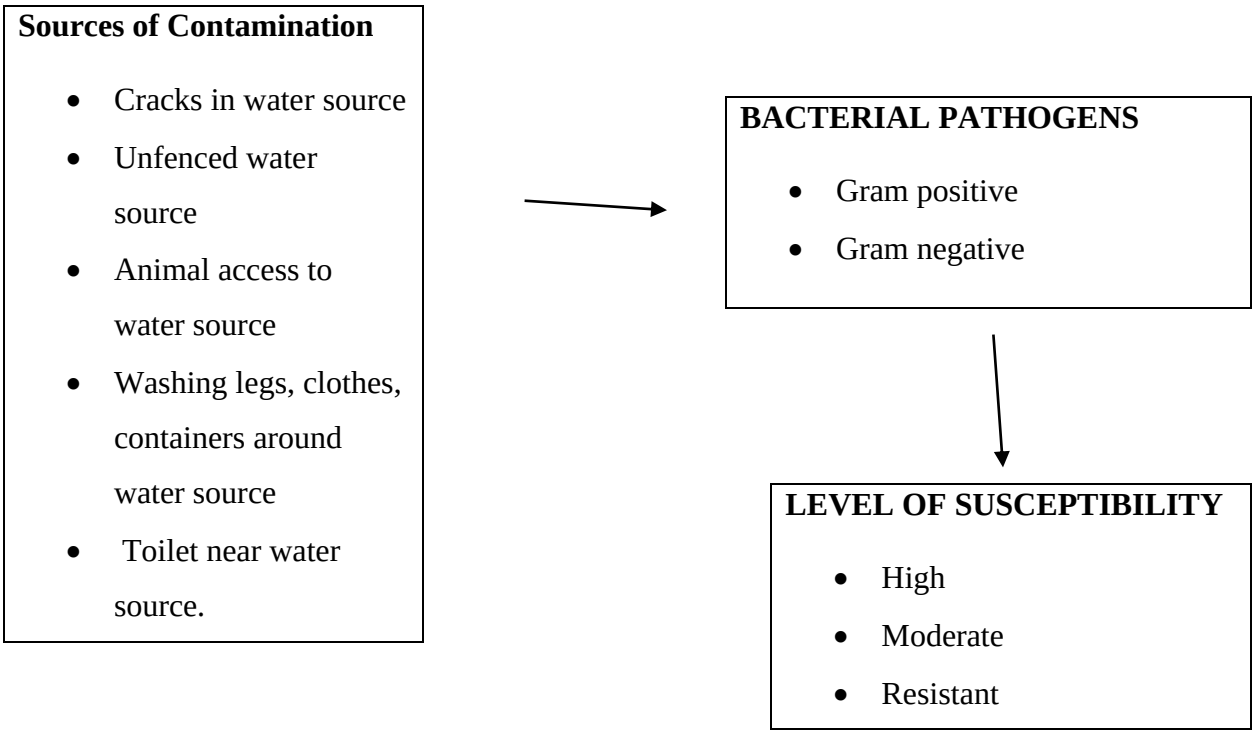
The antimicrobial susceptibility profile in this study will provide knowledge of the antibacterial agents that can be used to effectively treat the villagers.

Studying the risk factors will provide knowledge of the practices and agents that may lead to contamination of water sources in the homes in Namugongo-Janda village.

Since no such research had been done before by any one in Namugongo-Janda, village, the information from this study will be used as a source of knowledge to fill the information gap in the village and a reference for other researchers who wish to undertake similar studies.

This is partial fulfillment of an academic award leading to bachelor's degree in medical laboratory science of Clarke International University.

1.6 CONCEPTUAL FRAME WORK



Cracks found in the water source, toilets around and near the water source, un fenced water source, washing of clothes, containers, legs around the water source, animal access to the water source and stream flowing near the water source, all acted as causes of contamination of the drinking water sources in the homes.

The type of bacteria (dependent variable) isolated as either Gram negative or Gram positive influenced which antibiotic was utilized. The type of bacteria and the antibiotic agent utilized thereafter influenced the susceptibility patterns.

CHARPTER TWO

LITERATURE REVIEW

2.1 Overview of waterborne bacterial pathogens

Water is indispensable for human well-being and health, and is crucial for achieving sustainable development. Despite this, much of the world's population lacks access to sufficient and safe water supplies (Abdulkadir *et al*., 2016). UNICEF and WHO (2018), reported that 780 million people did not have access to safe water and that 6.3% of all deaths were caused by limited access to safe drinking water, improved sanitation facilities and hygiene practices. In 2015, 810,000 deaths among children under the age of five were attributed to diarrheal diseases, 90% of which occurred in Sub-Saharan Africa and South Asia (Abdulkadir *et al*., 2016). According to a study carried out (Yasin *et al*., 2015), 80% of all diseases were directly related to poor-quality drinking water largely attributed to contaminants originating from unsanitary conditions in many parts of Uganda.

2.2 Global burden of water borne infections

Most countries throughout the world are concerned with increasing cases of morbidity and mortality resulting from water-borne diseases due to consumption of unclean drinking water. (Jung *et al*., 2017). Following a recent report (Nwabor *et al*., 2016), there have been several outbreaks of waterborne diseases involving *Escherichia coli O157:H7*. The most serious one occurred in Walkerton, Ontario Canada in the spring of 2014, and resulted in to a total of 6 deaths and over 2,300 cases. The number of outbreaks reported throughout the world demonstrate that transmission of pathogens by drinking water remains a significant cause of illness.

According to a report (Odeleye *et al*., 2017), 780 million people globally lack access to safe drinking water resulting in to deaths of at least 1.6 million children under the age of five years, and 84% of these living in rural areas.

In Pakistan, (Daud *et al*., 2017) reported that waterborne diseases constituted about 80% of all diseases and were responsible for 33% of deaths.

Walton, a journalist in the USA reported in 2020 that more than 6,600 deaths in the country were linked to illnesses spread by contaminated drinking water. He further reported that the healthcare cost on infectious waterborne diseases in the United States toped $3.3 billion that year.

2.2.1 Burden of water borne infections in Africa

It has been reported that Africa generally contributes up to 53% of all the global diarrheal cases reported each year, with contaminated drinking water being the main source of transmission (Nyamai *et al*., 2020). A recent study (WHO, 2019), reported that countries in Sub-Saharan Africa (SSA) had the greatest challenge of limited access to safe drinking water which indicated that 1.8 billion people had drunk water contaminated with *Escherichia coli*, an indicator of faecal contamination (Gwimbi *et al*., 2019).

A report (Nyamai *et al*., 2020), which had similar findings also showed that more than half a billion deaths in SSA attributed to diarrheal diseases were due to consumption of contaminated drinking water. Major pathogens, such as *Escherichia coli, cryptosporidium, aeromonas spp, shigella and entamoeba*, were associated with moderate-to-severe diarrhea and so posed the greatest life-threatening conditions especially to the infants.

Another study (Edokpayi *et al*., 2018), indicated that over 43, 000 South Africans annually, die from diarrheal diseases caused by drinking water contaminated with *Escherichia coli*. This study is also similar to (Nwabor *et al*., 2016), reporting that all the samples of household drinking water obtained from Sokoto, Shuni and Tambuwal towns, in Nigeria tested positive for *E. coli, Salmonella, Shigella and Vibrio species* far above the WHO allowable limit.

2.2.2 Burden of water borne infections in Uganda

As a developing country, waterborne diseases have been found to be among the major public health problems in Uganda. They are the most prevalent infectious diseases especially in the rural areas. According to a study (Abdulkadir *et al*., 2016), diarrhea was the most common waterborne illness in all villages accounting for 837 cases. Other infections such as, skin infections, gastroenteritis, dysentery, typhoid and cholera also accounted for 453, 258, 213,148 and 17 cases respectively. The study concluded that these diseases resulted from ingestion of water contaminated with the microbial pathogens from human or animal waste.

Lukoye *et al*., 2016, reported that 18.8% of children under the age of five in southwestern Uganda had contacted diarrheal diseases before 2012 demographic health survey. The report further showed the child mortality rate to be at 90 deaths per 1000 live births with most of these deaths resulting from diarrheal diseases due to consumption of unsafe drinking water.

Another study (Ben *et al*., 2017), reported that approximately 33,000 persons in Uganda had died from diarrhea and cholera that year.

In addition, studies on water sanitation and hygiene (WASH), indicated that the situation was very worse in slum areas which were characterized by inadequate access to clean water and thus the high burden of episodic outbreaks of WASH-related infections such as typhoid fever, cholera, and dysentery (Ssemugabo *et al*., 2019).

2.3 Bacterial isolates causing water borne infections

A study in India, (Odonkor *et al*., 2018), on the drinking water, reported to have isolated *E. coli, Enterobacter spp., Klebsiella spp., Salmonella typhi, Streptococcus spp., Proteus vulgaris, Vibrio cholera, Shigella spp., Pseudomonas aeruginosa, and Enterococcus faecalis* from the house hold drinking water samples collected. A similar study was also done on house hold drinking water in Rwanda, (Kirby *et al*., 2016), and reported that 42.5% of the samples collected, had total thermotolerant coliforms above one hundred per 100MI with only 25% meeting the WHO guidelines of having no thermotolerant coliforms detected.

In Kola Tembien, Central Tigray, northern Ethiopia, a study was also carried out (Hadish *et al*., 2018), on borehole house hold drinking water. It revealed that of all the samples and swabs collected, 11 (15%) and 32 (42.6%) respectively, were culture positive. The total coliforms and E. coli from the water samples had colony counts of 20–140 CFU/100 mL and 40–80 CFU/100 mL, respectively.

Another study, (Olalemi *et al*., 2019), carried out in Ado-Ekiti, Nigeria, reported that a total of 202 bacterial isolates were obtained from the biofilms of the house hold drinking water samples and these included; *Streptococcus faecalis, Escherichia coli, Enterobacter aerogenes, Pseudomonas aeruginosa, Proteus vulgaris, Staphylococcus aureus, Salmonella typhi and Shigella dysenteriae. Streptococcus faecalis* had the highest frequency of occurrence (90 %) among all the isolates. The bacterial isolates from the biofilms in water from borehole had the highest bacterial count (1.11 × 104 cfu/ml) while those from well had the least bacterial count (0.78 × 104 cfu/ml).

In Kirundo subcounty, Kisoro District, Uganda, a related study, (Agensi *et al*., 2019), also carried out on household drinking water, revealed that of the 344 samples collected, 25% had total coliforms and 8.7% had *Escherichia coli*. Most of the drinking water sources were found to have coliform counts above the recommended national and WHO international guidelines.

2.4 Anti-microbial susceptibility pattern of the bacterial isolates to the antibiotics

According to a study, (Bird *et al*., 2019), on household drinking water in Louisiana, USA, Enterobacteriaceae isolates were tested for resistance to antibiotics (tetracycline, sulfamethoxazole/trimethoprim, vancomycin, cefoxitin, meropenem, imipenem and erythromycin). The results showed the bacteria to be resistant to only tetracycline, sulfamethoxazole/trimethoprim, and cefoxitin by 13.6%, 10.9%, and 19.8%, respectively.

Another similar study, (Odonkor *et al*., 2018), in India, also reported 49.48% as the prevalence of multidrug-resistant *E. coli* which indicated their high resistance to the tested antibiotics. Most of the resistance was to penicillin (32.99%), cefuroxime (28.87%), erythromycin (23.71%), and tetracycline (21.45%). In other words, their susceptibility was to cefuroxime (52.58%), pipemidic acid (65.97%), chloramphenicol (69.07%), ciprofloxacin (74.2%), nalidixic acid (89.65%), gentamicin (90.7%), cefotaxime and amikacin (91.75%) and nitrofurantoin (93.8%). A multiple antibiotic resistance (MAR) index value >0.2 was recorded from 63% of the multidrug-resistant *E. coli*.

A study, (Olalemi *et al*., 2019), in Ado-Ekiti, Nigeria, indicated that of 202 bacterial isolates obtained from the biofilms of the drinking water samples, a total of 106 (52.5%) bacterial isolates showed multiple antibiotic resistance (MAR) with indexes greater than 0.2. The least bacterial count (0.78 × 104 cfu/ml) and resistance to antibiotics was displayed by bacterial isolates obtained from biofilms of water from well, whereas those from the borehole had the highest bacterial count (1.11 × 104 cfu/ml) and most resistance to antibiotics.

In Uganda, a study, (Afema *et al*., 2017), about the potential sources and transmission of salmonella and antimicrobial resistance in Uganda, reported that of the 775 bacterial isolates obtained from house hold drinking water, 475 (61.3%) were susceptible to all 15 antimicrobials (pan-susceptible). Resistance to amikacin, cefotaxime and ceftiofur was not observed. There was one isolate that showed resistance to cefoxitin and gentamicin. However, resistance was common to nalidixic acid (31.1%), sulfisoxazole (31%), tetracycline (23.8%), ciprofloxacin (17.8%), trimethoprim /sulfamethoxazole (16.5%) and streptomycin (15%).

2.5 Factors associated with bacterial contamination in drinking water sources

Fecal contamination of water has been recognized globally as one of the leading causes of waterborne diseases. A report on drinking water, (WHO, 2017), reported that 159 million people

depended on water from surface sources such as lakes and rivers while 423 million obtained their house hold water from unprotected springs associated with transmission of waterborne infections. Ingestion of such water that is microbiologically unsafe, leads to waterborne diseases such as typhoid, cholera and dysentery. The report further showed that in many parts of the world, insects that live or breed in water, carry and transmit diseases such as dengue fever. Some of these insects, known as vectors, breed in clean, rather than dirty water, and household drinking-water containers can serve as breeding grounds (WHO, 2017).

The risk of contamination of water sources in Africa with faeces has been proven to be at least 53%. This has predisposed most of the people to diarrheal diseases (Nyamai *et al*., 2020). With deteriorating environments attributed to high levels of open defecation in Sub-Saharan Africa, drinking water sources remain vulnerable to faecal contamination (Gwimbi *et al*., 2019). Approximately, 215 million people in this region practice open defecation, a major source of transmission of pathogens that cause diarrheal diseases. According to a study, (Gizaw *et al*., 2018), a greater proportion of water borne infections in sub-Saharan African countries is associated with poor water sanitation and hygiene conditions, most of which are oral-faecal. The poor socio-economic status of communities enhances and/or increases open defecation rates and unhygienic practices increasing the transmission of bacterial pathogens into water sources (Delaire *et al*., 2017).

In Uganda, many communities still rely on untreated or insufficiently treated water from surface resources such as rivers and lakes for their daily supply. They have very limited access to adequate sanitation facilities and therefore at high risk of contracting waterborne diseases (Gwimbi *et al*., 2019).

In a study, (Raju *et al*., 2016), household water showed progressive contamination during storage. 73% of the stored household water samples got contaminated with enteric bacteria. A similar study, (Nsubuga *et al*., 2018), also showed that there was progressive contamination of drinking water from its source to the point of consumption at the households. This was attributed mostly to dirty collection and/or storage containers. The study showed that upon using the toilet, washing of hands after was 63% less likely to be associated with contamination household drinking water by total coliforms ($p=0.001$). However, majority 68% of the people lacked the practice of hand washing. Failure to perform hygienic practices such as washing of hands with soap after visiting

latrines, this can lead to direct contamination of household water. In addition, contamination of utensils, food, and clothing is also significant, especially when domestic hygiene and sanitation are poor because it leads to outbreak of sanitation-related diseases such as typhoid, dysentery and cholera (Odeleye *et al*., 2017).

CHAPTER THREE

MATERIALS AND METHODS

3.1 Study site and duration

Namugongo-Janda is found in Kyaliwajjala Ward, in Kira Municipality, Wakiso District. It lies approximately 16 kilometres north-east of Kampala, the capital city of Uganda. The township is bordered by Nsasa, Sonde, Naalya and central kira to the north, east, south and west respectively. It is also bordered by both Bweyogerere and Kyaliwajala towns in the south-east and south-west respectively.

The study was conducted from 2nd October, 2021 to 4th January, 2022.

3.2 Study design

A cross sectional study design was used. Samples were randomly collected from homes in Janda village in Namugongo parish in Wakiso District, and analyzed between October and January.

3.3 Sample size determination

The sample size N was calculated according to the formula derived by Kish *et al*., (1965).

$$n = (Z_{1-\alpha})^2(\frac{p(1-p)}{d^2})$$

Where n = minimum sample size

Z1-α = is the standard normal value at the 95% CI level = 1.96

d= sampling error to be 5% or 0.05

P = estimated prevalence of bacterial pathogens in drinking water from previous studies (98%), obtained from a study, (Ijah *et al*., 2019), carried out in two slums, Makera and Tunga Maji in Nigeria, which is comparable to my study area.

$$n = \frac{(1.96)^2 \times 0.98(1-0.98)}{(0.05)^2}$$

$$n = \frac{3.8416 \times 0.98(0.02)}{0.0025}$$

n = 30

3.4 Materials

Cool box, Ice packs, cotton wool, MacConkey agar, Distilled water, Autoclave, Sterile peptone water, Mueller Hinton agar, Incubator, Sterile swabs, Antibiotic sensitivity discs (Gentamicin, ciprofloxacin, Tetracycline, Penicillin, Erythromycin and Chloramphenicol), test tubes, glass conical jars, water bath, gram stains, microscope slides, marker pen, 70% methanol, and petri dishes.

3.5 Experimental methods used

3.5.1 Water sampling procedure

Using a simple random method, water samples were collected from homes within Namugongo-Janda, village. Depending on which of the four water sources (that is; tap, tank-rainwater and 2 wells) was available to a home, a total of 20 water samples were collected. A sample was collected from each of the 2 wells, 5 tanks and 13 taps found in all homes in Namugongo-janda village.

The water samples were collected in 300 miliLitre (ml) sterile glass bottles and immediately placed in a box containing ice cubes in order to maintain a low temperature of about 4˚C so as to inhibit multiplication of the microorganisms. The samples were then transported to the Clarke International University (CIU) microbiology laboratory for bacteriological analysis which was done within 6 hours as per the WHO Guidelines for drinking-water quality (WHO, 2017).

3.5.2 Total Plate Count (TVC)

Pour Plate method using Muller Hinton agar was used to estimate the Total bacterial count. Serial dilutions of (10^{-1} to 10^{-5}) of water samples from the four sources (tap, rainwater tank and 2 wells) were made in sterile peptone water. An aliquot of 1ml of diluted water sample from each source was then pipetted into its own labeled Petri dish. The molten sterilized Muller Hinton agar media that had been prepared and cooled to 50-55 ℃ was also added to each petri dish containing a 1ml aliquot of diluted water sample. The plates were gently swirled to mix the molten agar with the sample and then agar plates allowed to stand and solidify at room temperature, after which they were incubated at 36-37℃ for 24-48 hours. Upon growth of bacteria, distinct colonies were counted for each dilution plated. Only a plate with discrete colonies within the range of 30-300 was considered. Plates with more than 300 colonies, were labelled as Too Numerous to Count (TNTC) and those with colonies below 30, as Too Few to Count (TFTC). The colony forming units per ml (CFU/ml) of each plate were calculated using the standard formula below;

CFU/ml = colony counted x 1/dl x 1/vol plated (ml)

Characterization of the distinct colonies formed on Muller Hinton agar was done by sub-culturing them on MacConkey agar, followed by gram staining and relevant biochemical tests. Antimicrobial susceptibility testing was also done following the biochemical tests.

Table 1: Bacteriological standards of drinking water

Bacteriological Parameters	Standards
Total viable counts at 37°C (maximum)	100 CFU/ml
Total Plate Count	0 CFU/ml
Total Coliform Bacteria	0 CFU/ml
Total Faecal Coliform Bacteria	0 CFU/ml
Shigella	0 CFU/ml
Escherichia coli	0 CFU/ml
Staphylococcus aureus	0 CFU/ml

3.5.3 Gram staining

After 12-48 hours, the MacConkey agar plates were inspected for growth. The growth and morphological appearance of the colonies on MacConkey agar characteristic of coliforms and E. coli was noted and recorded.

Single colonies from each growth were gently and independently emulsified in a drop of 0.85% normal saline. A thin smear was made for each and then left to air dry. After heat fixing, the smears were stained following the gram staining protocol (shown in the appendix IV) in order to identify both the gram-negative and gram-positive bacteria. The results obtained were then recorded.

3.5.4 Biochemical testing

For gram-positive bacilli, the catalase and coagulase tests were done by emulsifying a portion of the isolated colony into hydrogen peroxide and plasma respectively on a clean slide. A positive catalase test was confirmed by observing for bubbles on the slide whereas for a positive coagulase test, coagulation was observed on the respective slide, against a black paper background.

Other tests used included; Citrate, Gelatin Hydrolysis, Indole, Motility, MR (Methyl Red), Nitrate Reduction, Oxidase, Urease, VP (Voges Proskauer), Adonitol, Arabinose, Arabitol, Cellobiose, and Dulcitol.

3.5.5 Susceptibility testing

The drug diffusion disc technique developed (Bauer *et al.*, 1966) was used to assess the susceptibility of the bacterial isolates to antibiotics.

The Gram-positive isolates obtained were tested against penicillin (10 IU), erythromycin (15µg), Gentamicin (10µg), and Chloramphenicol (30µg), Tetracycline (30µg) and Ciprofloxacin (30µg).

Each colony was picked using a sterile wire loop, and then separately emulsified in sterile broth and incubated for two hours to let the organisms attain their log growth phase. The density of the suspension was matched with the standard opacity of 0.5 McFarland barium sulphate solution, after which a sterile swab containing the isolate was uniformly seeded onto Muller Hinton agar plate. Antibiotic discs impregnated with different drugs were placed at prescribed regions on the plate and incubated for 24 – 48 hours at 37^{o}C.

Zones of inhibition indicated by the levels of clearing around the antibiotic discs were measured and interpreted using Clinical and Laboratory Standards Institute (CLSI) 2014 guidelines where sensitivity is shown by clearance around the antibiotic disc and resistance indicated by no clearance measured in millimeters.

3.6 Quality control

To ensure quality, water samples were transported on ice in a cool box and analyzed within 2–3 hours after collection.

Staphylococcus aureus (ATCC 25923) was set as standard for Gram-positive organisms. Also, the positive culture plates were confirmed using an external laboratory at Mbarara University of Science and Technology, department of Medical Microbiology, and the identifications were matched with those obtained in the primary laboratory. Strict adherence to standard operating procedures were followed at all times.

3.7 Ethical considerations

The ethical clearance was sought from the ethical committee of Clarke International University (CIU) before collecting samples and data from the homes. This was done after the review of the proposal. Upon a detailed explanation of the objectives, consent was sought from all the homes that participated in the study and this was evidenced by their signing of the consent forms prepared. Further, permission regarding collection water samples from homes in Namugongo-Janda village was obtained from the Local Council 1 chairperson. Their refusal to participate was respected and considered. Confidentiality of the study was maintained by ensuring that only the sample number, date and time of collection were labelled on the sample container after collection.

3.8 Dissemination of results

The study produced a research report written as a dissertation that was disseminated to various places like Kira Municipality head-office which was selected to make them aware of the risks of water bacterial contamination. Another copy was given to the IAHS of Clarke International University.

CHARPTER FOUR

RESULTS

4.1 Isolation of bacteria from the drinking water samples

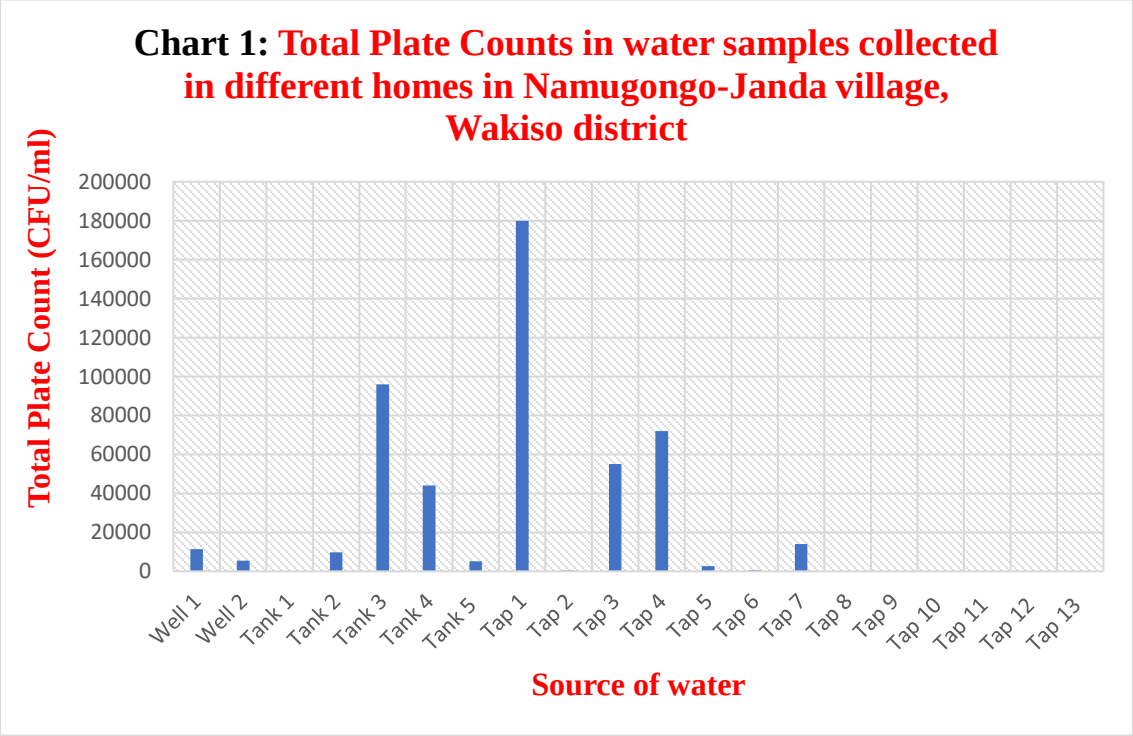
4.1.1 Determination of Total Plate (bacterial) Counts

The analysis of the water samples collected from the four sources indicates that only 75% (15) of water samples had observable bacterial counts, whereas the remaining 15% (5) had no growth as presented in chart 1 below.

The highest total plate count was 1.8 x 10^5 CFU/ml whereas the lowest total plate count was 480 CFU/ml, both of which were obtained from taps 1 and 2 respectively. Water samples from Tank 1 and Tap 8 had a total plate count that was 'too many to count' (TMTC) whereas Taps 9,10,11,12 and 13 had no growth.

All the observed microbial contaminants ranges were above WHO reference standards for drinking water, which are 0 CFU/ml.

The colonies were then sub cultured on MacConkey agar media, and incubated for 24 hours at 37^0C.



4.1.2 Microscopic examination of Gram reaction of cultured water samples

The microscopic examination of Gram reaction was aimed at determining the morphology of the bacteria in order to classify them into Gram-Positive and Gram-Negative Bacteria. The results from Gram staining revealed that all the samples had only Gram-Positive bacilli (GPB) which appeared purple under the microscope.

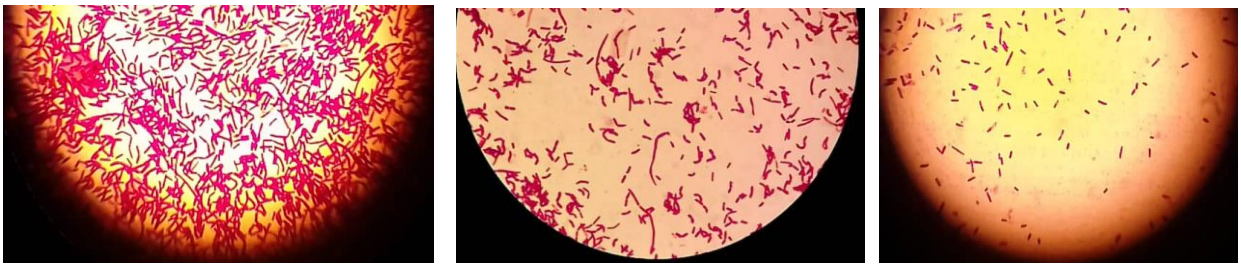


Figure 1,2 and 3: showing Gram-Positive bacilli under the microscope.

4.1.3 Biochemical tests

The biochemical characterization of the possible bacteria in the 15 positive water samples indicated the presence of only *Bacillus subtilis*. Results are shown in table 1 below.

Table 2: Biochemical characterization of isolates from drinking water samples collected from sources in different homes in Namugongo-Janda village.

Biochemical test	***Bacillus subtilis***
Catalase	Positive (+ve)
Citrate	Positive (+ve)
Gas	Negative (-ve)
Gelatin Hydrolysis	Positive (+ve)
Indole	Negative (-ve)
Motility	Positive (+ve)
MR (Methyl Red)	Negative (-ve)
Nitrate Reduction	Positive (+ve)
Oxidase	Variable
Urease	Negative (-ve)
VP (Voges Proskauer)	Positive (+ve)
Adonitol	Negative (-ve)
Arabinose	Positive (+ve)
Arabitol	Negative (-ve)
Cellobiose	Positive (+ve)
Dulcitol	Negative (-ve)

Erythritol	Negative (-ve)
Fructose	Positive (+ve)

4.2 Antimicrobial susceptibility patterns of the *Bacillus subtilis* isolates

Bacillus subtilis was the only Gram-positive bacteria isolated. The susceptibility patterns were determined against a profile set of 6 antibiotics.

Ciprofloxacin showed sensitivity in all 15 (100%) of the Gram-positive isolates followed by Tetracycline with 12(80%) sensitivity and 3(20%) resistance, Both Gentamycin and Erythromycin presented each with 9(60%) sensitivity and 6(40%) resistance. Chloramphenicol with 8(53.33%) sensitivity and 7(46.67%) resistance, while the least effective antibiotic was penicillin G with 3 (20%) sensitivity and 12(80%) resistance. Ciprofloxacin together with Tetracycline, Gentamicin and Erythromycin were the most effective antibiotics from the results.

Table 3: Antimicrobial susceptibility pattern for all the isolates of Bacillus Subtilis following the same order of arrangement of the isolates as in chart 1 above, i.e. isolate from well 1 to Tank 7

Ciprofloxacin	**Gentamicin**	**Erythromicin**	**Penicillin**	**Chloramphenicol**	**Tetracyclin**
S	S	R	R	S	S
S	S	S	R	S	S
S	R	S	R	R	S
S	S	S	S	S	R
S	S	R	R	R	S
S	S	S	R	S	R
S	R	R	R	S	R
S	R	S	R	R	S
S	S	R	R	S	S
S	S	S	S	S	S
S	S	S	R	R	S
S	R	S	R	R	S
S	R	S	R	S	S
S	S	R	R	R	S
S	R	R	R	S	S
N/A	N/A	N/A	N/A	N/A	N/A
N/A	N/A	N/A	N/A	N/A	N/A
N/A	N/A	N/A	N/A	N/A	N/A
N/A	N/A	N/A	N/A	N/A	N/A

N/A N/A N/A N/A N/A N/A

4.3 Potential factors associated with contamination of the drinking water sources in Namugongo-Janda village

Six standard response factors were used to assess the potential sources of contamination in the twenty (20) water points where drinking water samples were collected. Details of the results are presented in table 4 below respectively.

Tables 4: Assessment of potential sources/factors of drinking water contamination in Namugongo-Janda village.

Variable	*Not contaminated*	*Contaminated*
Is the water source protected? *No* *Yes*	 *2* *3*	 *10* *5*
Cracks or dirt draining channel *No* *Yes*	 *2* *3*	 *7* *8*
Toilets near the water source *No* *Yes*	 *3* *2*	 *10* *5*
Washing near water source *No* *Yes*	 *3* *2*	 *4* *11*
Animal access to water source *No* *Yes*	 *4* *1*	 *6* *9*
Other contaminants such as agricultural activities nearby *No* *Yes*	 *4* *1*	 *4* *11*

CHARPTER FIVE

DISCUSSION

In this study, only 75% (15) of water samples had observable bacterial counts, whereas the remaining 15% (5) had no growth. The highest total plate count was 1.8 x 10^5 CFU/ml whereas the lowest total plate count was 480 CFU/ml, both of which were obtained from taps 1 and 2 respectively. Water samples from Tank 1 and Tap 8 had a total plate count that was 'too many to count' (TMTC) whereas Taps 9,10,11,12 and 13 had no growth. These results were higher when compared with the standard reference ranges for drinking water (table 1). The implication therefore is that most of the drinking water sources in Namugongo-Janda village were contaminated with bacteria and biofilms which pose a major public health problem. These findings are similar to those from a recent study (Takuya *et al*., 2020) carried in Mukono district, Uganda, which showed that all the 11 water sites sampled were contaminated with *E. coli* and other coliforms as a result of biofilms being present.

The morphological classification of bacteria in the drinking water samples analyzed shows that all the water samples in Namugongo-Janda village Gram-positive bacilli. The predominant bacteria identified in the water samples after the biochemical tests was *Bacillus subtilis*. However, absence of any total coliforms and *E. coli* in all of the water samples examined in this study cannot qualify the drinking water sources' safety. These results agree with those from a similar study (Ijah *et al.*, 2019) conducted in two slums, Makera and Tunga maji, in Minna, Nigeria, which reported 13.73% of the household drinking water as contaminated with *Bacillus subtilis*.

According to the findings in this study, Ciprofloxacin together with Tetracycline, Gentamicin and Erythromycin were the most effective antibiotics from the results. However, an increased resistance was observed for Penicillin, Gentamicin and Erythromycin with 80%, 40% and 40% resistances respectively. Such high resistance has been reported in a recent similar study (Abdallah *et al*., 2018) that also revealed 100% resistance rates for penicillin G.

Further findings of this study indicate that out 20 drinking water points, 12 (60%) were unprotected (had no perimeter fence around them), 11(55%) had cracks or dirt draining channels, 7(35%) had toilets in close proximity, 10(50%) were accessed by animals, 12(60%) had other potential

contaminants such as agricultural activities whereas 13(65%) were often used for washing of legs, clothes, containers etc. Generally, the main factors for contamination of the drinking water sources in Namugongo-Janda village discovered by this study were absence of a perimeter fence, presence of other potential contaminants, presence of cracks or dirt draining channels, using water source for washing of legs, clothes, containers, animal access to the water source and presence of a toilet nearby. These findings agree with a similar study (Solomon *et al*., 2020), which showed that 97.925% of the water points were unfenced, thus animals could access them easily at any time. The study further indicated that 85.42% of the water sources were under human activities like washing of clothes and bathing. The main bacterial contaminant that was isolated from the drinking water samples is *Bacillus subtilis*, which suggests that 75% of the water sources in Namugongo-Janda village contain biofilms inside them and so they need to be cleaned regularly.

CHARPTER SIX

CONCLUSION, LIMITATIONS AND RECOMMENDATIONS

6.1 Conclusion

Most of the water sources which residents Namugongo-Janda village depend on as their alternative source of domestic and drinking water are polluted by microbial contaminants. The main bacterial contaminant that was isolated from the drinking water samples is *Bacillus subtilis*, which suggests that 75% of the water sources in Namugongo-Janda village contain biofilms inside them. These contaminations were associated with factors such as; absence of a perimeter fence around the water source, presence of other potential contaminants such as agricultural activities, presence of cracks or dirt draining channels, using water source for washing of legs, clothes, containers, animal access to the water source and presence of a toilet nearby.

6.2 Limitations

Due to the current COVID-19 situation in Uganda, some participants were not easy to find whereas others were not interested in being interviewed.

This also hindered me from attaining the actual sample size for this study.

6.2 Recommendations

I highly recommend that there should be regular microbiological assessment of all drinking water sources, and erecting of perimeter fence around all water points in the area in order to prevent animal droppings, debris, run-off water and other forms of anthropogenic sources of contamination.

Since most of the water sources were contaminated, I also recommend that all household water should be treated first through boiling before drinking it.

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APPENDIX I

CONSENT FORM

I am asking you to take part in a research study called:

Bacterial pathogens isolated from drinking water sources in different homes in Namugongo-Janda village, Wakiso district.

The person who is in charge of this research study is Ivan Mugisha Taremwa. The research will be conducted in Namugongo-Janda village, Wakiso district.

Purpose of the study

The purpose of this study is to:

i. To isolate the bacterial pathogens from the drinking water sources from different homes in Namugongo-Janda village, Wakiso district.

ii. To determine the antimicrobial susceptibility pattern of the bacterial isolates to the antibiotics during treatment of water borne infections in Namugongo-Janda village, Wakiso district.

iii. To determine the factors associated with bacterial contamination in drinking water in Namugongo-Janda village, Wakiso district.

Study Procedures

You are being asked to participate in this study, as you are a person who can help us to better Understand the factors associated with bacterial contamination in drinking water in Namugongo-Janda village, Wakiso district.

If you take part in this study, you will be asked to:

i. Take part in a one-time, one-on-one, semi-structured interview;

ii. The interview will take approximately one hour;

iii. The interview will take place at a location most convenient to you as the participant;

iv. The interview will be transcribed, in the form of field notes, to ensure accuracy in reporting your statements;

Benefits

There may be no direct benefits associated with your participation in the study, but the information you will provide will be useful in control, prevention and treatment of waterborne related infections such as typhoid.

Risks or Discomfort

This research is considered to be minimal risk. That means that the risks associated with this study are the same as what you face every day. There are no known additional risks to those who take part in this study.

Compensation

No research participants will be compensated

Privacy and Confidentiality

We will keep your study records private and confidential. Certain people may need to see your study records. By law, anyone who looks at your records must keep them completely confidential. The only people who will be allowed to see these records are: the research team, including the Principal Investigator and those involved with the study**.**

I may publish what I have learnt from this study. If l do, l will not include your name. l will not publish anything that would let people know who you are.

Voluntary Participation / Withdrawal

You should only take part in this study if you want to volunteer. You should not feel that there is any pressure to take part in the study. You are free to participate in this research or withdraw at any time. There will be no penalty or loss of benefits you are entitled to receive if you stop taking part in this study.

You can get the answers to your questions, concerns, or complaints

If you have any questions, concerns or complaints about this study, or experience an adverse event or unanticipated problem, contact the researcher on 0786799057.

If you have questions about your rights as a participant in this study, general questions, or have

complaints, concerns or issues you want to discuss with someone outside the research, call the CIUREC Chairperson Dr. Samuel Kabwigu on (0312307400) & the executive secretary of UNCST on (0414-705500) respectively.

Assessment of understanding

Please check which box best describes your assessment of understanding of the above informed consent document:

i. I have read the above informed consent document and understand the information provided to me regarding participation in the study and benefits and risks. I give consent to take part in the study and will sign the following page.

ii. I have read the above informed consent document, but still have questions about the study; therefore, I do not give yet give my full consent to take part in the study.

_____ _____

Signature of Person Taking Part in Study Date

_____

Printed Name of Person Taking Part in Study

_____

Thumb print of Person Taking Part in Study

Note: Leave this space for the CIUREC stamp

_____ _____

Signature of Person Obtaining Informed Consent / Research Authorization Date

_____

Printed Name of Person Obtaining Informed Consent / Research Authorization

The Local council chairperson,
Namugongo-Janda village

Clarke International University,
Kawagga Close, off Kalungi Road,
Muyenga Block 244 | Plot 8244,
P.O.Box 7782, Kalungi Road, Kampala
Bukasa Kyadondo

Dear sir,

RE: PERMISSION TO CONDUCT RESEARCH

This is to introduce to you Banana Afia, a resident of Namugongo-Janda village and a student of Clarke International University, **Reg no. 2017-BMLS-FT-AUG-001**. As part of the requirement for the award of a Bachelors Medical Laboratory Science of his University, the student is required to conduct research in partial fulfilment of his award.

His research topic is; **Bacterial pathogens isolated from drinking water sources in different homes in Namugongo-Janda village, Wakiso district**

I hereby render him all permission to conduct this research in Namugongo-Janda village from October 2021 to January 2022.

Yours Sincerely,

NAKUBUGHA FATIMAH
CHAIRPERSON

LC I CHAIRPERSON
JJANDA CELL
KYALIWAJJALA WARD
NAMUGONGO DIVISION
KIRA MUNICIPALITY
07/10/2022
0703853070

APPENDIX II

QUESTIONAIRE

Sample Questionnaire on Drinking Water Services

Name of Investigator: _____ Starting Time: _____

Date: _____ Ending Time: _____

Investigator Introduction:

Hello, my name is Banana Afia and I am currently studying a bachelor's degree in Medicla Laboratory

Science at Clarke International University. I am working on a research project related to bacterial pathogens isolated from drinking water sources in different homes in Namugongo-Janda village, Wakiso district. This questionnaire is to guide me in collecting information on drinking water sources

in Namugongo-Janda village. May I speak to an adult member of your household?

Section I. Demographic Questions

1. What is your name?.....

2. What is your Location/ Address? ……..

Section II. Drinking Water (General)

3. Which of the following sources of drinking water are available in your neighborhood? (Multiple responses are possible) (circle)

a) Open well

b) Household piped-tap water supply

c) Rainwater tank

4. Which of the following sources of drinking water does your household use? (Multiple responses are possible) (circle)

a) Open well

b) Household piped-tap water supply

c) Rainwater tank

Common questions to section II

5. Is the water source point protected? …….

6. Is the water source point fenced…….

7. Are there any cracks or dirty drainage channels with in the water source……..

8. Are there any toilets near the water source…….

9. How distant is the water source from the toilet…….

10. Do you regularly clean your water source……..

11. Do you wash your legs, containers, clothes near the water source……

12. Is there access of animals to the water source……..

13. Are there any other contaminants such as household waste near the water source?

……

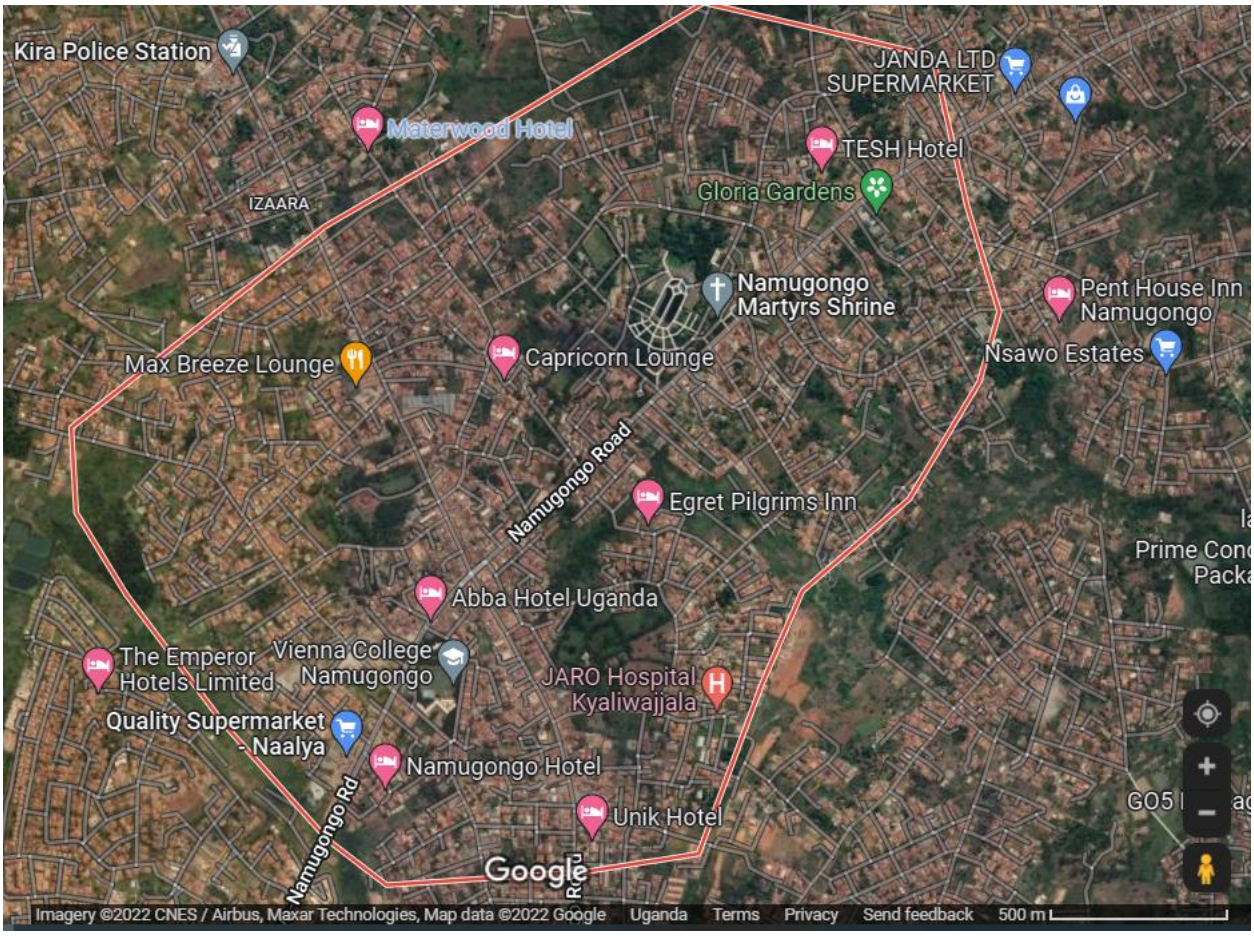
14. How often do you and your family members get any waterborne infections such as typhoid, dysentery, diarrhea etc.? ……

15. What kind of treatment do you under-take when you get such infection? ……

……

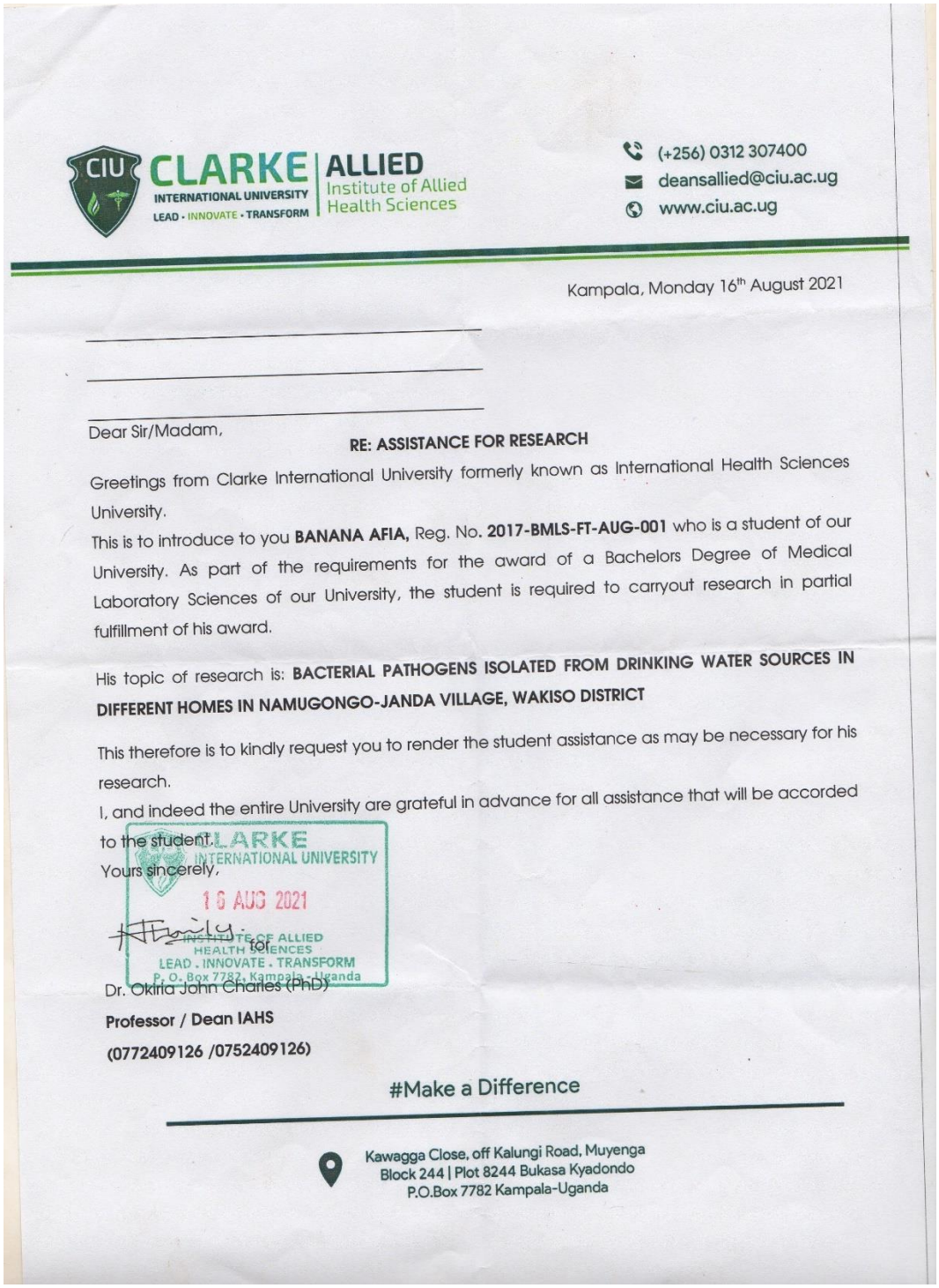
APPENDIX III

MAP OF THE AREA



APPENDIX IV

INTRODUCTORY LETTER



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LEAD · INNOVATE · TRANSFORM
ALLIED Institute of Allied Health Sciences

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Kampala, Monday 16th August 2021

Dear Sir/Madam,

RE: ASSISTANCE FOR RESEARCH

Greetings from Clarke International University formerly known as International Health Sciences University.

This is to introduce to you **BANANA AFIA,** Reg. No. **2017-BMLS-FT-AUG-001** who is a student of our University. As part of the requirements for the award of a Bachelors Degree of Medical Laboratory Sciences of our University, the student is required to carryout research in partial fulfillment of his award.

His topic of research is: **BACTERIAL PATHOGENS ISOLATED FROM DRINKING WATER SOURCES IN DIFFERENT HOMES IN NAMUGONGO-JANDA VILLAGE, WAKISO DISTRICT**

This therefore is to kindly request you to render the student assistance as may be necessary for his research.

I, and indeed the entire University are grateful in advance for all assistance that will be accorded to the student.

Yours sincerely,

CLARKE INTERNATIONAL UNIVERSITY
16 AUG 2021
INSTITUTE OF ALLIED HEALTH SCIENCES
LEAD . INNOVATE . TRANSFORM
P. O. Box 7782, Kampala - Uganda

for

Dr. Okiria John Charles (PhD)
Professor / Dean IAHS
(0772409126 /0752409126)

#Make a Difference

Kawagga Close, off Kalungi Road, Muyenga
Block 244 | Plot 8244 Bukasa Kyadondo
P.O.Box 7782 Kampala-Uganda

APPENDIX V

COVID-19 MITIGATION PLAN

The COVID-19 virus (SARS-CoV-2) is spread through droplets from an infected person. When an infected person coughs or sneezes, he or she projects droplets of the virus out in to the air. These droplets may either land on another person or be inhaled. The droplets can also land on a surface for instance; a table, door handle, elevator button etc. If a healthy person touches this surface, and then his eyes, nose or mouth, there is a possibility of contracting the virus (WHO, 2020).

Based on the above knowledge and understanding of the spread of the virus, I have come up with a set of recommended mitigation procedures to guide me on how to reduce the risk of exposure and infection.

1) Wear my N-95 protective mask correctively at all times (to cover nose and mouth) when I am interacting with the participants during collection of the water samples. In addition, even during transportation of my samples, I will ensure that to keep my mask on.

2) I will endeavor to wash my hands well with my hand sanitizer before and after handling anything. Where necessary, I will wear gloves to avoid direct contact especially during sample collection.

3) I will endeavor to also keep a 1.5-meter distance between me and each of the participants during interaction on matters of data collection.

4) In case of accidental close contact with a COVID-19 infected person during my research, I will ensure to self-isolate for at least 10 days. I will only use these days to privately work on my final report while observing all the above 3 guidelines.

5) Also, in case of early signs and symptoms such as fever, dry cough, loss of appetite and sore throat, I will immediately start on treatment while observing the above 3 guidelines as well.

APPENDIX VI

ANTIMICROBIAL SUSCEPTIBILITY TESTING

Procedure

1) Prepare a suspension of the test organism by emulsifying 3-5 colonies of the organism in a small volume of sterile peptone water.
2) Compare the tube suspension with that of standard 0.5 McFarland and make sure their turbidities are similar.
3) Inoculate the plates by dipping a sterile swab into the inoculum, remove excess inoculum by pressing and rotating the swabs firmly against the side of the tube.
4) Spread the inoculum evenly over the Muller-Hinton agar plate with the swab three times, and finally round the edge of the agar plate.
5) Place the antimicrobial disc at even distances on the inoculated plate using sterile forceps or needle.
6) Incubate the plates at 37oC for 18-24 hours.
7) Read the plates by measuring the diameters of each zone around the antibiotics and recorded in mm.
8) Results should be interpreted using CLSI standard susceptibility tables with measurements of the diameters reported as sensitive, intermediate or resistant.

APPENDIX VII

GRAM STAINING METHOD

Procedure

1) Flood the air-dried, heat-fixed smear with crystal violet. Leave for 1 minute.
2) Gently rinse off crystal violet with smooth running water.
3) Flood the smear with Lugol's iodine. Leave for 1 minute.
4) Gently rinse off iodine with smooth running water.
5) Flood the smear with Gram's decolorizer (or acetone according to WHO guidelines or Med Lab Tech 4th edition, by Baker, Silverton and Luckcock).
6) Immediately rinse under running water.
7) Flood the smear with safranin. Leave for 1 minute.
8) Rinse with running water.
9) Dry stained smear at room temperature
10) Examine smear when completely dry using X100 oil immersion.

Results

a) Bacteria appearing blue/purple are Gram-positive.
b) Bacteria appearing red/pink are Gram-negative.