ADDIS ABABA UNIVERSITY

COLLEGE OF GRADUATE STUDIES

SCHOOL OF ALLIED HEALTH SCIENCES

DEPARTMENT OF MEDICAL LABORATORY SCIENCE

BACTERIOLOGICAL AND PARASITOLOGICAL QUALITY AND SAFETY ASSESSMENT OF PUBLIC MUNICIPAL DRINKING WATER SOURCES IN ADDIS ABABA, ETHIOPIA

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This is to testify that the thesis prepared by Amsalu Mekonnen which is entitled with "*Bacteriological and Parasitological Quality and Safety Assessment of Public Municipal drinking Water Sources in Addis Ababa, Ethiopia*" and submitted in partial fulfillment of the requirements for the degree of Master of Clinical Laboratory Sciences (Diagnostic and Public Health Microbiology Specialty) complies with the regulations of the University and meets the accepted standards with respect to originality and quality.

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Approved by the Examining Board

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ABSTRACT

Background: In developing counties, disease-associated with poor water still have considerable public health and socio-economic development importance. Water of poor quality can cause water borne diseases by bacteria, viruses, protozoa and parasites. It has been frequently described responsible for millions morbidity and mortality. Therefore, quality and safety status of municipal drinking water of Addis Ababa should be regularly monitored in sustainable manner. **Objective**: The aim of this study was to assess the bacteriological and parasitological quality and safety status of treated and non-treated municipal drinking water sources in Addis Ababa City.

Methodology: A cross-sectional study was carried out on drinking water sources such as public taps, reservoirs, springs and wells managed by Addis Ababa Water and Sewerage Authority (AAWSA). 125ml drinking water of each 2951 samples were collected from water sources and analyzed for bacteriological by Presence/ Absence (P-A) culturing method and 11L drinking water of each were collected from 25 selected reservoirs for parasites identification by direct microscopy.

Results: This study revealed that there were 10% of all samples were positive for bacteriological parameters done by presence-absence method. Consequently, 7% and 3% were positive for total coliforms and faecal coliforms respectively. The bacterial distribution trend from 1 to 13 weeks of the first to end of wet season showed a slight increment of total coliforms. Inversely, there was a slight decrement for faecal coli forms. On the other hand, all parasitological tested samples from selected reservoirs were free from intestinal parasites.

Conclusion: Samples collected from municipal drinking water sources were positive for fecal coli forms, total coli forms organisms and indicator bacterial species during the study period. It needs continuous screening and treating water sources to utmost important for prevention and control of infectious diseases caused by water transmitted pathogen microorganisms. Therefore, regular and timely research on bacteriology and parasitology is mandatory to overcome crisis resulted from poor water quality.

Key words: Presence-Absence test, faecal coliforms, total coliforms, municipal drinking water quality and safety.

LIST OF ABBREVIATIONS

AAWSA	Addis Ababa City Administration water and sewerage Authority
AWD	Acute Watery Diarrhea
CDC	Centers for Disease control and prevention
DPD	Diethyl-P-Phenylene Diamine
EPA	Environmental protection agency
FDA	Food and Drug Administration
FMoH	Federal Ministry of Health
ISO	International Organization for Standardization
OECD	Organization for Economic Co-operation and Development
UNICEF	United Nations Children's Fund
USEPA	United States Environmental protection agency
WASA	Water and Sanitation Agency

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1. INTRODUCTION

1.1 . BACKGROUND

Microorganism contamination of drinking water has been caused serious illnesses and associated mortality worldwide (1). It serves as a mechanism to transmit communicable diseases such as diarrhea, cholera, dysentery, typhoid and guinea worm infection (2). In the developing world, diseases associated with poor water and sanitation still have imposed economical development in addition to threaten million lives (3). World Health Organization estimates that in 2008 diarrheal disease claimed the lives of 2.5 million people (2). In 2013, there were nearly 1.8 million deaths mainly with diarrhea and cholera caused by unsafe water supply in conjunction with inadequate sanitation and hygiene (4). Globally, diarrhea ranks second after respiratory infections. It causes incidence of 4,600 million episodes and 2.2 million deaths every year. Deaths of 1.33 million were children under five years represented 15% of overall mortality (3). This burden is greater than the combined burden of HIV/AIDS and malaria (2).

In Africa, roughly 40% of the population does not have access to improved water supply and sanitation (5). The safety of drinking water is challengeddue tocontaminants from natural and man-made situations at global scale (6).The quality and safety of the drinking water continues to be an important public health issue. Itis a pillar of primary prevention and control of pathogenic microorganisms such as bacteria, viruses, protozoa and helminthes. Those organisms contaminated drinking-water not only through faecal contamination but also some organisms grow in piped water distribution systems (2). Many of the diseases in communities are caused by micro-organisms carried indrinking-water. As a result, chlorination is by far applied as disinfecting methods anywhere.It destroys the membrane of many microorganisms and kills them.

On the other hand, there is no study conducted in the last nineteen years to assess the quality and safety status of the water from all service sources of Addis Ababa. Therefore, safe and quality of water supply is an important consideration in the protection of human health and well-being of our communities. Therefore, a microbiological aspect is one of the specific standards to provide a basis for determining quality of drinking water (7) which is free from any microorganisms known to be pathogenic and free from bacteria indicative of pollution with excreta (8).

1.2 . STATEMENT OF THE PROBLEM

Availability of safe and wholesome drinking water for all is one of the most significant challenges faced by the municipal authorities worldwide. A clean and treated water supply to each house may be the norm in Europe and North America, but in developing countries, access to both clean water and sanitation are not the rule, and waterborne infections are common (9).

According to World Health Organization (WHO), nearly 1.8 million deaths, due to diarrhea and cholera per year, are attributed to unsafe water supply in conjunction with inadequate sanitation and hygiene whereas improvement in water supply can help reduce morbidity by 6 to 25% (4). The most predominant waterborne disease, diarrhea, has an estimated annual incidence of 4,600 million episodes and causes 2.2 million deaths every year. In terms of global burden of disease, diarrhea ranks second after respiratory infections. Children under five years of age are most affected; some 1.33 million die each year of diarrhea, representing 15% of overall mortality in that age group (3).

Lack of safe drinking water is associated with high morbidity and mortality from excreta related diseases. Bacteria, protozoa, helminthes, fungi, yeasts, and viruses are ubiquitous in aquatic environments and may pose threats to water quality for both human and ecosystem health. Microbial risk assessment and management in the water sector is a focus of governmental regulation and scientific inquiry; however, stark gaps remain in their application and interpretation (10).

Information concerning Municipal drinking water quality and safety is not done recently; after 1997 in and around Addis Ababa where there is big change in population density, Infrastructure, expansion, development of the drinking water dams, new springs and wells.

Therefore, this study was hoped to fill the gap by conducting drinking water service sources of all sites and the consumers' taps from all representative places of the city for the bacteriological and parasitological quality assessment and safety of municipal drinking water of Addis Ababa city Administration.

1.3 . SIGNIFICANCE OF THE STUDY

- This study improves the scientific basis of drinking water, water quality and safety monitoring in Addis Ababa.
- This study will be an evidence for the AAWSA and the related stake holders to evaluate the quality and safety of public Municipal drinking water of the City, in regular basis.
- This study helps to evaluate how drinking water of the city is safely treated, handled and free from burden of microbiological contaminants.
- This study will be base-line information for further studies concerning the Municipal drinking water of the city.
- It will be for Planning and policy development for water quality and safety.
- It will be for drinking water Management and operational information.

2. LITERATURE REVIEW

The evaluation of the microbiological quality of drinking water aims to protect consumers from illness due to consumption of water that may contain pathogens such as bacteria, viruses and protozoa, and thus to prevent drinking-water related illness outbreaks.This has been, and still is nowadays an important challenge. For the past century, this evaluation has been performed through the analysis in finished drinking water of faecal pollution indicators, which are expected to predict the potential presence of pathogenic microorganisms in the water (6).Water is essential to life, but many people do not have access to clean and safe drinking water and many die of waterborne bacterial infections (9). Bacteria, protozoa and viruses are ubiquitous in aquatic environments and may pose threats to water quality for both human and ecosystem health (10). Microbial health risks of regulated drinking waters in the United States to help educate the public about the importance of access to safe drinking water and inform policy makers and the general public about issues such as water distribution systems, infrastructure repair, safe water availability, and EPA's regulation of public water systems for microbial contaminants. The study had shown that water quality breach notification differences means tap water drinkers would consume potentially hazardous drinking water before they are notified (1).

The use of contaminated water in drinking can expose human body to various water borne diseases hence water treatment and improving quality of water before drinking is required. The water samples collected from different sources of different regions in Indore were found to have significant impurities, considerable deterioration and remarkable variation. In the bacteriological analysis, coliform group of bacteria are differentiated by the presumptive test, confirmed test and completed test. The isolates were characterized and identified as *E. coli, Enterobacter, Klebsilla, salmonella* and *Shigella* (11).

The quality of drinking water supplied by water and sanitation agency (WASA) was determined. Sampling units were tube well (direct source) and tap (indirect source) localized in different areas/ towns of Lahore, Pakistan. Bacteriological study was recorded by finding total coliform colonies (4). An assessment study was undertaken to determine the quality and pollution status of source surface waters. A total of 228 water samples were collected from 12 sites and analyzed for one bacteriological quality indicators, using standard methods. The high FCC ranged from

2.0×10^1 to 1.6×10^6 MPN/100 ml and exceeded the WHO standards for drinking water (12). To evaluate the bacteriological analysis of drinking water supply, four samples were collected from Ramanpadu and koilsagar water which both of them were natural reservoir and filtered. For each sample bacteriological parameters were evaluated. The results indicated that the bacterial count was highest in Ramanpadu water (RP-736 CFU/ml) and the least count was found in koilsagar water (KS-06 CFU/ml) (13).

To isolate and identify the micro-organism responsible for the contamination of drinking water, a total of 30 drinking water samples were collected. Presumptive test by multiple tube method and confirmative test by identification of bacterial isolates was carried out. The level of faecal coliform in water distribution network of Jaipur was evaluated in 12 different areas of Jaipur city. Residual chlorine and faecal coliform bacteria was analyzed. It was found that residual chlorine was present in permissible limit in all areas, however, showing the presence of microbes and coliforms (14). The drinking water quality with respect to bacteriological examination by quantitative determination of total coliform and fecal coliform count and presence or absence for *Escherichia coli* were done for 32 numbers of drinkingwater (well, Tube Extended supply, pond and tube well)samples from tea estate areas. Standard methods were used for analysis of total coliform and fecal Coliform bacteria. The samples were examined and found bacterial levels were failed to meet water quality standards. Coliform contamination far exceeds the WHO standards in most cases (15). To determine the microbiological analyses for drinking water samples to match the results with the Sudanese and international standards for drinking water quality as well as identification of the dominating micro-flora in water samples. Samples were taken monthly from different places in Khartoum State and Wadmedani district. These samples were taken from different sites (groundwater, treated and untreated surface water). The microbiological analyses had shown that Wadmedani drinking water samples were highly contaminated with total coliform and fecal coliform compared to Khartoum drinking water samples, it also pointed out that Wadmedani groundwater samples were also highly contaminated with the same microbial groups, and this contamination decreased in the surface water samples (16).

Bacteriological Contamination of well Water was evaluated. According to the investigation a total of 15 Water samples were collected from hand dug Wells and analyzed for total Bacteria

count as it affects the Quality of drinking water for both wet and dry season. The analysis was done according to Standard Methods of Water Examination and as reported in WHO guide limit for drinking water. The investigation revealed that the wells examined were highly contaminated with bacteria (17). Forty eight water samples were collected randomly from different localities. Water samples were subjected to Bacteriological and Parasitological examination. Drinking water samples taken in the study are almost following the WHO and US -EPA standards. For bacteriological examination, 12% of water samples were exceeded the WHO guideline value (>10cfu/100ml), as the total coliform count was determined on each sample through the most probable number of colony forming units method. Parasitological examination revealed that *Giardia* cysts were detected in 25% of water samples and *C*. parvi oocysts were detected in 16.6 % of water samples by both microscopy and ELISA methods (18).

To assess the drinking water quality by analyzing some important biological parameters, standard methods were used to analyze and four microbiological water quality parameters at five selected sampling locations in the water supply system were studied. The samples from four sites were found to be microbiologically unfit for drinking due to the presence of *Escherichia coli* (range 18-96 ± 14 cfu/100 ml) (19). For bacteriological analysis of drinking water of new urban areas and compared the old historical, Ten areas for drinking water samples were selected and samples were collected from water supply, distribution system and storage tanks. Microbial analyses (Total and fecal coli form and *E. coli)* were conducted. According to the results, there were in bacteriological analysis, except one sample collected from the tube well, most samples were Total coliform positive. On the other hand, 6 samples of drinking water from distribution system were *fecal coliform* positive and 4 samples were *E. coli* positive (20). Water access and quality was assessed and water sample was taken from each water source and tested for a variety of microbiological substances. Only 38.9% of the water sources met WHO microbial safety requirements based on fecal coliform levels (21).

To assess bacteriological quality of drinking water, a total of 530 water samples were collected from different localities of whole of the Lahore city. All the samples were tested for contamination with bacteria using multiple tube method to determine most probable number of total coliforms and faecal coliforms using standard procedure. Among 530 water samples, 197 samples (37.2%) were positive for bacterial contamination (22). To isolate and identify the

micro-organism responsible for the contamination of drinking water a total of 30 drinking water samples were collected. Presumptive test by multiple tube method and confirmative test by identification of bacterial isolates was carried out. Out of 30 samples collected 10 (33.33%) were contaminated with either one or more than one type of organisms (23). To deal with the assessment of water supply from different sources which had been designed to assess the potability of the water sources of the city. Samples were collected in the wet, monsoon and winter seasons of two consecutive years for the study. The results of the two yearlong studies were then compiled to assess the actual water quality of the potable water being supplied in the city. During the investigation coliform bacteria were found positive in few of the municipal water supply samples (24).

A cross-sectional epidemiological method was adopted to investigate the four main urban water sources to assess their bacteriological characteristics and suitability for potable purposes (i.e. bottled, desalinated, surface, and well water). A total of 95 water samples from bottled, desalinated, surface, and well water were collected randomly from the study area using different gathering and analyzing techniques. The bacteriological examination of water samples included the most probable number of presumptive coliforms, faecal coliforms, and faecal streptococci. Faecal coliforms were detected in desalinated, surface, and well water, with percentages of 3.23, 60.0 and 87.88, respectively (25). Another cross-sectional study on drinking water quality was conducted. Twelve water samples were collected and analyzed by different microbiological analysis. Microbiological analysis of the samples showed the presence of different microorganisms when the samples were fresh mounted (26). To determine bacteriological quality of drinking water sources, 100 ml of water specimen was collected from water sources and a total of twenty four drinking water samples were analyzed. Twenty three out of the total (87.5%) have presumptive bacteria count above the permissible limits for drinking water (27).

Studies had showed that people on a globe are under tremendous threat due to the contamination of drinking water by different microorganisms. Therefore, the aim of this project was to assess the current bacteriological and parasitological quality and safety status of Addis Ababa municipal drinking water. It had given the recent and up-to-date information about the quality and safety status of the city's drinking water especially bacterial and parasitic contamination level.

3. OBJECTIVE

3.1 . GENERAL OBJECTIVE

To Assess the Bacteriological and Parasitological Quality and Safety status of all sources of Municipal drinking water of Addis Ababa City and identifying the dominating microorganisms in the Public Municipal drinking water Sources.

3.2 . SPECIFIC OBJECTIVES

- To determine the bacteriological quality of all sources of Public Municipal drinking water of the city.
- To determine the Parasitological quality of selected service reservoirs receiving from the three main water sources from where Public Municipal drinking water of the city is produced.
- To assess the safety status of the Public Municipal drinking water sources of the city according to the National, USEPA and WHO safety standards of drinking water quality.

4. MATERIALS AND METHODS

4.1. STUDY SETTING

The study was carried out at the Addis Ababa Water and Sewerage Authority water quality and drainage Administration Microbiology laboratory from June 2015 to October 2015. A total of Two thousand nine hundred fifty one water samples for bacteriological tests and twenty five water samples for parasitological tests were collected and tested from all service sources of public municipal drinking water.

4.2. STUDY AREA

Location

Addis Ababa is the capital city of Ethiopia and diplomatic capital of Africa. It is located in the heart of the country surrounded by mountains. The city covers about 540 Km^2 of which 18.2 Km^2 are rural. The city lies at the foot of the 3,000 meters high of Entoto Mountains. Addis Ababa enjoys a mild, Afro-Alpine temperate climate and it is located at the geographical coordinate of 9ºN and38º 45'E. The city is a base for the African Union and many other international Organizations.

Population

The population of the city is approximately about4 million live in 10 sub-cities and 116 districts divided for administrative purposes. The ten sub-cities are: Addis Ketema, AkakiKality, Arada, Bole, Cherkos, Gullele, KolfeKeraniyo, Lideta, Nefas silk Lafto and Yeka each with an average of 300,000 people.

4.3. STUDY DESIGN

A cross-sectional study design was conducted to assess the bacteriological quality and safety of public municipal drinking water samples from all the public municipal drinking water service sources in different localities of Addis Ababa city Administration, the parasitological quality and safety of Public Municipal drinking water samples were from twenty five service reservoirs

receiving the produced water from the three main water plants and an observational survey technique was held to determine how all water sources were handled, protected, treated and their vulnerability to contamination of public municipal drinking water sources of Addis Ababa City Administration. The laboratory analyses, investigations and observations were carried out by collecting water samples from different sources during June, 2015 to October, 2015.

4.4. STUDY PERIOD

The study had been conducting from October 2014 to June 2016. These include the entire period of title selection and approval, preparation of the proposal, submission and approval, pre-testing, data collection, data analysis, report preparation, and dissemination of the results.

However, the sample collection and laboratory analysis of this study was conducted from June 2015 to October 2015.

4.5. STUDY VARIABLES

4.5.1 DEPENDENT VARIABLES

Bacterial species in Public Municipal drinking water samples

Parasitological species in Public Municipaldrinking water samples

The safety status of public municipal drinking water samples according to the national, USEPA and WHO standards.

4.5.2 INDEPENENT VARIABLES

Type of water samples

Source of water samples

Location of water samples

4.6. SAMPLE SIZE DETERMINATION

All Public Municipal drinking water service sources (public taps, reservoirs, springs and wells) managed by AAWSA were included in to the study. These are: 141 representative public taps, all 33 reservoirs, all 11 springs and all 42 wells.

The sample size of the current study was determined as:

The city Administration was divided into 141 areas in order to represent all places of the city and based on the endpoints, hydraulic zone and topography of distribution system of water sources (reservoirs, springs and wells).

Then 1833 representative samples of Public taps were collected from cafes, factories, hotels, households, offices, restaurants, schools and university.

429 samples from service reservoirs, 143 samples from springs and 546 samples from wells were collected for this study during the wet season of the study area (June to August).

Therefore: 1833+429+143+546

2951 Samples were collected and analyzed for bacteriology and 25 water samples from selected service reservoirs for parasitology.

4.7. SAMPLING METHOD

Purposive sampling method was applied for the study to cover representative public taps, service reservoirs, springs and wells in the city. All service outlets/ reservoirs, all springs and all wells supplying municipal drinking water were included. Sampling sites were fixed and some public tap sites were changed agreed with the AAWSA. The drinking water samples were collected according to the USEPA and WHO sampling methods for bacterial analysis and parasitological examination (28, 29 and 30). The samples were collected between 9:00am to 11:00 am in sterilized glass and plastic containers for bacteriology and parasitology respectively and transported to laboratory in ice (28).

4.8. SAMPLE COLLECTION

The Public Municipal drinking water samples were collected from four service sources of the public municipal drinking water planted in different localities of the city by the principal investigator. Drinking water samples were collected from all service outlets of public municipal drinking water sources in Addis Ababa during June 2015 to October 2015. The sites were from all representatives of public taps, all service reservoirs, all springs and all wells outlets managed by AAWSA, Addis Ababa. All sites were named by unique codes and the results were also reported by their unique codes.

In the study area and sampling sites the water samples were collected from four types of water sources were used in this study. Therefore, in thirteen rounds of sampling, the water samples collected from Public taps were 1833, from service reservoirs 429, from springs 143 and from Wells were 546. A total of 2951 water samples for bacteriological analysis were collected from four Service sources: public taps, service reservoirs, springs and wells managed by AAWSA from June 2015 to August 2015. For bacteriological analysis drinking water samples of 125ml were collected from each type of water sources in sterilized glass bottles with 0.1ml of 3% sodium thiosulphate. Sodium-thiosulphate ($Na_2S_2O_3$) is a quenching agent or a reducing agent added to containers (the sample bottles) before the collection of water having residual chlorine (for treated samples) at the laboratory before sample collection to neutralize the Chlorine present in the sample. Aseptic Sample collection technique was maintained according to WHO and US-EPA standards (Annex 1). These water samples were transported to AAWSA water quality control laboratory case team. The water samples were handled aseptically in sterilized glass bottle, labeled with unique ID and kept in ice box during transportation.

For parasitological tests with the same sampling procedure, twenty five water samples were collected from 25 service reservoirs receiving produced public municipal drinking water from the three main water plants: Akaki, Gefersa and LegeDadi and tested in October 2015. Samples of 11L from each service reservoirs were collected in sterilized plastic jerry cans, labeled with unique ID and kept in ice box during transportation (the standard is 10-50L of water sample for parasitology (31, 32). From 33 service reservoirs 25 were from the three main water plants and the left eight service reservoirs were from wells and springs.

4.9. LABORATORY TESTING

SELECTING A BACTERIOLOGICAL ANALYTICAL TECHNIQUE

The main testing Method to determine the presence of bacteria species in Public Municipal drinking water is Presence/ Absence (P-A) Method.

The advantages of Presence/ Absence (P-A) method are that it is relatively inexpensive, quick and easy to use. Additional advantages include the possibility of examining a larger number of samples per unit of time. Therefore, it is selected for the current study.

Presence-Absence (P-A) Coliform Test

Principle

The presence-absence (P-A) test for the total coliform group is a simple modification of the multiple-tube procedure. Simplification, by use of one large test portion (100 ml) in five culture bottles to obtain qualitative information on the presence or absence of coliforms, is justified on the theory that no coliforms should be present in 100 ml of a drinking water sample after culturing in five culturing bottles (30, 33).

Parasitological Quality Methods

Principle

Ten up to fifty (10-50L) liters of water samples are filtered through a 47 mm diameter, 0.450 μm pore size membrane filters by vacuum pump. The sediment is then prepared for direct microscopy and using a 0.9% saline smears for parasite cysts, trophozoites and helminthes ova/ eggs (31 and 32).

The collected water samples were first filtered through a 47 mm diameter, 0.450 μm pore size membrane filters by a pressure of vacuum pump. The sediments collected on the filter membrane were first transferred into 15mlconical centrifuge tube containing distilled water and centrifuge at 5000rpm for 10 minutes. Finally, the sediment was prepared for direct microscopy using 0.9% saline smears for the detection of parasite cysts, trophozoites and helminthes ova/ eggs.

COLLECTION OF DRINKING WATER SAMPLES

To achieve 2951 samples in 90 days (seven days interval for each sample) for bacteriological analysis, 227 samples were collected from Monday to Sunday with the average of 33 samples per day for 13 rounds weekly. This was to determine the bacterial distribution in the drinking water sources during the whole wet in the city. Drinking water samples for parasitological tests were collected and filtered at September to October daily per sample since the vacuum pump was accounted 1 day and half (12 working hours) to filter and complete 11L of the sample. Finally, to achieve 25 parasitological testes 37 days were stayed.

4.10. QUALITY ASSURANCE

For water sample collection distilled water from the study laboratory was taken to all sample collection sites and carry along with the water sample back to the laboratory. Then both water samples were analyzed in parallel. Media, Reagents and Samples were run with Positive and Negative controls for the whole quality of the study under the whole supervision of Samplers of AAWSA, Environmentalist of AAWSA water quality case team, Biologists of AAWSA water quality case team.

4.11. STATISTICAL ANALYSIS

Basic descriptive summaries were used to describe measures of central tendencies and dispersion of microbial concentrations. The frequencies and percentages were calculated to evaluate statistical significance of the bacterial and parasitological qualities and safety statuses of each service sources of the water. The data were analyzed by Statistical Package for Social Science (SPSS) statistical software Version 20.0 computer software program. The results were displayed/ shown in tabular and graphic forms.

4.12. ETHICAL CONSIDERATION

Ethical clearance was obtained from the Ethical clearance and Research Review committee of Addis Ababa University College of Health Sciences. A Supporting Letter with Ethical clearance informing the Addis Ababa Water and Sewerage Authority about the objective of the study was obtained from the School of Clinical laboratory Science in collaboration with student research and publication office, College of Health Sciences, Addis Ababa University six days before prior to the actual data collection and laboratory testing.

All water samples were collected and tested with the unique identification code and all confidentiality of the results was maintained with great care. Therefore, the confidentiality of the results of all samples was kept from the time of sample collection up to the end of result dissemination.

5. RESULTS AND DISCUSSION

5.1 . RESULTS

Although there is no globally accepted composite index of water quality, identification of some human pathogenic microorganism indicators in drinking water sources helps to minimize risks associated from contaminants. A drinking water quality index was developed using both health (including microbial) and acceptability measurements. Water of poor quality can cause disease outbreaks and it can contribute to background rates of disease manifesting themselves on different time scales. Periodic researches on the quality and safety of drinking water do not only support public health but also promote socio-economic development and well-being. Therefore, we assessed quality and safety of drinking water in Addis Ababa based on their bacteriological and parasitological contaminants indicators.

5.1.1 BACTERIOLOGICAL ANALYSIS

A. Distribution of faecal coliforms and total coliform organisms in water sources

This study showed that drinking water sources of the municipal drinking water were contaminated with faecal coliforms and total coliforms (Fig 1). Microorganisms were detected in four sources of municipal drinking water sources of Addis Ababa. The overall bacterial result showed that the least results were shown in public taps and reservoirs while the highest results were shown in springs and wells.

Finally, 106 (6%) samples of all public taps, 26 (6%) samples of reservoirs, 34 (24%) samples of springs and 115 (21%) samples of wells were contaminated with bacterial contamination.

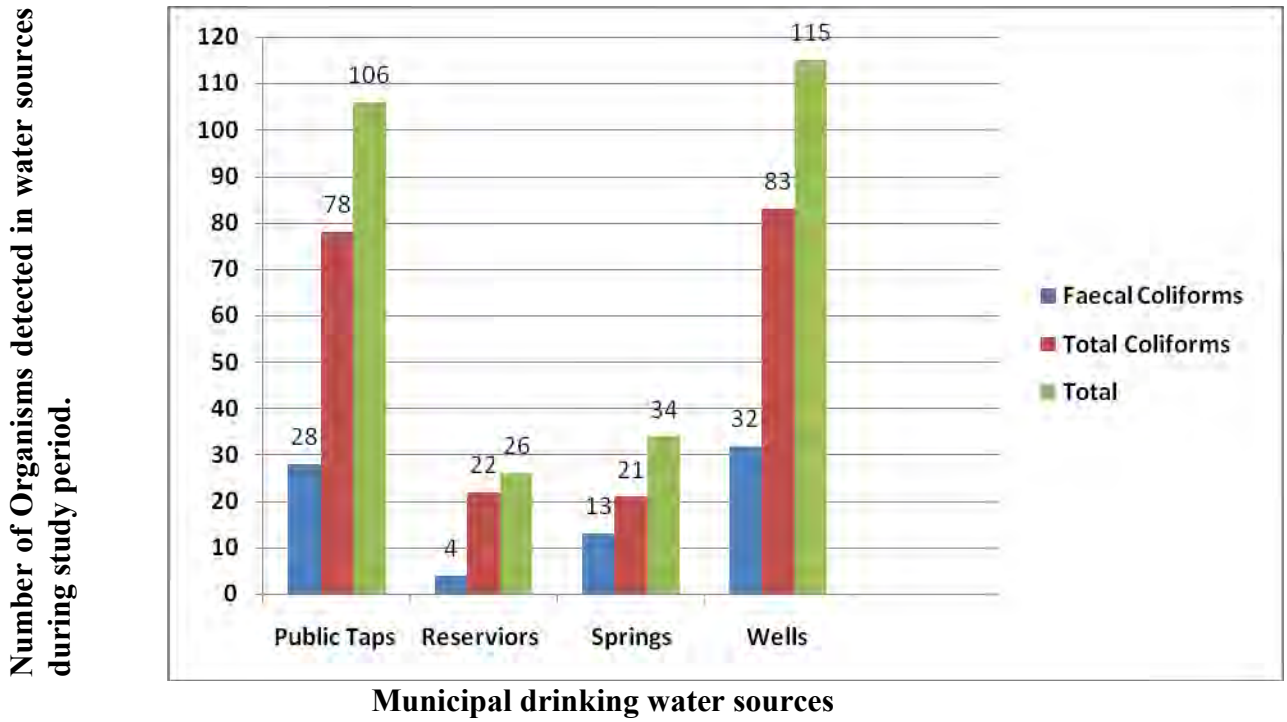

Fig. 1 Distribution of faecal coliforms and total coliform organisms in water sources

B. Distribution of faecal coliforms and total coliforms by week in wet season of Addis Ababa

The municipal drinking water was contaminated with both faecal coliforms and total coliforms throughout the sampling rounds and all weeks of the wet season. The highest faecal coliforms was observe in week 1 and 5 while the highest total coliform was observed in week 11 while (Figure 2). There was a general slight increment of Total coliforms from week 1 to week 13 of the wet season while the number of faecal coliformswas slightly decreasing from week 1 to week 13 of the wet season.

The more frequently the water is examined for faecal indicator organisms, the more likely it is that contamination will be detected. Frequent examination by a simple method is more valuable than less frequent examination by a complex test or series of tests. On the other hand, contamination indicators were slightly varied in weeks of the wet season. The nature and likelihood of contamination can vary seasonally, with rainfall and with other local conditions.

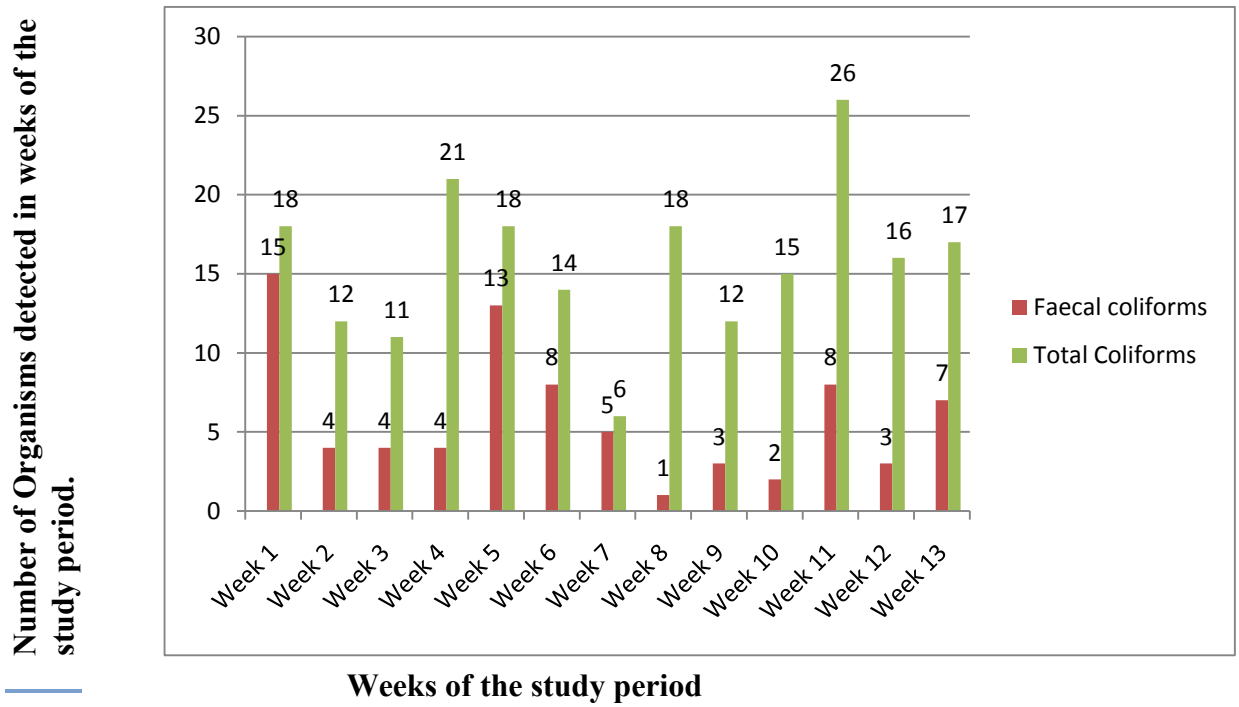

Fig.2 The trends of bacterial distribution from week 1 to week 13 of the first to the end of the wet season in the four sources of Addis Ababa

Table 1 The total summary results of four sources and all collected samples of public municipal drinking water samples in Addis Ababa (June to August 2015)

Sample Source	Total Negative	Total Positive	Total Coliforms	Faecal coliform Positives	Total Samples
Public Taps	1727 (59%)	106 (6%)	78 (4%)	28 (2%)	1833
Reservoirs	403 (94%)	26 (6%)	22 (5%)	4 (1%)	429
Springs	109 (76%)	34 (24%)	21 (15%)	13 (9%)	143
Wells	431 (79%)	115 (21%)	83 (15%)	32 (6%)	546
Total	2670 (90%)	281(10%)	204 (7%)	77 (3%)	2951

The Municipal drinking water samples of 2951 analyzed for bacteriology which accounts 281 (10%) were contaminated. Consequently, 204 (7%) samples were of total coliforms and 77 (3%) were faecal coliforms (table 1). About 90% of all samples were free from contamination and 10% were shown contamination according to this study during the study period and study site.

5.1.2 PARASITOLOGICAL TEST RESULTS

In this study, the parasitological examinations of twenty five selected reservoirs were negative for parasite by conventional microscopic examination as shown table 2. Therefore, the results of selected service reservoirs were free from parasite species. These reservoirs were receiving produced drinking water from Akaki, Gefersa and LegeDadi water plants.

Table 2 the results Parasitological tests of 25 selected service Reservoirs

Serial No	Reservoir Code	Water Capacity	Laboratory test Result
1	2001	5,000m^3	Negative
2	2002	5,000m^3	Negative
3	2003	500m^3	Negative
4	2004	10,000m^3	Negative
5	2006	5,000 m^3	Negative
6	2007	5,000m^3	Negative
7	2008		Negative
8	2009		Negative
9	2011	2,500m^3	Negative
10	2015	1,500m^3	Negative
11	2016	1,000m^3	Negative
12	2017		Negative
13	2018	5,000m^3	Negative
14	2019	7,500m^3	Negative
15	2020	3,000m^3	Negative
16	2021	2,500m^3	Negative
17	2022		Negative
18	2023		Negative
19	2025	5,000m^3	Negative
20	2026		Negative
21	2027	5,000 m^3	Negative
22	2029	500,m^3	Negative
23	2030	2,000 m^3	Negative
24	2031	20,000 m^3	Negative
25	2033		Negative

5.1.3 SAFETY STATUS OF ADDIS ABABA PUBLIC MUNICIPAL DRINKING WATER SOURCES

The safety status of Addis Ababa public municipal drinking water service sources were assessed based on the National, USEPA and WHO safety standards for microbiological quality (Annex 7.4).Water is considered safe for drinking if it contains no coliforms per 100 ml of drinking water. The occurrence of coliforms detected in water is a direct measurement of deleterious effects of pollution of human health. In laboratory, to confirm the water quality to drinking water standards, the actual number of coliforms was not reported but they are mentioned as positive.

There were 2951 municipal drinking water samples collected and analyzed from all municipal drinking water service sources (public taps, reservoirs, springs and wells). The bacterial contaminations were 281 (10%). Public taps account for 106 (6%), service reservoirs 26 (6%), service springs 34 (24%) and service wells account for 115 (21%) for both total coliforms and faecal coliformssuggesting significant number of the drinking water from service springs and service wells sources had recent contamination of these organisms while insignificant number of public taps and reservoirs of the drinking water sources were showed contamination.Finally, about 90% of all samples showed free from bacterial contamination. Therefore, the public municipal drinking water sources were needed great attention and sustainable follow up especially during wet season.

5.1.4 OBSERVATION

The quality and safety of a water supply depends upon proper construction and protection against contamination. A favorable bacteriological analysis alone should not be accepted as conclusive evidence of the quality and safety of a water supply therefore, all sites of water supply sources used for drinking purposes were observed on-site for defects.

Observational check lists for all sampling sites and/ or sources was done to evaluate how the sources were handled, kept, protected and treated by AAWSA and the city population (Annex 7.6). It was observed that most service reservoirs were well kept, well organized and well-

handled even though insignificant number of contamination was detected. This may be due to insufficient amount of chlorination or any other random and/ or technical errors. On the other hand, most springs were highly exposed to heavy rain, flood and microorganism contamination. It was also observed that most private taps were tied with ropes, plastic tubes and pieces of clothes which can harbor microorganisms and help to multiply. In some areas of the city the taps were old and exposed to breakages.

This study had showed that almost all service reservoirs were well organized, well built, well kept. Household pipes (public taps) were tied with ropes, plastic tubes, and pieces of tires which could contain dirt, microorganisms, fungal elements and some other small organisms. Almost all springs and wells of the drinking water sources were highly exposed to flood, rainfall and microorganism contamination.

5.2. DISCUSSION

The microbiological examination of drinking-water emphasizes assessment ofthe hygienic quality of the supply. This requires the isolation and enumeration of organisms that indicate the presence of faecal contamination. In certain circumstances, the same indicator organisms may also be used to assess the efficiency ofdrinking-water treatment plants, which is an important element of quality control.

This study revealed that about 10% of all bacteriological samples were positive for total coliforms and faecal coliforms (Table 1). This finding confirms previous similar studies conducted in different parts of the world that drinking water samples had been contaminated with microorganisms such as microbial with the percentages of 87.5%, 83.34%, 80%, 75%, 66.67%, 50%, 37.2%, 30% and 29.4% (27, 34, 17, 26, 14, 16, 22, 39 and 7), total coliforms with the percentages of 100%, 90%, 70%, more than 51%, 33.33%, 23% and 12% (41, 20, 35, 4, 37 and 18), faecal coliforms with the percentages of 100%, 73.94%, 70%, 61.1% and 40% (12, 25, 38, 35, 21 and 20), both total and faecal coliforms together with the percentages of more than 50%, 50%, 33.33% and 31.2% (40, 13, 23 and 36) and *Escherichia coli* with the percentages of 80%, 78.1%, 70%, 27.1%, 20% and significant number (19, 15, 35, 5, 20 and 11).

The similar study result which was conducted in Jimma by Zemene et al in 2011 had showed that 87.5% of total samples were positive for total coliform bacterial contamination which was done by multiple tube technique (27). On the other hand, the study conducted in Dire Dawa by Gobena et al in 2013 had showed that 83.34% of drinking water samples were contaminated with both total coliforms and faecal coliforms (34). Another similar study conducted in Lahore, Pakistan by Siddiqi et al in 2010 had showed that 37.2% samples were positive for bacterial contamination (22). A similar study conducted in Saudi Arabia in Tabuk different localities by El badawy et al in 2013 had showed that about 12% of bacteriological samples were total coliform positives so that exceeded the WHO guideline value (18). Similarly, the study conducted in Pakistan of the new Urban Peshawar by Khattack Khan in 2013 had showed that drinking water samples from different sources were contaminated with 60% faecal coliforms and 40% *Escherichia coli* (20). These similar study reports were greater than our study results. However, further in-depth study is very important in order to trace the root cause of the municipal drinking water sources contamination and the sanitary practice of the study area city.

This study revealed that 7% of the municipal drinking water samples were contaminated with total coliforms during the study period (Table 1). Coliform bacteria were detected in the sample tested. Sample is considered unsatisfactory for drinking water purposes.Similar studies were conducted in Peshawar, Pakistan by Ahmad et al in 2013, by Bhatnagar et al in 2012 in Jaipur, by Metgaud et al in 2011 in Karnataka and by Rana et al in 2014 in Bhilai and El badawy et al in 2013 in Tabuk, India had showed that 70%, 66.67%, 33.33%, 23% and 12% of drinking water samples were positive for total coliform bacteria contamination respectively (35, 14, 23, 37 and (18). In their study they had revealed that the water sources were highly polluted with pathogenic microorganisms.

This study also revealed that 3% of the municipal drinking water samples were contaminated with faecal coliforms during the study period (Table 1). Faecal coliform bacteria were also detected in the samples tested. Sample is considered unsatisfactory for drinking water purposes. Presence of faecal coliform bacteria indicates fecal contamination of the water supply has occurred. Similar studies conducted by Misra et al in Assam, India in 2010, by Ahmad et al in Peshawar, Pakistan in 2013, and by Stenger et al in 2012 in Bo, Sierra Leonehad showed that 78.1%, 70%and 61% of drinking water samples were contaminated with *Escherichia coli* and faecal coliforms bacteria contamination respectively (15,35and 21).Our study result is very lower than those studies done in different parts of the world. Similar studies conducted by Labhasetwar et al in 2014 in Nagpur, by Sallam et al in Sudan in 2012, by Metgaud et al in 2011 in Karnataka, India and El badawy et al in 2013 in Tabuk, Saudi Arabia had showed that31.2%, 27.1%,20% and 12% samples were contaminated with Faecal coliforms and *Escherichia coli*respectively (36, 5, 23and 18). Thesewere greater than our study result revealing that there were big differences of contamination between our study result and the study results revealed from different parts of the world.

In developing countries particularly in Ethiopia, drinking water is obtained from different sources. Such as taps, reservoirs, springs and wells. This study revealed that samples collected from the municipal drinking water sources of this study area were contaminated with microorganisms during the study period. Accordingly, 6% of drinking water samples collected from public taps (4% total coliforms and 2% faecal coliforms) positive for bacterial groups; and 6% of drinking water samples collected from service reservoirs (5% total coliforms and 1%

faecal coliforms) were positive for bacterial groups (Table 1). Similar studies conducted by Bhatnagar et al in 2012 in Jaipur, in Lahore, Pakistan by Siddiqi et al in 2010, by Traistaruin Cyprus in 2011, by El badawy et al in 2013 in Tabuk of Saudi Arabia had showed that 66.67%, 37.2%, 14%, 12% of bacteriological samples were contaminated with total coliforms (14, 22, 39, and 18). These study results were greaterthan our study results in big differences. Other similar studies had showed that most samples collected from reservoirs were both total and faecal coliforms positives whereas; in some reservoirs the contaminations were with similar bacterial groups (13 and 20).

This study also revealed that 24% samples from springs (14% total coliforms and 9% faecal coliforms) were positive for bacterial groups. On the other hand, 21% samples from wells (15% total coliforms and 6% faecal coliforms) were positive for bacterial groups. Similar studies by AlOtaibi, Saudi Arabia 2009,Rajasekaran et al, India 2011 and Woyessa et al, Jimma 2013had showed that 57.58%,12.0% and large number of diverse group of bacterial load of samples from well water were found positive for faecal streptococci and Microbial pollution was recorded during laboratory analysisrespectively (25, 7 and 40).

This study revealed that the highest total coliform was observed in week 11 while the highest faecal coliforms was observed in week 1 and 5 (Figure 2). There was a general slight increment of total coliforms from week 1 to week 13 of the wet season while the number of faecal coliforms was slightly decreasing from week 1 to week 13 of the wet season.Similar studies had showed that due to flash flooding and heavy rainfall were found microbiologically unfit for drinking due to the presence of *Escherichia coli*, *Shigella*, *Salmonella* and *Staphylococcus aureus* (19).A thorough study was done on the basis of prevailing seasons so thattotal coliforms in water sample in highest value were reported in wet season fromNarmada, India in 2013 (41).

This finding concise with many reports presented on drinking water affected flash flooding, by rainfall frequency, intensity, and/or duration leading to increased runoff. The study had showed by Young et al in 2016 that the impact of climate change on prevalence of water sources contamination with *Cryptosporidium* and *Giardia* were estimated to be equivalent because, drinking water quality was expected to be impacted primarilythroughincreased rainfall frequency, intensity, and/or duration leadingto increased runoff and higher levels of Cryptosporidium and Giardia insource water (42).

This study also revealed that all samples collected for parasitological quality and safety assessment were negative for parasitological species. Similar study by El badawy et al in 2013 in Tabuk, Saudi Arabia had showed that parasitological examination revealed that giardia cysts were detected in 25% of water samples and C. parvi oocysts were detected in 16.6 % of water samples by both microscopy and ELISA methods (18). This was may be due to the water sources differences where wells and surface water samples were used and/ or ELISA method while our sample sources were from treated service reservoirs and direct microscopy respectively.Similar study had showed by Rostami et al, 2015 in Shush, Iran 40% samples were infected with at least one of the active stages of parasitic organisms; out of these, 28.7% the protozoa and 18 11.2% were infected with the worm process of living organisms. 6.3% were related to the parasite *Entamoeba histolytica*. Therefore according to the study prevalence of 3.6% of drinking water in the city of Shush to *Entamoeba histolytica*, is remarkable, that need to health planning to reduce it (43). Even though, our study was similar methodology to the report from Shush, Iran, the differences were sample sources. According to Gobena et al in 2015 water analysis demonstrated that all water sources from Dire Dawa were contaminated by pathogenic parasites. From the recapitulate results, above (83.34%) of unprotected wells water sources, (50%-100%) from unprotected springs and protected wells, (33.34%-66.67%) from protected springs and (50%) from tap water were positive both for the presences of *Cryptosporidium* oocysts and *Girdia lamblia* cysts (44).

This study revealed that about 90% of the samples were free from bacteriological contamination. 90% of drinking water samples were total coliform and faecal coliform negative (total coliform and faecal coliform bacteria were not detected in the samples tested). Therefore, Samples were considered satisfactory for drinking water purposes.Relatively it was safer than different countries reports except Jordan. Studies had showed that different countries had reported their drinking water safety. Accordingly, Ethiopia 88%, Jordan 100%, Nicaragua 86%, Nigeria 77% and Tajikistan 88% (45). Hence, the majority of the samples were safe for drinking. Samples collected for parasitological tests revealed that all of themwere free from parasitic species. Samples were negative for parasite species.

5.3. CONCLUSION

The use of indicator organisms, in particular the coliform group, as a means of assessing the potential presence of water-borne pathogens in drinking water has been of paramount importance in protecting public health. The principle of the detection of selected bacteria that are indicative of either contamination or deterioration of water quality has been the foundation upon which protection of public health from water-borne diseaseshas been developed.

It was concluded that the contamination detected in the water sources were due to improper or insufficient chlorination, random and technical faults during operation of service reservoirs, poor handling and accidental contamination of public taps. On the other hand, the contamination detected in most springs and wells were majorly due to the rainy season of the study area and/ or study period as during the wet season heavy rain and flood can easily carry contaminants of human and animal wastes from the environment and contaminate the springs and wells. This study concluded that most Addis Ababa city drinking water sources had acceptable quality and were safe to drink.

The overall distribution of total coliform organisms throughout the wet season could be due to real contamination from flood, heavy rains, and their living characteristics i.e. in the environment, on animals and humans. The overall distributions of faecal coliforms were also determined throughout the wet season. However, they showed general decrement from the first week to the end of the season. This reveals that there were faecal contaminants as the immediate emergence of heavy rain and flood which brought about the contaminations of taps (where their facets were nearer to the ground), springs and wells.

These all performances, so much utilization of energy and resources were aimed to protect the public health from both bacteriological and parasitological contaminants that their contaminations harm the public health and its economy by disabling the public from production and service providing and also by costing the public for waterborne treatment. It was also to set a base-line for further researches to conduct on the area of drinking water as safe and quality water is mandatory for all living things.

5.4. RECOMMENDATION

Routine basic with strict follow up of operational and technical procedures in drinking water plants to safeguard the service reservoirs from any contamination of microbiological contaminants of drinking water.

The population of the city has to protect and/ or minimize the taps from microbiological contamination can be caused by human and animal contact. Not fixing, tying and hanging ropes, plastic tubes and pieces of clothes and plastics minimize the harboring and growing of microorganisms on faucets and nozzles. Therefore, the population has to practice keeping the faucets and nozzles neat and clean. The pipes of some city places are older and highly prone to breakage and leakage. Therefore, regular monitoring and maintenance is very important.

Protecting springs and wells of the drinking water sources by building safe them from human, animal, heavy rain and flood contact. Therefore, implementation is the critical strategy.

Regular monitoring of drinking water quality is essential as it is an important factor that has a direct effect on human health.

Operational research need to be conducted to check whether the trend of water quality and safety practices at utility, community and household levels are implemented.

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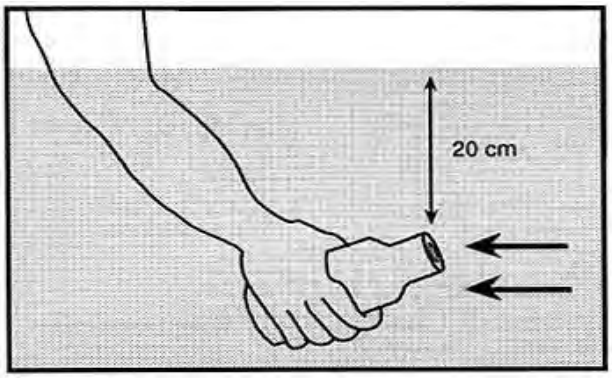
7. ANNEXES

7.1. WATER SAMPLING PROCEDURE

COLLECTION OF DRINKING WATER SAMPLES

Sampling from a tap or pump outlet



1. Clean the tap. Remove any attachments that may cause splashing from the tap. These attachments are a frequent source of contamination that may influence the perceived quality of the water supply. Use a clean cloth to wipe the outlet and to remove any dirt.

2. Open the tap. Turn on the tap to maximum flow and let the water run for 1-2 minutes. Turn off the tap.

Note*:* Some people omit the next two steps and take the samples at this stage, in which case the tap should not be adjusted or turned off, but left to run at maximum flow.

3. Sterilize the tap for 1 minute with a flame (from a gas burner, cigarette lighter or an alcohol-soaked cotton wool swab).

4. Open the tap before sampling. Carefully turn on the tap and allow water to flow at medium rate for 1 - 2 minutes. Do not adjust the flow after it has been set.

5. Fill the bottle. Carefully remove the cap and protective cover from the bottle, taking care to prevent entry of dust that may contaminate the sample. Hold the bottle immediately under the water jet to fill it. A small air space should be left to allow mixing before analysis. Replace the bottle cap.

7.2. PROCEDURE OF BACTERIOLOGICAL ANALYSIS

Presence-Absence (P-A) Coliform Test

The P-A test also provides the optional opportunity for further screening of the culture to isolate other indicators (fecal coliform, fecal streptococcus, and others) on the same qualitative basis. Additional advantages include the possibility of examining a larger number of samples per unit of time. Comparative studies with the membrane filter procedure indicate that the P-A test may maximize coliform detection in samples containing many organisms that could overgrow coliform colonies and cause problems in detection (31).

Since some non coliform bacteria can also ferment lactose, this first test is called a "presumptive" test. Bacteria from a positive tube can be inoculated into a medium that selects more specifically for coliforms, leading to "confirmed" results. Finally, the test can be "completed" by subjecting positive samples from the confirmed test to a number of additional identification steps. Each of the three steps (presumptive, confirmed and completed) requires 1-2 days of incubation. Typically only the first two steps are performed in coliform and faecal coliform analysis, while all three phases are done for periodic quality control or for positive identification of *E. coli* (31).

"P/A testing was developed for and is applicable where most tests provide a negative result. Where a significant proportion of tests provide a positive reaction quantitative testing is preferred in order to determine relative health risk and therefore relative priority of need for correction, such as by improved or greater treatment or by finding a higher quality source water for supply" (31, 34)

1. Presumptive Phase

A. Culture Media: **1. P-A broth**:This medium is commercially available in dehydrated and in sterile concentrated form.

Beef extract 3.0g Peptone 5.0g

Lactose 7.46g Tryptose 9.83g

Dipotassium hydrogen phosphate, (K_2HPO_4) 1.35g

Potassium dihydrogen phosphate, (KH_2PO_4) 1.35g

Sodium chloride, (NaCl) 2.46g	Sodium lauryl sulfate 0.05g
Bromcresol purple 0.0085g	Reagent-grade water 1L

Make this formulation triple (3×) strength when examining 100-ml samples.

Dissolve the P-A broth medium in water without heating, using a stirring device.

Dispense 50 ml prepared medium into a screw-cap 250-ml milk dilution bottle. A fermentation tube insert is not necessary.

Autoclave for 12 min at 121°C with the total time in the autoclave limited to 30 min or less. PH should be 6.8 ± 0.2 after sterilization. When the P-A medium is sterilized by filtration a 6× strength medium may be used. Aseptically dispense 20 ml of the 6× medium into a sterile 250-ml dilution bottle or equivalent container.

B. Lauryl Tryptose broth: Shake sample vigorously for 5s (approximately 25 times) and inoculate 100 ml into a P-A culture bottle. Mix thoroughly by inverting bottle once or twice to achieve even distribution of the triple-strength medium throughout the sample. Incubate at 35±0.5°C and inspect after 24 and 48 h for acid reactions.

C. Interpretation*:* A distinct yellow color forms in the medium when acid conditions exist following lactose fermentation. If gas also is being produced, gently shaking the bottle will result in a foaming reaction. Any amount of gas and/ or acid constitutes a positive presumptive test requiring confirmation.

2. Confirmed Phase

A. Culture Medium:Use brilliant green lactose bile fermentation tubes.

1. Transfer all cultures that show acid reaction or acid and gas reaction to brilliant green lactose bile (BGLB) broth for incubation at 35 ± 0.5°C.

B. Interpretation:Gas production in the BGLB broth culture within 48 ± 3 h confirms the presence of coliform bacteria.

Report result as presence-absence test positive or negative for total coliforms in 100ml of sample.

3. Completed Phase

Fecal Coliform Test (EC Medium)

The fecal coliform test is used to distinguish those total coliform organisms that are fecal coliforms.

EC medium:Tryptose or trypticase 20.0g Lactose 5.0g
Bile salts mixture or bile salts No.3 1.5g
Dipotassium hydrogen phosphate, (K_2HPO_4) 4.0 g
Potassium dihydrogen phosphate, KH_2PO_4 1.5g
Sodium chloride, NaCl 5.0g
Reagent-grade water 1L

Add dehydrated ingredients to water, mix thoroughly, and heat to dissolve. PH should be 6.9 ± 0.2 after sterilization. Before sterilization, dispense in fermentation tubes, each with an inverted vial, sufficient medium to cover the inverted vial at least partially after sterilization. Close tubes with metal or heat-resistant plastic caps.

Submit all presumptive fermentation tubes or bottles showing any amount of gas, growth, or acidity within 48 h of incubation to the fecal coliform test.

Gently shake or rotate presumptive fermentation tubes or bottles showing gas, growth, or acidity.

By using a sterile 3-or 3.5mmdiameter loop or sterile wooden applicator stick, transfer growth from each presumptive fermentation tube or bottle to EC broth.

Incubate inoculated EC broth tubes in a water bath at 44.5 ± 0.2°C for 24 ± 2 h.

Place all EC tubes in water bath within 30 min after inoculation.

Maintain a sufficient water depth in water bath incubator to immerse tubes to upper level of the medium.

Interpretation:Gas production with growth in an EC broth culture within 24 ± 2 h or less is considered a positive fecal coliform reaction. Failure to produce gas (with little or no growth)

constitutes a negative reaction. When using only one tube for sub-culturing from a single presumptive bottle, report as presence or absence of fecal coliforms.

Confirmation Phase

Use brilliant green lactose bile broth fermentation tubes for the confirmed phase.

Brilliant green lactose bile broth: Peptone 10.0 g Brilliant green 0.0133g

Oxgall 20.0g Lactose 10.0g

Reagent-grade water 1L

Add dehydrated ingredients to water, mix thoroughly, and heat to dissolve. PH should be 7.2 ± 0.2 after sterilization. Before sterilization, dispense, in fermentation tubes with an inverted vial, sufficient medium to cover inverted vial at least one-half to two-thirds after sterilization.

Close tubes with metal or heat-resistant plastic caps.

Submit all presumptive tubes or bottles showing growth, any amount of gas, or acidic reaction within 24 ± 2 h of incubation to the confirmed phase. If active fermentation or acidic reaction appears in the presumptive tube earlier than 24 ± 2 h, transfer to the confirmatory medium; preferably examine tubes at 18 ± 1 h. If additional presumptive tubes or bottles show active fermentation or acidic reaction at the end of a 48 ± 3- h incubation period, submit these to the confirmed phase.

Gently shake or rotate presumptive tubes or bottles showing gas or acidic growth to resuspend the organisms. With a sterile loop 3.0 to 3.5 mm in diameter, transfer one or more loopfuls of culture to a fermentation tube containing brilliant green lactose bile broth or insert a sterile wooden applicator at least 2.5 cm into the culture, promptly remove, and plunge applicator to bottom of fermentation tube containing brilliant green lactose bile broth. Remove and discard applicator.

Repeat for all other positive presumptive tubes. Incubate the inoculated brilliant green lactose bile broth tube at 35 ± 0.5°C. Formation of gas in any amount in the inverted vial of the brilliant

green lactose bile broth fermentation tube at any time (e.g., 6 ± 1 h, 24 ± 2h) within 48 ± 3 h constitutes a positive confirmed phase.

2. Confirmed Phase

A. Culture medium:Use brilliant green lactose bile fermentation tubes.

1. Transfer all cultures that show acid reaction or acid and gas reaction to brilliant green lactose bile (BGLB) broth for incubation at 35 ± 0.5°C.

B. Interpretation:Gas production in the BGLB broth culture within 48 ± 3 h confirms the presence of coliform bacteria.

Report result as presence-absence test positive or negative for total coliforms in 100ml of sample.

3. Completed Phase

Faecal Coliform Test (EC Medium)

The fecal coliform test is used to distinguish those total coliform organisms that are fecal coliforms.

EC medium: Tryptose or trypticase 20.0g Lactose 5.0g
Bile salts mixture or bile salts No.3 1.5g
Dipotassium hydrogen phosphate, (K_2HPO_4) 4.0 g
Potassium dihydrogen phosphate, KH_2PO_4 1.5g
Sodium chloride, NaCl 5.0g Reagent-grade water 1L

Add dehydrated ingredients to water, mix thoroughly, and heat to dissolve. PH should be 6.9 ± 0.2 after sterilization. Before sterilization, dispense in fermentation tubes, each with an inverted vial, sufficient medium to cover the inverted vial at least partially after sterilization. Close tubes with metal or heat-resistant plastic caps.

Submit all presumptive fermentation tubes or bottles showing any amount of gas, growth, or acidity within 48 h of incubation to the fecal coliform test.

Gently shake or rotate presumptive fermentation tubes or bottles showing gas, growth, or acidity.

Using a sterile 3 or 3.5-mmdiameter loop or sterile wooden applicator stick; transfer growth from each presumptive fermentation tube or bottle to EC broth.

Incubate inoculated EC broth tubes in a water bath at 44.5 ± 0.2°C for 24 ± 2 h.

Place all EC tubes in water bath within 30 min after inoculation.

Maintain a sufficient water depth in water bath incubator to immerse tubes to upper level of the medium.

Interpretation:Gas production with growth in an EC broth culture within 24 ± 2 h or less is considered a positive fecal coliform reaction. Failure to produce gas (with little or no growth) constitutes a negative reaction. When using only one tube for sub-culturing from a single presumptive bottle, report as presence or absence of fecal coliforms.

ISOLATION MEDIA	**USES**	**INCUBATION TEMPERATURE**	**REMARKS**
Lactose broth	Total or thermo-tolerantcoliforms	48 hours at 35 ± 0.5 °C or 37 ± 0.5 °C for total coliforms and 24 hours at 44 ± 0.25 °C or 44.5 ± 0.25 °C for thermo tolerantcoliforms	Prepare single strength medium by diluting double strength medium with distilled water. Each tube or bottle should contain an inverted fermentation (Durham) tube.
Brilliant green lactose bile broth	Total or thermo-tolerant coliforms (gas production)	44.5 ± 0.25 °C for thermo-tolerant coliforms	As above
EC medium	Thermo-tolerant coliforms (indole production)	44.5 ± 0.25 °C for thermo tolerant coliforms	As above

7.3. PROCEDURE OF PARASITOLOGICAL QUALITY METHODS

Filter Ten up to fifty (10-50L) liters of water samples through a 47 mm diameter, 0.450 μm pore size membrane filters by the pressure of vacuum pump.

Mix the sediment with 5 ml distilled water and centrifuge for direct microscopy and using a 0.9% saline smear the sediment for parasite cysts, trophozoites and helminthes.

Wet mount preparation

Place a drop of suspended sediment on a clean glass slide;

Cover with cover slip and examine microscopically using x10 and x40 objective lenses.

Place a drop of suspended sediments on a drop of iodine solution using Pasteur pipette.

Cover the mixture with cover slip and examined microscopically using x10 and x40 objective lenses.

7.4. SAFETY STANDARDS OF DRINKING WATER SOURCES

Based on the National standard, ES 261:2001:

- Any treated water shall not contain faecal and coliform organisms when tested with the corresponding methods.
- Any treated water shall not contain any faecal streptococci when tested with the corresponding methods (3).

Organism	Maximum Permissible level
Total viable Organisms, colonies per ml	Must not be detectable
Faecal Streptococci per 100 ml	Must not be detectable
Coliform organisms, number per 100 ml	Must not be detectable
E. coli, number per 100 ml	Must not be detectable

Based on the USEPA Standard: (1)

Microbiological Contaminants	Microbiological Containments Level (MCL)
Total coliform	No MCL in source water No MCL in finished water
Escherichia coli (E. coli)	No MCL in source water None detected in finished water None detected in any of the follow-up samples if initial sample is positive

Based on the WHO Standard (4):

Organisms	**Guideline value**
All water directly intended for drinking *E. coli* or thermo-tolerant coliform bacteria	Must not be detectable in any 100 ml sample
Treated water entering the distribution system *E. coli* or thermo-tolerant coliform bacteria	Must not be detectable in any 100 ml sample
Treated water in the distribution system *E. coli* or thermo-tolerant coliform bacteria	Must not be detectable in any 100 ml sample

7.5. SUMMARIES OF THE ADVANTAGES AND DISADVANTAGES OF THE COMMONLY USED CULTIVATION TECHNIQUES(46)

Technique	Advantages	Disadvantages
Presence/Absence (P/A) test using liquid media	• Flexible sample volume range • Applicable to all kinds of samples • Allows resuscitation and growth of injured organisms. • Usually easy interpretation of test results and no special skills required. • Minimal time and effort needed to start the test. • The precision and sensitivity can be chosen by selection of volumes analyzed, number of dilution levels and number of replicate tubes. • Media often inexpensive.	• In routine application, when few replicates are used, the precision is often low. • Confirmation steps involving new cultivations are usually needed, which increase costs and time. • When the selectivity of the medium is not adequate, the target organisms can be masked due to the growth of other microorganisms. • Sample may contain inhibitors affecting the growth of the targetorganisms. • For the isolation of pure cultures, further cultivation on solid media is necessary. • If big sample volumes are studied costs of media increase and large space for incubation is needed. • No information on level of concentration of target organisms.
Most probable number (MPN) using liquid media	• Flexible sample volume range • Applicable to all kinds of samples • Allows resuscitation and growth of injured organisms • Usually easy interpretation of test results and no special skills required • Minimal time and effort needed to	• In routine application, when few replicates are used, the precision is often low • Confirmation steps involving new cultivations are usually needed, which increase costs and time • When the selectivity of the medium

	start the test • The precision and sensitivity can be chosen by selection of volumes analyzed, number of dilution levels and number of replicate tubes • Media often inexpensive	is not adequate, the target organisms can be masked due to the growth of other microorganisms • Sample may contain inhibitors affecting the growth of the target organisms • For the isolation of pure cultures, further cultivation on solid media is necessary • If big sample volumes are studied costs of media increase and large space for incubation is needed.
Pour plate	• Simple and inexpensivemethod	• The sample volume analyzedroutinely is a maximum of 1 ml • Thermal shock, caused when melted agar is poured on the sample, inhibits sensitive organisms • Scoring of typical colonies not easy.
Spread plate	• Strictly aerobic organisms are favored because colonies grow on the agar surface (unless anaerobic conditions are applied). • Differentiation of the coloniesis easier than from pour plates	• The sample volume analyzedroutinely is a maximum of 0.1ml • Scoring of typical colonies not always easy.
MembraneFiltration	• Flexible sample volume range enabling the use of large sample volume and therefore increased sensitivity • Water soluble impurities interfering with the growth of target organisms separated from the sample in the	• Quality of membranes varies. • Solid particles and chemicalsadsorbed from sample to themembrane during filtration may interfere with the growth of the target organism.

	filtration step. • Quantitative result and goodprecision if the number of colonies grown adequate. • Further cultivation steps notalways needed, which lowers the costs and time needed for the analysis • When confirmation is needed,isolation from well separated colonies on membrane is easy.	• Not applicable to turbid samples. • Scoring of typical colonies not always easy.
Liquid enrichment + confirmation and/or isolation on solid media	• Liquid enrichment in favorable media and incubation temperature allows resuscitation of injured or stressed cells. • Streaking of a portion of enrichment culture on an agar medium allows isolation of separate colonies. • Differentiation and preliminary identification is possible on selective solid media. • Detection and identification of organisms occurring in low numbers possible (*e.g. Salmonella*).	• Many cultivation steps increase costs of media, labour, skills needed and duration of the test.

7.6. QUESTIONS FOR THE SANITARY RISK OBSERVATION (47)

I. PUBLIC TAP (PIPED WATER)

1. Is the tap sited outside the house (e.g. in the yard)? YES NO

2. Are any taps leaking or damaged? YES NO

3. Are any taps shared with other households? YES NO

4. Is the area around the tap unsanitary? YES NO

5. Are there any leaks in the household pipes? YES NO

6. Do animals have access to the area around the pipe? YES NO

9. Is there any exposed pipeline to outside and old looking pipes? YES NO

II. RESERVOIR

A. TREATMENT PROCESS

1. Are there evident cracks in the pre-filters? YES NO

2. Are there leaks in the mixing tank? YES NO

3. Is the mixing tank in an unsanitary condition? YES NO

5. Is any sedimentation tank in an unsanitary condition? YES NO

7. Are there mud balls or cracks in any of the filters? YES NO

8. Are there evident cross-connections between backwashed and treated water? YES NO

9. Are free residual chlorine concentrations (minimum 0.2 mg/l) not being achieved? YES NO

B. DISTRIBUTION SYSTEM

1. Do any taps or pipes leak at the sample site? YES NO

2. Does water collect around the sample site? YES NO

3. Is the area around the tap unsanitary? YES NO

4. Is there a sewer or latrine within 30m of any tap? YES NO

5. Is the supply main pipeline exposed in the sampling area? YES NO

6. Is the supply tank cracked or leaking? YES NO

7. Are the vents and covers on the tank damaged or open? YES NO

8. Is the inspection cover or concrete around the cover damaged or corroded? YES NO

III. SPRING

1. Is the collection/ spring box absent or faulty? YES NO

2. Is the backfill area behind the retaining wall absent or eroded? YES NO

3. Does spilled water flood the collection area? YES NO

4. Is the fence absent or faulty? YES NO

5. Can animals have access within 10 m of the spring? YES NO

6. Is there a latrine uphill and/or within 30m of the spring? YES NO

7. Does surface water collect uphill of the spring? YES NO

8. Are there any other sources of pollution uphill of the spring (e.g. solid waste)? YES NO

IV. WELL

1. Is there a latrine within 10 m of the well? YES NO

2. Is the nearest latrine uphill of the well? YES NO

3. Is there any source of other pollution within 10m of the well (e.g. animal breeding, cultivation, roads, industry, etc)? YES NO

4. Is the drainage absent or faulty, allowing ponding within 3m of the well? YES NO

5. Is the drainage channel absent or cracked, broken or in need of cleaning? YES NO

6. Does spilt water collect in the apron area? YES NO

7. Is the hand pump loose at the point of attachment, or for rope-washer pump: is the pump cover missing? YES NO

8. Is the well-cover absent or unsanitary? YES NO

9. Is the well prone to flood, rainfall and contamination? YES NO

7.7. OPERATIONAL DEFINITIONS

Bacteriological water analysis: is a method of analyzing water to estimate the numbers of bacteria present and, if needed, to find out what sort of bacteria they are. It represents one aspect of water quality. It is a microbiological analytical procedure which uses samples of water and from these samples determines the concentration of bacteria.

Microbiological water quality test: Microbiological contamination of drinking water is the existence of organisms in water including viruses, bacteria, protozoa and algae deteriorating water quality.

Potable Water: is defined as having acceptable quality in terms of its physical, chemical, and bacteriological characteristics so that it can be safely used for drinking and cooking. Is a water that is safe to drink, pleasant to taste and useable for domestic purposes.

Safe drinking water:is water with microbiological, chemical and physicalcharacteristics that meet WHO guidelines or national standards on drinking waterquality.
Service Sources: Are drinking water producing sources such as public taps, reservoirs, springs and wells.

Thermo-tolerant (Fecal Coliforms) (TTC/FC): Thermo-tolerant/ Fecal coliforms bacteria are a subset of the total coliforms group, with the same definition as total coliforms bacteria except that they grow at 44.5^{o}C.

Total coliforms bacteria: Are classically defined as "All facultative anaerobic, gram-negative, non-spore forming, oxidase-negative, rod-shaped bacteria that ferment lactose to acid and gas within 48 hours at 35^{o}C. Total coliforms are the ones that are commonly measured as indicator bacteria for drinking water quality. They are defined as aerobic and facultatively anaerobic non spore forming bacteria that ferment lactose at 35 to 37^{o}C with the production of acid and gas within 24-48 hours.

Unsafe water: Water that contains disease causing microorganisms and presence of indicators of disease-causing organisms.

DECLARATION OF PRINCIPAL INVESTIGATOR

I the undersigned agree to accept all responsibilities for the scientific and ethical conduct of the research project. I provided timely progress report to my advisors and seek the necessary advice and approval from my primary advisors in the course of the research. I communicated timely to my advisors and all stakeholders involved in the study including any source of funding for this research.

Name of the Student: Amsalu Mekonnen

Signature: _____

Date: _____

Approval of the Advisors

Name of Advisors:

Gebru Mulugeta (MSc, Assistant Professor)

Signature: _____

Date: _____

Kassu Desta (MSc, Assistant Professor)

Signature: _____

Date: _____