ADDIS ABABA UNIVERSITY

COLLEGE OF HEALTH SCIENCES

SCHOOL OF ALLIED HEALTH SCIENCE

DEPARTMENT OF MEDICAL LABORATORY SCIENCES

VIROLOGICAL AND BACTERIOLOGICAL QUALITY OF SELECTED DRINKING WATER SAMPLES REFERRED TO ETHIOPIAN PUBLIC HEALTH INSTITIUTE

Principal Investigator: Tesfaye Legesse (BSc)

A THESIS SUBMITTED TO THE DEPARTMENT OF MEDICAL LABORATORY SCIENCES OF ADDIS ABABA UNIVERSITY IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF MASTERS IN CLINICAL LABORATORY SCIENCES (DIAGNOSTIC AND PUBLIC HEALTH MICROBIOLOGY)

ADVISOR: Kassu Desta: Assistant Professor of Medical microbiology, Department of Medical Laboratory science, School of Allied Health Science, Addis Ababa University.

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Name of investigator	Tesfaye Legesse
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Address of investigator	Tel: 0912139197
	Cell phone: 0112771053
	Fax: 251112758634
	P.o.Box: 1242
	Email: tesfayelegesse21@gmail.com
Name of Advisor(s)	Mr Kassu Desta (phD Fellower), Walelign Dessie (MSC)

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Principal investigator name: Tesfaye Legesse (BSc)

Signature: _____ Date: _____

Examiner 1 name: _____

Signature: _____ Date: _____

Examiner 2 name: _____

Signature: _____ Date: _____

Advisor name Mr. Kasu Desta (BSc, Msc, PhD candidate, Assistant professor)

Signature _____ Date: _____

Chairman of the school/post graduate coordinator name_____

Signature: _____ Date: _____

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List of Abbreviations

CDC	US-Centre for disease Control and Prevention
CFU	Colony forming unit
DNA	Deoxyribonucleicacid
E. coli	*Escherichia coli*
E. coli	*Escherichia coli*
EC broth	*Escherichia coli* broth
EPA	United States Environmental Protection Agency
EPHI	Ethiopian Public Health Institute
HPC	Heterotrophic plate count

ISO	International Standards Organization
$MgCl_2$	Magnesium chloride
ML	Milliliter
MPN	Most probable number
PCA	Plate count agar
PFU	Plaque-forming unit
RADWQ	Rapid assessment of drinking water quality
RNA	Ribonucleic acid
Rpm	Revolution per minutes
SAL	Single agar layer
TSA	Tryptic soy agar
WHO	World Health Organization

Operational definitions

Coliforms: Gram-negative, non-spore-forming, lactose and gas producer rod shaped bacteria at 37 °C within 48 hours.

Coliphages: viruses (bacteriophages) that infect coliform bacteria.

Drinking water: water comes from improved or unimproved sources and used for drinking.

Drinking water of good quality: Water contains no any viral or bacterial indicative of faecal pollution.

Famp *E. coli*: resistant to streptomycin and ampicillin (host)

Host bacteria: bacteria that allow the bacteriophage to penetrate and replicate within them.

Indicator organisms: non pathogen found in large numbers in the faeces used to indicate pathogens.

Male *E. coli*: bacterial cells that express small appendages called pili.

Male-specific coliphages (F+): RNA or DNA viruses that infect via the F-pilus of male strains of *E. coli.*

Plaque: Circular zones of clearing (1 to 10 mm in diameter) in lawn of host bacteria in SAL after incubation.

Somatic coliphages: DNA viruses that infect host cells via the outer cell membrane.

Abstract

Background: Water Quality monitoring is assessed widely using different indicators. Since there is no universal indicator that has been identified yet, assessing water quality using bacterial and viral indicators that would provide a more complete picture of water quality.

Objective: To assess the quality of drinking water using bacterial and cophages indicators.

Methodology: A cross sectional prospective study was conducted on 218 drinking water samples of various sources collected from some regions of Ethiopia from February to June 2016 to determine coliphages by the help of CB390 *E. coli* host using plaque assay technique; most probable number for coliforms and pouring for hetrotrophic plate count at Ethiopian Public Health Institute. The data was analyzed using SPSS 20 statistical package.

Results: Hetrophilic bacteria, total and thermotolerant coliforms, *E. coli* and coliphages were detected in 72.9 %, 51.8%, 38.5%, 23.9% and 2.3 % of total water samples respectively. HPC > $1.0x10^2$ Cfu/ml were noted in 41 (18.8%) water samples and detections of total coliforms, thermotolerant coliforms and *E. coli* in 38 (17.4%), 24 (11.0%) and 10 (4.6%) samples respectively and no detection of phages in chlorinated waters. While, HPC > $1.0x10^2$ Cfu/ml were observed in 100 (45.9%) water samples and detections of total and thermotolerant coliforms, *E.coli* and coliphages in 75 (34.4%), 60

(27.5%), 42 (19.3%) and 5 (2.3%) samples respectively for the untreated waters. Rho values between *total coliphages* and other indicators, HPC, total coliforms, thermotolerant coliforms *were* 0.202, 0.232 and 0.269 respectively. Total heterophilic plate count**,** total coliforms, thermotolerant coliforms, *E.coli* and total coliphages were statistically differed by region; sources and treatment type (P value < 0.05). *Coliphages* were not detected in all waters except rivers.

Conclusion: Majority of the waters, mainly untreated sources contained bacterial and viral indicators above the standard limits. This indicates that the sources are contaminated with environmental and fecal contaminants signifying poor quality of water and it is a potential threat for human health. Hence regular monitoring of water source using coliphages and other bacterial indicators should be a priority agenda by all stake holders.

Keywords: Indicators, drinking water, water sources, coliforms, coliphage

1. Introduction

1.1. Background

Quality and safety assessment of drinking water is used to investigate and to compel action to respond to and rectify incidents of contamination-caused outbreaks of waterborne disease or other threats to public health. Safe drinking-water is essential to health, a basic human right and a component of effective policy for health protection (1), yet safe drinking water for all is one of the major challenges of the 21st century (2). According to WHO report, 663 million people rely on unimproved sources, including 159 million dependent on surface water by 2015 globally. Over 40 percent of all these people are Sub-Saharan Africans (3).

In Ethiopia, 63% of the population relied on unimproved water sources and only one fifth was served by utility piped supplies (4); 52.1% still used unimproved sanitation facilities in 2014 and 35.6% practiced open defecation (5).

Even where the source is good, water can be contaminated while being transported in environments where sanitation is inadequate. A substantial proportion of water supplied through pipes is contaminated, especially where water supply is intermittent or treatment is inadequate. (3).

Water quality monitoring is assessed widely using indicators. Coliforms have been used for routine monitoring of water quality, because they are easier and less expensive to detect than the pathogens themselves (2). Since there is no universal indicator has been identified (6), testing of water using coliforms and coliphages would give a more complete picture of water quality. Due to cost constraints and the complexity of earlier coliphages methods, requiring testing for both indicators was previously not considered economically feasible (7).

Coliforms measure levels of fecal contamination in water distribution systems. Their existence reflects the presence or absence of pathogens. They are facultative anaerobic, rod-shaped, Gram-negative bacteria, lactose fermenters and produce acid and gas within 48 hours at 35-37^{o}C (8). Since coliforms respond to environment and water treatments, much less similar, and they have larger size compared to viruses (9), they are not representative of viral contamination (10). Even

if total coliforms are mainly used for the assessment of sanitary water quality after the water has been treated and disinfected, they are not feces-specific. Thermotolerant coliforms are more closely related to fecal pollution. *Escherichia coli (E. coli*) is the most specific to fecal contamination (11).

Another fecal contamination monitoring tools in drinking water are Coliphages. They have been proposed as a good indicator of human enteric viruses, because they resemble enteric viruses in their structure, composition, survivability in the environment, persistence in treatment processes compared to coliform and found in higher numbers than enteric viruses in contaminated water (12).

Coliphages are bacteriophages that infect and destroy *E. coli* and other coliforms. The phages are often found in the intestinal tract of humans and animals (13). They have a head or "capsid" that contains genetic material, a tail or "sheath", and tail fibers used to attach the phage to the host cell (14). Two main groups of coliphages are male-specific and somatic coliphages. Male-specific coliphages are either DNA or RNA viruses that infect *E. coli via* pilli. They are also known as, F+, and F RNA coliphages. Somatic coliphages are DNA viruses that infect bacterial hosts by direct attachment to cell walls (15). Phages infection of bacterial cells occurs when the phage first has to attach itself to the host cell where it then separates its nucleic acid from the protein coat and enters. Using the replication mechanisms of the host, they then begin making new viral particles, taking over the cell's functions. Eventually the phage causes lysis of the host cell (16).

Plaque assay is a typical culture-based technique used for enumerating virus particles (17,18). This method has been applied to various water sources (19-22). The Single- agar layer (SAL) method is the recommended over other method of coliphage detection. It requires fewer steps to perform, takes less time to give results and that such simultaneous detection be on total coliphage host *E. coli* CB390 (23), thus reducing cost and workload to detect and quantify total coliphages in water (24).

A secondary indicator employed to evaluate water quality and sanitation standard is heterotrophic plate count (HPC). Heterotrophic bacteria are aerobic and facultative anaerobic organisms encompass an extremely broad range of genera in water. HPC is important because the large number of bacteria may suggest the presence of opportunistic pathogens of non faecal origin,

potential increased possibilities of taste, odour and corrosion problems in the distribution system (25).

1.2. Statement of the problem

Water quality is a major issue of all countries. Inadequate access to safe water and sanitation services negatively impact health, nutrition and economy (26- 28). In terms of human health, the most dangerous water pollutants are pathogenic microorganism (29). Infectious diseases caused by pathogenic bacteria, viruses and parasites (e.g. protozoa and helminths) are the most common and widespread health risk associated with drinking-water (1). The greatest risk to public health from microbes in water is associated with consumption of drinking-water that is contaminated with human and animal excreta (1). Every year, more people die from the consequences of unsafe water than from all forms of violence, including war (30). Waterborne pathogens transmit diseases to around 250 million people each year resulting in 10 to 20 million deaths around the globe (31). These waterborne pathogens continue to occur as outbreaks (32) and contribute 80% of health problems in developing countries (33).

Ethiopia is such a country facing water quality problems due to Poor water supplies and sanitation facilities. As a result, majority of the health problems in Ethiopia are due to communicable diseases. There is little information on water quality assessment using cost effective coliphages detection worldwide and there is no single study on water quality assessment using coliphages in Ethiopia context. It is therefore essential to assess quality of various drinking water sources using various indicators and this research will fill the gap and sensitize further research to determine water quality in the country.

1. 3. Significance of the study

virological and bacterial quality assessment of drinking water has a vital role in the protection of consumers from illnesses due to the consumption of water that may contain pathogenic microbes such as bacteria, viruses and protozoa, thus prevent water-related disease outbreaks. Frequent examination of these indicator organisms is very important to assess the sanitation conditions of water. Although, virological quality of water hasn't been assessed in most countries including Ethiopia and the results of this research can contribute to provide the base line data on water

safety from that provided by bacterial indicators on quality of drinking water. Since bacterial indicators currently used for water quality monitoring are not indicators of viruses and the results obtained can help in risk assessment of drinking water quality by indicating Viral and fecal contaminations. This research involved the use of single host (*E. coli* CB390) which could improve our understanding of the method that minimizes resource and time to perform the tests in simultaneous detection of both somatic and male-specific coliphages.

2. Literature Review

As literature has revealed, coliforms and coliphages should be included in water safety testing as each indicates a different microbial risk (34). Of all Waterborne Outbreaks frequently occurred worldwide, more than 50% illness are caused by enteric viruses. As a result, coliphages were added as another fecal indicator of viral surrogate (35). Coliforms are used globally to assess the microbiological safety of waters. However, waterborne viral outbreaks have occurred in drinking water systems despite negative bacterial results. Conversely, no discernible public health outcomes have occurred in systems with positive coliform results due to the large number of environmental organisms like *Klebsiella* species (35). Therefore, phages are increasingly being used as indicators to confirm human fecal contamination presence in waters and have been shown to be more highly correlated to pathogen presence in a variety of waters than coliforms. Even though there are some drawbacks using total coliforms as indicators, detection of these indicators in drinking water may indicate failure in the treatment system, regrowth or infiltration in the distribution system which might have serious health implications (36).

A study shows, Coliphages are present in large numbers in sewage (approximately 10^8 plaque forming units [PFU] per milliliter [mL]) (37). They are considered reliable indicators of human enteric viruses in faecally contaminated water (38) due to coliphages and enteroviruses are removed at comparable rates during treatment processes. Coliphages are at least as resistant to environmental stresses and to chlorination as enteroviruses, and that coliphages exhibit a seasonal variation similar to that of enteroviruses. Also, both coliphages and enteroviruses have been found in chlorinated drinking water, sometimes in the same samples (39).

In a study carried out by Jeanine D, 2014 on a total of 72 treated and untreated surface water and ground water in the United State of America, total coliforms detected in 21 (29.2%), *E. coli* in 14 (19.4%) and total coliphages in 21% with maximum count of 880 pfu/100 mL of the samples. The presence of viruses was high in the presence of coliphage than coliform (37).

A study was performed involving 40 samples from different potable water sources for water quality analyses by Mookerjee S et al., 2014, in Municipality of West Bengal, India. Altogether, 30% water samples were contaminated with coliform and 5% with *E. coli*, 55% outbreak samples with coliphage contamination, the study revealed that 80%, double than that of bacteriological identification rate, were contaminated with coliphages (4-20 pfu/10 ml (40).

Similar study carried out by Keitumetse L, 2015 on 16 potable water samples of the North West Province of South Africa showed a serious problem in the availability of safe and clean water. In this study large numbers (189 PFU/100 mL) of somatic bacteriophages plagues in 15 of the 16 potable water samples and F-RNA bacteriophages in two of the samples was detected which indicated that these water samples did not comply with the standards (9).

Further study by Marthie ME et al., on 10 different bottled water from different sources in South Africa, Egypt, and three brands of bottled drinking water were tested for total coliform, fecal coliform, and coliphage populations. The results indicated that 8/10 of the bottled water samples analysed, met the requirements set for HPC in bottled water of less than 100 counts per ml. However, in two bottled water samples the average HPC bacteria counts were 2.64 x 102 cfu·ml-1 and 8.89 x 103 cfu·ml-1 respectively. Total and faecal coliform bacteria bacteriophages were not detected in any of the ten bottled water samples analysed. (41).

As a study indicated, using multiple indicators including both a coliphages indicators as well as bacterial indicators, rather than measuring either one alone can more efficiently determine water sources vulnerability to fecal contamination (42).

One study evaluated the use of a single *E. coli* host strain CB390 which was preferred in detecting both somatic and male-specific coliphages when compared to the sum of Famp and CN13 coliphage totals tested individually since sample preparation requires less time and resources in order to effectively quantify the phage present within a given water source. SAL allowing a more fast and direct approach to experimental results (43).

As reviled in some literatures, heterotrophic plate count is used as an indicator of drinking water microbiological quality and the spectrum of organisms detected by HPC testing includes organisms sensitive to disinfection processes, such as coliform bacteria; organisms resistant to disinfection, such as spore formers; and organisms that rapidly proliferate in treated water in the absence of residual disinfectants. The test can be useful in operational monitoring as a treatment and disinfectant indicator, where the objective is to keep numbers as low as possible. In addition, HPC measurement can be used in assessing the cleanliness and integrity of distribution systems and the presence of biofilms (1).

As research indicated, faecal streptococci are also used as indicators of the microbiological quality of drinking-water. They survive longer in a water environment and are more resistant to drying and chlorination and are recommended for monitoring groundwater subject to receiving contaminated recharge water and for monitoring water quality in chlorinated distribution systems (4)

As drinking water guidelines indicated, for Protozoa and helminthes, among the most common causes of waterborne infection that have a major public health and socioeconomic impact due to their resistant to conventional environmental conditions or treatment technologies, it may be desirable to include more resistant microorganisms, such as coliphages and/or *Clostridium perfringens*, as indicators of persistent microbial hazards. *(1)*

3. Objectives and hypothesis

3.1. General Objective

To assess quality of drinking water *in selected water samples in Ethiopia using coliphages and bacterial indicators.*

3.2. Specific objectives

- To determine the quality of drinking water by the presence and concentration of somatic and F-RNA bacteriophages.
- To monitor the quality of drinking water using *E .coli*, thermotolerant coliform and total coliform.
- To determine quality of water using total heterotrophic bacteria.

3.3. Hypothesis

There is no difference in water quality using colliphages and other bacterial indicators.

4. Methods and Materials

4.1. Study Area

The study was carried out on treated (chlorinated) and untreated drinking water samples from treated and untreated piped, dug well, river and bottled drinking waters from different locations of Addis Ababa city administration and Oromia, Amhara, Southern Nations, Nationalities, and Peoples' Region (SNNPR) and Afar regional states of Ethiopia.

4.2. Study design and period

A cross sectional prospective study was conducted from February to June 2016.

4.3. Sample size and sampling technique

All 218 drinking water samples referred to EPHI, Public Health Microbiology laboratory, between February and June 2016 from health offices for the assessment of microbial quality of water were included in the study. Convenience sampling technique was used.

4.4. Measurement

4.4.1. Dependent Variables

Water quality interms of Coliphages level, Mesophilic plate count, total coliforms, thermotolerant coliforms enumerates and *E. coli* detection.

4.4.2. Independent Variables

Water sources: Treated and untreated Piped water, well water, bottled drinking water, river water; Chlorinated water and non chlorinated water and regions.

4.5. Data Collection procedures

The data was collected using pre-developed template containing all information regarding water sample analysis. The type of water whether it was chlorinated or not, water source, time of collection, place of collection, sample code, type of laboratory tests and all necessary information was filled using data collection form.

4.5.1. Sample collection

Samples were collected from piped water (123 samples), from dug well (69 samples), from bottled water (13 samples) and from river) (13 samples) by trained Environmental Health Officers from the water quality monitoring units in respective health offices of various regions. A 500mL of drinking water was sampled. The Health Officers were well informed to use aseptic techniques to collect the samples and sterile container provided by the Central Laboratory. A format developed with the facility name, location, date and time of collection, name of analytical facility, contact, and phone number, sample number and sample location information for each sample was maintained. Latex gloves or hand washing as thoroughly as possible with soap and hot water was used to collect water. When collecting the sample, the interior or mouth of the container, or the container caps were never be touched with fingers, clothing or unsterile objects. The collection of water samples from different water sources were collected into 500 ml bottles and transported on ice to public Health Microbiology laboratory in Ethiopian Public Health Institute. Water samples containing residual chlorine were neutralized by adding pre-sterilized 0.5 mL sodium thiosulphate (3% w/v) per 500 mL of water sample. The samples were stored at 2°C–8°C in a dark area to avoid changes in microbial count until analysis. Microbiological investigations were performed within 24 hours after collection (44).

4.5.2. Laboratory methods

All samples were tested for heterophlic plate count, total coliforms, fecal coliform, *E. coli*, male-specific coliphages, and somatic coliphages using accepted methodologies from Standard Methods or the US Environmental Protection Agency (45- 47).

4.5.2.1. HPC Assay

Water samples were Pipetted into the sterile petri dish. After thoroughly mixing, the melted plate count agar (PCA) was Poured into the dish by gently lifting the cover just high enough to pour. The lid was replaced when finished. The melted medium was mixed thoroughly with the sample in the petri dish by swirling in a figure-eight motion with the petri dish on the bench top. The plates were placed on a level surface and let them solidify. The plates were inverted and placed in a plastic bag, sealed and placed in an incubator. The plates were incubated for 48 ± 3 hours at 35 ± 0.5 °C by inverting (45).

4.5.2.2. Coliform assay

The total coliform count test was based on the multiple tube fermentation method to estimate the most probable number (MPN) of total coliform, fecal coliform and *E. coli* in 100 mL of water. *The* coliforms were estimated per 100 ml of water. The test was carried out by incubated for 48 hours at 37°C of measured quantities of sample water (50, 10, 1 mL) into tubes of double- and single-strength MacConkey broth (Difco). The tubes showing gas formation were considered to be presumptive coliform positive. For the confirmed stage, tubes showed acid and gas in the presumptive test were inoculated in brilliant green lactose bile broth to observe gas production after 48 hours of incubation at 37°c. Then, *E. coli broth and nutrient broths were inoculated and incubated at 44.5^{0}c for 48 hours for gas production and indole test respectively (46).*

4.5.2.3. Coliphages Assay

For the simultaneous detection of both types of male-specific and somatic Coliphages, single agar layer plaque assay using *E. coli* coliphage host CB390 log phase containing 0.15% ampicillin was applied with magnesium chloride (MgCl2) in double strength tryptic soy agar (TSA) [Difco]. The Coliphages were incubated for 16-24 hours (47).

4.5.3. Interpretation of results

4.5.3.1. HPC

All colonies on plates were promptly counted after incubation using Colony Counters feature a built-in grid to simplify counting. A back and forth pattern, moving down the grid way was followed to count the colonies, and all colonies were counted as colony-forming units (CFU)/mL. Results were rounded to two significant digits to avoid creating false precision. If plates from the plates have no colonies, the count was reported as less than one (<1) CFU/mL (45). Drinking water samples with HPC above permissible limit of >1.0x10^2 cfu/ml when incubated at 37°c had low quality (48).

4.5.3.2. Coliforms

and incubated at 44.5°c for 48 hours for gas production and indole test respectively.(46)

The presence of coliforms was confirmed by the production of gas from lactose at 37°C, and that of *E. coli* was confirmed by the production of gas from lactose and indole from tryptophan at 44°C. *The* coliforms were estimated per 100 ml of water using MPN tables (46). Total coliforms, thermotolerant coliforms and *E. coli* above Ethiopian drinking-water standard CES 58: 2013 and WHO guideline value of "no detections/100ml" for drinking water was unsafe (49,1).

4.5.3.3. Coliphages

Coliphages were enumerated in water sample after overnight incubation that infected host cells produce localized areas of infection of the confluent growth of *E. coli* in the agar medium, resulting in the formation of clear zones of lysed *E. coli* cells called plaques. From the number of plaques counted in the culture plate and the volume of sample analyzed in the culture plate, the coliphage counted was computed per 100 mL of the sample (47) and total coliphages above WHO guideline of >1 pfu/100ml was unsafe (1).

4.6. Quality Assurance

All developed formats containing different information about water samples were checked and registered on log book prepared for this purpose. All materials such as water samples bottles, sodium thiosulphate and culture media were sterilized. The samples were run within 24 hours of collections. Refrigerators, incubators and water baths were monitored for their performance. Sterility and performance tests were done for all culture media. For each activity standard operational procedure was followed. MS2 stock coliphage (ATCC#15597-B1) and *E. coli* Famp were used for quality control and host *E.coli* CB390 strain for quality control and running the procedure. All data were check for completeness and representativeness prior to entry. Negative and positive controls were used for both bactrriological and virological indicators, method blanks as negative control and MS2 stock titer as positive controls for Single Agar Layer Plaque asay.

4.7. Data Management

All information filled in the request formats and test done was checked for completeness every time. Each data collected was handled by registering in triplicate.

4.8. Data Analysis procedures

The data was entered, cleaned and analyzed using SPSS statistical package version 20.0. To reduce bias in the statistical analyses, values different from zeros (0.5) were used for results of no detections (50). The non parametric Kruskall-Wallis test was used to find out the differences in indicators values by regions, water sources and treatment types and to observe the associations among the various indicators; the Spearman Rank Correlation were used because of non- normal distributed data and unequal size of the samples among the datasets. To check the normality of data and the presence of outliers, Shapiro-Wilk test was used. The significance level was set at p value $\leq$ 0.05. The results of bacteriological and virological analyses were compared with established Ethiopian standards and WHO guidelines of drinking water.

4.9. Ethical consideration

Written authorization to carry out the study was obtained from Ethiopian Public Health Institute using an agreement letter prepared from departmental research and ethical Review committee of Addis Ababa University, College of Health Sciences, Department of Medical Laboratory Sciences. The results about water analyzed were reported immediately to water quality team of respective health offices of the regions. All information that was obtained about the subject was kept confidential.

4.10. Dissemination of results

The results were disseminated to respective regions of health offices by filling water analysis result forms. To the research community, the results will be disseminated through presenting at conferences and publication in peer-reviewed scientific journals and to the general public through different media.

5. Results

5.1. Characteristics of the variables

A total of 218 water samples were tested from Addis Ababa (n=105), Oromia (n=101), Amhara (n=6), SNNPR (n=5) and Afar (n=1) regions of Ethiopia between February and June 2016. Out of these, 123 samples were from piped waters, 69 samples from dug wells, 13 samples from bottled waters of eight brands and 13 samples from rivers. One hundred thirteen (51.8%) of the waters samples were untreated. Out of the total samples collected, 86 samples, 15 samples and four samples were piped, dug wells and bottled waters from Addis Ababa; 33 samples, 51 samples, nine samples and eight samples were piped, dug wells, bottled and rivers waters from Oromia; four samples and two samples were piped and wells water from Amhara respectively; five samples were rivers waters from SNNPR and one sample was well from Afar region (Table 5. 1). The results included total heterotrophic plate count**,** total and thermotolerant coliforms, *E.coli* and total coliphages detections.

Table 5. 1: ***Treatment status of source water type for various regions of Ethiopia included in the study*** **between February and June 2016**

Regions	**Treatment**	**Water sources**				Total
		Piped	Well	BW	River	
AA	Treated	69	0	4	0	73
	untreated	17	15	-	0	32
Oromia	Treated	22	0	9	0	31
	untreated	11	51	-	8	70
Amhara	Treated	1	0	0	0	1
	untreated	3	2	-	-	5
SNNPR	untreated	0	0	-	5	5
Afar	untreated	0	1	-	0	1
Total		123	69	13	13	218

BW–Bottled water, AA–Addis Ababa, SNNPR– Southern Nations, Nationalities, and Peoples' Region.

5. 2. Microbial quality of water samples

Of the total 218 water samples tested from various water sources, hetrophilic bacteria, total and thermotolerant coliforms, *E. coli* and total coliphages (male and somatic) were detected in 72.9

% (n=159), 51.8% (n=113), 38.5% (n=84), 23.9% (n=52) and 2.3 % (n=5) of the water samples respectively. Heterotrophic plate counts (HPC) of greater than $1.0x10^2$ Cfu/ml were observed in 141 (64.7%) water samples. High counts (>$3.0x10^2$ cfu/ ml) were found in 22.5% (n= 49) of all samples for HPC; greater than 180 MPN/100ml in 17% (n=37) of the samples for total coliform and 11.5% (n=25) of the samples for thermotolerant coliforms.

Of the total water samples for 92 chlorinated water samples, HPC > $1.0x10^2$ Cfu/ml were observed in 41 (18.8%) samples. Detections of total coliforms, thermotolerant coliforms and *E. coli* in 38 (17.4%), 24 (11.0%) and 10 (4.6%) samples respectively and no detection of total coliphages. While, for 113 untreated waters, HPC > $1.0x10^2$ Cfu/ml were observed in 100 (45.9%) samples. Detections of total and thermotolerant coliforms, *E.coli* and total coliphages in 75 (34.4%), 60 (27.5%), 42 (19.3%), and 5 (2.3%) samples respectively. In all the total coliphages detected samples, all indicators, heterotrophic bacteria, total coliforms, thermotolerant coliforms and *E. coli* were also detected, inversely in the absence of thermotolerant coliforms or *E. coli*, there were no total coliphages detections.

The detections of heterotrophic bacteria, total coliforms, thermotolerant coliforms by treatment types of water samples were shown in *table 5.2 to table 5.4.*

***Table 5. 2**: Total heterotrophic bacteria counts in treated and untreated drinking water samples using pouring method in different regions of Ethiopia between February and June 2016*

	***Heterotrophic* plate count (cfu/ml)**

Type of water water	<1	1-1.0x10^2	1.01x10^2-2.0x10^2	2.0x10^2-3.0x10^3	>3.0x10^2	Total	%	ES/ WHO guideline
chlorinated	41	10	26	6	9	92	42.2	<100cfu/ ml
Unchlorinated	8	5	50	10	40	113	51.8	
Bottled water	10	3	0	0	0	13	6	
Total	59	18	76	16	49	218	100	
%	27.1	8.3	34.9	7.3	22.5	100	100	

Cfu: colony forming unit WHO: world health organization ES: Ethiopian standard

Table 5. 3*: Estimation of total coliforms in treated and untreated drinking water samples using MPN method in different regions of Ethiopia* between February and June 2016

Type of water water	Total coliforms count (MPN/100ml)						
	<1	1-10	10-100	101-180	>180	Total	ES/ WHO guideline
chlorinated	4	16	14	3	5	92	<1mpn/100 ml
Un-chlorinated	8	18	23	2	32	113	
Bottled water	3	0	0	0	0	13	
Total	15	34	37	5	37	218	

MPN–most probable number, WHO: world health organization, ES: Ethiopian standard

Table 5.4: Estimation of thermotolerant coliform in treated and untreated drinking water samples using MPN method in different regions of Ethiopia between February and June 2016

Type of water	Thermotolerant coliforms (mpn/100ml)						
	<1	1-10	10-100	101-180	>180	Total	ES/ WHO guideline
chlorinated	68	21	2	1	0	92	<1mpn/100 ml
Un-chlorinated	53	23	11	1	25	113	
Bottled water	13	0	0	0	0	13	
Total	134	44	13	2	25	218	

MPN –most probable number WHO: world health organization ES: Ethiopian standard

The results of Spearman's rank correlation coefficient r of HPC, total coliforms, thermotolerant coliforms *and total coliphages* were shown in Table 5.5.

Table 5. 5: Rho values of HPC, total coliforms, thermotolerant coliforms and total coliphages in water samples between February and June 2016

Parameters	HPC	Total coliforms	Thermotolerant coliforms	Phage
HPC	1			
Total coliforms	0.754	1		
Thermotolerant coliforms	0.677	0.816	1	
Phage	0.202	0.232	0.269	1

P-values for HPC, total coliforms, thermotolerant coliforms and total coliphages using non parametric Kruskall-Wallis test for drinking water samples by region, source and treatment type were < 0.05.

Regionally, HPC >$1.0x10^2$ cfu/ml were recorded in 55 of 105 (52.4%) water samples and total coliforms detected in 42 of 105 (40%) water samples; thermotolerant coliforms in 31 of 105 (29.5%) water samples; *E. coli* in 14 of 105 (13.3%) water samples and no total coliphages in water samples in Addis Ababa city administration. HPC >$1.0x10^2$ cfu/ml were recorded in 78 of 101 (77.2%) water samples and total coliforms detected in 63.4% (64 of 101) water samples; thermotolerant coliforms in 46 of 101 (45.5%) water samples; *E. coli* in 32 of 101 (31.7%) water samples and total coliphages in 2 of 101 (2%) water samples in Oromia region. HPC >$1.0x10^2$ cfu/ml were recorded in 2 of 5 (40%) water samples and total coliforms, thermotolerant coliforms and *E. coli* detections in One of five (33.3%) water samples and no total coliphages detection in water samples in Amhara region. All water samples in the SNNPR (5 of 5) had HPC >$1.0x10^2$ cfu/ml and positive for total coliforms, thermotolerant coliforms and *E. coli and* total coliphages were detected in two of the five samples (40%). One of one water sample of Afar had HPC >$1.0x10^2$ cfu/ml and positive for total coliforms and thermotolerant coliforms, but negative for *E .coli* and total coliphages.

5. 2. 1. Bacteriological and virological indicator detection in treated water sources

Of 92 chlorinated drinking waters, 41(44.6%) samples had HPC >$1.0x10^2$ cfu/ml and 38 (41.3%), 24 (26.1%), and 10 (10.9%) samples were positive for total coliforms, thermotolerant coliforms and *E. coli* respectively and no detection of total coliphages. Out of 69 treated piped waters in Addis Ababa city administration, HPC >$1.0x10^2$ cfu/ was recorded in 25 (36.2%) samples and 23 (33.3%), 17 (24.6%) and 6 (8.7%) samples were positive for total coliforms, thermotolerant coliforms and *E. coli* respectively and no detection of total coliphages in the samples. Of 22 treated piped waters in Oromia, HPC >$1.0x10^2$ cfu/ml was recorded in 16 of 22 (72.7%) samples and 15 of 22 (68.2.4%), 7 of 22 (31.8%) and 4 of 22 (18.2%) samples were positive for total coliforms, thermotolerant coliforms and *E. coli* respectively and no detection of total coliphages in the water samples. The details of HPC enumeration ranges, *total coliforms, thermotolerant coliforms estimation ranges and E. coli detection in various regions, sources and treatment types of waters were shown in figure 5.1 to figure 5.4.*

All eight brands of 13 bottled drinking water samples in Addis Ababa (N=4) and Oromia (N=9) had HPC < 100 cfu/100; total and thermotolerant and *E. coli* of <1mpn/100ml and total coliphages of <1 pfu/100ml.

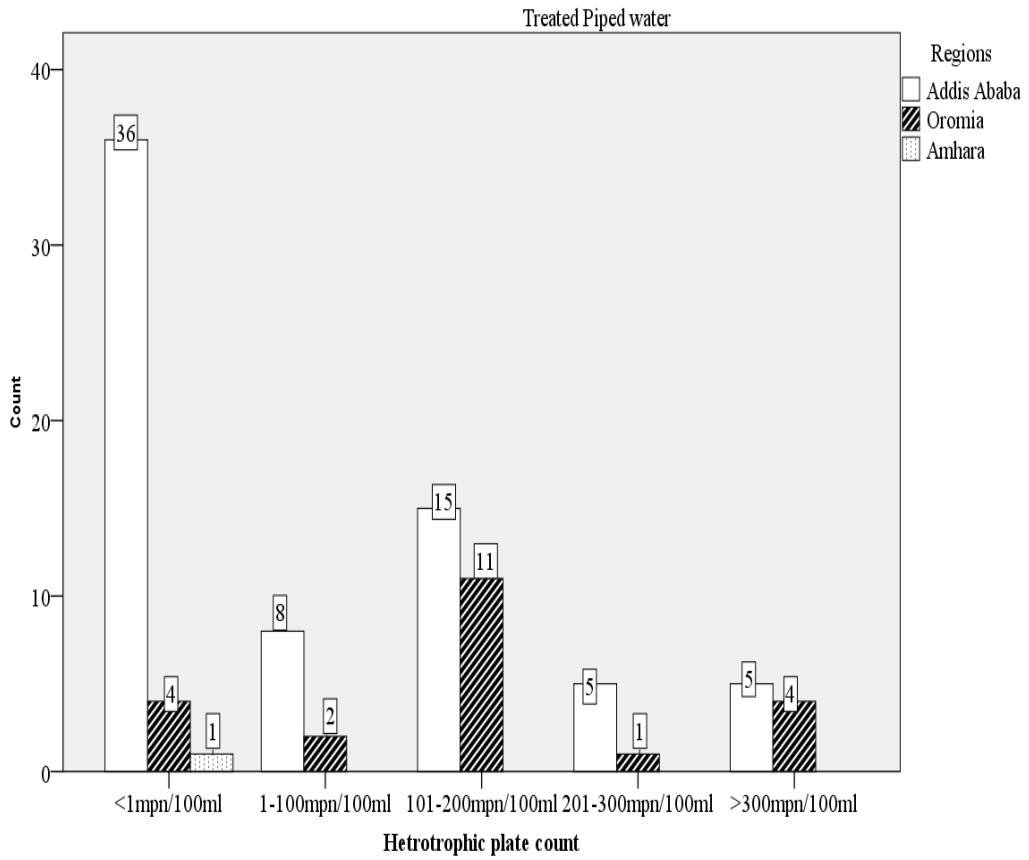

Fig.5.1: ***Enumeration of total heterotrophic bacteria in various treated piped water sources in different regions of Ethiopia*** **between February and June 2016**

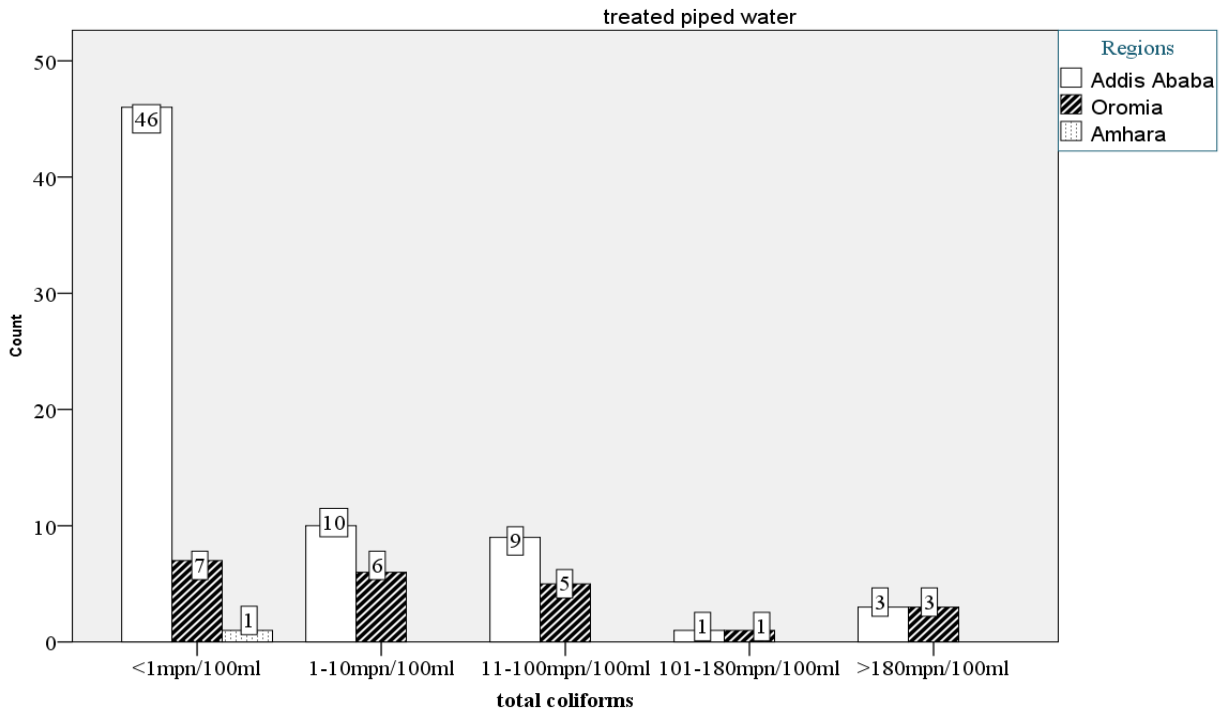

Fig.5.2: ***Estimation of total coliforms in treated piped drinking water source samples using MPN method in different regions of Ethiopia*** **between February and June 2016**

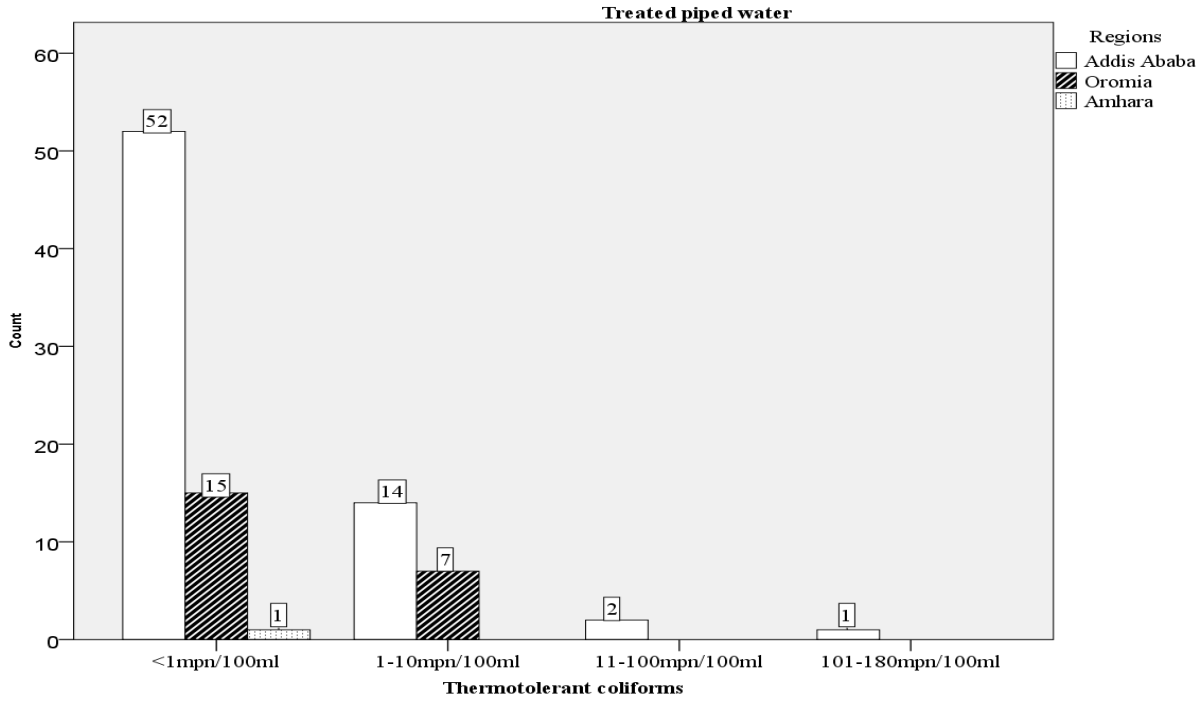

Fig.5.3*: Estimation of thermotolerant coliforms in treated piped water samples using MPN method in different regions of Ethiopia* **between February and June 2016**

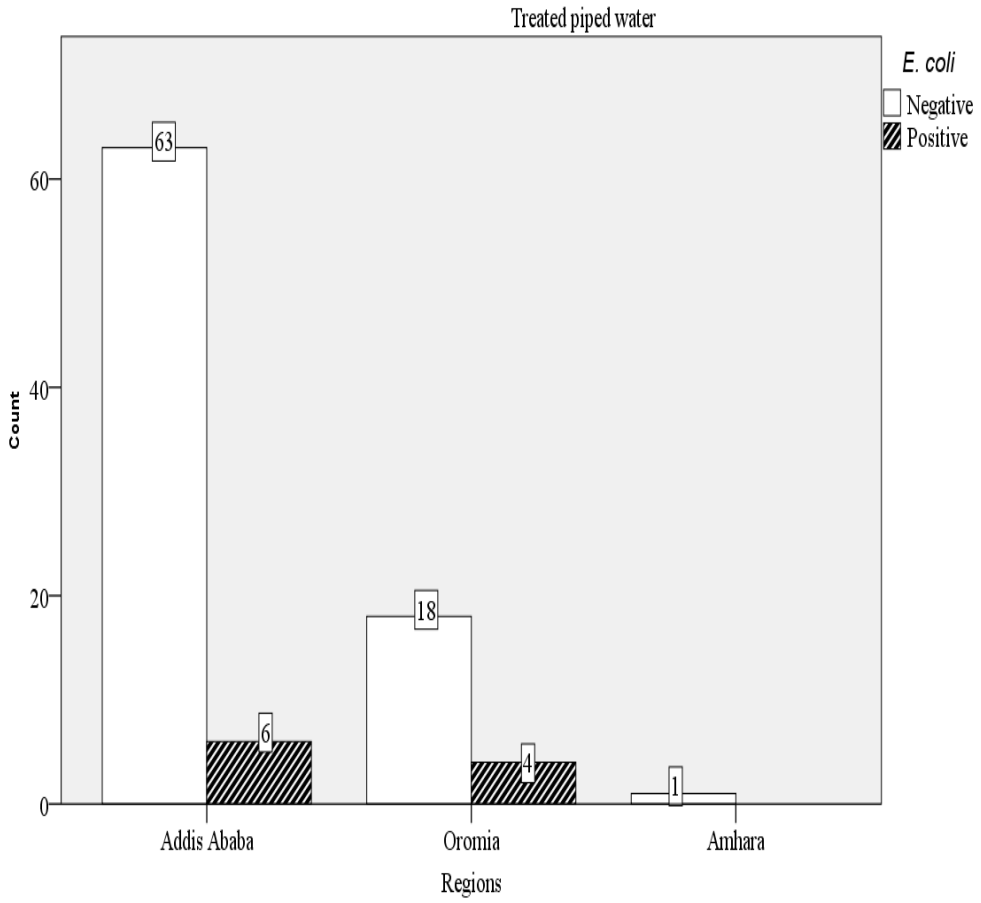

Fig.5.4: ***Estimation of E. coli in treated piped water samples using MPN method in different regions of Ethiopia*** **between February and June 2016**

5.2. 2. Bacteriological and virological indicator detection in untreated water sources

Of 113 untreated drinking waters, 100 (88.5%) samples had HPC >$1.0x10^2$ cfu/ml and 75 (66.4 %, 60 (53.1 %), 42 (37.2 %) and 5 (4.4%) samples were positive for total coliforms and thermotolerant coliforms, *E. coli* and *total coliphage* respectively. un-treated water sources had HPC ranging from 100% for river to 80.6% for untreated piped water, total coliforms ranging from 100% for river to 60.9% for well water, thermotolerant coliforms ranging from 92.3% for river to 45.1% for piped waters, *E. coli* ranging from 84.6% for river to 29% for well waters and total coliphages ranging from 38.5% for river waters to no detection for piped and well waters .

Detections of HPC, *total coliforms, thermotolerant and E. coli in various regions, sources and treatment status of waters were shown in table 5.6 to table 5.9.*

Of 31 un-chlorinated piped drinking waters, 25 (80.6%) samples had HPC >1.0x10^2 cfu/ml; 21 (67.7%), 14 (45.1%) and 11 (35.5%) samples were positive for total coliforms, thermotolerant coliforms and *E. coli* respectively and no detection of total coliphages in the water samples. Of 17 un-treated piped waters in Addis Ababa city administration, HPC >1.0x10^2 cfu/ was recorded in 15 (88.2%) samples and 13 (76.5%), 9 (52.9%) and 6 (35.3%) samples were positive for total coliforms, thermotolerant coliforms and *E. coli* respectively and no detection of total coliphages in the water samples. Of 11 untreated piped waters in Oromia, HPC >1.0x10^2 cfu/ml was recorded in 9 (81.8%) samples and 7 (63.6%), 4 (36.4%) and 4 (36.4%) samples were positive for total coliforms, thermotolerant coliforms and *E. coli* respectively and no detection of total coliphages in the samples. One out of three untreated piped water in Amhara had HPC >1.0x10^2 cfu/ml and total coliforms, thermotolerant coliforms and *E. coli* detections and no detection of total coliphages in the samples.

Of 69 un-chlorinated well waters, 62 (89.9%) samples had HPC >1.0x10^2 cfu/ml; 42 (60.9%) samples, 34 (49.3%) samples and 20 (29%) samples were positive for total coliforms, thermotolerant coliforms and *E. coli* respectively and no detection of total coliphages in the water samples. Of 15 un-treated well waters in Addis Ababa city administration, HPC >1.0x10^2 cfu/ml was recorded in 15 (100%) samples and 6 (40%), 5 (33.3%) and 2 (13.3%) samples were positive for total coliforms, thermotolerant coliforms and *E. coli* respectively and no detection of total coliphages. Of 51 untreated well waters in Oromia, HPC >1.0x10^2 cfu/ml was recorded in 45 (88.2%) samples and 35 (68.6%), 28 (54.9%) and 18 (35.3. %) samples were positive for total coliforms, thermotolerant coliforms and *E. coli* respectively and no detection of total coliphages. One of two well water samples in Amhara has HPC >1.0x10^2 cfu/ml and one of one well water sample in Afar has HPC >1.0x10^2 cfu/ml and positive for total coliforms, thermotolerant coliforms and negative for *E. coli*.

Of 13 un-chlorinated river waters, all samples had HPC >1.0x10^2 cfu/ml and 12 (92.3%), 12 (92.3%), 11 (84.6%) and 5 (38.5%) samples were positive for total and thermotolerant coliforms, *E. coli* and total coliphages respectively. Of eight untreated river waters in Oromia, HPC >1.0x10^2 cfu/ was recorded in all samples and 7 (87.5%), 7 (87.5%), 6 (75%) and 3(37.5%)

samples were positive for total coliforms, thermotolerant coliforms, *E. coli* and total coliphages respectively. All SNNPR river water samples were positive (n=5) for HPC, total and termotolerant coliforms and *E. coli and coliphages were detected in* 2 (40%) of the water samples. The enumeration of total coliphages analyzed in water samples from river sources were ranged between $2.2x10^{1}$and $5.3x10^{2}$ pfu/ 100.

Table 5. 6*: Enumeration of total heterotrophic bacteria in various untreated water sources in different regions of Ethiopia* between February and June 2016

Regions	Sources	Heterotrophic plate count (cfu/ml)

	(Untreated)	<1cfu/ml	1-1.0x10^2	1.0x10^2-2.0x10^2	2.0x10^2-3.0x10^2	>3.0x10^2	Total	**ES/ WHO guideline**
AA	Piped	1	1	9	0	6	17	<100cfu/ml
	well	0	0	8	2	5	15	
Oromia	Piped	2	0	6	1	2	11	
	well	3	3	24	7	14	51	
	River	0	0	1	0	7	8	
Amhara	piped	2	0	1	0	0	3	
	well	0	1	0	0	1	2	
SNNPR	River	0	0	1	0	4	5	
Afar	Well	0	0	0	0	1	1	
Total		8	5	50	10	40	113	

cfu–colony forming unit, **AA**–Addis Ababa, SNNPR– Southern Nations, Nationalities, and Peoples' Region, WHO: world health organization, ES: Ethiopian standard

Table 5. 7*: Estimation of total coliforms in untreated water source samples using MPN method in different regions of Ethiopia* between February and June 2016

Site	**Source (untreated)**		**Total coliforms (MPN/100ml)**					**ES/ WHO guideline**
		<1	1-10	<100cfu/ml	101-180	>180	Total	<1mpn/100ml
	Pipeded	4	5		0	5	17	

AA	well	9	1		1	2	15	
Oromia	Pipeded	4	4		0	1	11	
	well	16	8		1	12	51	
	River	1	0		0	7	8	
Amhara	Piped	2	0		0	0	3	
	well	2	0		0	0	2	
SNNPR	River	0	0		0	5	5	
Afar	Well	0	0		0	0	1	
Total		38	18	23	2	32	113	

MPN: most probably number**, AA**–Addis Ababa, SNNPR– Southern Nations, Nationalities, and Peoples' Region, WHO: world health organization, ES: Ethiopian standard

Table 5. 8*: Estimation of thermotolerant coliform in untreated water source samples using MPN method in different regions of Ethiopia* between February and June 2016

Site	**Sources (untreated)**		**Thermotolerant coliforms (MPN/100ml)**					**ES/ WHO guideline**
		<1	4-10	10-100	101-180	>180	Total	<1mpn/100 ml
AA	Piped	8	4	0	0	5	17	
	well	0	3	0	0	2	15	
Oromia	Piped	7	3	0	0	1	11	
	well	23	12	10	0	6	51	
	River	1	0	0	1	6	8	
Amhara	piped	2	1	0	0	0	3	

	Well	2	0	0	0	0	2	
SNNPR	River	0	0	0	0	5	5	
Afar	Well	0	0	1	0	0	1	
Total		53	23	11	1	25	113	

MPN– most probably number**, AA**–Addis Ababa, SNNPR– Southern Nations, Nationalities, and Peoples' Region, WHO: world health organization, ES: Ethiopian standard

Table 5. 9*: Estimation of E. coli in untreated water source samples using MPN method in different regions of Ethiopia* between February and June 2016

Source (unchlorinated)			**Regions**					Total	**ES/ WHO G**
			AA	Oromia	Amhara	SNNPR	Afar		
Pipe	*E. coli*	Negative	11	7	2			19	No detection
		Positive	6	4	1			11	
Well	*E. coli*	Negative	13	33	2		1	49	
		Positive	2	18	0		0	20	
River	*E. coli*	Negative		2		0		2	
		Positive		6		5		11	
Total			32	70	5	5	1	113	

WHO: world health organization, ES: Ethiopian standard, G: guidelines

6. Discussion

In the present investigation, virological and bacteriological quality of drinking water was assessed from various water sources in five regions of Ethiopia. As expected, majority of the water samples (64.7%) contained hetrophilic bacteria above permissible limit of $>1.0x10^2$ cfu/ml when incubated at 37°c (66) and total coliforms (51.8%), thermotolerant coliforms (38.5%) and *E. coli* (23.9%) above Ethiopian drinking-water standard CES 58: 2013 and WHO guideline value of " no detections/100ml" for drinking water (49,1) and total coliphages above WHO guide line of <1 pfu/100ml (1) in 2.3 % of the total water samples. The bacteriological and virological indicators contained above the permissible limits in various drinking water sources in this study may be due to soil and agricultural runoff, infiltration of grazing animal fecal matter, leakage from septic tanks, atmospheric deposition, lack of sewage and solid waste disposal systems which were the main threats to water resources (51,52). The presence of these indicators may not cause illness, but used as one of the indicators of pathogenic microbes that can cause intestinal infections, dysentery, hepatitis, typhoid fever, cholera and other illnesses (53).

The findings observed in the present study about the incidences above the permissible limits of HPC and total coliforms in the total water samples were lower than in some studies done by Tista P. et al., 2007 on various drinking water samples in Nepal (82.6% and 92.4%) (54), *Pakistan (both 100%), Javed A. et al., 2011* (55) and Lesotho (71.4% and 97%), Gwimbi P, 2011 (56) and higher than in one study in Pakistan (45% and 25%), Abdul H, 2010 (57).

HPC contained in the total samples is used to estimate the total amount of bacteria in water and indicates the overall microbial status of the water (58). The presence of total coliforms in the chlorinated drinking water samples indicates a serious treatment failure; distribution system may be vulnerable to contamination or may be experiencing bacterial re-growth (36, 59). Out of water samples positive for thermotolerant coliforms in the present study, 28.6% were treated drinking waters which indicate inadequate treatment and disinfection, bacterial re-growth, or infiltration in the distribution system (60). The presence of *E. coli* in samples indicates recent faecal contamination and was more serious than other coliforms alone and possible presence of pathogenic microorganisms mainly bacteria (11, 60), while the detection of total coliphages in the total water samples indicates the presence of entroviruses (37).

The non-comply of 38.5% drinking water samples for coliform indicator more closely related to fecal pollution, thermotolerant coliforms, is higher than the national survey, rapid assessment of drinking water quality in the federal democratic republic of Ethiopia (RADWQ) that tested drinking water quality for thermotolerant coliforms and found 28% of 1602 samples non potable. According to this rapid assessment of drinking water quality in different sources, the major sanitary risk factors identified are cracks in the infrastructure; leaks; unsanitary conditions around the source; a latrine, sewer or other potential source of pollution nearer to the water supply; animal access to the water source; and a poor drainage system (4).

The finding observed in the present study about the presence of thermotolerant coliforms or *E. coli* above the recommended limits were comparable to similar study conducted by Parvez AK et al., *2016* in Bangladesh on a total of 106 samples of various water sources from 37 districts (61) and lower than other studies in *Pakistan (80% and 66%) (*Javed A. et al., 2011) (55), in Lesotho (71% *E. coli*) (Gwimbi PG, 2011) (56) and in India (100% and 78.1%) (Madhab B, 2010) (62). Though, in one study, the detections of these bacterial indicators were higher than that from Lithuania (16.7% *E. coli*) (Mindaugas M et al., 2007) (63).

The detections above permissible limits for HPC, total and thermotolerant coliforms, *E.coli* and total coliphages in untreated water samples were 2.7 times, 2.0 times, 2.5 times, 4.2 times, and 4.4 times more than the detections for chlorinated water samples respectively. This indicates the effectiveness of chlorination methods in microbial reduction (1). All the treated water samples were free from virological indicators, total coliphges. This is consistent with the study done in United States of America on ground water by Jeanine D et al., 2014 (37).

The detection of coliphages in the absence of *E. coli* was inconsistent with the study done in Massachusetts, USA by Long SC & Dewar KG, 2008 (34). Spearman Correlation test indicated statistically significant positive correlations between total coliphages and all other bacterial indicators and agreed with a study done in the United States of America on drinking water samples (38). HPC, total coliforms, thermotolerant coliforms were strongly related to each other. Though, the non parametric Kruskall-Wallis test showed that HPC, coliforms and total coliphages differed statistically by region, water source and treatment type (p value < 0.05).

Regionally, the non compliance level of bacteriological and virological indicators for Oromia was higher than that of Addis Ababa may be due to the difference of water supplied by technology considered to be improved (4). The detection of HPC, total coliforms, thermotolerant coliforms and *E. coli* above permissible limit in treated water samples in Oromia was almost twofold higher than treated water samples in Addis Ababa. Thermotolerant coliforms for water samples in Addis Ababa (29.5%) and Oromia (45.5%) were higher than water in Addis Ababa and Oromia (17.4%) in the previous national survey of RADWQ (4). This indicates the deterioration of drinking water sources in Oromia through time followed by Addis Ababa.

In the present study, the non- compliance of samples collected from treated (chlorinated) piped water for thermotolerant coliforms was high (26.1%) compared to 22.4% in the RADWQ national survey (4). The presence of coliforms suggests inadequate treatment or post-treatment contamination (64) and the major means of contaminations are sanitary risk factors such as lack of maintenance, leaks in piped-water distribution system; Poor site selection and failure to minimize sanitary risks (A latrine or source of pollution close to piped water distribution systems) and Poor sanitary conditions (unsanitary mixing and sedimentation tanks and unsanitary piped distribution systems) (4).

Compulsory Ethiopian Standard for bottled drinking water, CES 99 (48) and WHO guidelines (1) for drinking water set the maximum acceptable limits at 100 cfu per ml for HPC and no detectable organism per 100 ml for total coliforms, thermotolerant coliforms and *E. coli*, while the guidelines (1) set coliphages at <1 pfu per 100ml. All the 13 samples from the eight brands of bottled water met these criteria and thus it is bacteriologically and virologically potable. This good quality is may be due to the current follow up and enforcement of the bottled water companies to adhere to the mandatory Ethiopian specifications by the regulatory body. This is in consistent with the studies done by Addo et al., 2009 in Ghana (65) and Eed L, et al., 2009 (66) in Saudi Arabia which found all coliforms none detectable in any of the examined brands of bottled drinking waters.

Some investigations done in different countries have contained HPC, total coliforms, thermotolerant coliforms and coliphages in bottled drinking water. In Ethiopia, 325 bottled water samples of 11 domestic and two imported brands contained HPC above the permissible limits in 16.9% of the samples (Tafere W. et al., 2014) (67). In India, Jaipur city by Gangil R, 2013, 40 samples of various brands of bottled drinking water were tested for HPC, total coliforms and *E. coli* and reported 50%, 45% and 20% respectively (68). In one study done in Dar es Salaam, Tanzania by Kassenga GR, 2007 on 13 brands of 130 bottled drinking water reported 92% HPC, 4.6% total coliforms and 3.6% fecal coliforms (69).

As expected, HPC non compliance for the treated water sources, treated piped water, was lower than untreated water sources, because chlorine prevents microbial contamination. However, the HPC figure observed in the treated piped water source is due to problem with water treatment, a change in quality of the water source, prior to treatment or bacterial re-growth in the distribution system (70). Non Compliance of thermotolerant coliforms for treated piped water in Addis Ababa was lower than non Compliance of thermotolerant coliform in Oromia piped water but consistent with the previous national survey of RADWQ (4).

Large non-comply of untreated water sources for HPC (88.5%), total coliforms (66.4 %), thermotolerant coliforms (53.1 %), *E. coli* (37.2 %) and total *coliphages (*4.4%) signified the suggestion of WHO states that surface water or shallow ground water should not be used as a source of drinking-water without sanitary protection or treatment (1).

As expected, HPC, total coliforms, thermotolerant coliforms, *E. coli* and total coliphags non-compliance was considerably high which exceeded Ethiopian standard (49) or WHO guidelines (1).

The contamination of HPC, total and thermotolerant coliforms and *E coli* in untreated piped water was higher than that of treated piped water. The presence of *E coli* in untreated piped water (35.5%) was 3.3 times higher than in treated piped water samples. This shows the ability of the treatment in reduction of microbes. The detections of total and thermotolerant coliforms and *E coli* in 60.9%, 49.3% and 29% respectively in well water samples were lower than studies done on well water samples by Eed L, et al., 2009 in Saudi Arabia (100% total coliform, 87.88% thermotolerant coliforms) (66) and Nwachukwu E., 2013 in Nigeria (100% total coliform and **65% *E. coli*)** (71). On the other hand, in some studies, the detections of total and thermotolerant coliforms and *E coli* were higher than that from Turkey by Aydin A, 2007 (25% total coliform, 17.5% thermotolerant coliforms and 15% *E. coli)* (72) and from Pennsylvania State, USA by Bryan R, 2009 (33% total coliform and 14% *E. coli***)** (73).

Thermotolerant coliforms in Protected dug well waters (49.3%) were higher than untreated and treated piped waters and comparable with the national survey of RADWQ (4). The percent of permissible values for total coliforms, thermotolerant coliforms and *E coli in Oromia well water was higher than* that of Addis Ababa Protected dug well waters. Non Compliance of thermotolerant coliforms in Addis Ababa Protected dug well waters (33.3%) and Oromia Protected dug well waters (54.9%) was higher than the national survey of RADWQ (25%) for both regions (4). This level of contaminations may be due to a source of contamination is nearby, otherwise groundwater sources are usually bacteriologically safe (48). The closeness of the well waters to latrine and their existence of on equal altitude to the latrine houses along with short depth were the major risk factors (74).

The none-compliance of HPC, total and thermotolerant coliforms *E coli* and total coliphages in 100%, 92.3%, 92.3%, 84.6% and 38.5% respectively in river water samples indicates rivers are the most contaminated waters of all the sources and unfit for consumption. The contamination of the rivers is may be as a result of industrial discharge, municipal waste disposal and surface run-off (75). River samples in SNNPR were more contaminated with the indicators than river samples in Oromia in the present study. The presences of total coliphages in the river water provide an indicator of faecal pollution and hence the potential presence of enteric viruses and possibly also

other pathogens (1). The Bacteriological and virological quality of river waters were poor and unsuitable for human consumption and consistent with the study done in South Africa by Obi CL et al (76).

7. Strength and limitation of the study

7.1. Strengths of the study

This study used multi indicators, total heterotrophic plate count**,** total and thermotolerant coliforms, *E.coli* and total coliphages, to assess the quality of drinking water sources in some regions of Ethiopia that could provide water quality information. It can also improve our understanding of the method that minimizes resource and time to perform the tests in simultaneous detection of both somatic and male-specific coliphages, since single host (*E. coli* CB390) was used.

7. 2. Limitation of the study

Water results in some regions, Afar, SNNPR and Amhara may have been affected by the small number of samples and sampling technique may affect the result of some water sources. Some other regions, seasonal variations and gram stain tests haven't been included in the study.

8. Conclusion

This study assessed bacterial and viral indicators in drinking water sources in some regions of Ethiopia. Majority of the water samples, mainly untreated sources contained bacterial indicators and river water source samples contained viral indicators above the standards permissible limits. This indicates drinking water sources are contaminated with environmental and fecal contaminants which are threats for human health. Bacterial indicators were detected in all water sources except bottled drinking water. As the results of this study indicate, most water sources were unsafe and river waters are the most contaminated sources for human consumption. This shows the high risk of infection for consumers and calls for immediate action. All indicators were differed statistically by region, water source and treatment type. These water quality variations among regions, water sources and treatment type need regular national surveillance programs, selection and treating drinking water sources.

The contamination of HPC, total and thermotolerant coliforms, *E coli* and coliphages in untreated water sources was significantly high compared to that of treated water, thus without assessing water quality, sanitary protection or treatment should not be used as a source of drinking-water. The Large levels of contaminations of the sources indicate a serious treatment failure; distribution system may be vulnerable to contamination or may be experiencing bacterial re-growth or infiltration; a source of contamination is nearby, the closeness of the source to latrine and their existence of on equal altitude to the latrine houses along with short depth. Unless actions are taken, these poor and unsuitable waters for human consumption pose a serious risk.

9. Recommendations

The following recommendations are provided considering the findings of this study.

1. All water sources should be protected and treated regularly to safeguard the health of the consumers.
2. Regular water quality assessments using bacterial and coliphages indicators are urgently needed to take timely action and secure the safety of all drinking water sources.
3. Using untreated water sources such as rivers, wells, un-chlorinated piped water for human consumption may lead to human and economic loss, thus intervention is required by the responsible government bodies.
4. Large scale future studies are needed for the comprehensive risk assessment of water sources using coliphages, human enteric viruses and bacterial indicators.

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Annexes

Annex A: Data Collection Form

Every sample was managed and information about the sample was filled in this format.

Code of water sample _____ Study Serial Number _____

Date/time of received _____/_____

Site; region_____ Town_____Kifle Ketema _____Kebele_____

Type of water sample: _____chlorinated,_____Non-chlorinated

Sourse of water_____

Date of collection_____

Time of collection_____

Date/ time of received _____/_____

Annex B: Laboratory test Procedure for virological and bacteriological water sample Collection and Processing

Water sample collection

The samples were collected according to the Standard Methods for Examination of Water and Wastewater.

Procedure:

Preparation

1. Date, sample location and sample time were recorded on an unopened sample container using marker.
2. Rubber gloves were put on before opening sample container.

Distribution System

1. If it was piped without attachments, a piped that was supplying water from a service pipe directly connected with the main was selected and not served from a cistern or storage tank.
2. The piped was disinfected with an alcohol wipe or flaming the piped to sterilizing the faucet or immersing the end of the faucet in the strong solution of chlorine or bleach for a couple of minutes
3. Piped was opened fully and water let run to waste for at least 3 minutes to rinse any foreign material that dislodged and the sample was collected without rinsing the container.
4. The container was filled slowly to the line as indicated on the container. The container was not let overflow if sodium thiosulfate was used.

Rivers and Wells

1. A bottle was grasped by its base and plunged into the water source with the neck facing down not from too near the bank or too far from the point of drain off, or at depth above or below the point of drain off.
2. The bottle was turned until the neck pointing slightly upward and the mouth was directed toward current (if any) and allowed to fill. After filling, the bottle was removed from water with the neck pointing up.
3. The bottle was capped tightly.

Transportation

1. The sample was placed in the cooler and a sufficient amount of gel ice packs were placed to keep the sample cold during transport to the laboratory.
2. The samples were transport to the laboratory.

Drinking bottled water

Appropriate number of bottles (at least five) was selected from the stock of the same brands.
The batch was Sufficient to give a representative sample.

The sample was brought to the laboratory within one hour of collection or in ice box from different manufacturers.

Annex C: Culture for virological indicator

Single Agar Layer Assay

Grow up overnight *E. coli* hosts in TSB

Materials required:

1x TSB

Appropriate 100x antibiotics

Wire loop for cell transfers

Sterile flasks or bottles

Procedure

To prepare 25 mL A 25 mL TSB and 0.25 mL ampicillin was labeled with *E. coli* host CB390 and added to flask. (for proper growth conditions not less than 25mL)

Using a sterile wire loop, a small amount (1 loop) of frozen *E. coli* cells was scraped from a frozen stock and inoculated in the TSB.

The flask or bottle was caped and incubated at 37^{o}C on a shaker tray (90-100 rpm) for 18-24 hours.

Day 2:

Preparation of log-phase host bacterial cultures was done 1.5 to 2 hours prior to assay.

Materials

100mL 1x TSB

Appropriate 100x antibiotics

250 mL sterile bottles or flasks

Overnight *E. coli* host stock

Procedure:

- A 250 mL bottles or flasks were labeled with each *E. coli* host, date & time, log phase, and

initials.

- A 100mL TSB and 1 mL appropriate antibiotic was added to each bottle or flask.
- Mixed overnight *E. coli* host culture of 1 mL was added to the bottles or flasks.
- The mixture was incubated at 37ºC on shaker tray for 1.5 - 2 hours, (4 hours if from frozen stock *E.coli*) or until culture was visibly turbid.
- The log phase was stored at 4°C to slow replication until ready for use best within 6 hours.
- The remaining loge-phase was Stored at 4°C overnight for the preparation of new one.

Single Agar Layer Assay procedure

Materials

Labeled 125mL bottles or flasks, sterile

Labeled plates, sterile

Log phase *E. coli* host broth cultures

100x antibiotics

4M $MgCl_2$

Procedure:

- ✓ A sterile 4 M magnesium chloride (Mg cl_2) of 2.5mL was added to 100 mL water sample in to 250-mL flask and placed in a 37°C water bath.
- ✓ Method blanks were prepared.
- ✓ The water sample with Mg cl_2 bottle was transferred from 37ºC water bath to 45ºC water bath and removed after 5 minutes.
- ✓ A 2ml sterile antibiotic was added to 100 mL double tryptic soya agar (2X TSA) in 45-48°C water bath to equilibrate until used in the SAL assay.
- ✓ Then 10 mL of *E. coli* host log phase was added to the sample water and immediately placed in 45°C water bath for 3-5 minutes.
- ✓ The sample *E. colli* CB390 mixture was added to the 100 mL of 2X TSA containing ampcillin
- ✓ The contents were gently mixed and poured into five petri- dishes at 40 mL per 150-mm-diameter dish per 100-mL sample. The plate was mixed very gently by tilting and swirling to

avoid bubbles, which can mimic or obscure plaques.

- ✓ The plates were dried for 15 minutes under the hood to reduce condensation in the plates, which mimic plaques on the agar.
- ✓ The plates were covered, inverted, and incubated at $37^{O}C$ for 18-24 hours.

Interpretation

Circular zones of clearing (typically 1 to 10 mm in diameter) in lawn of host bacteria in SAL plates after 16 - 24 hours was considered to be plaques and Counted per plate series and recorded as PFU / 100 mL. For each sample the total number of plaques were counted from all plates.

Total number of plaques per 100 mL sample = PFU / 100 mL

Annex D: Quality Control for SAL

Preparation of method blanks

- ➢ Method blank was analyzed to demonstrate freedom from contamination. One method blank (reagent water sample containing no coliphage) with each analytical batch (all samples analyzed during a single day, up to a maximum of 20 samples).
- ➢ A reagent water sample containing no coliphage was prepared and analyzed using the same procedure as used for analysis of the sample.

Preparation of Positive Controls

- ➢ Positive Control was analyzed for each analytical batch to ensure the analytical batch was in control (all samples analyzed during a single day, up to a maximum of 20 samples per coliphage type).
- ➢ A 100mL samples were dispensed in to separate sterile 250mL screw cap bottle.
- ➢ A 100 mL of sterile distilled water was dispensed into separate sterile 250mL screw cap bottle.
- ➢ A 31.3 µL ~ 80PFU of MS2 phage was added to the positive controls.

Annex: E. Laboratory diagnosis of Coliforms (most probable number)

Materials

Water samples

Lactose broth.

Bunsen burner

Pipettes

Test tubes

Procedure

Laboratory test for MPN

procedure

A. Fifty ml of water sample was added to 50 ml of double-strength broth tube.

B. Ten ml of water was added to each of five tubes of 10 ml of double-strength broth tubes.

C. One ml of water was added to each of five tubes of five ml of single-strength broth tubes.

D. The tubes were mixed by inverting.

E. The tubes were incubated at 35-37°C and noted the numbers of tubes showing acid and gas after 48 hours

Confirmatory and termotolerant tests

Procedure

- Each tube was label with the source of its water sample.
- Presumptive test positive, the tubes of MacConkey broth showing acid and gas was inoculated to brilliant green bile and EC broths.
- The tubes were incubated at 37°C for 24 hours and 44.5°C for 24 h in respectively.
- Gas formation was observed and positive tubes were tested for indol test.

Interpretation of MPN

The MPN tables were consulted and read to report the results of the most probable number of *presumptive* coliform bacilli/100 ml of water.

Annex table 1*:* Most probable Number table of coliforms in water analysis

Number of positive tubes

1×50ml	5×10ml	5×1ml	MPN/100ml
0	0	0	0
0	*0*	*1*	*1*
0	*0*	*2*	*2*
0	*1*	*0*	*1*
0	*1*	*1*	*2*
0	*1*	*2*	*3*
0	*2*	0	0
0	2	1	3
0	2	2	4
0	3	0	3
0	3	1	5
0	4	0	5
1	0	0	1
1	0	1	3
1	0	2	4
1	0	3	6
1	1	0	3
1	1	1	5
1	1	2	7
1	1	3	9
1	2	0	5
1	2	1	7
1	2	2	10
1	2	3	12
1	3	0	8
1	3	1	11
1	3	2	14
1	3	3	18
1	3	4	20
1	4	0	13
1	4	1	17
1	4	2	20
1	4	3	30
1	4	4	35
1	4	5	40
1	5	0	25
1	5	1	35
1	5	2	50
1	5	3	90

1 5 4 160
1 5 5 >180 (46)

Annex table 2: Most probable Number result formats of coliforms in water analysis

Tests	**Volume of macConkey broth initially used (ml)**										
	50	10	10	10	10	10	5	5	5	5	5
PCC											
CCC											
TTC											
E. coli type 1											

PCC- pesmptive coliform count CCC- Confirmed coliform count

TTC- Thermotolerant coliforms *E. coli- Escherichia coli*

Total coliforms_____mpn/100ml

Thermotolerant coliforms_____mpn/100ml

E. coli- Present/Absent_____

Comment_____

Date ____/_____/_____Signature_____

Annex F: Declaration

Title of project: ***VIROLOGICAL AND BACTERIOLOGICAL QUALITY IN SELECTED DRINKING WATER SOURSES IN ETHIOPIA***

I, the undersigned, declare that this MSC research project is my original work. It has not been presented for a degree in any other University. False statements could be cause for invalidating this research project and may lead to other administrative or legal action.

Name of Principal investigator Tesfaye Legesse (BSc)

Signature: _____ Date: _____

Name of advisor Mr. Kasu Desta (BSc, Msc, PhD candidate, Assistant professor)

Signature _____ Date: _____