SCREENING OF COLIFORM FROM DIFFERENT NATURAL WATER SOURCES AVAILABLE IN VARIOUS SITES OF DHARAN

A

Project Work Submitted to

Department of Microbiology,

Central Campus of Technology, Tribhuvan University.

In Partial fulfillment for the Award of the Degree of

Bachelor of Science in Microbiology

Submitted by

Sajuta Shrestha

Department of Microbiology, CCT, Tribhuvan University

Hattisar, Dharan

September, 2016

RECOMMENDATION

This is to certify that **Ms. Sajuta shrestha** has completed this project work entitled **"Screening of coliform from different natural water sources available in various sites of Dharan"** as a part of partial fulfillment of the requirements of B.Sc. degree in Microbiology under my supervision. To my knowledge this work has not been submitted for any other degree.

.....

Mr. Suman Rai

Supervisor

Department of Microbiology

Central Campus of Technology

Hattisar, Dharan, Nepal

Date:

CERTIFICATE OF APPROVAL

On the recommendation of **Mr. Suman Rai**, this project work of **Ms. Sajuta Shrestha** entitled **"Screening of coliform from different natural water sources available in various sites of Dharan"** has been approved for the examination and is submitted to the Tribhuwan University in Partial fulfillment of the requirements for B.Sc. degree in Microbiology.

.....

Mr.Suman Rai

Department of Microbiology

Central Campus of Technology

Hattisar, Dharan, Nepal

Date:

BOARD OF EXAMINERS

Recommended by:

…….

Mr. Suman Rai

Supervisor

…….

Mr. Hemant Khanal

Co- supervisor

Approved by:

.....

Mr. Shiv Nandan Sah

(Assistant Professor)

Head of Department

Department of Microbiology

Examined by:

…….

(Internal Examiner)

…….

(External Examiner)

Date:

ACKNOWLEDGEMENTS

I am glad to be able to acknowledge the people without whom our project work wouldn't have been possible.

First and foremost, I would like to express my gratitude to my respected supervisor **Mr. Suman Rai**, academic and laboratory supervisor, for his guidance throughout my work. His constructive suggestions throughout the study and writing helped to get me to its completion. I would also like to thank my co-guide **Mr. Hemant Khanal** for his help and support. I am also thankful to **Mr. Shiv Nandan Sah**, HOD of microbiology for providing me ideas for the work and help on the literature. I would also like to thank **Mr. Prince Subba** for his help throughout my work. I am also thankful to my entire teaching and non-teaching staffs of microbiology department for their guidance, teaching and encouragement over the last 4 years without which I would not have had the opportunity, the confidence, or the ability, to embark on an undertaking such as this.

I would also like to express my gratitude to: **Mr. Aindra Bahadur Karki** and **Kamana Bantawa Rai**, Laboratory persons of the microbiology lab of Central Campus of Technology for their continuous help regarding the equipment's and other materials during the study.

My loving and respected parents, **Mr. Gokarna bahadur Shrestha** and **Mrs. Radhika Shrestha** and husband **Mr. Deependra k.c** for their love, support and encouragement to make me stand here today.

My very dear friends: **Sneha Shrestha, Tin Maya Rai, Sushmita Phattepuri,** and **Deepa Rai** for their great support and encouragement during the study.

Above, I have gratefully acknowledged people who have been invaluable to me in completing this thesis. However, all opinions expressed in this thesis, and all errors, are mine.

Sajuta Shrestha

ABSTRACT

The study was carried out to isolate and characterize the water borne bacteria isolated from various water samples taken from the different sites of Dharan and adjoining areas. A total of 8 water samples were collected, of which 6 were from naturally running spouts and 2 were from river which are distributing drinking water to the locality. All the eight samples were isolated by membrane filter technique in EMB agar and all samples were isolated by following spread plate technique in SS agar and TCBS agar. *Enterobacter aerogens, Proteus vulgaris, Salmonella typhi, Klebsiella pneumoniae, Pseudomonas aeruginosa* were isolated and characterized.

Key words: Water, Water borne bacteria, membrane filter technique

Contents

LIST OF TABLES

LIST OF ABBREVIATIONS

1. **EMB** = Eosine Methylene Blue
2. **SS** = Salmonella Shigella
3. **TCBS** = Thiosulphate Citrate Bile salt Sucrose

LIST OF PHOTOGRAPHS

CHAPTER I

INTRODUCTION

1.1 Background of the study

Water, the most vital resource for all life on this planet, may be adversely affected qualitatively & quantitatively by different human activities. Public & environmental health protection requires safe drinking water, which means that it must be free from pathogenic bacteria. Clean & safe water is an absolute need for health & productive life. Water has a profound influence on human health and quality of the water supplied is important in determining the health of individuals and whole communities. Safe drinking water is a major concern with reference to public health importance as health and well being of the human race is closely tied up with the quality of water used (Sharma et al 2005).

Today most of the surface and ground water receive millions of liters of sewage, domestic waste, industrial & agricultural effluents containing substances varying in characteristics from simple nutrients to highly toxic substances. Changes in water quality are reflected in its physical, chemical and biological conditions; and these in turn are influenced by physical and anthropogenic activities (ADB/ICIMOD 2006).

Earth consist of approximately 70% surface area covered with water and remaining is land which have only 2% water which is drinkable (Lim et al 1999). Water is an important chemical molecule containing feature of life it can be dissolved into organic compounds, salts, inorganic compounds and gases that are involved in metabolic processes because it is universal solvent and due to that it provides stability to membrane system, macro molecules, hemostatis, transportation and thermal regulation of body **(**Bourne and Seager 2001). All cells of body contain water as an important component. Water content of a single cell is 45% to 95% and microorganism contains 80% of body weight as water and

human contains water i.e. 70% of their body weight. It is thermal regulator of human body and normal human body contains 42 liters of water in them (Anthony et al 1980).

Water is equally important and critical for both humans and environment and it is a key issue in form of drinking water (Solley et al 1998). Dams, canals and wells show importance of water and the impact of human beings on water cycle. Environmental effects like migration of peoples and animals, land losses, change of environmental factors, depletion of biological resources shows that these activities are noticeable (Boktin and Keller 2005).

Supply of safe drinking water is now becoming a global concern since there are still more than one billion people who lack access to clean drinking water &more than two third of world population don't have access to proper sanitation. Pathogenic contamination of water is also important threat for living organisms. The lack of proper purification and sanitation of drinking water in developing countries leads to the scarcity of safe drinking water among one third of the total population along with the increased prevalence of water borne diseases, diarrhea being major cause for death, mostly among the children under the age of five years (WHO 2007).There is a vital connection between water and health. Water, though is an absolute necessity for life, can also be a carrier of many water borne diseases such as typhoid, cholera, hepatitis, dysentery& other diarrheal related diseases(WHO 2007).

It is believed that poor quality of water i.e. microbial pollution directly related to public health impact. The microbiological quality of water can be accessed through the analysis of coliform group of organisms which are also called indicator organism of water quality. Therefore, the study has been designed with the aim for Screening of coliform from different natural water sources available in the various sites of Dharan by membrane filtration technique.

The membrane filter (MF) technique is fully accepted and approved as a procedure for monitoring drinking water microbial quality in many countries. This method consists of filtering a water sample on a sterile filter with a 0.45-mm pore size which retains bacteria, incubating this filter on a selective medium and enumerating typical colonies on the filter**.** Many media and incubation conditions for the MF method have been tested for optimal recovery of coliforms from water samples (Grabow and Preez 1979)

1.2: Statement of problem

Water is one of the sources of transmission of various waterborne diseases such as cholera, typhoid, dysentery, worm infection, polio, hepatitis etc. Such types of diseases are mainly related with the water which is mixed up with fecal materials, dungs, droppings, soil contaminants & other various sources of pathogenic microorganisms.

The water sources which are open & found in natural condition are most susceptible to the water pollution. It is assumed that 60% of the total diseases cause to human beings is waterborne diseases. Drinking water is indispensable for human existence. Dharan suffers a severe drinking water crisis particularly in the dry seasons every year. So people are force to use the water from the different nearly available natural running spouts, rivers, ponds etc. to fulfill their works related for different purpose. The growing imbalance between supply and demand has led to chronic shortages & competition that have resulted in pollution & environmental degradation (Rai 2070)**.** Apart from quantitative shortages, the quality of drinking water is becoming a serious public health issue for the Dharan people.

1.3: Objectives of the study

1.3.1. General objective:

- To isolate and identify coliform from different water sample.

1.3.2. Specific objectives

- To know about coliforms.

- To perform biochemical test for the isolation and identification of coliforms found in water.

1.4: Rationale of the study

Since peoples are using for sustainable life and are practicing from generation to generation. However, the technologies behind its effects are not studied well in Dharan sub-metropolitan.

Nepal is second rich in water resources but lack of proper use of resources some people misuse & pollute the resources which makes people far from getting proper benefits. The principal reasons of the pollution of drinking water are due to inadequate sanitation, dumping of wastes, poor drainage system and irregular supply of drinking water in the pipeline. Besides that the contamination may be either due to the failure of the disinfections of the raw water at the treatment plant or because of the infiltration of contaminated water (sewage) through cross connection and leakage points. All natural water sources, such as wells, stone spouts and ponds are neither treated nor protected properly. Thus, deteriorating water quality is the major problem and it has created serious threat to human health and environment (Aryal et al 2010). The findings will support to isolate and characterize the microorganisms present in the sources which make people concern about cleanliness & maintain safety measures to use water by applying different purifying techniques.

1.5: Limitation of the study

Some of the limitations of this research are as follows:

1. Different natural sources are available in Dharan. Use of all sources as a sample couldn't be taken due to lots of sources and less time.
2. As the major portion of the works is laboratory based & will depends on continuous electricity supply. Nepal is highly victim of load shedding so most of work will delay and proper result may not achieved.
3. Due to lack of sufficient amount of budget & equipment species of isolated organism couldn't be identified by molecular study.

CHAPTER II
LITERATURE REVIEW

2.1: Introduction of water

Water, the most vital resource for all life on this planet, may be adversely affected qualitatively & quantitatively by different human activities. Public & environmental health protection requires safe drinking water, which means that it must be free from pathogenic bacteria. Clean & safe water is an absolute need for health & productive life (Sharma et al 2005). Today most of the surface and ground water receive millions of liters of sewage, domestic waste, industrial & agricultural effluents containing substances varying in characteristics from simple nutrients to highly toxic substances (ADB/ICIMOD 2006).

.

Supply of safe drinking water is now becoming a global concern since there are still more than one billion people who lack access to clean drinking water and more than a two third of world population do not have access to proper sanitation. The WHO estimates that 1.15 billion population in developing world lack access to improved water supplies (WHO 2007). The lack of proper purification and sanitation of drinking water in the developing countries leads to the scarcity of safe drinking water among one third of the total population along with the increased prevalence of water borne diseases, diarrhea being the major cause for death, mostly among the children under the age of five years (WHO 2007).

Diarrheal diseases are still recognized as a major problem of Nepalese children, being recorded as the second most prevalent diagnosis in out-patience services. Today 72% of the nationwide disease burden is related to poor quality of drinking water & around 75 children die each day from diarrhea alone (Rai 2070). According to the WHO, diarrheal disease accounts for an estimated 4.1% of the total daily global burden of disease. It has also been estimated that approximately 4 billion cases of diarrhea each year cause 2.2 million deaths, mostly among

children under the age of five which is equivalent to one child dying in every 15 seconds. Water, sanitation and hygiene interventions reduce diarrheal disease on average by between one-quarter and one –third (WHO 2007).

Like many developing countries, Nepal faces a plethora of problems regarding both its drinking water quality and availability. Throughout Nepal, people are exposed to severe health threats resulting from water contamination by sewage, agriculture, and industry. Owing to the impact of sewage, typhoid, dysentery, and cholera are endemic every summer (Sherpa 2003). These diseases account for 15% of all illness and 8% of total deaths, but those numbers increase to 41% of all illness and 32% of all deaths in children up to 4 years old (Khadka 1993). In the Kathmandu Valley, the main urban center of Nepal, the chief concern is contamination from sewage lines, septic tanks, open pit toilets (Sharma 1990), and from surface water that has been polluted by direct disposal of sewage waste (Jha et al 1997; Khadka 1992). Surface water in Kathmandu Valley is also polluted with direct disposal of industrial waste, possibly leading to contamination of the shallow aquifer (Khadka 1992; Karn and Harada 2001) Approximately 50% of the water supply in the Kathmandu Valley is derived from groundwater sources (Sharma 1990; Khadka 1993). Because of the insufficient Municipal supply, fed by a combination of surface and Groundwater, people use a variety of other groundwater sources including dug wells, tube wells and *dhunge dharas* (Karn and Harada 2001).

Dhunge dharas (literally stone spouts or water taps) are the primary alternative to the municipal, piped water supply in the Kathmandu Valley (Khatiwada et al 2002). They are located throughout the valley, both in dense urban and village settings. *Dhunge dharas* are historic and revered sources that derive much of their water supply from shallow groundwater (1–5 m below the ground surface) or from groundwater that may be artificially high because it is fed by shallow canals (Conan 2004). In urban settings in Kathmandu, Patan and Bhaktapur, *dhunge dharas* are usually located in low-lying areas and are excavated rectilinear brick-

lined pits that tap the groundwater system and channel the groundwater to a spout or series of spouts. Some *dhunge dharas*, especially many built in the seventeenth century, bring water from a distant surface-water source or reservoir via a network of canals. *Dhunge dharas* in the valley periphery occasionally tap natural springs where water flows to the surface on terraced banks (Dixit and Upadhya 2005). *Dhunge dharas* have been used over the past 15 centuries; the oldest one known was built in 554 and is still in use today (Shrestha et al 1996). The water from *dhunge dharas* is often considered to have religious significance and people generally consider the water clean enough to drink although some do boil or filter the water before drinking. The water is used for washing the body and face, drinking, healing, purification of deity images, and laundry (Moench et al 2003).

2.2 Water quality

Water quality reflects the composition of water as affected by natural causes and man's cultural activities, expressed in terms of measurable quantities and related intended water use. Water quality is perceived differently by different people, for example, a public health official is concerned with the bacterial and viral safety of water used for drinking and bathing while aquatic scientist is concerned with the health of aquatic habitats, including fish plankton and other plants and organisms. The term pollution, contamination, nuisance and water degradation are often used synonymously to describe faulty conditions of surface and ground water.

Water pollution is a change in bacteriological, biological & psysico-chemical quality of the surface or ground water resources is caused by man activities that is injurious to existing intended or potential use of resource. Water quality can be determined & measured by comparing physical, chemical, biological, microbiological & radiological quantities & parameters to a set of standards & criteria (Becker- Ritterpatch 1990; Ballence 1996). The quality of water for drinking has deteriorated because of the inadequacy of treatment plants, direct discharge of untreated sewage into rivers & inefficient management of the piped water distribution system. Safe drinking water is a major concern with reference

to public health importance as health & well-being of the human race is closely tied up with the quality of water used (Khadka 1993).

Study on Microbial Quality of Drinking Water in Nepal

Studies on microbiological quality of drinking water in Nepal have been carried out by different researchers. Some of the relevant studies are depicted as follows.

Shrestha analyzed a total of 95 water samples for bacteriological parameters from various sources. The maximum count of coliform was observed from all raw, settled and reservoir water distribution point Balkhu and Kuleshwor. The study also found 85.26 % of the samples to have exceeded WHO guideline value for total coliform (Shrestha 2002).

Similarly, Joshi and Baral also analyzed 160 samples randomly collected from 86 tube wells and 77 open wells in urban areas and reported that more that 87 % of analyzed ground water samples of tube well and open well was contaminated (Joshi and Baral 2004).

Warner sampled water from over 100 sources in Kathmandu and examined for contamination from sewage, agriculture, or industry. Total coliform and Escherichia coli bacteria were present in 94 and 72% of all water samples respectively (Warner et al 2007).

Diwakar analyzed the drinking water of Bhaktapur Municipality Area in premonsoon season. The analysis of 116 water sample from different sources revealed the presence of total coliform in 96 (82.76%) of samples. This study has pointed out that the drinking water quality of city water supply has not been improved and traditional sources like stone spouts and tube well water are also not free from contamination. Such circumstances are responsible for spreading water borne outbreaks. The waterborne diseases are closely related with the conditions of living and environmental sanitation in the community. So, it can be

effectively controlled by appropriate water management and safe disposal of excreta (Diwakar et al 2008).

2.3. Coliform bacteria

Coliform bacteria are a commonly used indicator of sanitary quality of foods and water. They are defined as rod-shaped Gram-negative non-spore forming and motile or non-motile bacteria which can ferment lactose with the production of acid and gas when incubated at 35-37ºC (APHA 1995). Coliforms can be found in the aquatic environment, in soil and on vegetation; they are universally present in large numbers in the feces of warm-blooded animals. While coliforms themselves are not normally causes of serious illness, they are easy to culture, and their presence is used to indicate that other pathogenic organisms of fecal origin may be present. Such pathogens include disease-causing bacteria, viruses, or protozoa and many multicellular parasites. Coliform procedures are performed in aerobic or anaerobic conditions (APHA 1995). Some of the examples of coliform bacteria found in the water are *Escherichia coli, Salmonella typhi, Shigella, Proteus vulgaris, Proteus mirabilis, Pseudomonas aeruginosa, Klebsiella pneumoniae, Enterobacter aerogenes, Citrobacter freundii etc.*

2.4. Description of *Enterobacter Aeroginosa*

The Enterobacteriaceae family includes genera of *Escherichia, Shilgella, Salmonella, Enterobacter, Klebsiella, Serratia, Proteus*, amongst others. The gram-negative bacteria reside in soil, water, dairy products and inhabit a natural flora in the gastrointestinal tract of animals as well as humans. The rod shaped Enterobacteriaceae exists in a variety of sizes; are not spore forming; are both motile (with peritrichous flagella) and nonmotile; grow both aerobically and anaerobically.

Habitat

Enterobacter are found in the soil, water, dairy products, and in the intestines of animals as well as humans. *Enterobacter aerogenes* has been plated on several different media and have been observed under several types of testing. The results

are as follows- *E. aerogenes* tested negative when treated with: Indole, Methyl red, Hydrogen sulfide (by way of TSI), Urease. Delayed positive results were obtained from: Gelatin (22°C) (Janda et al 2006).

2.5. Description of *Klebsiella Pneumoniae*

Klebsiella pneumoniae is a gram-negative, non-motile, lactose fermenting, rod-shape organism. *K. pneumoniae* is able to grow either with or without free oxygen, deeming it a facultative anerobes. This organism is also surrounded by a capsule, which increases its virulence by acting as a physical barrier to evade the host's immune response. This capsule also protects the cell from dessication. *K. pneumoniae* is a home-grown microorganism in that it is resides in the microbiota of humans. It can be found in the mouth, skin, and intestinal tract, where it initially does not cause disease. Although found in the microbiota, *K. pneumoniae* can progress into severe bacterial infections leading to pneumonia, bloodstream infections, wound infections, urinary tract infections, and meningitis (UniProt).

2.6. Description of *Pseudomonas Aeruginosa*

Pseudomonas aeruginosa is a gram-negative, rod-shaped, asporogenous, and monoflagellated bacterium that has an incredible nutritional versatility. It is a rod about 1-5 µm long and 0.5-1.0 µm wide. *P. aeruginosa* is an obligate respirer, using aerobic respiration (with oxygen) as its optimal metabolism although can also respire anaerobically on nitrate or other alternative electron acceptors. *P. aeruginosa* can catabolize a wide range of organic molecules, including organic compounds such as benzoate. This, then, makes *P. aeruginosa* a very ubiquitous microorganism, for it has been found in environments such as soil, water, humans, animals, plants, sewage, and hospitals. In all oligotropic aquatic ecosystems, which contain high-dissolved oxygen content but low plant nutrients throughout, *P.aeruginosa* is the predominant inhabitant and this clearly makes it the most abundant organism on earth (Costerton and Anwar 1994).

P.aeruginosa is an opportunistic human pathogen. It is "opportunistic" because it seldom infects healthy individuals. Instead, it often colonizes immunocompromised patients, like those with cystic fibrosis, cancer, or AIDS (Botzenhardt and Doring 1993).

2.7. Description of *Salmonella Typhi*

There are over 2,000 various groupings (serovars) that comprise S. enterica, each very closely related to each other making *Salmonella typhi* a prime example of a serovar. *Salmonella typhi* is a gram negative bacterium that causes systemic infections and typhoid fever in humans. This rod-shaped, flagellated organism's sole reservoir is humans. It has caused many deaths in developing countries where sanitation is poor and is spread through contamination of water and undercooked food. Eradication seems highly unlikely due to recent emergence of multi drug resistance strains. *Salmonella typhi* strain Ct18 was originally isolated from a patient in a hospital in Vietnam. The chromosome sequence is 4,809,037 bp in length with a G+C content of 52.09%. The chromosome was sequenced though the method of shotgun sequencing with 97,000 shotgun reads. Since then, *Salmonella typhi* has undergone evolutionary change and has become resistant to antibiotics (Kita et al 1973).

CHAPTER III

METHODOLOGY

3.1: Site of the study

The study was carried out in Dharan, Sunsari.

3.2: Lab set up

The laboratory work of this study was carried out in the microbiology lab of Central Campus of Technology, Hattisar, located in Dharan 14. The laboratory was provided with all the necessary materials and equipment that were required to carry out this study.

3.3: Research design

The study was basically descriptive type of research design & qualitative nature. This study was mainly focused on obtaining information about the coliforms found in water.

3.4: Population and sample

3.4.1: Sample: Water

3.4.2: Description of the research site

The study was conducted by collecting different water samples from various sites of Dharan sub-metropolitan. The sample collection sites were Ek-dhara, Dui-dhara, Tin-dhara, Shiva dhara, Khatri dhara, Gau dhara, Kali khola and sera khola.

3.4.3: Sample collection

A total of 8 drinking water samples were collected from different sites of Dharan sub-metropolitan. Four water samples from Ek-dhara, dui-dhara, Tin-dhara, were collected in different sterile plastic bottles on 15th May 2016. Samples from Shiva

dhara, Khatri dhara, Gau dhara were collected in the same way on 17th May 2016. Similarly, samples from Sera khola and Kali khola were collected on 19th May 2016.

3.4.4: Transportation of the sample

All the water samples were transported from the site of collection to the laboratory aseptically and without any mechanical and chemical damage. The water samples were poured aseptically through membrane filter and the filter was transferred into selective media within the 6 hours of the sample collection.

3.5: Isolation and Identification

3.5.1: Media preparation

Different selective media and biochemical test media were prepared for this study in order to isolate and identify the coliforms found in water. The media were prepared according to the protocol of the media.

Following are the list of the selective media prepared for isolation process of this study:

i. Eosine Methylene Blue Agar medium (EMB)
ii. Salmonella Shigella Agar medium (SS)
iii. Thiosulphate Citrate Bile Salt Sucrose medium (TCBS)

Similarly, following are the list of biochemical test media prepared for the identification process of the study:

i. Citrate Agar
ii. MRVP Broth
iii. TSI Agar
iv. Tryptone Broth
v. Urease Agar
vi.Gelatin liquefaction

3.5.2: Sample processing

After the samples were collected, the water sample was taken to the laboratory and was then processed as follows:

i. Membrane filtration

- First of all sterile membrane filtration apparatus was fitted on suction flask.
- An electric motor pump was fitted on a suction flask.
- Then the filter of pore size 0.45nm was kept on the filtration apparatus.
- Funnel was placed on the top of filter paper aseptically and held tightly the funnel with the help of clip.
- Then measured 100ml of water was poured into the funnel.
- Allow to filter all water through the help of motor.
- After filtration funnel was opened and membrane filter was transferred to selective media with the help of sterile forceps.

ii. Culture

After the filtration of 100ml water through membrane filter, the membrane filter was transferred to the Eosine Methylene Blue (EMB) media plate with the help of the sterile forceps. Let the filter to absorb the media and incubated at 37^{o}C, for 24 hours. Again 0.1ml sample was transferred to the both Salmonella Shigella (SS) and Thiosulphate Citrate Bile salt media plate respectively with the help of sterile pipette following the spreading of the sample into the media by spread plate technique. Both SS and TCBS media plates were incubated at 37^{o}C, for 24 hours.

iii. Observation

After the 24 hours of incubation, the culture plates were observed for the growth of the colonies on each of the media plates. The colonies were observed for their color, shape, size, configuration, elevation and margin. The number of colonies was counted on the individual culture media plates.

iv. Sub culture

After observation, the individual type of bacterial colonies of different characteristics from each of the media plates were further sub cultured on the nutrient agar plates by streaking method and were again incubated at37^{o}C, for 24 hours.

v. Biochemical test

After the subculture of organism was performed, they were then tested biochemically for their identification. The process of biochemical test for identification involved the following steps:

a. Gram staining

It is the first step of the biochemical test which is performed in order to differentiate bacteria i.e. whether the bacteria is gram positive or gram negative bacteria.

b. IMViC tests

IMViC tests consists of four different tests viz; Indole production, Methyl Red, Voges-proskauer and citrate utilization.

Indole production

The indole test was carried out by inoculating the isolates into 1% tryptone broth. After incubation at 37^{o}C, 1ml Kovac's reagent was added to the broth containing the isolates and was shake well and allowed the tubes to stand for few minutes for cherry- red ring on the top; indicates positive.

Methyl Red test

The methyl red test was carried out by inoculating the isolates into MRVP broth. After incubation at 37^{o}C, 5 drops of 0.1% MR

reagent was added to the broth containing the isolates and was shake well and allowed the tubes to stand for few minutes for the development of red color; indicates positive test.

Voges- proskauer test

The VP test was carried out by inoculating the isolates into MRVP broth. After incubation at 37^{o}C12 drops of VP reagent-I(alpha Napthol solution) and 3 drops of VP reagent-II (40% KOH) were added to the broth containing the isolates and was shake well and allowed the tubes to stand for few minutes for the development of pink red color; indicates positive test.

Citrate utilization test

Citrate utilization test was performed by streaking the organisms in the slant of simmon's citrate agar media.

c. Catalase test

This test is primarily used in order to demonstrate the presence of catalase enzyme in the bacteria.

d. Oxidase test

The basic principle of this test is to determine the ability of bacteria to produce oxidase enzyme that will catalyse the transport of electrons between electron donors in bacteria and redox dye, 1% tetramethyl-p-phenylene-diamine, cytochrome oxidase, in the presence of atmospheric oxygen, oxidizes the reagent to form blue-purple colored compound gives the positive test. This test was performed by dry filter paper method.

e. Urease test

Some bacteria have the ability to produce the enzyme urease which break down the urea with liberation of ammonia resulting raise in pH of the medium which changes the color of phenol red indicator from yellow too pink-red; indicates positive.

f. TSI test

TSI stands for the Triple sugar iron test. TSI test is used for the determination of carbohydrate fermentation and hydrogen sulphide production. This test is carried out in the slant of TSIA media which consists of three sugars; lactose, sucrose and a very small amount of glucose. This test is generally used to identify enteric pathogen.

g. Gelatin hydrolysis test

Gelatin hydrolysis test is used to detect the ability of an organism to produce gelatinase (proteolytic enzyme) that liquefy gelatin.

CHAPTER IV

RESULTS

A total of 8 drinking water samples were collected from different sites of Dharan sub-metropolitan, of which 6 were from naturally running spouts and 2 were from river which are distributing drinking water to the locality. All the eight samples were isolated in EMB agar performing membrane filtration by transferring membrane filter to the media aseptically and also isolated each sample in SS agar and TCBS agar by following spread plate technique. All the samples isolated on EMB agar developed the colonies in membrane filter but out of eight samples only 3 i.e. Ek-dhara, Dui-dhara and Tin-dhara developed colonies on both SS and TCBS media.

The results of the study are categorized as the result of the culture, subculture and results of the biochemical tests, separately for each sample in each media, which are represented in the table below:

4.1 Colonial, cultural and biochemical characteristics of the isolates of samples on EMB media:

According to the table no.1, all the samples isolated on EMB media gave the similar morphological characteristics i.e. irregular, convex, entire, mucoid and opaque and gave cultural characteristics Gram negative rods and are motile. And also have similar biochemical characteristics i.e. Indole (-), MR (-), VP (+), Citrate (+), Urease (-), Catalase (+), Gelatin hydrolysis (+) and Oxidase (-) indicates that *Enterobacter aerogens* is present in the entire water sample.

4.2 Colonial, cultural and biochemical characteristics of the isolates of samples Ek-dhara, Dui-dhara, Tin-dhara on SS media:

According to the table 2, 3 and 4 only three samples isolates gave colony on SS media. These three samples gave two different types of colony pink and pink with dark center. Three sample having pink colony gave similar morphological characteristics i.e. round, umbonate, undulate, mucoid, opaque and gave cultural characteristics Gram –ve rods and are non-motile. And also have similar biochemical characteristics i.e. Indole (-), MR (-), VP (+), Citrate (+), Urease (+),

Catalase (+), Gelatin hydrolysis (-) and Oxidase (-) indicates that *Klebsiella pneumonia* is present in Ek-dhara, Dui-dhara, Tin-dhara water samples.

Again, three samples having pink with dark center colony gave similar morphological characteristics i.e. round, raised, entire, dull, smooth, opaque and cultural characteristics Gram –ve rods and motile. And also have similar biochemical characteristics i.e. Indole (-), MR (+), VP (-), Citrate (+), Urease (-), Catalase (+), Gelatin hydrolysis (+) and Oxidase (-) indicates that *Salmonella typhi* is present in Ek-dhara, Dui-dhara, Tin-dhara water samples.

4.3 Colonial, cultural and biochemical characteristics of the isolates of samples Ek-dhara, Dui-dhara, Tin-dhara on TCBS media:

According to the table 2, 3 and 4 only three samples isolates gave colony on TCBS media. These three samples gave two different types of colony green and yellow. Three samples having green colony gave similar morphological characteristics i.e. oval, umbonate, wavy, mucoid, opaque and gave cultural characteristics Gram –ve rods and are motile. And also have similar biochemical characteristics i.e. Indole (-), MR (-), VP (-), Citrate (+), Urease (-), Catalase (+), Gelatin hydrolysis (+) and Oxidase (+) indicates that *Pseudomonas aeruginosa* is present in Ek-dhara, Dui-dhara, Tin-dhara water samples.

Again, three samples having yellow colony gave similar morphological characteristics i.e. round, convex, entire, muciod, opaque and cultural characteristics Gram –ve rods and motile. And also have similar biochemical characteristics i.e. Indole (+), MR (+), VP (-), Citrate (-), Urease (+), Catalase (+), Gelatin hydrolysis (+) and Oxidase (-) indicates that *Proteus vulgaris* is present in Ek-dhara, Dui-dhara, Tin-dhara water samples.

Hence, the isolated bacteria from the above eight samples on EMB plates were identified as *Enterobacter aerogens* as the rose pink colony on EMB. Similarly, the isolated samples from three samples ek-dhara, Dui-dhara and Tin dhara were identified as *Salmonella typhi* as pink with dark centered colony and *Klebsiella pneumoniae* as the pink colony on SS agar plates. *Pseudomonas aeruginosa* as the green colony and *Proteus vulgaris* as the yellow colony on TCBS agar plates.

CHAPTER IV

DISCUSSION

In this study, the coliforms present in water were isolated and identified. Water is life. This colorless, odorless and tasteless liquid is essential for all forms of growth and development human, animal and plant. Also, water is a fundamental basic need for sustaining human economic activities. Not only water supports a wide range of activities, it also plays a central symbolic role in rituals through out the world and is considered as a divine gift by many religions. Availability of water in the desired quantity and quality, at the right time and place, has been the key to the survival of all civilizations. No other natural resource has had such an overwhelming influence on human history.

As the human population increases, People express their desire for a better standard of living, and as economic activities continue to expand in scale and diversity, the demands on fresh water resources continue to grow. Clean & safe water is an absolute need for health & productive life. Today most of the surface and ground water receive millions of liters of sewage, domestic waste, industrial & agricultural effluents containing substances varying in characteristics from simple nutrients to highly toxic substances.

Coliform bacteria are a commonly used indicator of sanitary quality of foods and water. They are defined as rod-shaped Gram-negative non-spore forming and motile or non-motile bacteria which can ferment lactose with the production of acid and gas when incubated at 35-37^{o}C. Some of the examples of coliform bacteria found in the water are *Escherichia coli, Salmonella typhi, Shigella, Proteus vulgaris, Proteus mirabilis, Pseudomonas aeruginosa, Klebsiella pneumoniae, Enterobacter aerogenes, Citrobacter freundii etc.*

This study result indicates that the most sources of water of Dharan are of good quality. Aryal J reported the absence of *E.coli* in the study performed in myagdi which resembles with the present study.

Gyawali assessed the water quality of Kathmandu, taken from seven different sources. All the water samples showed the growth of coliform bacteria and Salmonella spp which resembles with the present studies, sample taken from eight different places of Dharan (Gyawali 2007).

The bacterial species identified from the contaminated water samples were mainly *E.coli*, *Klebsiella* spp, *Citrobacter* spp, *Vibrio* spp which was presented by Dhanya. Out of which *Salmonella* and *Klebsiella* were identified in the present study. Dhanya et al has studied microbial quality of water from different sources. Of all water sources tested, well water was found to be highly contaminated (Dhanya et al 2013). However in the present study it was found that some natural running spouts whose surroundings are dirty were more contaminated as there is presence of coliform.

CONCLUSION

From this study, it is concluded that various coliform can be found in water. Among them *Enterobacter aerogens, Klebsiella pneumoniae, Salmonella typhi, Pseudomonas aeruginosa, Proteus vulgaris* were isolated and identified as coliforms present in taken water samples.

RECOMMENDATIONS

1. There are different water sources available in Dharan. More samples can also be taken to carry out further study.
2. Public awareness programs should be effectively conducted for improving sanitation condition of Dharan.
3. Coliforms were observed in natural sources of water. So, regular monitoring of water should be recommended.

REFERENCES

ADB/ICIMOD (2006). Environmental Assessment of Nepal, Emerging Issues and challenges. Asian Development Bank and International Centre for Integrated Mountain Development, Kathmandu pp 55-64.

Anthony FG, Elizabeth JR, Gaudy T (1980). Microbiology for Environmental Scientists and Engineers. Mc Graw Hill Book company pp 2 and 667.

Aryal J, Sapkota N & Gautam B (2010). Assessment of Drinking Water Quality at Myagdi District, Western Nepal Ram Shah Path, Kathmandu, Nepal. Nepal Health Research Council.

Bourne LT and Seager JR (2001). Water the neglected nutrient, S Afr. J Clin.Nutr 14(3) pp 64-70.

Botkin DB and Keller EA (2005). Water supply use and management in Environmental Science, 5th ed. John Willey and sons pp 406-415.

Becker-Ritterspach ROA (1990). Dhunge-dharas in the Kathmandu Valley: an outline of their architectural development. Ancient Nepal 116–118:1−9.

BARTRAM, Jamie and Richard Ballence eds (1996). Water Quality Monitoring [A practical guide to the design and implementation of freshwater quality studies and monitoring programmes]. London: E and FN Spon.

Botzenhardt K and Doring G (1993). Ecology and epidemiology of *Pseudomonas aeruginosa*. *Pseudomonas aeruginosa* as an Opportunistic Pathogen, p1-7.

Conan H (2004). Small piped water networks: helping local entrepreneurs to invest. Water for All Publication Series, vol 13, Asian Development Bank, Manilla, Philippines 47–154.

Costerton W and Anwar H (1994). Pseudomonas aeruginosa: The Microbe and Pathogen. *Pseudomonas aeruginosa* Infections and Treatment, p 1-17.

Diwakar J, Yami KD, Prasai T (2008). Assessment of Drinking Water of Bhaktapur Municipality Area in Pre-Monsoon Season. Scientific World 6(6):94-96.

Dixit A, Upadhya M (2005). Augmenting groundwater in Kathmandu Valley: challenges and possibilities. Nepal Water Conservation Foundation, Kathmandu, Nepal.

Dhanya VC, Anitha J, Dhanashree B (2013). Bacteriological Screening of water in Mangalore, India.

Grabow WOK and DU Preez M (1979). Appl Environ. Microbiol 38: 351-358.

Gyawali R (2007). Study of Drinking Water Quality of Kathmandu Metropolitan Areas and Evaluation of Antibacterial property of Medicinal plants against Isolated Bacteria; MSc dissertation, Central Department of Microbiology, Tribhuvan University, Kathmandu.

Jha MG, Khadka MS, Shrestha MP, Regmi S, Bauld J, Jacobson G (1997). The assessment of groundwater pollution in the Kathmandu Valley, Nepal: report on Joint Nepal-Australia Project 1995–96, Australian Geological Survey Organisation, Canberra, pp 1–64.

Joshi DR, Baral MP (2004). Chemical and Microbiological Quality of Ground Water of Kathmandu Valley. A paper presented in Fourth national Conference on Science and Technology, March, pp 252.

Janda J, Michael, Sharon L Abbott (2006). The Enterobacteria 2nd ed. Washington D.C ASM press.

Khadka MS (1993). The groundwater quality situation in alluvial aquifers of the Kathmandu Valley, Nepal. AGSO J Aust Geol Geophys 14: 207-211.

Khadka MS (1992). Nepal: groundwater quality. In: Groundwater quality monitoring in Asia and the Pacific. Water Resources Series, United Nations, New York 70:202–204.

Karn SK, Harada H (2001). Surface water pollution in three urban territories of Nepal, India and Bangladesh. Environ Manage 28 (4):483–496.

Khadka MS (1993). The groundwater quality situation in alluvial aquifers of the Kathmandu Valley, Nepal. AGSO J Aust Geol Geophys 14: 207-211.

Khatiwada NR, Takizawa S, Tran TVN, Inoue M (2002). Groundwater contamination assessment for sustainable water supply in Kathmandu Valley, Nepal. Water Sci Technol 46(9):1

Kita Hiroshi and Nikaido Hiroshi (1973). Structure of Cell Wall Lipopolysaccharide from Salmonella typhimurium IV. Anomeric Configuration of l-Rhamnose Residues and Its Taxonomic Implications. Journal of Bacteriology. Volume 113:672-679.

Lim LC, Low JA and Chan KM (1999). Chryseobacterium meningosepticum (flavobacterium meningosepticum) a report of five case in local hospital. AnnAcad Med Singapor. 28 pp 858-860.

Moench M, Dixit A, Janakarajan S, Rathore MS, Mudrakartha S (2003). The fluid mosaic: water governance in the context of variability, uncertainty and change, a synthesis paper. National Water Conservation Foundation, Kathmandu, Nepal and the Institute for Social and Environmental Transition, Boulder, CO, USA.

Rai S (2070). A textbook of Environment Microbiology, First edition. Page no 143-149.

Solley WB, Pierce RR and Peralman HA (1998). Estimated use of water in United States 1995.US Geological Survey Circular 1200.

Sharma S, Bajracharya R, Sitaula BK and Merg J (2005). Water quality in the Central Himalaya, current science 9(5): 782

Sherpa MG (2003). Environment and Public Health Organization, Kathmandu pp:23.

Sharma CK (1990). Chemical pollution of the soil and groundwater in the Kingdom of Nepal. Ground Monit Manage 173:391–397.

Shrestha SD, Karmacharya R, Rao GK (1996). Estimation of groundwater resources in Kathmandu Valley, Nepal. J Ground Hydrol 38:29–40.

Shrestha (2002). Assessment of Drinking Water Quality Supplied by NWSC and Identification, Antibiotic Sensitivity Pattern and Serotyping of Isolated E-coli. M.Sc dissertation, Central Department of Microbiology, TU.

UniProt. Taxonomy: Species *Klebsiella pneumoniae*. Available at http://www.uniprot.org/taxonomy/990925.

Warner NR, Jonathan l, Karen H, Frank F (2007). Drinking Water Quality in Kathmandu valley, Nepal: A Survey and Assessment of Selected Controlling Site characteristics, Hydrology journal (Available at: http//www.springerlink.com).

WHO (2007). Health through safe drinking water and basic sanitation. World Health Organization, Geneva.

APPENDICES

APPENDIX I: COMPOSITION OF MEDIA USED

1. Eosine Methylene Blue Agar (pH-7.2)

Peptone	10.0 g
Lactose	10.0 g
Dipotassium hydrogen phosphate	3.5 g
Sodium sulphite	2.5 g
Basic fuchsin	0.4 g
Agar	15.0 g
Distilled Water	1000.0 ml

2. Salmonella Shigella Agar Media

Lactose	20.0gm
Bile salt No.3	8.50gm
Sodium citrate	8.50gm
Sodium thiosulphate	8.50gm
Beef extracts	5.0gm
Proteose peptone	5.0gm
Ferric citrate	1.0gm
Brilliant Green	0.33mg
Neutral red	0.025gm
Agar	13.50gm
Distilled water	1000.0ml

3. Thiosulphate Citrate Bile Salt Sucrose Agar

Sucrose	20.0gm
Dipeptone	10.0gm
Sodium citrate	10.0gm
Sodium thiosulphate	10.0gm
Sodium chloride	10.0gm

Yeast extracts	5gm
Oxbile (oxgall)	5gm
Sodium cholate	3gm
Ferric citrate	1gm
Bromothymol blue	0.04gm
Thymol blue	0.04gm
Agar	15gm

APPENDIX III: TABLES

Table I: Colonial characteristics of the isolates of samples on EMB Media

S.N	Samples	Color	Form	Elevation	Margin	Texture	opacity
1.	Ek- dhara	Rose pink	Irregular	Convex	Entire	Mucoid	Opaque
2.	Dui- dhara	Rose pink	Irregular	Convex	Entire	Mucoid	Opaque
3.	Tin- dhara	Rose pink	Irregular	Convex	Entire	Mucoid	Opaque
4.	Khatri dhara	Rose pink	Irregular	Convex	Entire	Mucoid	Opaque
5.	Gau dhara	Rose pink	Irregular	Convex	Entire	Mucoid	Opaque
6.	Shiva dhara	Rose pink	Irregular	Convex	Entire	Mucoid	Opaque
7.	Sera khola	Rose pink	Irregular	Convex	Entire	Mucoid	Opaque
8.	Kali khola	Rose pink	Irregular	Convex	Entire	Mucoid	Opaque

Table II: Morphological characteristics of the isolates of samples on EMB

S.N	Gram's Staining	Shape	Motility	TSI	H_2S	Gas
1.	-ve	Rod	Motile	A/A	+	+
2.	-ve	Rod	Motile	A/A	+	+
3.	-	Rod	Motile	A/A	+	+
4.	-	Rod	Motile	A/A	+	+
5.	-	Rod	Motile	A/A	+	+
6.	-	Rod	Motile	A/A	+	+
7.	-	Rod	Motile	A/A	+	+
8.	-	Rod	Motile	A/A	+	+

Table III: Biochemical characteristics of the isolates of samples on EMB

S. N.	Biochemical Characteristics							
	Indole	MR	VP	Citrate	Gelatin hydrolysis	Urease	Catalase	Oxidase
1.	-	-	+	+	+	-	+	-
2.	-	-	+	+	+	-	+	-
3.	-	-	+	+	+	-	+	-
4.	-	-	+	+	+	-	+	-
5.	-	-	+	+	+	-	+	-
6	-	-	+	+	+	-	+	-
7.	-	-	+	+	+	-	+	-
8.	-	-	+	+	+	-	+	-

Table IV: Colonial characteristics of the isolates of sample Ek- dhara

S.N	Media	Color	Form	Elevation	Margin	Texture	Opacity
1.	Salmonella-Shigella Agar	Pink	Round	Umbonate	Undulate	Muciod	Opaque
2.	Salmonella-Shigella Agar	Pink with dark center	Round	Raised	Entire	Dull, smooth	Opaque
3.	Thiosulphate Citrate Bile salt Sucrose Agar	Green	Oval	Umbonate	Wavy	Mucoid	Opaque
4.	Thiosulphate Citrate Bile salt Sucrose Agar	Yellow	Round	Convex	Entire	Muciod	Opaque

Table V: Morphological characteristics of the isolates of sample Ek- dhara

S.N	Gram's Staining	Shape	Motility	TSI	H_2S	Gas
1.	-ve	Rod	Non motile	A/A	-	+
2.	-ve	Rod	Motile	A/Alk	+	-
3.	-	Rod	Motile	Alk/Alk	-	+
4.	-	Rod	Motile	Alk/A	+	+

Table VI: Biochemical characteristics of the isolates of sample Ek- dhara

S. N.	Biochemical Characteristics							
	Indole	MR	VP	Citrate	Gelatin hydrolysis	Urease	Catalase	Oxidase
1.	-	-	+	+	-	+	+	-
2.	-	+	-	+	+	-	+	-
3.	-	-	-	+	+	-	+	+
4.	+	+	-	-	+	+	+	-

Table VII: Colonial characteristics of the isolates of sample Dui- dhara

S.N	Media	Color	Form	Elevation	Margin	Texture	Opacity
1.	Salmonella-Shigella Agar	Pink	Round	Umbonate	Undulate	Muciod	Opaque
2.	Salmonella-Shigella Agar	Pink with dark center	Round	Raised	Entire	Dull, smooth	Opaque
3.	Thiosulphate Citrate Bile salt Sucrose Agar	Green	Oval	Umbonate	Wavy	Mucoid	Opaque
4.	Thiosulphate Citrate Bile salt Sucrose Agar	Yellow	Round	Convex	Entire	Muciod	Opaque

Table VIII: Morphological characteristics of the isolates of sample Dui-dhara

S.N	Gram's Staining	Shape	Motility	TSI	H_2S	Gas
1.	-ve	Rod	Non motile	A/A	-	+
2.	-ve	Rod	Motile	A/Alk	+	-
3.	-	Rod	Motile	Alk/Alk	-	+
4.	-	Rod	Motile	Alk/A	+	+

Table IX: Biochemical characteristics of the isolates of sample Dui- dhara

S. N.	Biochemical Characteristics							
	Indole	MR	VP	Citrate	Gelatin hydrolysis	Urease	Catalase	Oxidase
1.	-	-	+	+	-	+	+	-
2.	-	+	-	+	+	-	+	-
3.	-	-	-	+	+	-	+	+
4.	+	+	-	-	+	+	+	-

Table X: Colonial characteristics of the isolates of sample Tin- dhara

S.N	Media	Color	Form	Elevation	Margin	Texture	Opacity
1.	Salmonella-Shigella Agar	Pink	Round	Umbonate	Undulate	Muciod	Opaque
2.	Salmonella-Shigella Agar	Pink with dark center	Round	Raised	Entire	Dull, smooth	Opaque
3.	Thiosulphate Citrate Bile salt Sucrose Agar	Green	Oval	Umbonate	Wavy	Mucoid	Opaque
4.	Thiosulphate Citrate Bile salt Sucrose Agar	Yellow	Round	Convex	Entire	Muciod	Opaque

Table XI: Morphological characteristics of the isolates of sample Tin- dhara

S.N	Gram's Staining	Shape	Motility	TSI	H_2S	Gas
1.	-ve	Rod	Non motile	A/A	-	+
2.	-ve	Rod	Motile	A/Alk	+	-
3.	-	Rod	Motile	Alk/Alk	-	+
4.	-	Rod	Motile	Alk/A	+	+

Table XII: Biochemical characteristics of the isolates of sample Tin- dhara

S. N.	Biochemical Characteristics							
	Indole	MR	VP	Citrate	Gelatin hydrolysis	Urease	Catalase	Oxidase
1.	-	-	+	+	-	+	+	-
2.	-	+	-	+	+	-	+	-
3.	-	-	-	+	+	-	+	+
4.	+	+	-	-	+	+	+	-

PHOTOGRAPHS

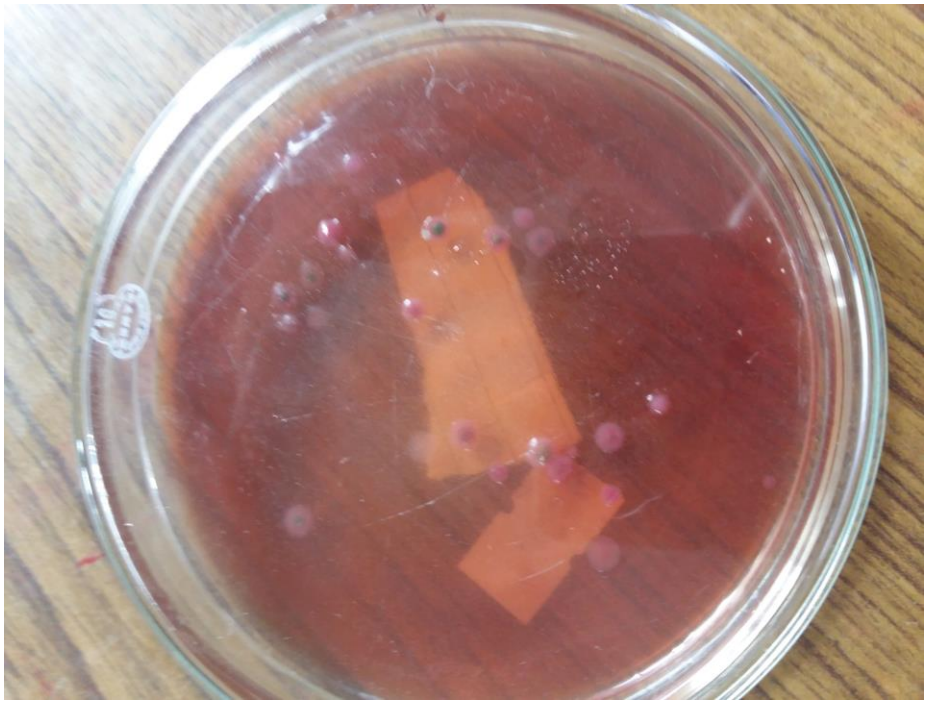

Photo 1: Colony growth on the Salmonella Shigella media plate

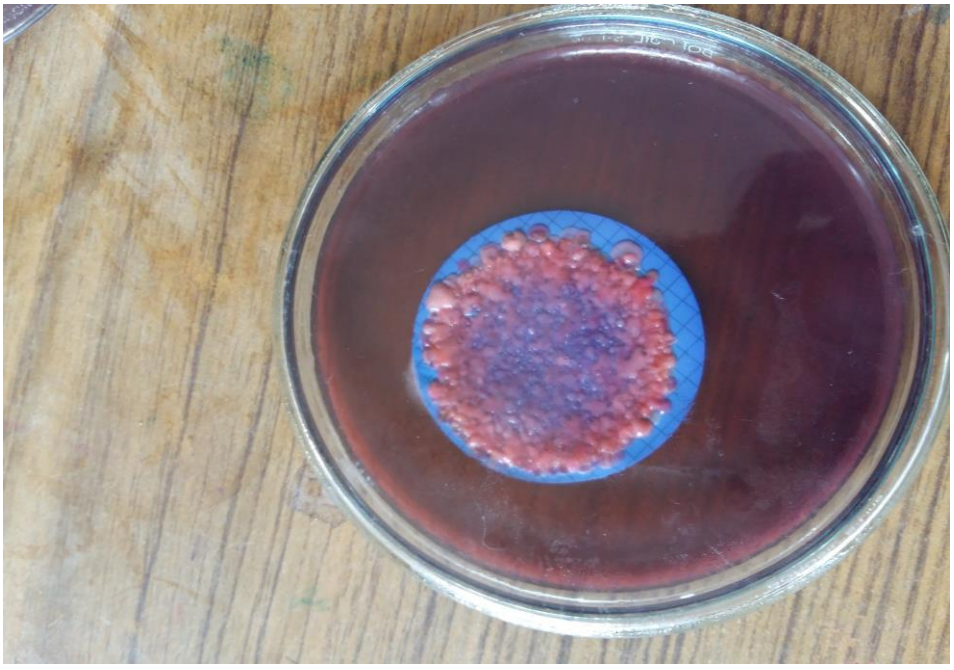

Photo 2: Colony growth on EMB media plate

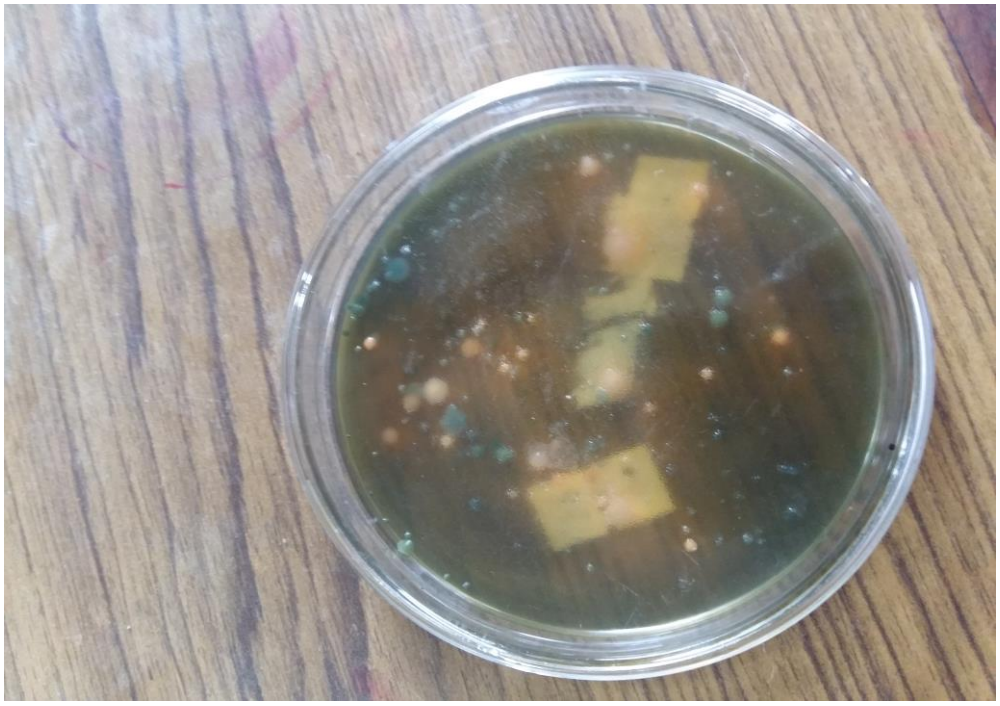

Photo 3: Colony growth on TCBS media plate

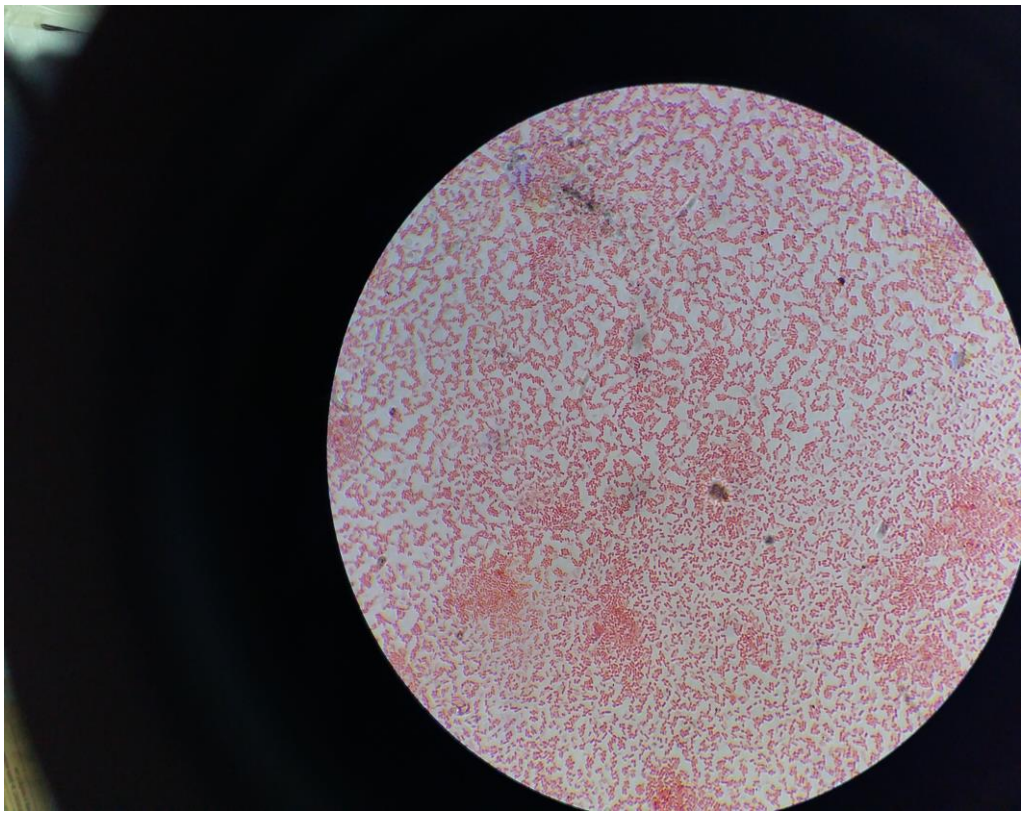

Photo 4: Gram negative rod shaped cells coliform visualized under light microscope after Gram's staining.