Addis Ababa University

School of Graduate Studies

Faculty of Medicine

Department of Microbiology, Immunology & Parasitology

MSc Thesis

Bacteriological quality of drinking water at Arba Minch town, Southern Ethiopia

By:-

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A thesis submitted to the school of Graduate Studies of Addis Ababa University in partial fulfillment of the requirements for the degree of Master of Sciences in Medical Microbiology

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Acronyms

AAU= Addis Ababa University

APHA = American Public Health Association

Cfu/ml= Colony forming unite per milliliter

E.col= Escherichia coli

EHNRI= Ethiopian Health and Nutrition Research Institute

FC/TTC= Fecal/Thermotolerant coliform

FDRE, MoWR= Federal Democratic Republic of Ethiopia, Ministry of Water Resources

FDRE, MoH= Federal Democratic Republic of Ethiopia, Ministry of Health

IWSC= International Water and Sanitation Center

Lab= Laboratory

LES= Lewis Experimental Station

LDC= Lysine Decarboxylase

M- Endo Agar= Membrane- Endo Agar

MF= Membrane Filters

MDG= Millennium Developments Goal

NGO's= Non-Governmental Organizations

NTU= Niphlometric Turbidity Unit

SS agar= Salmonella Shigela agar

SNNPRE= South Nations, Nationality and People of Republic of Ethiopia

TC= Total Coliform

WHO= World Health Organization

W/V= Weight by Volume

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Abstract

Background: Globally, 1.1 billion people rely on unsafe drinking water sources from lakes, rivers, and open wells. Many studies have confirmed that water related diseases not only remain a leading cause of morbidity and mortality worldwide but also that the spectrum of disease is expanding and the incidence of many water related microbial diseases are increasing. Thus, water becomes contaminated with faecal material due to inadequate protection of the source, unhygienic practice of the community at the source and poor household handling practices.

Objectives: The aim of this study was to assess bacteriological quality of drinking water and to survey the possible sanitary risk factors at household level in Arba Minch town, Southern Ethiopia.

Methods: A cross- sectional study was carried out in Arba Minch town from November, 2010 to January, 2011. A total of 126 water samples were collected from main source, reservoir, pipe and household water in three rounds to determine bacteriological quality of drinking water. The bacteriological analysis was conducted at Arba Minch Regional Laboratory. Consequently, level of contaminations was determined on the bases of total coliforms and fecal coliforms existence.

Results: Water analysis of the study area demonstrated that 100% and 55% of household water samples were contaminated with total and faecal coliform bacteria respectively. Similarly, 10% and 5% of water samples taken from pipes were again contaminated with total and faecal coliform bacteria respectively. But, 100% of water samples taken from both main source and reservoir were free from any indicator bacteria. Accordingly, 100% of water samples taken from both main source & reservoir and 95% of water samples taken from pipes meet WHO guidelines. But, 5% of pipes and 100% of household water samples were found above the limits of WHO standards set for drinking water quality.

Conclusions: The majority of water samples in the study area were safe for drinking purpose from bacteriological point of view. But some water samples taken from pipes and almost all water samples taken from households were not recommended for drinking purpose. Therefore, training of local people to look after the water supply system, extension of hygiene and health education on sanitation could have a notable impact for the provision of safe water supply at community/household level.

Key words: Water quality, indicator bacteria, pathogenic bacteria, hygiene, main source water, reservoir water, pipe water and household water.

1. Introduction

1.1 Background

Access to safe water is a fundamental human need and, therefore, a basic human right. Contaminated water jeopardizes both the physical and social health of all peoples. According to WHO, more than 80% of diseases in the world are attributed to unsafe drinking water or to inadequate sanitation practices (WHO, 2003a). Globally, 1.1 billion people rely on unsafe drinking water sources from lakes, rivers, and open wells (WHO, 2000). In Ethiopia, drinking water coverage was less than or equal to 21% for the rural, 84% for the urban and 30% for the country level. The capital per day water consumption ranged from 3 to 20 liters with median of 8.5 liters (Kume and Ali, 2005).

Several studies have confirmed that water-related diseases not only remain a leading cause of morbidity and mortality worldwide but also that the spectrum of diseases is expanding and the incidence of many water-related microbial disease is increasing (WHO, 2003a). The diversity and severity of water borne diseases is greatest in tropical environments. Since most countries in tropical climates are under-developed, with poor medical services, and large populations that are under-nourished and ill-housed, water- borne diseases may have a much greater effect on public health in the tropics than in temperate areas (Jesuis and Terry, 1987).

Diarrhea remains a major killer in children and it is estimated that 80% of all illnesses in developing countries are related to water and sanitation; and that 15% of all child deaths under the age of 5 years in developing countries results from diarrheal diseases (WHO, 2003a; 2004b).

In Ethiopia, three-fourth of the health problems of children is communicable diseases arising from the environment, especially water and sanitation, and 46% of the mortality rate in children less than 5 years is due to diarrhea in which water-related diseases occupy a high proportion (IWSC, 1989). The Ministry of Health of Ethiopia estimated that almost 6000 children die each day from diarrhea and dehydration in Ethiopia (FDRE, MoH, 1997).

In rural areas and villages of Ethiopia, water for human consumption, drinking, washing (bathing, laundry), for preparation of food etc, is obtained from rivers, streams, shallow wells, springs, lakes, ponds, and rainfall. Unless water is made safe or treated for human consumption, it may be hazardous to health and transmit diseases. The main contaminants of these water sources are human excreta, animal waste and effluent because of open field defecation practices. Thus, the majority of rural communities use water from contaminated or doubtful sources, which expose the people to various water-borne diseases (FDRE, MoWR, 2004).

Detection, differentiation and enumeration of Entrobacteriaceae are of primary importance in the microbiological quality control of water. Indicator bacteria are used to evaluate the potability of drinking water because it would be impossible to accurately enumerate all pathogenic organisms that are transmitted by water (Paccker *et al*., 1995). The use of indicator organisms, in particular the coliform group, as a means of assessing the potential presence of water-borne pathogens 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 diseases has been developed (Barrell *et al*., 2002).

Thermotolerant coliforms are the group of coliform organisms; their use in assessing water quality is considered acceptable for routine purposes (WHO, 2003b). The group contains mainly type 1 *Escherichia coli* (*E.coli*) at about 95%, which are almost exclusively derived from human and animal feces and many other bacterial species that have an environmental source (example *Citrobacter* or *Klebsiella* species). Thermotolerant coliforms other than *E.coli* may also originate from organically enriched water such as industrial effluents or from decaying plant materials and soils (FDRE, MoH, 2006).

Bacteriological techniques employed to distinguish between human and animal fecal pollution are a valuable tool in water pollution control programs, because they are useful in tracing the source of pollution of drinking water supplies, and they can help in assessing the overall adequacy of protection rendered to small rural water supplies (Mara and Oragui,1985).

Management of fecal contamination of water would be improved if its sources could be accurately identified through water analysis. Irrespective of the relative risks, the ability to identify sources would assist in overall management of microbial water quality (Sinton *et al.*, 1998).

According to WHO (2004a) and FDRE, MoWR (2002) recommendation, drinking water should contain zero total coliforms and fecal coliforms/100 ml of water. Therefore, this study was evaluated two bacterial indicators of drinking water quality (total coliforms and fecal/thermotolerant coliforms) in addition to isolation of pathogenic bacteria mainly *Salmonella, shigella and Vibrios* from main source, reservoir, pipes and household drinking water at Arba Minch town, Southern Ethiopia.

1.2 Statement of the problem

Assessing water quality is significantly contributed to reduce water borne-diseases, to increase hygiene status of the society and to improve quality life for both rural and urban populations. However, quality of drinking water is being questioned, due to reduction in sanitation and increment of environmental pollutions. Access to clean potable water in most of Ethiopia is estimated at about 15% and most people are required to drink water from unprotected sources (MoWR, 1998).

On basis of the above evidences, in this study we tried to confirm the presence of undesirable bacteria in drinking water in the study area. Thus, the problem has negative effectiveness and efficiency of health service facilities and more importantly, the people who are the economic engines are at great risk. Accordingly, this study tried to solve the research gaps that were conducted on water quality both in rural and urban areas of Ethiopia by Said *et al.,* 2003 in Tehuledere woreda (South Wollo), by Mengesha *et al.*, 2004 in North-Gonder, by Atsnaf, 2006 in Worebabo district (South Wollo) and by Milkiyas *et al.,* 2011 in Bahir Dar city. Therefore, most of the above mentioned studies were not focused on urban areas particularly in Arba Minch town. As a result, this study mainly evaluated the bacteriological quality of drinking water at main source, reservoir, along the pipe and household level respectively and it also assessed the possible sanitary risk factor at individual home in Arba Minch town, Southern Ethiopia.

2. Literature Review

2.1 Water and Sanitation

Water and sanitation are about much more than health (Cairn cross *et al*., 2003). Access to safe drinking water is important as a health and development issue at national, regional and local levels. In some regions, it has been shown that investments in water supply and sanitation can yield a net economic benefit, since the reductions in adverse health effects and health care costs outweigh the costs of undertaking the interventions. This is true for major water supply infrastructure investments through water treatment in the home. Experience has also shown that interventions in improving access to safe water favor the poor in particular, whether in rural or urban areas, and can be an effective part of poverty alleviation strategies (WHO, 2004b).

The WHO report indicated that globally, the percentage of people served with some form of improved water supply rose from 79% (4.1 billion) in 1990 to 82% (4.9 billion) in 2000 (WHO, 2000). The same report indicated that over the same period, the proportion of the world's population with access to excreta disposal facilities increased from 55% (2.9 billion people served) to 60% (3.6 billion). Hence, at the beginning of 2000, one-sixth (1.1 billion people) of the world's population was without access to improved water supply and two-fifths (2.4 billion people) lacked access to improved sanitation. The objective of the United Nations Millennium Development Goals (MDGs) is to reduce persistent poverty and promote sustainable development worldwide especially in developing countries. Improvement of drinking water supply and sanitation is a core element of poverty reduction (Thompson, *et al*., 2000).

The MDG target for water is to halve by 2015 the proportion of people without sustainable access to safe drinking water and basic sanitation. WHO estimated that if these improvements were to be made in sub-Saharan Africa alone, 434,000 child deaths to diarrhea would be averted annually (WHO, 2004b). However, some scholars suggested that the provision of water supply in developing countries may not be sufficient because of (a) high population growth, (b) conflict and political instability, and (c) low priority given to water and sanitation programs (Thompson et al., 2000).

Several authors have indicated that diseases caused by contaminated water are the major threat in developing countries. Esrey and his colleagues (1985) have noted that contaminated water sources can serve as a vehicle for the transmission of pathogens to human. More than one third of deaths in developing countries are caused by drinking water especially from highly contaminated sources (Ngoma, 1992).

Of the 37 major diseases in developing countries, 21 have been reported to be related to water and sanitation. In Ethiopia, the World Health Organization reported that some 4.2 million people suffered from acute lack of water (WHO, 2004b).

The lack of access to safe drinking water and sanitation places a heavy burden on children who are especially vulnerable to diarrheal disease. According to the Ethiopian Ministry of Health (FDRE, MoH, 2001), diseases related to water, sanitation and hygiene problems are among the leading causes of morbidity and mortality, accounting for a large portion of the deaths of 500,000 children each year.

The study of Esrey and Habicht (1986) described a matrix of factors necessary at the community level to positively influence community health. Water quality was recognized as the foundation for any health improvement strategy, accompanied by programs for increased water quantity and sanitation. However, in the absence of changes in personal behavior and hygiene practices, the incidence of water-related diseases, for which the fecal-oral route is a major source of disease transmission, is likely to remain high in contaminated environments.

2.2 Major biological contaminants of water

While water is essential for life, unfortunately not all water helps man to survive. Water from contaminated sources may cause numerous diseases and untimely deaths. As a result, people suffer from water-borne diseases especially in Ethiopia. The contaminants are mainly biological pathogens. In rural villages and urban areas of Ethiopia, the main contaminants are from human excreta, animal waste, liquid waste from factories, flourmills, garages, pesticides from different sources. Water sources contaminated with these wastes is not fit for human use, unless it is made safe or treated (FDRE, MoH, 2006).

A large variety of bacterial, viral and protozoan pathogens are capable of initiating water borne infections. The enteric bacterial pathogens include early recognized agents, such as *Salmonella* and *Shigella* species and newly recognized pathogens from fecal sources such as *Campylobacter jejuni* and enterohaemorrhagic *E.coli*. Several bacterial pathogens, such as *Legionella spp*, *Aeromonas spp*, *Pseudomonas aeruginosa* and *Mycobacterium avium*, have a natural reservoir in the aquatic environment and soil (Leclerc, 2003).

The most common manifestation of water-borne illness is gastrointestinal upset (nausea, vomiting, and diarrhea), and this is usually of short duration. However, in susceptible individuals such as infants, the elderly, and immunocompromised individuals, the effects may be more severe, chronic (e.g., kidney damage) or even fatal. Other pathogens may infect the lungs, skin, eyes, central nervous system, or liver (Health Canada, 2006).

The risk to human health from water-based infections in natural watershed systems is most easily determined by assessing the concentrations of certain indicator bacteria in the water, such as fecal coliforms and streptococci. Bacteria originating from human and animal wastes also provide a clear indication of waste disposal in watershed systems and help to identify management strategies for providing clean water to the watershed residents (Monzer *et al*., 2005).

2.3 Indicator organisms and their degree of pollution in water samples

The concentration of pathogens (disease causing bacteria) in natural waters is generally very low; methods for their detection and enumeration are often complex and expensive. Alternative organisms that are consistently present in fecal material, survive reasonably well in water compared to pathogens, and are easier to detect, have therefore become widely used as fecal pollution "indicators" (Mara and Oragui, 1985).

The most commonly used indicator organisms are the coliform bacteria, including their subset, the fecal coliforms. Coliform bacteria, thermotolerant (fecal) coliforms and *E. coli* have, for almost a century, been used as indicators of the bacterial safety of drinking-water (Leclerc *et al*., 2001).

The term “coliform organisms” refers to Gram-negative, rod-shaped bacteria capable of growing in the presence of bile salts or other surface-active agents with similar growth-inhibiting properties and able to ferment lactose at 35-37 °C with the production of acid, gas, and aldehyde within 24-48 hours. They are also oxidase-negative and non-spore-forming (Barrell *et al*., 2002; WHO, 2003b).

Originally, total coliform bacteria were considered to be from four genera of the family Enterobacteriaceae that could all ferment lactose. These genera were *Escherichia*, *Klebsiella*, *Enterobacter* and *Citrobacter* (Stevens *et al.,* 2003). Thermotolerant coliforms are defined as “the group of coliform organisms that are able to ferment lactose at 44-45°C”; they comprise the genus *Escherichia* and, to a lesser extent, species of *Klebsiella*, *Enterobacter*, and *Citrobacter* (WHO, 2003b).

A survey of the microbiological quality of a natural spring, located in an area with little interference by human and animals, was conducted in Seoul, South Korea. Total coliforms were detected in all samples and the mean density of total coliforms was up to a maximum of 228 cfu/ml. Detectable *E. coli* are found in 78% of all samples and the mean densities of *E. coli* varied from a minimum of 0 cfu/ml to a maximum of 15 cfu/ml in all samples (Youn-Joo and Breindenbach, 2005).

In a study conducted in Gondar, Ethiopia, 75% of the samples taken from unprotected wells and springs were contaminated by fecal coliforms, especially *E.coli.* The authors further reported that 50% of the samples in both cases had a coliform count of 180/100 ml and above. No sample in either case had a coliform count of less than 10/100 ml. The least coliform count seen was 13 coliform /100 ml and on the basis of these, they concluded that the majority of the drinking water sources were either of unacceptable quality or grossly polluted (Mengesha *et al*., 2004).

A French study identified higher rates of illness among inhabitants of villages whose drinking water failed European directive standards than among inhabitants of villages whose drinking water satisfied the standards. The marker mostly closely associated with illness was the entrococci count, although fecal coliforms were also independently associated with illness (Barrell*et al*., 2000).

The degree of bacterial pollution in any water samples were summarized in the following table according to WHO and FDRE, MoWR.

Table 1: Standards set by World Health Organization (WHO, 2004) and Ethiopian, Ministry of Water Resources (MoWR, 2002).

Ranges of Coliform bacteria (Tc & Fc) Per 100 ml of water sample	Standards
0 cfu/100 ml	'safe water' range
1-10 cfu/100 ml	'acceptable/a reasonable water quality' range
11-100 cfu/100 ml	'unacceptable/polluted' range
101-1000 cfu/100 ml	'dangerous' range
> 1000 cfu/100 ml	'very dangerous' range

Table 2: Categorization of drinking water systems based on compliance with performance and safety targets set by World Health Organization (WHO, 2008).

Quality of water	Proportion (%) of samples negative for E.coli		
	Population size		
	<5000	5000-100,000	>100.000
Excellent	90	95	99
Good	80	90	95
Fair	70	85	90
Poor	60	80	85

2.4 Sources of water pollution

The primary source of microbial pollution in agricultural watersheds is fecal matter from livestock production. The microbial loading potential from point sources, such as storage facilities and feedlots, and from non-point sources, such as grazed pastures and rangelands, is substantial (Jamieson *et al*., 2004). Although a variety of protozoa and bacteria can be shed by livestock and transmitted to humans through water, animal agriculture is likely responsible for a percentage of many of the pathogens we find in surface water, but whether that percentage is 5%, 50% or 95% compared to non-agricultural sources such as humans or wildlife is unknown at this time for most watersheds (Edward, 1995).

2.5 Environmental factors that influence pathogens

Once a pathogen leaves the host environment, it must adjust to external conditions that are different and stressful. The length of survival is extended from days to possibly months in relatively protected (lack of predators, toxins, sunlight, and cooler), moist, nutritive environments such as sediments and deep soils (> 40cm). It is assumed that gastrointestinal pathogens in fecal matter will die at the same rate as the indicator bacteria; therefore, high concentrations of fecal indicator bacteria indicate an increased likelihood of pathogens being present (Cabelli *et al*., 1982).

The survival of most pathogens is highly variable depending upon the receiving water, particularly turbidity, temperature, oxygen levels, presence of nutrients and pesticides, pH, organic matter, and solar radiation (Moore *et al*., 1988).

Temperature, pH, moisture, nutrient supply and solar radiation seem to have the greatest effect on enteric bacterial survival. Lower temperatures appear to increase survival time as noted by (Kibbey *et al*., 1978). This investigator also indicated that temperature extremes seem to be most disruptive to bacterial survival. Most bacterial pathogens are sensitive to temperatures exceeding 60^{O}C. Bacterial pathogens can produce resistant endospores or thick-walled cells and only be killed by high temperatures in excess of 100^{O}C. The normal pH range for most water bodies is close to 7 (neutral) and would not affect bacteria survival. Only at extreme pH (< 4.5 or > 8.2) can cell die-off be expected (James, 1999).

Turbidity in addition to temperature and pH can also influence the microbiological quality of drinking water. Therefore, these are recommended in water monitoring programs as they may influence disinfection efficiency and microbial survival (FDRE, MoH, 2006). Turbidity in water is caused by suspended and colloidal matter such as clay, silt, finely divided organic and inorganic matter, and plankton and other microscopic organisms. Turbidity caused by high levels of organic matter can protect microorganisms from the effects of disinfection. It even can stimulate bacterial growth. Higher turbidity levels are often associated with higher levels of disease-causing microorganisms such as viruses, parasites and some bacteria. The maximum contaminant level turbidity for public potable water is 1 NTU (Environmental Monitoring and assessments, 2006).

3. Significance of the study

Water-borne diseases are a global problem with multi facet out comes. The problem is well pronounced in developing countries. In our country, effective water quality checking programs such as assessing Bacteriological quality of drinking water, the one established in the developed world intended to reduce water-related disease, is not done regularly due to lack of infrastructure and resource facilities (Kume and Ali, 2005).

Health status of the community is a priority criterion in the MoH of Ethiopian government. Consequently, increasing water quality of the community is taken as a means to increase health status of the people, food handling practices and self-sufficiency of the rapidly increasing population of the country. At present, the transmission of water related diseases are a global public health problem and result in increased illnesses, deaths, and health-care costs (FDRE, MoH, 1997).

Because of the need of recent and up-to-date information on bacteriological quality of drinking water, there comes the need to assess water quality of the study area in order to increase water quality of the community which inturn taken as a means to increase health status of the people. For that matter, the primary intent of this study was assessing bacterial contamination level among selected sites and surveying sanitary risk factors at home level about drinking water in Arba Minch town.

Therefore, this study could play a significant role for different stakeholders who are involved directly or indirectly in the quality of water supply in the study area. It also creates awareness among households about hygiene of the water and also be used as an input by the city officials for policy implementation and decision making regarding to quality of water supply in Arba Minch town, Southern Ethiopia.

4. Objectives of the study

4.1 General Objective

- To assess the bacteriological quality of drinking water at main source, reservoir, along pipes and household level and to survey the possible sanitary risk factors at individual household level in Arba Minch town, Southern Ethiopia.

4.2 Specific Objectives

- To assess and compare indicator bacterial (TC and FC) contamination of drinking water at main source, reservoir, along pipes and household level.
- To determine water contamination indicator bacteria such as total coliforms (TC) and faecal coliforms (FC) and
- To isolate pathogenic bacteria mainly *Salmonella*, *Shigella* and *V.cholerae*.

5. Materials and Methods

5.1 Description of the Study area

The study was conducted in SNNPR region, Gamo Gofa zone, Arba Minch town, South Ethiopia. It is located at about 505 km South of Addis Ababa and 270 km away from Hawassa, capital city of SNNPR region. The study area has uneven topography with upland, mid-slopes and bottom lands. It receives mean annual rainfall ranging from 800-1000 mm and has an average temperature of 29.7°C. This high temperature is because of the area is found in "East African Great Rift Valley Region". The altitude of the area ranges between 1300 and 1500 meter above sea level. On global map, the town is located 6.04^0N Latitude and 36.4^0E Longitude. Its land coverage is more than 2184 hectares and its total population is 82,000 (**Source:** Municipality of Arba Minch town and Arba Minch Branch Meteorology Observatory Office, 2010). The mapping of water resources in Arba Minch town showed that the community had around 50 water sources including springs, rivers and lakes.

5.2 Study Design and Period

A cross-sectional study was carried out to assess bacteriological quality of drinking water at main source, reservoir, along pipe and household level in Arba Minch town (Sikela and Secha Sub-cities) from November, 2010 to January, 2011.

5.3 Source and Study population

The source and study population for this study was the water that is available in Arba Minch town and main source water (untreated spring water before distribution), reservoir (treated spring water before distribution), pipes water (treated water after distribution) & household water (treated water after collection and storage) in selected sites respectively.

5.4 Sampling techniques and Sample size determination

Both probability and non- probability sampling techniques were applied to conduct this study. The probability sampling technique was employed to take water samples from pipes and households. To determine the sample size from households and pipes, the two sub- cities were divided in to 4 different strata based on kebeles. Then after, one kebele per each stratum was selected through simple random sampling techniques. After that from selected kebeles, 100 households were taken to investigate their water handling practice by systematic random sampling method based on the proportion of total population of each selected kebeles for the

purpose of questionnaire. Similarly, samples for bacteriological water analysis from pipes and households were taken from every 5th (fifth) systematically selected households which already chosen for questionnaire survey.

On the other hand, convenient non- probability sampling technique was applied to take samples from main source and reservoir water. Besides, concerned governmental city officials were also interviewed to get information about past-research findings on water quality in the town.

As there is no reliable estimate on the proportion of main source, reservoir, pipes and household drinking water quality in the study area, a 50% proportion which leads to the highest possible sampling size was used (Recommended by Daniel, 2005).

Therefore, the estimate could be designed to become 5% margin of error and 95% confidence interval. Since, source population is less than 10,000, we would use correction formula.

Sample size calculation:

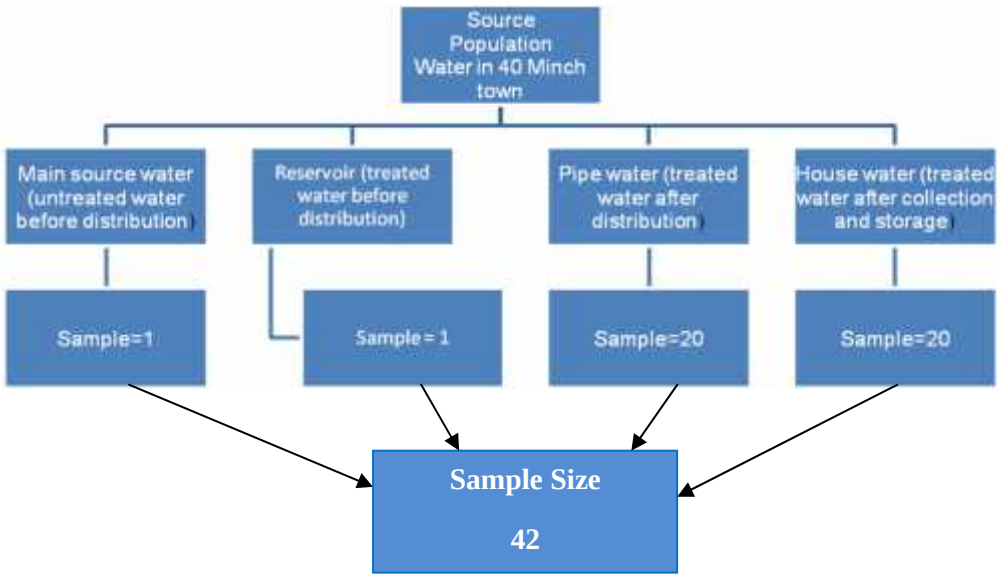
$$nf = \frac{no}{1+no/N} \longrightarrow nf = 42$$

Where nf - final sample size

no - initial sample size (384)

N - Source population (48)

Sampling frame work:



5.5 Eligibility criteria

1. Exclusion criteria

- Water samples were not taken from a broken sample collection bottle.

2. Inclusion criteria

- In the case of pipes, samples were taken from the households who have water-meter.

5.6 Operational definitions

1. Pollution indicators

- Are organisms used to measure faecal contamination level of water and its efficiency of treatment. Most commonly used indicator organisms are the coliform bacteria.

2. Total coliform

- Group of bacteria that able to ferment lactose at 35-37 °C with the production of acid and gas within 24-48 hours. These are genus *Klebsiella, Enterobacter* and*Citrobacter.*

3. Thermotolerant/Faecal coliforms

- Group of coliforms that are able to ferment lactose at 44-45°C within 16-18 hours and they comprise mainly genus *Escherichia coli* (*E.coli*).

4. Potable water

- Drinking water that is recommended to contain zero total coliforms and fecal coliforms/100 ml of water according to WHO guidelines.

5. Main source water

- Protected spring water that has a distribution site somewhere near the protection or collection boxes/chamber that is used to allow water to be entering distribution line.

6. Reservoir water

- Protected spring well water that is properly covered by stone masonry, internally plastered at least 3 meters above and with distribution system that used to deliver treated water to the town.

5.7 Variables

Both dependent and independent variables will be used to conduct this study.

Independent variables

- Sites
- Date of sampling (Rounds)
- Socio-economic status
- Educational background

Dependent variables

- Bacterial Counts

5.8 Sample collection and transportation

5.8.1 Sample Collection

Both primary and secondary data were collected to carry out laboratory water analysis for confirmation of bacteriological contamination level of drinking water at the selected sites.

Primary data were compiled through pre-designed & pre-tested questionnaire survey and water sample collection methods **(annex 1**). Questionnaire were distributed to the selected households to get relevant information about the sanitation (handling practices) of household drinking water (**annex 1-I)**. Similarly, data from the concerned city officials were also obtained through questionnaire to get further information about the treatment of water and awareness of the community about sanitation of drinking water before this study **(annex 1-II)**. Finally, Water samples taken from main source, reservoir, pipes and households (200 ml from each site) for three rounds were used to determine bacteriological quality of drinking water **(annex 1- III)**.

On the other hand, secondary data such as assessing both bacteriological and physicochemical parameters of the drinking water of the town which were obtained in the form of written documents from Arba Minch Municipal Water Supply Enterprise Office and it was used as supplementary information for gathering primary data.

5.8.2 Sample Transportation

Immediately after sample collection, water samples were placed in an insulated cold box for transport to a water testing laboratory (Arba Minch Regional Lab). Then after, water samples were examined within 6 hours of interval of sample collection. However, in the case of water samples that were taken from reservoir which contain chlorine or chloramines, 0.1-0.2 ml of thiosulphate ($Na_2S_2O_3.5H_2O$) was added to each collection bottle to neutralize these substances (**annex 1-IV**).

5.9 Laboratory Procedures

After sample collection and transportation, the water samples were processed in Arba Minch Regional Laboratory to assess bacteriological quality of drinking water according to standard culture and examination methods **(annex 2)**.

5.9.1 Culturing the Organisms

All water samples were cultured and examined for total coliforms, thermotolerant/faecal coliforms and pathogenic bacteria in Arba Minch Regional Laboratory.

a) Membrane Filtration Techniques

To determine the presence of total coliform and thermotolerant/faecal colifrom in the water samples, standard membrane filtration methods were used as described in membrane filtration techniques (APHA, 1998)**.** This technique involved filtering water through a membrane that retained total coliforms & thermotolerant/fecal coliforms; incubating this membrane on a growth promoting medium and then, counting the result of TC and TTC units (**annex-2A**).

b) Enrichment and Streak Plate Methods

This method was used to enrich culture and identify pathogenic bacteria mainly *Salmonella, Shigella and V.cholarae* (**annex-2B**).

5.9.2 Identification of Organisms

Verification test or identification process was done by transferring grown organisms from each colony and placed them in their specific biochemical test category. As a result, Citrate utilization test, Indole test, Oxidase test, LDC test, and Motility test were used in this study (**annex-2C**).

5.10 Quality control

The quality of data was managed/controlled according to the guidelines of World Health Organization (WHO, 2004).

A) Reference Strains

Standard reference strain for *E. coli* (ATCC-25922) and *P. aeruginosa* (ATCC-27853) from the EHNRI laboratory stock were used as a quality control throughout culturing process and biochemical testing methods.

B) Other quality controls

These include:

- Training data collectors.
- Taking a care during sample collection, handling, transportation & culturing time.
- Monitoring temperature, storage conditions and moisture requirements.
- Checking sterility of culture media, membrane filters and all laboratory equipments.

5.11 Data Analysis

Data entry and analysis was done using SPSS (17^{th} version) computer package systems. After that, results of water analyses were compared against standards set by (WHO, 2004) and (MoWR, 2002). Then after, T-test at 5% level of significance was used to compare the quality of water between all sampled sites. As a result, a *p*-value of <0.05 was considered indicative of a statistically significant difference. Beside to this, descriptive, Bivariate and multivariate linear logistic tests were also applied to analyze this study.

5.12 Ethical considerations

This MSc thesis was ethically cleared by the Department of Microbiology, Immunology and Parasitology (DMIP) of the Faculty of Medicine of Addis Ababa University (FoM, AAU) before implementing the actual study. Sampling of water was carried out with full consent of the head of the households. Before each sampling, the study objectives were clearly explained to the head of households that the aim of this study is neither to evaluate the performance of the individual nor to blame anyone for weakness, but to gather information that might lead to eventual improvement in the situation. Each head of household was also assured that the information provided will be confidential and used only for the purpose of research (**annex-3**).

6. Results

6.1 Sanitary risk factors at household level

The questionnaire survey was done before starting the laboratory water analyses. The household were selected by systematic random sampling technique in order to investigate water handling practice at individual home level. For that matter, total of 100 households were interviewed by using pre-tested questionnaire survey method (**annex 1-I**). In similar manner, the city officials were also interviewed to get pre-research findings on water qualities in the study area (**annex 1-II**). Accordingly, the following results were obtained.

The data from the respondents (**table 3**) indicated that:-

- Most 80 (80%) of the households said that they use stored water for at least more than 2 days.
- 75 (75%) of the households responded that they use bucket for water collection and storage process.
- Similarly, 75 (75%) of the households reported that they practice dipping method to transfer drinking water from bucket (storage container).
- Comparably, 50 (50%) of the households responded that they don't wash their hands before water collection and
- Similarly, 50 (50%) of the households answered that they practice placing/putting water drinking utensils on floor.

The following questionnaire results were also obtained from the city officials (**table 4)**. According to their response, the following results were obtained. These are:

- All (100%) of the city officials said that they have practices of assessing the quality of pipes water for both bacteriological and physicochemical aspects within three (3) months of interval.
- Again, 100% of them responded that they don't have any practice of testing the quality of household water for bacteriological as well as physicochemical parameters.
- All (100%) of the city officials also reported that they don't have any trends of giving awareness regarding to hygiene/sanitation practice drinking water at both community and household level.

Table 3: Summary of major sanitary risk factors as identified from house hold level by using questionnaire survey (n=100) at Arba Minch town in November, 2010.

Sanitary risk factors	% of respondents	
	Yes	No
Collecting water using Bucket	75	25
Washing hands before water collection	50	50
Storing water at home for more than 2 days	80	20
Transferring water using dipping method	75	25
Placing water drinking utensils on floor	50	50

Table 4: Summary of trends of checking/testing quality of drinking water as interviewed from the city officials by using questionnaire survey (n= 5) at Arba Minch town in November, 2010.

Checking quality of drinking water	% of respondents	
	Yes	No
Assessing the quality of pipe water before this study	100	0
Assessing the quality of household water before this study	0	100
Creating awareness water handling practices at community level	0	100
Creating awareness water handling practices at household level	0	100

6.2 Bacteriological analysis of water samples at selected sites

In the present study, assessment of contamination level drinking water by Total coliforms (TC) and Faecal coliforms (FC) as well as presence/absence of pathogenic bacteria from main source, reservoir, pipes and household level were studied. A total of 126 water samples were collected from the above selected sites for three rounds. Consequently, all water samples were analyzed for bacteriological qualities.

6.1.1 Detection of indicator and pathogenic bacteria in sampled water

a) Detection of indicator bacteria

Bacteriological analysis of water samples from four selected sites at Arba Minch town showed that most water samples that were taken from household level and some water samples which were taken from pipes were positive for TC and FC bacteria. But those from main source and reservoir were negative (zero count) for both TC and FC bacteria in three (3) sampling rounds (**table 5**).

Table 5: Bacteriological analysis of water sample from main source, reservoir, pipe and household level in Arba Minch town between November, 2010 and January, 2011.

Types of Bacteria	Water sampled sites							
	Main source (n=1)		Reservoir (n=1)		Pipe (n=20)		Household (n=20)	
	No. of +ve Samples	%	No. +ve samples	%	No. +ve samples	%	No. +ve samples	%
TC	0	0	0	0	2	10	20	100
TTC/FC	0	0	0	0	1	5	11	55
Pathogenic Bacteria	0	0	0	0	1	5	4	20

Note: 1) No. = Number

2) +ve = Positive

Indicator bacteria (TC and FC) (**Fig. 1**) were often encountered in most of household water and in some of pipe water. But, no occurrences of indicators were observed in water samples that were taken from main source and reservoir.

Figure 1: Picture of TC/FC bacterial colonies on Membrane Filtration (MF) at sampled sites (pipe and household water).

b) Detection of pathogenic bacteria (*Salmonella and Shigella spp*)

Similar to indicator bacteria, there were no occurrences of pathogenic bacteria in water samples that were taken from main source and reservoir. But fewer occurrences of pathogenic bacteria (**Fig. 2**) were observed in household water and very less (1/20) pathogenic bacteria were seen in pipes water (**table 5**).

Figure 2: Picture of *Salmonella/Shigella spp* on SS (Salmonella- Shigella) agar in sampled sites (pipe and household water).

6.1.2 Concentration ranges of indicator bacteria in sampled water

The highest average bacteriological counts were observed in household water. That was, 14.50 cfu/100 ml for total coliform (TC) and 2.68 cfu/100 ml for faecal coliform (FC) bacteria. While the lowest mean counts were observed in main source and reservoir water. That was, 0 cfu/100 ml for both TC and FF bacteria (**table 6**).

Table 6: Mean and Standard Error (S.E) of bacteriological counts of total coliform (TC) and fecal coliform (FC) from main sources, reservoir, pipe and household water in Arba Minch town between November, 2010 and January, 2011 (n =1 for main source & reservoir whereas n=20 for pipe & household water).

Source/sites	Mean & S.E of TC counts/100 ml	Mean & S.E of FC counts/100 ml	P*
Main source	0.00 ± 0.000	0.00 ± 0.000	
Reservoir	0.00 ± 0.000	0.00 ± 0.000	
Pipe water	0.75 ± 0.301*****	0.20 ± 0.100******	
Household water	14.50 ± 0.952*****	2.68 ± 0.389******	P<0.05
Total	**7.26**	**1.37**	

Note: 1) * Mean and S.E of TC bacteria counts from pipe and household water.

2) ** Mean and S.E of FC bacteria counts from pipe and household water.

3) P* = T-test for significance differences between the means of pipe and household water.

Table 6 above shows average analyses of water samples for TC and TTC/FC bacteria counts from main source, reservoir, pipe and household water. The result revealed that average TC and FC bacteria counts from both main source and reservoir (n=1 for each site) were 0 cfu/100ml. While, average TC bacteria counts from pipe and household water (n=20 for each site) were 0.75 & 14.50 cfu/100 ml respectively. Similarly, average FC bacteria counts again from pipe and household water (n=20) were 0.20 & 2.68 cfu/100 ml respectively.

Table 7: Degree of bacterial pollution ranges in sampled water of Arba Minch town between November, 2010 and January, 2011.

Sites	Pollution classes (bacterial cfu/100 ml)				
	0	1-10	11-100	101-1000	> 1000
Main source					
TC	100	0	0	0	0
FC	100	0	0	0	0
Reservoir					
TC	100	0	0	0	0
FC	100	0	0	0	0
Pipe					
TC	90	10	0	0	0
FC	95	5	0	0	0
Household					
TC	0	0	100	0	0
FC	45	55	0	0	0

Note: 0 = Safe/Excellent water, 1-10 = Reasonable/acceptable water, 11-100 = Polluted water, 101-1000 = dangerous water and > 1000 = Very dangerous water ranges.

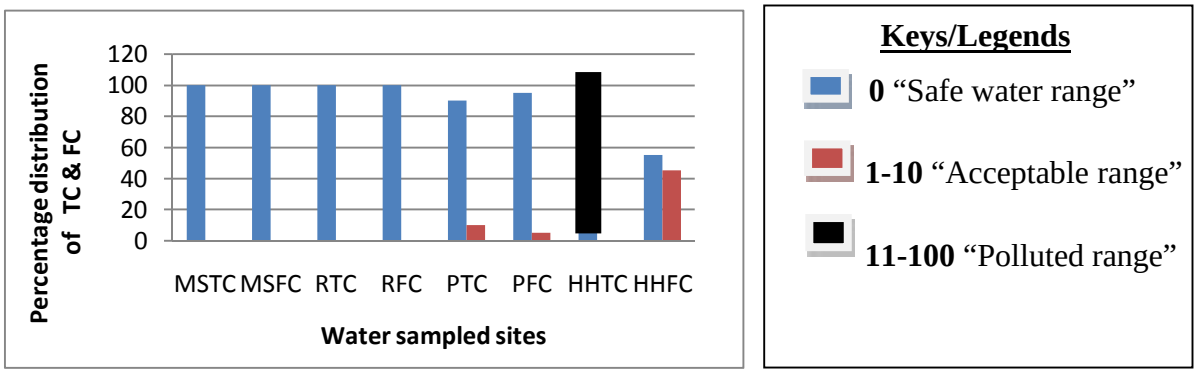


Figure 3: Ranges of TC and FC bacteria from Main Source (MS), Reservoir (R), Pipe (P) and Household (HH) water.

Table 7 and Figure 3 above show percentage distribution of water sample in each range of TC and TTC/FC bacteria from main source, reservoir, pipe and household water. The result indicated that 1(100%) of water samples that were taken from both main source and reservoir had 0 ranges for TC & FC bacterial counts. Similarly, 18(90%) and 2 (10%) of pipe water had 0 & 1-10 ranges for TC bacteria counts respectively. While, 19 (95 %) and 1 (5%) of pipe water again had 0 & 1-10 ranges for FC bacteria counts respectively. Whereas, 20 (100%) of water samples that were taken from home container had 11-100 ranges for TC bacteria counts. Moreover, 9 (45%) and 11 (55%) of household water had 0 & 1-10 ranges for FC bacteria counts respectively.

6.1.4 Trends of water contamination level over study period

Contamination levels of the indicators at each sampling site were not similar over the three sampling rounds (**Fig. 4**). Consequently, average concentrations TC and TTC/FC bacterial counts in all selected sampling sites (means of bacterial indicators were square root transformed) were not consistent over time. In most cases, the highest average indicator bacteria concentrations in both pipe and household were observed in November, 2010 (1st sampling rounds). While the lowest average counts were mostly recorded in January, 2011 (3rd sampling rounds).

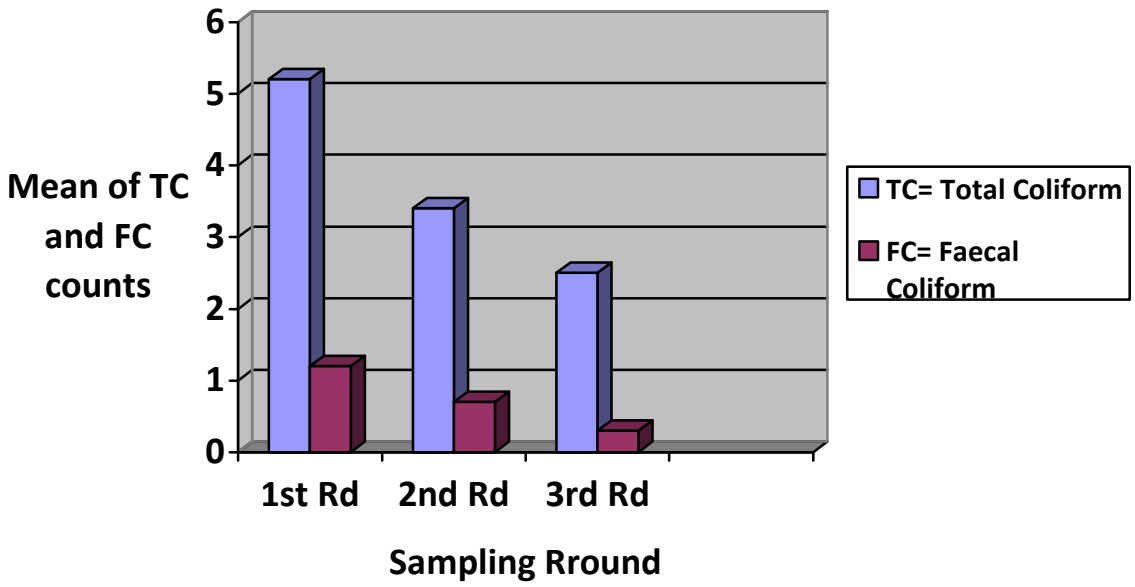


Figure 4: Trends of indicator bacteria concentration in sampled water during the three sampling rounds.

6.1.5 T-test analyses for TC and FC bacteria counts in sampled water

T- test was used to test whether the average counts of TC and TTC/FC bacterial counts were the same or not between all water sources at 5 % level of significance. The results of **t-test** for differences between water samples that taken from household and pipe water for TC and TTC/FC bacterial counts were summarized in **table 6.** The result of t-test showed that the difference in total coliform (TC) and faecal coliform (FC) bacterial counts were significant in household water ($P< 0.05$) at the 5 % level of significance. That is, water sample were taken from home storage containers had high concentration of total and thermotolerant/ faecal coliforms than pipe water (**table 6**).

Note: T-test can't be computed for water samples that taken from main source and reservoir. This is because of their standard deviation is zero.

6.1.6 Comparing water qualities between selected water sampling sites

Mean values of observations of total coliform (TC) and faecal coliform (FC) counts from water samples of main source, reservoir, pipe and home storage containers were compared using multivariate linear logistic test method (especially Bonferroni's multiple comparison method) to see significance differences between individual pairs of means (**table 8**). The result showed that the difference in total coliform (TC) and faecal coliform (FC) bacterial counts were significant in household water ($P<0.05$) at the 5% level of significance. That is, water samples taken from home storage containers had high concentration of total coliforms and feacal coliforms (FC) than other sources (main source, reservoir and pipe). Similarly, water samples taken from pipe had high concentrations of TC and FC bacterial counts than main source and reservoir water. But it had less/almost none concentration of TC and FC than that of household water.

Table 8: Multiple mean comparisons by using Bonferroni's method among all water sampled sites in Arba Minch town between November, 2010 and January, 2011.

Dependent Variable	(I) Site	(J) Site	Mean Difference (I-J)	Std. Error	Sig.	95% CI
TC	HH	MS	14.50(*)	3.181	.000	[5.97 - 23.03]
		P	13.75(*)	.982	.000	[11.12 - 16.38]
		R	14.50(*)	3.181	.000	[5.97 - 23.03]
	MS	HH	-14.50(*)	3.181	.000	[-23.03 - (-) 5.97]
		P	-.75	3.181	1.000	[-9.28 - 7.78]
		R	.00	4.390	1.000	[-11.77 - 11.77]
	P	HH	-13.75(*)	.982	.000	[-16.38- (-) [11.12]
		MS	.75	3.181	1.000	[-7.78 - 9.28]
		R	.75	3.181	1.000	[-7.78 - 9.28]
	R	HH	-14.50(*)	3.181	.000	[-23.03 - (-) 5.97]
		MS	.00	4.390	1.000	[-11.77 - 11.77]
		P	-.75	3.181	1.000	[-9.28 - 7.78]
FC	HH	MS	2.68	1.279	.228	[-.75 - 6.11]
		P	2.48(*)	.395	.000	[1.42 - 3.54]
		R	2.68	1.279	.228	[-.75 - 6.11]
	MS	HH	-2.68	1.279	.228	[-6.11 - .75]
		P	-.20	1.279	1.000	[-3.63 - 3.23]
		R	.00	1.766	1.000	[-4.74 - 4.74]
	P	HH	-2.48(*)	.395	.000	[-3.54 - (-)1.42]
		MS	.20	1.279	1.000	[-3.23 - 3.63]
		R	.20	1.279	1.000	[-3.23 - 3.63]
	R	HH	-2.68	1.279	.228	[-6.11 - .75]
		MS	.00	1.766	1.000	[-4.74 - 4.74]
		P	-.20	1.279	1.000	[-3.63 - 3.23]

Note: 1) * The mean difference is significant at the .05 level.

2) CI= Confidence Interval.

3) I-J= The mean difference between i^{th} site observation and j^{th} sites observation (there are a total of 3 j^{th} sites).

7. Discussion

Health is determined by many factors, including income, environmental conditions like access to adequate sanitation and safe drinking water supplies, behavioral change and availability of health services. Therefore, indicator bacteria such as total coliforms and thermotolerant/ faecal coliforms have been used to measure water quality and personal hygiene standards in a variety of settings (Kaltenthaler *et al*., 1996).

In this study, the bacterial total coliforms and thermotolerant/ faecal indicator organisms were used to provide an insight in to the water quality from main source, reservoir, pipe and home storage containers in Arba Minch town. Coliform bacteria may not cause disease but can be used as indicators for faecal contamination. In addition to bacteriological quality, present study also surveyed sanitary risk factors at household level.

In the present study, the sanitary survey was able to indicate the condition of water sanitation practices in Arba Minch town mainly in relation to socio-economical background, education level, family size and hygienic practices. The condition in most cases was very poor. This may be due to socio-economical, cultural and related knowledge barriers inherent in the study area. Unsafe water handling practices which were surveyed at household level in the study area revealed that the majority of the households (75%) use bucket for water storage purposes in the home. In study that was conducted in Northeast, Ethiopia showed that 72% of households use bucket (Seid *et al.,* 2003), which is less than the present study that was 75%. Metens (1990) showed that the proportion of faecal ciliform positive water samples were higher on the use of wider-necked water container than narrow-necked containers by preventing hands from entering the water container and contaminating the contents. Therefore, this storage container may contribute for the presence of better indicator bacterial counts than other storage containers.

It was also found that the majority of the households (75%) use dipped out rather than poured method when taking water from storage container. A study from Zambia showed that 80% of the households use dipping out method when taking water from storage container (Sultton and Dominic, 1989), which higher than the present study that was 75%. In another study that

was conducted in Northeast, Ethiopia indicated that 72% of the households use dipping method (Seid *et al.,* 2003), which is less than the present study that was 75%. Therefore, this method may contribute for the existence of undesirable bacteria in home stored water. This might be due to there was a chance of introducing infected/soiled fingers in to stored water.

Another sanitary risk factor for the contamination of stored water at home was placement of drinking utensils on the floor. A study from Northeast, Ethiopia showed that 73.4% of the households put drinking utensils on the floor (Seid *et al.,* 2003), which is higher than the present study that was 50% only. As a result, this method may also contribute for contamination of drinking water in the home. This might be due to there was a chance of transferring contaminated soil when water drawing utensils are placed on the floor.

Another sanitary risk factor for the contamination of stored water at home was hand washing practices before water collection. A study from Northeast, Ethiopia showed that 64.1% of the households wash their hands before water collection (Seid *et al.,* 2003), which is again higher than the present study that was only 50%. Therefore, hand washing practices found to have a positive association with the bacteriological quality of household drinking water from collection/storage containers.

Based on the above evidences, water handling practices at household level in the study area were very poor. This might be due to poor health awareness, poor level of personal hygiene, differences in socio-economic & education background and having different family size among individual home level.

On the other hand, this study demonstrated that adequate protection of water sources could improve their bacteriological quality by effectively preventing TC and FC bacteria from entering water system prior to their deliver point. The bacteriological analysis of main source (protected untreated spring water) and reservoir (protected treated spring water) in this study clearly indicated that they found to have an excellent bacteriological quality. That was, they had 0 TC and FC bacterial counts/100 ml of water. A similar study that was conducted in Northeast, Ethiopia showed that out of five (5) protected spring water, three (3) of them had 2 FC bacterial counts/100 ml water (Seid *et al.,* 2003), which is higher than the present study that was 0 cfu/100 ml. Another study that was conducted on protected spring in North-

Gonder, Ethiopia demonstrated that 35.7% of water samples had 5 FC bacterial counts/100 ml (Mengesha *et al.*, 2004), which is again higher than the present study of Arba Minch town that was 0 FC bacterial count/100 ml of water. Another study in Uganda that was conducted on protected spring water showed that 50% of water samples had 8 and above FC bacterial counts/100 ml of water (WHO, 1998), which is again higher than the present study that was 0 FC bacterial counts/100 ml of water. The above mentioned differences of indicator bacteria among stidied area were might be because of differences in treatment/disinfection, regular supervision, proper maintenance and source of contaminants.

The bacteriological analysis of pipe water (treated water after distribution) in the study area also revealed that it has an excellent bacteriological quality. That was, it had an average of 0.75 TC and 0.2 FC bacterial counts/100 ml of water. A similar study that was conducted on water line (pipe water) in North-Gonder, Ethiopia showed that 21% of water samples had 18 & above TC counts/100 ml and 20% of water samples had 5-10 FC bacterial counts/100 ml (Mengesha *et al.*, 2004), which is higher than the present study of Arba Minch town that was 10% of water had 10 & below TC counts/100 ml and 5% of water had 0-4 FC bacterial counts/100 ml of water. In another study that was conducted on tap water in Nepala indicated that 53.6% water samples had 16 & above FC bacterial counts/100ml water (Tista *et al.*, 2007), which is again higher than the present study. The possible reason for differences in TC & FC bacterial counts in the above described studied area was there might be due to inadequate treatment or water treatment system was not operated satisfactorily.

The bacteriological analysis of household water (treated water after collection and storage) in the study area also revealed that it has an inferior bacteriological quality. That was, it had an average of 14.50 TC and 2.68 FC bacterial counts/100 ml of water. A study that was conducted on storage vessel in Northeast, Ethiopia indicated that 55% of water samples had 11-100 TC counts/100 ml and 45% of water samples had 1-10 FC bacterial counts/100 ml water (Seid *et al.*, 2003), which is less than the present study of Arba Minch town that was 100% of water had 11-100 TC counts/100 ml and 55% of water had 1-10 FC bacterial counts/100 ml of water. The highest proportion of inferior water quality in household water in present study was due to there might be cumulative effect of the way in which water collection & drawing technique, the method of transporting drinking water to home and the type of storage container in the house/home.

In the present study, percentage distribution of water samples in each range of TC and TTC/FC bacterial counts was also studied. The result revealed that 100% of water samples that were taken from both main source and reservoir had 0 TC & FC bacterial counts/100ml of water and they are categorized under 'safe water' range according to standards set for drinking water by (WHO, 2004) and (MoWR, 2002). A similar study that was conducted on protected water in North-Gonder, Ethiopia showed that 71.4% of water samples had 11-100 TC counts/100 ml and categorized under 'polluted' range. While, 50% of water samples had 1-10 FC bacterial counts/100 ml and categorized under 'acceptable/"B" category' range (Mengesha *et al.*, 2004), which is higher than the present study of Arba Minch town.

Percentage distribution from pipe water indicated that 90% of pipe water in the study area had 0 TC bacteria counts/100ml and it is found under 'safe water' range. Similarly, 95% of pipe water had 0 FC bacterial counts/100 ml which is also found under 'safe water/Excellent' range according to (WHO, 2004) and (MoWR, 2002) guidelines. A similar study that was conducted on water line (pipe water) in North-Gonder, Ethiopia showed that 57% of pipe water had 1-10 TC counts/100 ml and categorized under 'acceptable/"B" category' range. While, 50% of water samples had 1-10 FC bacterial counts/100 ml and categorized under 'acceptable/"B" category' range (Mengesha *et al.*, 2004), which is again higher than the present study of Arba Minch town. Another study that was conducted on pipe line (water) in Central Sudan indicated that 71% of pipe water had TC & FC bacterial counts/100 ml of water and 'unfit' for drinking purpose according to Sudanese standards and international standards (WHO, 1992) for drinking water (Abdel *et al.*, 2009), which is again higher than the present study. Accordingly, 90% of pipe water for TC bacteria counts and 95% of pipe water for Fc bacteria counts in the study area (Arba Minch town) meets WHO guidelines value (0 cfu/100 ml) for drinking water as compared to pipe water of North-Gonder and Central Sudan which crossed WHO guidelines.

Percentage distribution of indicator bacteria was also demonstrated in household water. The result revealed that 100% household water had 11-100 TC bacteria counts/100 ml of water and categorized 'unacceptable/polluted' range. Similarly, 55% of household water had 1-10 FC bacteria counts/100 ml which is in 'acceptable/a reasonable water quality' range according to standards set by (WHO, 2004) and (MoWR, 2002). A similar study that was conducted in Northeast, Ethiopia on home storage vessel showed that 82.5% of home storage

vessel had 11-100 TC bacteria counts/100 ml and categorized under ‘polluted/”C” category’ range. While, 55% had 1-10 FC bacteria counts/100 ml water and categorized under ‘acceptable/”B” category’ range (Seid *et al.,* 2003). Accordingly, home stored water in both studied area exceeds WHO guidelines and ‘unfit’ for the drinking purpose from bacteriological point of view.

Based on WHO standards which are set for drinking water, the water samples that had 0 TC/FC bacterial counts or they categorized under ‘safe water’ range recommended that they are not risky to human health. But the water samples that had FC bacterial counts indicated that the water has been faecally contaminated and therefore, presents a potential risk of excreta related diseases.

In the present study, mean values of total coliform and faecal coliforms were also compared between water sampled sites. The result showed that home stored water had high concentration of TC and FC bacteria than other sites (main sources, reservoir and pipe). This high contamination of water for TC and FC bacteria in household water may have arisen because of water drawing cups placed on the ground prior to being dipped in to the storage container, water could be contaminated during collection, transportation, storage in open vessels, and/or in vessels that are not washed regularly and immersing dirt (soiled) hands when drawing water might be the possible reasons for the significant difference in TC and FC bacteria in household water. Similarly, mean of TC and FC bacterial counts in pipe water were also high as compared to main source and reservoir water. The possible reason for contamination of pipe water was there might be corrosion/leakage of pipe along distribution line or the pipe could be old. Therefore, this condition may facilitate the contamination of the water with the soil or environment.

Similar study conducted in Thailand (Pinfold and Horan, 1991) indicated that there was a significant difference in stored water samples contaminated with total and faecal coliforms, which is in agreement with the present findings. The presence of faecal coliforms in drinking water shows that the water has been fecally contaminated and therefore, presents a potential risk of excreta related diseases. Contamination after collection and during transportation and storage is increasingly being recognized worldwide as an issue of public health importance (Lindskog, 1988; Genthe and Strauss, 1997).

Different researchers have documented water quality variation between the source and storage in household. The deterioration of water quality between the source and its use is the result of poor water handling practice (Jim *et al*., 2004), which is similar with the present study. The World Health Organization (WHO) survey team observed that drinking water drawn from relatively safe supply was stored in earthen jars for subsequent use but was later found totally contaminated with faecal matter indicating the rate of unhygienic containers contribute to increase the number of diarrhea cases (Boot *et al*., 1988), which is in agreement with the present findings.

In addition to indicator bacteria, the present study also covered the detection of pathogenic bacteria mainly *Salmonella, Shigella and Vibrios* from selected sites. From sampled sites, only the household and pipe water were positive for pathogenic bacteria especially for *Salmonella* and *Shigella*. Accordingly, 5% of pipe water had *Salmonella* and *Shigella spp*. Similarly, 20% of household water had *Salmonella* and *Shigella spp*. A study that was conducted on tape water in Nepala indicated that 3% of water samples had *Salmonella spp*, 4% had *Shigella spp* and 1% had *V.cholerae* (Tista *et al*., 2007), which is higher than the present study of Arba Minch town that was 2% of *Salmonella spp*, 3% of *Shigella spp* and 0% of *V.cholerae* respectively.

Compassion of pathogenic bacteria interms of percentage distribution was also made between pipe and household water in the present study. Consequently, 20% of household water had pathogenic bacteria as compared to pipe water that had only 5%. The possible reason for such differences between pipe and household water might be there was high chance of household water to be contaminated during collection, transportation, storage in open vessels, and/ or in vessels that are not washed regularly. And using communal cups and immersing dirty hands when drawing water also contaminate the household water. Even if the samples that taken from household and pipe water were positive for *Salmonella* and *Shigella spp*, their colony dose was not significant to cause the disease. This is because of the concentrations their colony in sampled water (pipe and household water) were not in infectious dose (10^5-10^8 bacteria) to cause symptomatic diseases like typhoid fever, dysentery, gastroenteritis and other illness according to WHO guidelines for contaminated drinking water.

The present study also tried to see trends of water contamination level at each sampling rounds. Consequently, contamination levels of all indicator bacteria at each sampling site over three sampling rounds were not consistent over time. In most cases, the highest average indicator bacterial concentrations in both household and pipe water were observed in November, 2010 (1^{st} round). This might be due to this month is considered as high rainy season as compared to December and January (2^{nd} and 3^{rd} rounds) respectively. In contrast, lower average counts of indicator bacteria were mostly recorded in December and January, months of the dry season in which the flood contamination would not be expected. Therefore, seasonal variation has an impact on contamination level of drinking water that means the more dry the season, the less contamination level of the drinking water.

Limitations of the Study

- This study didn't comprise all pathogenic bacteria rather than *Salmonella spp, Shigella spp* and *V.cholerae* due to shortage of time, budget and resource facilities.
- It didn't also include enough sample size because of shortage of time, budget and resource facilities.

8. Conclusions and Recommendations

8.1 Conclusions

The improvement of water quality is closely associated with man-environment relationships. There should be a dialogue between all actors and the community when undertaking water and sanitation activities. For positive results and better sustainability, the community should be involved and participate at all stages of water development and environmental sanitation schemes.

A combination of safe drinking water, adequate sanitation and hygiene practices like hand washing is a pre-requisite for morbidity and mortality rates reduction, especially among under five years old children in developing countries. To reduce the incidence and prevalence of diarrheal diseases, improvements in the quality of water, and improvements in personal and environmental hygiene/sanitation generally is required. Therefore, assessing bacteriological quality has been recognized as an appropriate method for drinking water quality in both urban and rural areas. It is recognized as a suitable technique to categorize drinking water as 'safe/potable' water, 'acceptable/ reasonable quality' water, 'unacceptable/ polluted' water and 'deteriorated/grossly polluted' water ranges by standards of WHO guidelines.

In this study, bacteriological quality of drinking water at main source, reservoir, pipe and household level were studied. Consequently, water analyses from both main source and reservoir as well as from majority of pipe water showed that they are safe for drinking purpose from bacteriological point of view. But water samples that taken from household level were polluted with indicator bacteria and not used for drinking purpose from bacteriological point of view.

In light of results obtained so far, the following conclusions are drawn**:**

- The result of questionnaire survey revealed that there was a big gap regarding to water handling practice among individual household level.
- Bacteriological quality of most sampled water sources in Arba Minch town did meet National or International guidelines for drinking water except water sample that taken from home stored container.

- The overall bacterial count assessment indicated that the majority of water sources in Arba Minch town could be classified as 'safe/excellent water' range except those from home stored container which could be classified under 'polluted' range according to (WHO, 2004) and (MoWR, 2002) guidelines.
- High counts of indicator bacteria in household water as compared to other water sources in the study area suggested that the presence of pathogenic bacteria (mainly *Salmonella* and *Shigella spp*) that may contribute a threat to anyone consuming these water sources.
- The contamination of water sources with indicator bacteria can be explained in part due to:
    - Lack of sanitation/hygiene in the case of household water.
    - Corrosion/leakage of pipe along distribution line in the case of pipe water.
- Concentrations of pollution indicator bacteria in the selected sites for water sampling process were much less than what has been reported in the literature review part.

8.2 Recommendations

Based on the research findings, the following recommendations can be formulated:

- As indicator bacterial counts in household water have exceeded the guidelines set for human use, there is an information-gathering tool called the KAP (Knowledge, Attitude and Practice) about hygiene/sanitation of water at community/household level is needed.
- Since some water samples that taken from pipes had indicator bacterial counts, pipes should be checked regularly whether they have corrosion/leakage in distribution line/system.
- Drinking water that is stored in home storage container should be boiled or treated with safeguard before it is used for the drinking purpose.
- Further, more detail and in depth study on assessment of microbial contamination (bacteria, viruses and protozoa) of drinking water is needed/required.

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10. Annexes

10.1 Annex- 1

I. Questionnaires used to study handling practices of drinking water at the household level at Arba Minch town.

Respondent Name ----------

Sub-city----------

Code No. ----------

Date of visit----------

A. Socio- economic and educational background questions.

1. What is your education level?

No education ☐ Elementary complete ☐ High school complete ☐

Certificate ☐ Diploma and above ☐

2. How many family members do you have?

<3 ☐ 3-5 ☐ >5 ☐

3. What is your occupation?

Farmer ☐ Merchant ☐ Driver ☐ Student ☐

Private employee ☐ Government employee ☐ others ☐

4. How much is your income per month?

<500 ☐ 500-1000 ☐ >1000 ☐

B. Water handling practices of the households.

1. What type of water collection container do you use?

Clay pot ☐ Bucket ☐

Jerricans ☐ Others ☐

2. Do you wash your hands before water collection?

Yes ☐ No ☐

3. How often do you collect water?

Every other day ☐ twice a day ☐

Once a day ☐ More than twice a day ☐

4. How long the stored water stay at home?

A day and less ☐ More than a day ☐

5. Do you use cover for water storage container?

Yes ☐ No ☐

6. What method do you use to transfer water from storage container?

Pouring ☐ Dipping ☐

7. How do you place water drinking utensils at home?

Tables/shelves ☐ storage cover ☐ Hang on wall ☐ Floor ☐

II. Questionnaires used to obtain pre-research finding data on drinking water quality from the concerned bodies at Arba Minch town.

Respondent Name ----------

Date of interviews----------

1. Have you ever assessed the quality of water supply of the town?

   Yes ☐ No ☐

2. If your answer for question no. 1 is 'yes', what the research reveals?

3. Have you ever created awareness among the households about the sanitation of drinking water at home?

4. Is there any NGO's that are working on the quality of water supply of the town?

III. Water sample collection procedures for Bacteriological analysis

A. Procedures used for collecting a water sample from Main source

(Collecting a water sample from a chamber)

1. 1st, choose a chamber that allows a water to enter a distribution pipe.
2. Then, sterile the mouth of drawing cup/collection bottle with a cigarette lighter.
3. Next, draw the water in opposite direction with the current of the water that entering the distribution pipe.
4. Then after, screw down the drawing cup aseptically.
5. Finally, deliver the collected water sample to diagnostic Lab within 6-8 hours of interval.

B. Procedures used for collecting a water sample from Reservoir

1. 1st, tie up the mouth of the drawing cup with long rope that reach the surface of the water inside Reservoir.
2. Then, throw down the tied and unscrewed drawing cup inside the reservoir.
3. Next, allow/make the water to be entering in to the drawing cup.
4. Then after, pull up the drawing cup that contain water sample.
5. Finally, place the screw on drawing cup aseptically and deliver the collected water sample to diagnostic Lab within 6-8 hours of interval.

C. Procedures used for collecting a water sample from home storage containers

1. Wipe the drawing cup using a clean cotton pad.
2. Sterilize the drawing cup with a flame from ignited alcohol-soaked cotton.
3. Open the storage container, draw the water and fill the sterilized bottle by water sample.
4. Finally, place the stopper in the bottle and deliver the samples to Lab within 6-8 hrs.

D. Procedures used for collecting a water sample from Pipe

1. Remove any attachment from the tap.
2. Using a clean cloth outlet of the tap wipe to remove any dirt.
3. Turn on the tap for maximum flow and the water may run for two minutes.
4. Outlet of the tap is sterilized by means of flame from cigarette lighter.
5. Tap again opened to flow for 1 to 2 minutes at medium flow rate.
6. Sterile 250 ml plastic bottle is taken for sample collection.
7. Carefully unscrew the cap and immediately hold the bottle under the water jet and fill.
8. Water filled up to 200 ml and a small air space is left to make shaking before analysis.
9. Deliver collected water samples to Laboratory within 20 to 30 minutes of interval.

IV. Transporting chlorinated water samples to a water testing Lab

1. 1st, add 5 gram of Sodium thiosulphate to 500 ml of distilled water according to manufacturer rule.

2. Then, mix up the solution and boil until it dissolves.

3. Next, sterilize the solution at 121°c for 15 minutes.

4. Then after, add 0.1-0.2 ml of Sodium thiosulphate (3% w/v) to 200 ml sterile collection bottle.

5. Finally, deliver the chlorinated water samples that contain Sodium thiosulphate to diagnostic Lab within 6-8 hours of interval.

Source: Cheesbrough Monica. District Laboratory Practice in Tropical Countries Part 2 Second ed. Cambridge University press, 2005.

10.2 Annex-2

Laboratory Procedures for Bacteriological analysis of water

A. Membrane Filtration Method

1. Collect approximately 200 ml of water to be tested from study sites.

2. Mix thoroughly the sample of water by inverting the bottle several times.

3. Using sterile blunt-ended forceps, place a sterile membrane filter (0.45µm pore sizes, 47 mm diameter, sterile and gridded from WAGTECH) on the filter base/funnel.

4. Pour 100 ml of water from the sample into a filtering funnel.

5. Apply suction (using hand, water, or electric device) to draw the water sample through the filter membrane.

6. Using sterile blunt-ended forceps, aseptically remove the membrane from the filtration funnel.

7. Place the membrane in a Petri dish containing M-Endo Agar LES (No. 0756) which is a growth medium for total coliforms/thermocoliform bacteria.

8. Leave for 1 hour (4 hours for chlorinated samples) before incubating the sample(s) at the temperature of 44.5 ºC (TTC) or at 35 ºC (TC) for one day (24 hours).

9. Place the Petri dishes (lids uppermost) in the incubator.

10. Examine the membrane for Yellow/Pink colonies for TTC and TC counts respectively by using dissecting microscope/hand lenses.

11. Finally, calculate the presumptive TTC and TC colony count/100 ml of water sample by using colony counter.

Source: APHA, 1998 and Cheesbrough Monica. District Laboratory Practice in Tropical Countries Part 2 Second ed. Cambridge University press, 2005.

B. Enrichment and Streak Plate Method

i) Enrichment Method

a) Enriching *Salmonella spp*

1. Take 10 ml from the original water sample.
2. Then, add it to a test tube containing 10 ml of sterile selenite broth (Oxoid, Ltd).
3. Finally, incubate it at 37 ºC for 24 hours.

b) Enriching *Shigella spp*

1. Take 10 ml from the original water sample.
2. Then, add it to a test tube containing 10 ml of sterile nutrient broth (Oxoid, Ltd).
3. Finally, incubate it at 37 ºC for 24 hours.

c) Enriching *V.cholerae*

1. Take 10ml from the original water sample.
2. Then, add it to a test tube containing 10 ml of sterile alkaline peptone water.
3. After that, incubate the samples at 37 ºC for 4-6 hours.
4. Finally, Examine growth (turbidity) on and just below the surface of the medium.

ii) Streak Plate Method

a) Culturing Salmonella spp

1. Take a loopful growth of *Salmonella spp* from a well- enriched broth.
2. Then, sub-culture on SS (Salmonella Shigella) agar using streak plate method.
3. Incubate the culture at 37 ºC for 24 hours.
4. Observe black color colonies on center.

b) Culturing Shigella spp

1. Take a loopful growth of *Shigella spp* from a well- enriched broth.
2. Then, sub-culture on SS (Salmonella Shigella) agar (Oxoid, Ltd) using streak plate method.
3. Incubate the culture at 37 ºC for 24 hours.
4. Observe non- black color colonies on center.

c) Culturing V.cholerae

1. Take a loopful growth of *V.cholerae* from a well- enriched broth and sub-culture on TCBS (Thiosulphate Citrate Bile Salt) agar (Oxoid, Ltd) using streak plate method.
2. Incubate the culture at 37 ºC for 24 hours.
3. Observe yellow sucrose-fermenting colonies after 18-24 hours of incubation.

C. Biochemical Tests

1. Citrate test

(Citrate utilization using Simmon's agar)

- Used to confirm/verify pollution indicator bacteria mainly total coliforms (TC).

1. Prepare a slope of the medium in bijou bottles as recommended by the manufacturer.
2. Using a sterile straight wire, 1st streak the slope with a saline suspension of the test organism and then stab the butt.
3. Incubate at 35–37 ºC for 48 hours.
4. Look up for a bright blue color in the medium for Positive citrate test.

Note: 1) Bright blue…….Positive Citrate test

2) No change of the color in the medium…….Negative Citrate test

2. Indole test

(Detecting Indole production by using tryptone water)

- Used to confirm/isolate mainly *E.Coli*.

1. Inoculate the test organism in a bijou bottle that contains 3 ml of sterile tryptone water.
2. Incubate at 35–37 ºC for 48 hours.
3. Test for indole production by adding 0.5 ml of Kovac's reagent.
4. Shake gently and finally, examine a Red color in the surface layer within 10 minutes for Positive indole test.

Note: 1) Red surface layer…….Positive Indole test

2) No red surface layer…….Negative Indole test

3. Oxidase test

(Using fresh reagent method)

- Used to verify/isolate mainly *Vibros spp* (*V.cholerae*).

1. Place a piece of filter paper in a clean Petri dish and add 2 or 3 drops of freshly prepared oxidase reagent.
2. Using a piece of stick or glass rod, remove a colony of the test organism and smear it on the filter paper.
3. Finally, look for a blue-purple color for Positive Oxidase test within 10 seconds.

***Note*:** 1) Blue-purple color (within 10 seconds)…….Positive Oxidase test

2) No blue-purple color (within 10 sec)…….Negative Oxidase test

3) Ignore any blue-purple color that develops after 10 seconds.

4. Lysine Decarboxylase (LDC) test

(Using lysine agar)

- Used for identification/verification of *Salmonella* and *Shigella*.

1. 1st, prepare a dense suspension of the test organism in 0.25 ml physiological saline in a small tube.
2. Next, add an LDc/Lysine reagent.
3. Then after, add 3 drops of paraffin oil*.
4. Incubate at 35–37 ºC for 3–4 hours (or overnight).
5. Finally, read the Lysine Decarboxylase (LDC) reaction which produce Purple color in the medium for positive test.

Note: 1) Purple color…….Positive LDC test (*Salmonella*)

2) Yellow color…….Negative LDC test (*Shigella*)

3) *The oil over layer provides the anaerobic conditions required for the LDC reaction.

5. Motility test

- Used to identify/isolate motile bacteria from non-motile.

1. 1st, add 2-3 ml of already prepared motility medium in a small test tube.
2. Next, using sterile straight wire, inoculate the test organism in the medium.
3. Then, incubate at 35–37 ºC for 18-24 hours.
4. Finally, examine turbidity formation in the medium.

Note: 1) Turbidity formation in the medium….Positive motility test (*Salmonella)*

2) Clearance of the medium…… .Negative motility test (*Shigella*)

Source: Cheesbrough Monica. District Laboratory Practice in Tropical Countries Part 2 Second ed. Cambridge University press, 2005.

10.3 Annex-3

Consent form

A) English Version

The objective of this study is to gather information about Bacteriological quality of drinking water in Arba Minch town, South Ethiopia.

Water-borne diseases cause devastating consequences in the socio-economics of our community in general. The water will be assessed for the presence of bacterial contamination and its associated risk factors during the study period.

This Cross-sectional study has the potential to point out measures to reduce the incidence of cases in water borne diseases, significantly increased health care cost, and the possibility of longer period of hospitalization with final goal of improving community health conditions.

Therefore, here we request your kindly participation in this study which requires your willingness to give water samples for laboratory analysis, to respond to an interview and questionnaires

You have full right and free choice to either participate or not in this study and it will never affect your right of getting appropriate community service. Results will be confidential and reported to the concerned officials in the town.

I _____, after being fully informed about the purpose of this study, hereby give my consent to participate in this study.

_____	_____	_____
Name of Head of households	date	signature
_____	_____	_____
Name of Principal investigator	date	signature

B) <u>Amharic Version</u>

የስምምነት መግለጫ ቅፅ

የዚህ ጥናት ዋነኛ ዓላማ በአርባ ምንጭ ከተማ የንፁህ መጠጥ ውሀ አቅርቦት ላይ ለሚደረገው ጥናት የሚረዱ መርጃዎችን ለመሰብሰብ ሲሆን ማህበረሰቡ በተለያዩ ውሀ ወለድ በሽታዎች ምክንያት ማህበራዊና ኢኮኖሚያዊ ችግሮች ሲደርሱበት ይታያል፡፡ በመሆኑም ይህ ጥናት ከንፁህ መጠጥ ውሀ አቅርቦት ጋር በተያያዘ የሚከሰቱትን ውሀ ወለድ በሽታዎችን፣ተያያዥ የሕክምና ወጪዎችን እንዲሁም ረዥም የሆስፒታል ቆይታን ለመቀነስ የሚረዱ የመፍትሔ እርምጃዎችን ይጠቁማል፡፡

ስለዚህ ይህ ጥናት ለቤተ-መከራ ትንተና የሚያገለግሉ የውሀ ናሙናዎችን እንዲሁም ቃለ መጠይቆችንና የፅሁፍ መጠይቆችን ይፈልጋል፡፡ ስለሆነም ጥናቱ የእርስዎን ሙሉ ፈቃደኝነትና ትብብርን ይሻል፡፡

በዚህ ጥናት ውስጥ ለመሳተፍም ሆነ ላለመሳተፍ ሙሉ መብትና ምርጫ ያለዎት ሲሆን ባለመሳተፍዎ ተገቢውን ማሕበራዊ አገልግሎት አይከለከሉም፡፡ ስለሆነም የጥናቱ አጠቃላይ ውጤትም ሚሰጥራዊነቱ የሚጠበቅና ለተገቢው የአስተዳደር አካል የሚቀርብ ይሆናል፡፡

እኔ ---------- የዚህን ጥናት ዓላማ በግልፅ ስለተረዳሁ በጥናቱ ለመሳተፍ መስማማቴን በፊርማዬ አረጋግጣለሁ፡፡

_____	_____	_____
የተሳታፊው ስም	**ቀን**	**ፊርማ**
_____	_____	_____
ጥናቱን የሚያካሄደው ሰው ስም	**ቀን**	**ፊርማ**

10.4 Annex-4

Laboratory Results

a) Table showing total coliforms (TC in cfu) and thermotolerant/ faecal coliforms (TTC/FC, in cfu) per 100 ml of water sample and presence/absence of pathogenic bacteria in three sampling rounds from main source & reservoir (where n=1 for each site) in Arba Minch town between November, 2010 and January, 2011.

Rounds	Water sampled sites									
	Main source					Reservoir				
	TC	FC	Pathogenic bacteria			TC	FC	Pathogenic bacteria		
			Salm	*Shig*	*vibrios*			*Salm*	*Shig*	*vibrios*
1st	0	0	-	-	-	0	0	-	-	-
2nd	0	0	-	-	-	0	0	-	-	-
3rd	0	0	-	-	-	0	0	-	-	-

Note: 1) **+/-** = presence/absence

2) HH No. = Household number

3) *Salm. = Salmonella*

4) *Shig. = Shigella*

5) patho. = pathogenic

b) Table showing TC and FC bacteria per 100 ml of water sample and present/absent of pathogenic bacteria in three sampling rounds from pipe & household water (where n=20 for each site) in Arba Minch town between November, 2010 and January, 2011.

	1st round analyses										2nd round analyses									
	Water sampled sites										Water sampled sites									
HH No.	Pipe water					Household water					Pipe water					Household water				
	Tc	Fc	Patho. bacteria			Tc	Fc	Patho. bacteria			Tc	Fc	Patho. bacteria			Tc	Fc	Patho. Bacteria		
			Salm.	*Shig.*	*Vib.*			*Salm.*	*Shig.*	*Vib*			*Salm.*	*Shig.*	*Vib.*			*Salm.*	*Shig.*	*Vib.*
1	9	3	+	+	-	45	10	+	+	+	7	2	+	+	-	19	6	+	+	-
2	10	4	+	+	-	40	8	+	+	-	8	3	+	+	-	16	7	+	+	-
3	0	0	-	-	-	23	6	+	+	-	0	0	-	-	-	12	3	-	-	-
4	0	0	-	-	-	17	4	-	-	-	0	0	-	-	-	14	2	-	-	-
5	0	0	-	-	-	17	3	-	-	-	0	0	-	-	-	12	0	-	-	-
6	0	0	-	-	-	25	5	-	-	-	0	0	-	-	-	20	3	-	-	-
7	0	0	-	-	-	19	7	+	+	-	0	0	-	-	-	16	0	-	-	-
8	0	0	-	-	-	19	9	+	+	-	0	0	-	-	-	18	7	+	+	-
9	0	0	-	-	-	17	2	-	-	-	0	0	-	-	-	9	0	-	-	-
10	0	0	-	-	-	19	4	-	-	-	0	0	-	-	-	15	0	-	-	-
11	0	0	-	-	-	23	5	-	-	-	0	0	-	-	-	18	0	-	-	-
12	0	0	-	-	-	21	6	-	-	-	0	0	-	-	-	16	5	-	-	-
13	0	0	-	-	-	11	0	-	-	-	0	0	-	-	-	9	0	-	-	-
14	0	0	-	-	-	25	9	+	+	-	0	0	-	-	-	15	8	+	+	-
15	0	0	-	-	-	10	0	-	-	-	0	0	-	-	-	8	0	-	-	-
16	0	0	-	-	-	8	0	-	-	-	0	0	-	-	-	5	0	-	-	-
17	0	0	-	-	-	9	0	-	-	-	0	0	-	-	-	8	0	-	-	-
18	0	0	-	-	-	17	6	-	-	-	0	0	-	-	-	13	4	-	-	-
19	0	0	-	-	-	21	4	-	-	-	0	0	-	-	-	16	3	-	-	-
20	0	0	-	-	-	12	0	-	-	-	0	0	-	-	-	5	0	-	-	-

Rounds…..…….. Cont'd

	3rd round analyses									
	Water sampled sites									
HH No.	Pipe water					Household water				
	Tc	Fc	Pathogenic bacteria			Tc	Fc	Pathogenic bacteria		
			Salm.	*Shig.*	*Vibrio*			*Salm.*	*Shig.*	*Vibrio*
1	5	0	-	-	-	15	4	+	+	-
2	6	0	-	-	-	13	5	.+	+	-
3	0	0	-	-	-	11	2	-	-	-
4	0	0	-	-	-	9	0	-	-	-
5	0	0	-	-	-	10	0	-	-	-
6	0	0	-	-	-	16	2	-	-	-
7	0	0	-	-	-	13	0	-	-	-
8	0	0	-	-	-	10	3	-	-	-
9	0	0	-	-	-	8	0	-	-	-
10	0	0	-	-	-	12	0	-	-	-
11	0	0	-	-	-	10	0	-	-	-
12	0	0	-	-	-	14	3	-	-	-
13	0	0	-	-	-	8	0	-	-	-
14	0	0	-	-	-	13	6	-	-	-
15	0	0	-	-	-	6	0	-	-	-
16	0	0	-	-	-	4	0	-	-	-
17	0	0	-	-	-	8	0	-	-	-
18	0	0	-	-	-	11	0	-	-	-
19	0	0	-	-	-	13	0	-	-	-
20	0	0	-	-	-	4	0	-	-	-

Note: 1) +/- = presence/absence 2) HH No. = Household number

3) *Salm.* = *Salmonella* 4) *Shig.* = *Shigella* 5) patho. = pathogenic

Declaration

I, the undersigned, declare that this MSc thesis is my original work, has not been presented for a degree in this or any other University. I also declare that all sources of materials used for the thesis have been duly acknowledged.

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This thesis has been submitted for examination with my approval as University Advisors.

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