

CHAPTER – I

1. INTRODUCTION

Water is the basic and primary need of all vital life process. Water has crucial and usual properties which play a vital role for all living system because water constitutes about 80% of the protoplast. Total quantity of water available in our planet is 140 crore cu Km (1 cr. cu km = 10^{12} lit). Safe drinking water is only 3% of the total available water in the world.

Water is mostly utilized primarily for domestic use, public purposes, irrigation power generation and industrial purpose.

Water intended for human consumption should be safe and wholesome. Safe drinking water is defined as water free from microorganisms and chemicals in concentration, which could cause adverse effect on human health. Drinking water should not be only safe but also be agreeable to use or be wholesome. Such water may be termed potable water. Therefore improved health is possible by means of adequate, safe drinking water and good sanitation provision (Park,1994).

The main sources of water are atmosphere water, surface water and ground water .The moisture present in clouds and precipitated as snow, sleet, hail and rain constitutes atmospheric water. It consists of microorganisms from the dust particles suspended in them.

The water present on earth surface is known as surface water which is present in the form of rivers, streams, oceans, lakes. Their forms are contaminated by the microorganism present in soil and by anthropogenic activities.

Ground water is the water found below the water table in the pores of the soil. Spring and wells are the examples of ground water. Bacteria as well as suspended particles are removed by filtration action of the soil layers. Bacteriologically, wells and springs have good quality water if properly maintained.

Water pollution and its effect on mankind at present scenario of the world is one of the most serious public health issues all over the world. Actually, water is pure and free from external contaminants at the origin of source however with increasing mobilization, population growth and industrialization, water sources have been contaminated with industrial as well as domestic wastes.

Water pollution is any physical, chemical or biological changes in water that can adversely affect living organisms. Polluted water contains solids and dissolved organic compounds, poisonous chemical compounds, infective and parasitic microorganisms from industrial agricultural water or sewage. Pollution of water is responsible for a very large number of mortalities, morbidities and incapacitations in the world. As a main agent in the formidable transmission of several diseases, it causes deaths of thousands of people. According to WHO estimate, about 80% of the third world diseases are transmitted by polluted water. Due to the lack of safe and protective water supply and sanitation, more than 15 million children below five years die each year (UNICEF, 1997) According to public health department of HMG Nepal (1990) about 443,000 children die every year due to gastroenteritis by drinking contaminated water.

Nepal water supply Corporation undertakes the responsibility of drinking water supplies. Laboratory of NWSC located at Bansbari, under Maharajgunj branch carries out regular monitoring of water quality along with treatment of water in plants.

The present study was conducted with an aim to assess the quality of different drinking water viz. raw water, treated water, stone spout, well and tap water. Since emergence of drug resistant bacteria is common, this study also helps to find out possible epidemics of water borne diseases caused by consumption of contaminated water and their sustainable preventive measures.

CHAPTER-II

2. OBJECTIVES

2.1 General Objective

To assess the chemical and bacteriological quality of drinking water in Kathmandu and antibiotic susceptibility pattern of the isolates.

2.2 Specific Objectives

1. To determine total coliform and fecal coliform load in different water samples.
2. To isolate and identify the pathogenic bacteria from water samples.
3. To determine chemical quality of drinking water on the basis of some physicochemical parameters.
4. To perform antibiotic susceptibility pattern of isolated bacteria.

CHAPTER – III

3. LITERATURE REVIEW

3.1 Water pollution

Water pollution is any physical, chemical or biological changes in water that can adversely affect living organisms. At the origin of source, water is pure, free from external contaminants and hence safe for drinking, however, the anthropogenic activities and other environmental factors make the water contaminated when it flows away from its origin. As a result of the increasing industrialization, urbanization and development activities, water pollution is caused and has brought a water crisis. Polluted water may contain infective and parasitic agents, poisonous chemical substances, sewage, and industrial waste. Therefore, pollution of water is responsible for a very large number of mortalities and incapacitation of the world diseases are transmitted by polluted water.

3.1.1 Sources of water pollution

Point and non-point source

A point source is a source that discharges pollutants, or any effluent, such as waste water through pipes, ditches and sewers into bodies of water at specific location. Most water pollution control efforts have concentrated on reducing discharges to surface water from industrial and municipal point source.

A non-point source of water pollution is a source that is one of many widely scattered source that discharges pollutants over a large area such as run-off into surface water and seepage into ground water from croplands, urban and suburban

lands, parking lots, roadways, livestock feedlots etc. Control of water pollution from non-point sources is difficult.

3.1.2 Water pollutants

Biological, chemical and physical forms of water pollutants can be classified into following types.

1. Disease - causing agents (bacteria, viruses, protozoa, parasites)
2. Oxygen demanding wastes (sewage, manure, biodegradable organic wastes)
3. Water soluble on organic chemicals (acids, salts, toxic metal)
4. Water soluble nitrates and phosphate salts.
5. Organic chemicals (insoluble and water soluble)
6. Soil, salt and clay
7. Radioactive substances such as uranium, radium etc.
8. Heat

3.2 Source of water

Natural water is commonly grouped into four-well marked classes.

-) Atmospheric water
-) Surface water
-) Stored water
-) Ground water

3.2.1 Atmospheric water

Rain and snow are included under the atmosphere water. Microorganisms in the form of cells dormant propagate and dust particles remain suspended in water and snow considerable number of bacteria can be isolated from rainwater. After heavy

rain or snowfall, dust particles and bacteria are washed from the atmosphere. Therefore, atmosphere for sometime remain free from microorganism.

3.2.2. Surface water

The water present on earth surface is known as surface water. It is found in the form of several water bodies such as rivers, streams, oceans, lakes etc. These type of sources become contaminated by the microorganisms present in soil. Total microbial number in water depends on microbial population of soil, types of soil, and types of organic material. Moreover, microorganisms of water are governed by climatic, chemical and biological conditions of water. Microbial pollution is also influenced by anthropogenic activities.

3.2.3 Stored water

Inland waters held in ponds, lakes or reservoirs represent stored water. Storage generally reduces the number of organism in water. A certain degree of purity and stability is established during storage.

3.2.4. Ground water

As water seeps through the earth, microorganisms as well as suspended particles are removed by filtration in varying degrees. This depends on the permeability characters of the soil and the depth to which the water penetrates. Ground water brought to the surface by springs or deep wells contain few microorganisms. So during construction of a well the nature of soil, and underlying pores strata, the nature of water table and nature distance, direction of local sources of pollution must be taken into consideration. Ground water is subterranean water that occurs where all pores in the soil or rock containing materials are saturated. Springs consist of groundwater that reaches the surface through a rock fissure or exposed porous

soil. Wells are made by sinking a shaft into the ground to penetrate the ground water level. Wells and springs that are properly located produce water of good quality.

3.3 Bacterial flora of water

Generally surface water may contain following types of bacteria:

- Natural or normal flora: *Micrococcus*, *Acinetobacter*, *Pseudomonas*, *Alkaligenes*, *Flavobacterium*, *Chromobacterium*, *serratia*, *cytophaga* etc.
- Soil dwelling bacteria: *Bacillus subtilis*, *B. megaterium*, *Klebsiella* spp.

From Fecal Contamination

- Intestinal bacteria: *Escherichia coli*, *Klebsiella pneumoniae*, *Streptococcus fecalis*, *Clostridium perfringens* etc.
- Sewage bacteria proper: *Proteus vulgaris*, *Clostridium sporogenes*, *Nocardia* spp etc (Dubey and Maheshwori, 2000).

3.4 Causative factors of pollution

The problem of water pollution due to discharge of domestic and industrial wastes into aquatic systems has already become a serious problem in the country. A great number of nation's population is exposed to unsafe drinking water. In addition, most of the water resources receive millions of liters of sewage, agricultural effluents consisting of simple nutrients to highly toxic substances. As a result, enteric disease often reaching epidemic proportions, devastate several parts of the country. The origin of these problems as disgusting odour, lethal effects to aquatic organisms can be attributed to many source and type of pollutants.

Pollution causing factors are as follows:

I. Oxygen demanding waste

Dissolved oxygen (DO) is essential for sustaining the plant and animal life in aquatic system. Aquatic organism require a minimum of DO level at least 5mg/ml. There are four processes that affect DO content in water: re-aeration, photosynthesis, respiration and oxidation of wastes. The bacterial and algal respiration is responsible for the production of CO₂ and subsequent depletion of dissolved oxygen. Combination of the three effects of respiration, photosynthesis and aeration result in variation of DO.

The primary cause of deoxygenation of aquatic system is the presence of organic substances called oxygen-demanding waste. The more oxygen required for the breakdown of organic substance, the greater will be deoxygenation of water way. The pollution results when oxygen demand exceeds available oxygen.

Biochemical oxygen demand (BOD)

BOD is a measure of the oxygen utilized by microorganisms during oxidation of organic material. The demand for the oxygen is directly proportional to the amount of organic waste, which has to be broken down.

II. Disease causing agents

Water is a potential carrier of pathogenic microorganisms. These pathogens are carried into the water bodies by sewage and wastes from various industries. Some bacteria are water borne, and these include those responsibly for causing cholera, typhoid, bacillary dysentery and gastroenteritis. Viruses are also found in water, including strains which are responsible for polio, infectious hepatitis and coxsackie's fever. Water may also contain intestinal parasite including round worm, tape worm, *Entamoeba*, *Giardia* etc.

All these types of organisms occur in faces and so are present in the sewage water also plays an indirect role in transmission of diseases like yellow fever, filariasis and schistosomiasis (Rao, 1991).

III. Synthetic organic compounds

These include pesticide synthetic organic chemicals and detergents, which are non-biodegradable and may persist for long periods. Most of them are accumulative toxic poisons and ultimately may reach objectionable level in water.

IV. Plant nutrients

Nitrogen and phosphorus are essential elements, which are required by plants for growth and metabolism. In wastewater, these are present in abundance as phosphate, nitrate and ammonia. These compounds enter water bodies directly from fertilizer, processing of food or textiles or via domestic sewage treatment plants.

V. Inorganic chemicals and minerals

These pollutants include inorganic salt, minerals acid, waste and their compounds. Mine drainage is an important source of increased acidity in natural water and other source as acid rainfall. Fresh water salinity is due to industrial effluent, minerals in irrigation, salt brine, oil wells, and ocean salt.

VI. Sediments

This includes soil, sand and mineral particles washed into the aquatic environment by storms and floodwaters.

VII. Radioactive substances

Damage to aquatic environment from wastes of uranium and thorium from nuclear power plants, mining and refining process. Radium a significant waste product is a hazard in drinking water.

VIII. Thermal discharges

Used coolant water from power plants and industry is discharged directly temperature of water bodies with deleterious consequences for aquatic inhabitants.

3.5 Impact of water pollution

The effect of chemical pollutants from the industrial and agriculture waste is alarming for the living organism. Introduction of chemicals in drinking water show toxic effects, in addition poisons food animals (due to those organisms having bioaccumulated toxins from the environment over their life span). Moreover, such pollution creates unbalanced river and lake ecosystems that can no longer support full biological diversity, deforestation from acid rain and many other effects. The chlorinated organic pesticides are hazardous mainly due to their concentration in the food chain. They've high stability, low vapor pressure and very low solubility in water. As a result of biomagnifications, calcium metabolism in predatory birds and this cause birds laying eggs with thin shells.

Mine drainage is an important source of increased acidity in natural waters. Acids cause corrosion of metals and concrete and can be fatal to fish. Alkalis discharged by industries such as textile, tanneries can also destroy aquatic life. Hard water can

cause scale formation in pipes, boiler tubes. Chloride concentration in excess of 400 ppm can be fatal to fresh water fish.

Lead from industrial waste is toxic to aquatic organisms. It concentrates primarily in the bones. Cadmium generated in waste streams from pigment works, textiles, electroplating caused itai- itai disease in Japan as well retarded plant growth. Mercury discharged from industrial effluents as from paint and paper, chlorine and caustic soda, fertilizer are pollutants and gets accumulated in food chain and reduce rate of photosynthesis of phytoplanktons. Silver is also a cumulative poison and chronic.

Many of the chemicals that enter the water with run off and seepage are toxic to human health and can alter ecosystems. Acid rain deposition can impair plant growth alters soil composition and effect reproduction capacities of many species of fish, insects aquatic plants and bacteria.

Another effect is the acceleration of eutrophication processes of water. It is aging of a lake by biological enrichment. With time, streams draining into lake, introduce nutrient as nitrogen and phosphorous which encourage growth of aquatic organisms.

Biological impact

In water receiving sewage, domestic waste and industrial waste, a plethora of pathogenic organisms may be present. The vectors of water borne disease include bacteria, virus, protozoa and the helminthes. Pathogenic bacteria that have been transmitted by water or wastewater include *Salmonella* spp. causing typhoid fever, *Shigella* spp. causing bacillary dysentery, *Campylobacter*, enteropathogenic *Escherichia coli*, *vibrio cholerae*, *Leptospira* and *Yersinia*.

Typhoid is characterized by mild pyrexia to a fatal fulminating disease. The onset is usually gradual, with headache, malaise, anorexia, coated tongue and abdominal discomfort.

Shigella spp. causing dysentery is a clinical condition characterized by passage of loose blood stained mucopurulent stools along with abdominal cramps, fever and vomiting.

Cholera caused by *Vibrio cholera* is associated with major epidemics causing invasive disease and mild to severe form of diarrhea. It is autochthonous flora to brackish and estuarine water contributing to waterborne transmission.

Polluted water also contains several pathogenic viruses, prominent of them being that of infectious hepatitis (jaundice) and poliomyelitis. Some protozoan diseases like amoebic dysentery are caused, by ingesting polluted water. In stagnant water 'schistosomiasis' causing organisms (helminthes) flourish on molluscs in certain areas.

When sufficient water is not available for the proper hygienic practice then the pathogens are spread to causes disease. eg: - Scabies and Trachoma.

Some of the pathogens spend part of their life cycle in water. When come in contact with human beings cause various diseases. eg:- Schistosomiasis.

Water related insect vectors

- 1) Water biting: - Gambian sleeping sickness.
- 2) Water breeding: - Malaria, yellow fever

It is highly essential to examine the presence of pathogenic organisms in distributing water for potable purposes.

Besides pathogens, unwanted biological growth of algae, fungi and angiospermic plants is of utmost importance. Nutrient enrichment hinders with recreation, spoil its aesthetic value and cost more in water treatment.

3.6 Water quality parameter

Water quality can be, acknowledged on the basis of a large number of parameters that are physical, chemical and microbiological standards. A regular monitoring and surveillance is essential for the prevention of diseases and hazards to happen and also checks the water resources from being polluted.

3.6.1 Microbial indicators

The most important common source of infection is water. Even water that looks clean and pure may be sufficiently contaminated with pathogenic microorganisms to be a health hazard. Therefore some means are necessary to confirm that drinking water is safe. It is not usually practical to examine water for the various pathogenic organism that may be present. So, some "indicator organisms" are chosen for the examination of bacteriological quality of the water.

The primary bacterial indicator recommended for this purpose is the "coliform group" as a whole. Supplementary indicator organisms such as fecal streptococci and sulfite reducing clostridia are used to determine origin of fecal pollution as well as in assessing the efficiency of water treatment process. The indicator organisms are usually associated with the intestinal tract and their presence in water indicates the possible fecal contamination of water source.

3.6.1.1 Coliform and faecal coliform group

The coliform groups of bacteria includes all the aerobic and facultative anaerobic, gram-negative, nonsporulating bacilli that produce acid and gas from the fermentation of lactose at 37°C. Typical examples of the coliform group are *Escherichia coli*, *Enterobacter aerogenes*, *Klebsiella pneumonia* and *Citrobacter* spp.

The coliform group of organisms are suitable as indicators because.

- © Coliform organisms are constantly present in the intestinal tract of both humans and warm-blooded animals.
- © They are present in intestinal tract in large numbers, eg: *E. coli*, is present in numbers 10^9 /gm of faeces.
- © These organisms generally live longer in water than pathogens.
- © They are relatively easy to detect and assay in water.
- © The presence of coliforms in water is regarded as a warning signal; the water is subject to potentially dangerous pollution.

Escherichia coli

E.coli is a member of family Enterobacteriaceae and was discovered in 1885 by Theodor Escherich. It is characterized by possession of enzyme B- galactosidase and grows on 44-45°C on media, ferment lactose with the production of acid and gas; produce indole from tryptophan. They don't produce oxidase or hydrolyze urea. They are motile and give positive methyl red reaction, doesn't utilize citrate and liquefy gelatin (Collee et al. 1989).

Thermotolerant coliform bacteria

These are the group of coliform organisms that are able to ferment lactose at 44.5°C in 24 hrs and comprise genus *Escherichia* and to a lesser extent species of *Klebsiella*, and *Enterobacter*.

3.6.1.2 Faecal streptococci

Faecal streptococci regularly occur in feces, but in much smaller numbers than *E. coli*. In doubtful cases, the finding of faecal streptococci in water is regarded as important confirmatory evidence of recent faecal pollution of water.

They are highly resistant to drying and may be valuable for routine control testing often laying new means in distribution system or for detecting pollution by surface run-off to ground or surface waters. These all give a positive reaction with lancefield Group D antisera.

3.6.1.3 *Clostridium perfringens*

They also occur regularly in faeces, however in much smaller number than *E. coli*. They are anaerobic, spore forming organisms; the spores are capable of surviving in water for a longer time than organisms of the coliform group and usually resist chlorination at the dose normally used in water works practices. The presence of spores of *Clostridium perfringens* in natural water suggests that faecal pollution has occurred and their presence suggests that faecal contamination occurred at some remote time.

3.6.2 Enterobacteriaceae

The members of Enterobacteriaceae are gram-negative, aerobic or facultative anaerobic, non-spore forming, often motile by means of lateral flagella or non-motile, catalase positive, oxidase negative bacilli, and cells contain a characteristic

antigen called the enterobacterial common antigen and have simple nutritional requirements They reduce nitrate to nitrite, ferment glucose with production of acid or gas and are fermentative. They can be found in the intestinal tract of human and animal and in soil and water. Some are commensal and others few are pathogenic for human.

The members of Enterobacteriaceae family are as follows:

Escherichia

The major species *E.coli* occur in the lower portion of the intestine of humans and warm-blooded animals, where it is part of the normal flora, some strains can cause gastroenteritis, other can cause urinary tract infection.

Shigella

This genus is closely related to Escherichia moreover, all strains are pathogenic, causing bacillary dysentery in humans.

Salmonella

All strains of *Salmonella* are pathogenic for humans, causing enteric fever (as typhoid and paratyphoid fever) gastroenteritis, and septicaemia over, 2,000 antigenic types of *Salmonella* occur.

Enterobacter

Enterobacter spp. grow best at 30 C rather than 37°C. They occur mainly in water sewage, soil meat, plants and vegetables. They occur in human and animal faeces, and some can be opportunistic human pathogens.

Erwinia

They are often plant pathogen; causing disease as blights, canker, die back.

Serratia

They are widely distributed in soil, in water, and on plant surface. They can be opportunistic human pathogens.

Proteus

These organisms show swarming on agar media. They occur in the intestine of humans and a wide variety of animals, in polluted water, and in soil, and they can be opportunistic human pathogens. *P.mirabilis* is one of the leading causes of urinary tract infections in humans.

Yersinia

These are parasites of animals but can also cause infections on human. *Y.pestis* the causative agent of plague and *Y.enterocolitica* is a frequent cause of gastroenteritis in children.

Citrobacter

These are isolated from humans, wound infection, sepsis, fecal flora.

3.7 Microbiological test

The original test for the presence of coliform in water is done by standard multiple tube fermentation technique and membrane filter technique.

3.7.1 Most probable number (MPN) method or multiple tube method

Presumptive test

A series of fermentation tubes each containing lactose of known concentration with indicator are inoculated with known amount (10ml, 1ml, 0.1ml) of water. These tubes are incubated for 48 hrs. at 37°C for the production of acid and gas, if the coliforms are present. MPN is estimated with the help of number of tubes showing positive results from the statistical table for MPN.

The confirmed test

The confirmed test is done by sub culturing each presumptive positive tube in two tubes of Brilliant Green lactose bile (BGLB) broth, one of which is incubated at 37°C for 48 hours and the other is incubated at 44.5°C for 24 hours. (for faecal coliforms)

Alternatively, the subculturing can be done by streaking on Eosin-Methylene Blue (EMB) agar, and by observing for the production of greenish metallic sheen, the presence of coliforms can be confirmed.

The completed test

From the EMB agar plates, some typical colonies are selected and inoculated into lactose broth for production of acid and gas and slant for gram-staining and biochemical tests of each organism. By the completed test, identification of organism can be done.

3.7.2 Membrane filter technique

A measured volume generally 100 ml of water sample is filtered through sterile membrane filter. (made of cellulose ester and pore size 0.45 micrometer). Then membrane is placed on an absorbent pad saturated with suitable medium and incubated at 37°C for 24 hours. Production of metallic sheen confirms the presence of coliforms.

Advantages to membrane filter technique

- © It permits small number of bacteria from large quantities of water.
- © It doesn't allow to spread combination of any number of bacteria.
- © It allows the direct counting of microorganisms instead of counting most probable member.
- © It is time saving method.

3.8 Standards of water pollution

Drinking water standards are regulations that are set to control the level of contamination in the national drinking water. These standards are a part of the safe drinking water Act, which include assessing, and protecting drinking water resources, protecting wells and collecting system.

There are two categories of drinking water standards

I. National primary drinking water regulation

This is a legally enforceable standard that applies to public water systems. It protects drinking water quality by limiting level of specific contaminants that can adversely affect public health.

II. Secondary standard

This is a non-enforceable guideline regarding contaminants that may cause aesthetic and cosmetic effects in drinking water. Classification of drinking water according to biological tests:

Class	Quality	Presumptive coliform/100 ml	<i>E.coli</i> count/100ml
1.	Excellent	0	0
2.	Satisfactory	1-3	0
3.	Suspicious	4-10	0
4.	Unsatisfactory	> 10	1 or more

3.9 Purification of water

Purification of water depends on amount and quantity of water. It is essential before its consumption so that disease cycle of pathogenic microorganisms can be broken thereby prevention of pathogen to reach the human body. Therefore it is done with prospect of making it satisfactory in appearance, taste, and free from pathogens. There are three chief methods used for purification of drinking water in municipal supplies, which are as follows:

a. Sedimentation

It is done when water consists of large sized organic materials such as leaves and gravel which have run-off from the soil. Suspended particles settle down depending on their size and weight and conditions of the stored water. It is done in large reservoir or on settling tank. The rate of sedimentation is enhanced by adding alum, iron, salts, which acts as coagulants. The suspended materials and microorganisms are entrapped by coagulants and settle down rapidly. This procedure is called coagulation or flocculation. Sedimentation provides partial reduction of microorganisms in water due to there setting down on bottom.

b. Filtration

It is the second step of purification. The water is purified by passing it to filtration unit. It is the effective means of removing microorganisms and the other suspended material from the water. There are two types of sand filters used in purification purpose as slow sand filter and rapid sand filter.

Slow sand filter

In slow sand filtration plants the rate of filtration of water is slow, hence requires a considerable area. This plant consists of concrete floor containing draining tiles. The tile is covered first with coarse sand and finally sand at the top of the plant. Water passes slowly through this filter and collected by tile pipes at the bottom, which is pumped, into a reservoir. If water is turbid, slow sand filter are clogged soon. Therefore turbid water to be filtered should be clarified first by sedimentation thereafter passed through filter. The capacity of slow sand filter plant is to filter 5 million gallons of water per acre per day.

Purification is done by physiological mechanisms support by microorganisms. In the surface layers of fine sand, a colloidal material consisting of bacterial, algae and protozoa is attached. This makes the pores more effective by closing the pores between the sand grains. Sand grains have positive charges and bacterial cell walls have negative charge. Therefore bacteria are adsorbed on the surface of sand. Protozoa ingest bacteria. Due to intense microbial interaction, chemical concentration of water is reduced. Through this plant *Giardia* and its cysts are even filtered from water.

Rapid sand filter

The rapid sand filter is also constructed as that of slow sand filter. Before filtration water is treated with alum or ferrous sulphate in a settling tank where precipitates settle down, thereafter, water is allowed to pass through rapid sand filter. This plant depends on physical trapping of fine particles and flocs or coagulants. The pores, if clogged have to be cleaned by forcing clean water back ward. An about 99% bacterium one removed by this plant, but doesn't remove *Giardia* cysts. This plant operates about 50 times faster than slow sand filter plant, and can deliver about 150 to 200 millions gallons of water per acre per day. It requires less land area, less cost and less maintenance.

c. Disinfection

Disinfection of public water supply is done as bacteria pass through the filter which must be killed. It is the final step in which chlorination of public water supply. The amount of chlorine required depends on organic matter and time to act upon. The amount of chlorine required for disinfections is called chlorine demand. Water is chlorinated to contain 0.1 to 0.2 ppm of residual chlorine after 20 min. of addition. Dealing with the mechanism, often reacting with water chlorine is converted into hypochlorous acid, which in turn release nascent oxygen, which oxidizes the cellular components of microorganisms as well as organic matter. The other gas, which behaves like chlorine, is ozone.

3.10 Causes of water quality deterioration

Water quality can be contaminated due to various factors:

1. Faeces

When you are killing thousands of people through infectious diseases due to small blunder of fecal contamination in water you are excused due to unawareness of the

fact. A recent UNICEF report shows that each gram of faeces contains upto 10,000 viruses 1,000,000 bacteria, 1000 parasite cysts and 100 parasites eggs.

2. Flows

Even when the treated water supplied from the service reservoir is safe and pure, water gets contaminated when it flows through complicated network. Old and loose connected pipe in some congested places where there are more chances of contamination.

3. Fetching

In fetching water, one should be very careful about the hygienic concept. Some times we can see that water of satisfactory quality (0-4 coliform /100 ml) collected from tap can be converted to unsatisfactory due to unhygienic and unsatisfactory practices eg: - washing the container with potentially contaminated soil instead of sterile biocidal ash.

4. Flies

Even if the collected water is pure, the water becomes contaminated if the storage condition is not proper. If the storing container is not properly covered various flies and insects can contaminate the water.

5. Fingers

Your water can be contaminated lastly even in the drinking glass if your fingertips are not free from the contaminants. Thus, to wash your hand is 50 % to be free from contamination.

3.11 Water related diseases

Infectious diseases related to water and sanitation can be categorized into four classes

- a) Water borne b) Water based c) Water-washed
- d) Water related insect vectors

a) Water borne

Diseases caused by contaminated water and practices are:

I) Classical

Bacterial dysentery (*Shigella dysentery*) dysentery with blood or mucus, Typhoid Fever (*Salmonella typhi*), Cholera (*Vibrio cholera*), amoebic dysentery (*Entamoeba histolytica*) bulky stool 10-12 times Diarrhea (*E. coli*) .

II) Non-classical

Infectious hepatitis. It can be controlled by improving water quality and hygienic practices.

b) Water based

I) Water multiplied percutaneous

The pathogens spend part of their life cycle in water when come in contact with human beings cause various diseases. eg: - Schistosomiasis.

II) Ingested

Guinea worm and lung fluke infection. To prevent from this disease, excreta disposal must be controlled.

c) Water-washed

I) Superficial

When sufficient water isn't available for the proper hygiene practice then the pathogens are spread to cause different water washed diseases. eg: - Scabies and Trachoma.

II) Intestinal shigellosis

To prevent from these diseases, one should adapt the good hygiene practice using soap and plenty of water.

d) Water related insect vectors

I) Water biting

Gambian Sleeping sickness

II) Water breeding

The pathogen is spread by insects that feed or breed in water (flies and mosquitoes)
For eg: - Malaria, yellow fever. To prevent these diseases, the environment should be clean and sufficient water supply must be available for cleaning drainage, which control insect breeding as well.

3.12 Physico-chemical parameters of water

The good quality of water should be allowable in terms of sight, color, smell, temperature and taste. Urban run off, municipal and industrial waste water , soils and rocks with which water has been in contact affect the water quality.

The chemical parameters that affect the quality of water are described as follows:

Temperature

Temperature is basically important for its effects on the chemistry and biological reactions in the organisms in water. A rise in temperature of the water leads to the speeding up of the chemical reactions in water, reduces the solubility of gases and amplifies the taste and odour. The temperature of fresh water normally varies from 0 to 35°C to 95°C, depending on the source, depth and season. High water temperature enhances the growth of microorganisms.

Turbidity

Turbidity of water is actually the expression of optical property in which light is scattered by the particles present in water. Turbidity in natural water is caused by clay, silt, organic matter, phytoplankton and other microscopic organisms. It is highly dependant upon the size, shape and refractive index of the particles. Turbidity makes the water unfit for domestic purposes, food and beverage industry and many other industrial uses. A reduction in turbidity is associated with a reduction in suspended matter and microbial growth. Turbidity in natural water restricts light protraction for penetration (Trivedy and Goel, 1986).

p^H

p^H is the measure of the intensity of acidity or alkalinity and measures the concentration of hydrogen ions in water. Most natural waters are generally alkaline due to presence of sufficient quantities of carbonates. p^H of water gets drastically changed with time due to exposure to air, biological activity and temperature changes. Significant changes in p^H occur due to disposal of industrial wastes, acid mine drainage etc. p^H of water also change diurnally and seasonally due to variation in photosynthetic activity which increase p^H due to consumption of CO₂ in the process. Determination of p^H is one of the important objectives in treatment of wastes. p^H has no direct adverse effects on health, however, a lower value below 4 produces sour taste, higher value above 8.5 an alkaline taste. Acids contribute to

corrosiveness and influence chemical reaction rates, higher p^H value hasten, the scale formation in water heating apparatus and also reduce the germicidal properties of chlorine. In the water supplies, p^H is also an important factor in fixing alum dose in drinking water treatment.

Alkalinity

Alkalinity of the water is its capacity to neutralize a strong acid. It is the sum of all the titrable bases. In the natural and polluted water, there are many other salts of weak acids as silicate, borate which cause alkalinity in addition to that of carbonates and bicarbonates. The water supplies less than 100 mg/l are desirable for domestic use. Its value is also important in calculating the dose of alum and biocides in water. Alkalinity producing substances such as sodium bicarbonate are added to check corrosion in soft water supplies and important in controlling water and waste water treatment process. Raw domestic water has an alkalinity less than or slightly greater than that of water supply (APHA, 1995).

Acidity

Acidity of a water is its quantitative capacity to react with a strong base. Strong mineral acids, weak acids such as carbonic and acetic, and hydrolyzing salts such as iron or aluminium sulfates may contribute to the measured acidity. It contributes to corrosion, chemical speciation and biological processes.

Conductivity

Conductivity is a numerical expression of the ability of an aqueous solution to carry an electric current. This ability depends on the presence of ions, their total concentration, mobility, valence, and temperature of the measurement. Solutions of most inorganic acids, bases and salts are relatively good conductors. Conversely,

molecules of organic compounds that don't dissociate in aqueous solution conduct current very poorly.

Hardness

Hardness is the property of water, which prevents the lather formation with soaps and increase the boiling point of water. Principal cations imparting hardness are calcium and magnesium. The anions responsible for hardness are mainly bicarbonate, carbonate, sulphate, chloride, nitrates and silicates. Hardness is called temporary if it is caused by bicarbonate and carbonate salts of the cations, since it can be removed by boiling. Permanent hardness is caused by sulphates and chlorides of the metal. Hardness has its role in heart disease; it is undesirable due to the formation of heat retarding insulating scales in the boilers and other heat exchange equipments. The hard water is not suitable for domestic use in washing, cleaning and laundering. It prevents the corrosion in pipe by forming a thin layer of scale and reduces entry of heavy metal from pipes to water

Calcium

The presence of calcium in water supplies result from passage through or over deposits of limestone, dolomite, gypsum. Small concentration of CaCO_3 combat corrosion of metal pipes by laying down a protective covering. Chemical softening treatment, reverse osmosis or ion exchange is used to reduce calcium and associated hardness (APHA, 1995).

Magnesium

It is a common constituent of water. Magnesium salts are important contributors to the hardness of water and breakdown when heated, forming scale in boilers. Concentration greater than 125 mg/l can also have cathartic and diuretic effects (APHA, 1995).

Iron

Iron is one of the most abundant elements of the rocks and soil. All kinds of waters including ground water have appreciable quantities of iron. Iron has more solubility at acidic p^H , therefore large quantities of iron are leached out from the soils by acidic water (Eg: - acid mine drainage). In ground water most of the iron remains in ferrous state due to lack of oxygen. When the ground water with the higher concentration of iron is tapped, it quickly oxidizes to ferric state in the form of insoluble ferric hydroxide, a brown substance. In addition to the natural sources of iron, corrosion of pipes, pumps can also increase concentration in distribution system. Iron has got a microbial significance since a few microorganisms such as *Crenothrix*, *Leptothrix* and *Gallionella* are able to utilize dissolved iron. This gives a rusty appearance to the water. Iron in excess of 0.3mg/l cause staining of clothes and utensils. The higher concentration of iron is also not suitable for food processing, beverage, dying, bleaching etc.

Ammonia

The most important sources of ammonia is the ammonification of organic matter sewage has large quantities of nitrogenous matter. Thus its disposal tends to increase the ammonia content of water. Occurrence of ammonia in the waters can be accepted as the chemical evidence of organic pollution. If only NH_3 is present, pollution by sewage must be recent. Ammonia in higher concentration is harmful to fish and other biota. It is also toxic to man at higher concentrations. The toxicity of ammonia increases with p^H because at higher p^H most of ammonia remains in gases form.

Chloride

Chloride occurs naturally in all types of waters. In natural freshwater, its concentration remains quite low. The most important source of chlorides in the waters is the discharge of domestic sewage. Man and other animals excrete very high quantities of chlorides. About 8-15 gms of NaCl is excreted by a person per day.

Therefore, the chloride concentration serves as an indicator of pollution by sewage. Industries are also important source of chloride. It is harmless up to 1500mg/l concentration but produce a salty taste at 250-500mg/l. It can also corrode concrete by extracting calcium in the form of calcite concrete by extracting calcium in the form of calcite and can corrode boilers.

Dissolved oxygen

DO is one of the most important parameters in water quality assessment and reflects physical and biological processes prevailing in the water. Its presence is essential to maintain higher forms of life in the water; Non-polluted surface waters are normally saturated with dissolved oxygen. Other inorganic reductants such as hydrogen sulphide, ammonia, nitrites and other utilizable substances also tend to decrease dissolved oxygen in water. Low oxygen in water can kill fish and other organisms present in water. Low O₂ concentration are generally associated with heavy contamination by organic matter.

Biochemical oxygen demand (BOD)

BOD is the amount of oxygen utilized by microorganism in stabilizing the organic matter. The demand for oxygen is proportional to the amount of organic waste to be degraded aerobically. BOD approximates the amount of oxidizable organic matter present in the solution and BOD can be used as a measure of waste strength. It is

highly important to know the amount of organic matter present in the waste treatment system. BOD test is also useful in stream pollution control management and in evaluating self-purification capacities of streams. BOD test has been developed for 5 days at 20°C.

3.13 Antibiotics and bacterial resistance to antibiotics

Antibiotics refer to a metabolic product of one microorganism that in very small amounts exert antimicrobial activity. The significance of this antagonistic action exerted by one microorganism to other was not realized until 1929, when Alexander Flemming discovered penicillin. Some antibiotics are inhibitory to many different species ie. broad spectrum, while some are inhibitory to only a few species of microorganism. To determine the effectiveness of various antibiotics, susceptibility tests are performed. Waksman in 1945, according to him, those chemical substances of microbial origin which in small amount act against various microbial species are antibiotics. These tests are commonly done by following Kirby Bauer method in which small paper disc impregnated with antibiotics are placed on the surface of an agar plate heavily seeded with test organism. The plate is incubated for 24 hrs at 37 C. A zone of inhibition surrounding the filter paper disc is observed if the test organism is sensitive to the antibiotic.

Antibiotics have the following qualities:

1. They should have the ability to destroy or inhibit many different species of pathogenic microorganisms.
2. They should prevent the ready development of resistant forms of the parasite.
3. They should not produce undesirable side effects in the host, such as allergic reactions, nerve damage etc.

4. They should not eliminate the normal microbial flora of the host, because doing so may upset the balance of nature.

Some of the antibiotics are bactericidal ie, they can kill the bacteria and others are bacteriostatic ie, they inhibit the growth of bacteria. A way of classifying antibiotics is on the basis of their mode of action. The major point of attack of antibiotics on microorganisms include:

- i. Inhibition of cell wall synthesis: Penicillin, Cephalosporin, Cycloserine, Vancomycin, Bacitracin.
- ii. Damage to cytoplasmic membrane: Polymyxins, Gramicidines, Tyrocidines.
- iii. Inhibition of nucleic acid: Actinomycin D, Rifamycin, Mitomycin.
- iv. Inhibition of protein synthesis: Tetracycline, Aminoglycosides, Chloramphenicol, Erythromycin.
- v. Inhibition of specific enzymes: Sulfonamides.
- vi. Antifungal antibiotics: Nystatin, Griseofulvin.

Davies and Smith have classified the possible types of mechanisms of resistance to antimicrobial agents as follows:

- i. Alteration of the target site.
- ii. Blocking of antibiotic transport.
- iii. Enzymatic detoxification or inactivation of antibiotic.
- iv. Bypass mechanism in which a metabolic step inhibited by the antimicrobial agent is replaced.
- v. Saturating the drug by increasing the level of the inhibited enzyme.
- vi. Production of inhibition antagonistic metabolite.

- vii. Decreasing the metabolic requirement of the cell for the pathway or reaction inhibited by drug.
 - i. In this mechanism the binding of drug to the target site is reduced or eliminated by alteration of the site. In gram-positive bacteria methylation of 23s RNA results in erythromycin resistance.
 - ii. Bacteria have active transport systems designed to carry useful molecules as antibiotics into the cell. Certain plasmid coded substance prevent entry of antibiotics into the bacterial cells, thus conferring resistance to the cell.
 - iii. Resistance Mechanisms involving resistance to Penicillin and Cephalosporins are concerned with enzymatic detoxification. The enzyme B-lactamase catalyze the hydrolysis of B-lactam ring and detoxify the drug. B-lactamases are determined both chromosomally and by plasmid. Plasmid determined B-lactamases are the primary cause of bacterial resistance to penicillin and cephalosporin.
 - iv. A metabolic step, which is inhibited by an antimicrobial agent, may be replaced by another compound. Resistance to Sulphonamides and to Trimethoprim develops when the chromosome-coded enzyme is replaced by entirely new metabolic enzyme. This permits the blocked pathway to function in presence of drug.

3.14 Microbiological studies on drinking water in Nepal

Most water pollution studies have examined the quality of drinking water supplied to Kathmandu Valley. The extents of drinking water contamination in the areas covered by pollution studies suggest that the problems is nation wide.

Sharma (1986) in a study examined water samples from different sources of Kathmandu and Lalitpur. He found contamination level up to 4800-coliform/100 ml.

Adhikari *et al.* (1986) carried out coliform test in 100 samples of drinking water from various areas in Kathmandu Valley. He found that most of the unsatisfactory water samples had more than 1800 coliforms per 100 ml of water,

DISVI (1989) carried out a more detailed and comprehensive study to assess the quality of Kathmandu Valley.

CEDA (1989) study found out that all samples were contaminated with fecal materials.

DISVI (1990) carried out a drinking water quality in seven rural areas of Ilam in Eastern Nepal. Study found that unacceptable levels of fecal coliform bacteria ranging from 2 to 2400 cells 100ml.

CEDA (1989) reported that 5% of Kathmandu population used from dugwells and stone sprouts.

Sharma (1986) studied the water quality from these sources and coliform bacteria counts from 0-460/100ml during summer.

ENPHO/DISVI (1990) investigated bacteriological quality of ground water in Kathmandu. The lowest average fecal coliform were observed in samples from Bhatbhateni (8 cells/100ml), Balajutar (1 cell/100ml) and sundhara (19 cells/100ml) In all, 81% of the samples contained an average density of more than 100ml.

Sharma (1993) carried out microbiological examination water from samples of major cities of Nepal. He found that the highest coliform count was 2400 cells/100ml in Kathmandu 48,000 cells/100ml in Biratnagar, 4800 cells/100ml in Birgunj and 4800 cells/100ml in Pokhara.

Thapa (1997) examined water quality of 9 different sites in Baluwa VDC, near to the Kathmandu city. He found all the samples were contaminated during all the

season. The bacterial densities, ranged from 43-210 cells/100ml during winter, 75-240 cells/100ml during summer and 150-460 cells/100ml during rainy season.

3.15 Studies of physico-chemical parameters of drinking water

CEDA (1989) carried out study from 13 sites including 3 reservoirs-Balaju, Maharajgunj and Mahankal Chour. The chemical parameters tested included p^H , DO, BOD, COD, ammonia, nitrate, phosphate and chloride.

DISVI (1989) studied a detail chemical analysis of drinking water, which corrode water pipelines, affect the quality of water, and indicate contamination by domestic sewage.

DISVI (1990) studied in seven rural areas of Ilam in Nepal found that water samples from 6 sources were acidic with p^H values outside acceptable limit set by WHO. Total hardness and chlorides were also not within the desirable level, other parameter as conductivity, ammonia, nitrate, manganese, iron and phosphate were found to be within WHO standards.

DISVI (1990) studied seven rural areas in eastern districts, found that chemical parameters of the most water samples within WHO standards for drinking water quality. Study found that only 3% of the samples collected from project tube wells and home of the samples collected from other sources were above WHO guideline.

3.16 Studies on bacterial resistance to antibiotics

El – Zanfaly *et al* (1987) conducted a study on the incidence of antibiotic resistant bacteria in drinking water in Egypt. Most isolated strains appeared to be Ampicillin resistant (89.7%) Out of total isolates 62.4-98% exhibited resistance to 2 or more antibiotics and a total of 363 multiple antibiotic resistant strains were identified.

Honsy *et al* (1988) examined 101 isolates from underground water in Cairo for their resistance towards four antibiotics. They found 32 and 18 isolates resistant to Tetracycline and Chloramphenicol respectively.

Ramtete *et al* (1991) studied coliform isolates from different drinking water resources for antibiotic resistance. They found that resistance to Ampicillin was found more prevalent among the coliform isolates.

Pandey and Musaret (1993) studied antibiotic resistance of coliforms bacteria isolated from drinking water in urban area of India. Multiple drug resistant *E. coli* was isolated, and the frequency of drug resistance was determined, to be high to Ampicillin and Tetracycline.

Pathak and Gopal (1994) studied antibiotic resistance among coliforms isolated from about 49.4% of drinking water samples in India. Out of total coliform 30.4% exhibited resistance for varying number of antibiotics. Resistance for Ampicillin (56.8%) was found maximum. Half of isolates exhibited multiple antibiotic resistances.

In a study conducted by Bissonette *et al* (1995) examined 265 isolates from rural groundwater. All of the non-coliform and 87% of coliforms were resistance to at least one antibiotic.

CHAPTER – IV

4. MATERIALS AND METHODOLOGY

4.1 Materials

4.1.1 Equipments

Autoclave

Incubator

Hot Air oven

Regulator Hot Plate

Microscope

Refrigerator

p^H meter

Conductivity meter

Colorimeter

Comparator

Culture media

MacConkey broth

Nutrient Agar

Eosin Methylene Blue (EMB) Agar

Mueller Hinton Agar (MHA)

S-S-Agar

Selenite-F broth

Diagonistic Media (Bio-chemical Media)

O/F (oxidative/Fermentative Media)

Sulphide Indole motility agar (SIM Media)

Simmons citrate agar

Urea agar

MR-VP media

Triple Sugar Iron agar (TSIA) media

Chemical reagent

Crystal violet

Safranin

Gram's iodine

H₂O₂

Ethanol

Tetramethyl-Paraphenylene

Diamine

Dihydrochloride

Paraffin

Saline

Apparatus

Test tubes, beaker, conical flask, Petri dishes,

Inoculating loop, forceps, dropper, pipettes

Slides, glass rod.

Antibiotics used and their concentration

Ampicillin (A) 10 mcg

Chloramphenicol (C) 30 mcg

Tetracycline (T), 30 mg

Cephalexine (CP) 30 mcg/disc

Ofloxacin (Of) 5 mcg.

4.2 Methodology

4.2.1 Study area

Laboratory analysis was conducted in Bansbari treatment plant lab. The samples were collected randomly from the area of Maharajgunj, Basundhara, Balaju, Chetrapati, Lazimpat and that of the water treatment plant of Bansbari.

4.2.2 Sample collection

Grab water samples for microbial examination were collected in the pre-sterilized glass bottle (121°C for 15 min at 15 lbs. Pressure) and for physico-chemical examination water samples were collected in clean plastic bottle, washed and rinsed 3-4 times with water to be tested.

While taking samples from tap water, it was sterilized using alcohol and ignited and let the water run for 2 min and then bottle was filled from gentle flow of water. While taking samples of raw water, the bottle was placed against natural the flow of water and then filled and cap tightened.

While sampling from the inlet and outlet tank of the plant, the bottle was lowered in the tank at a depth of 15 to 30cm in water and tilt and filled with water to let it fill completely.

4.2.3 Sample transportation and preservation

After collection, samples were transported to laboratory by keeping within an ice box and transported as soon as possible.

4.3 Microbial examination of water sample

Following microbial examinations were carried out according to standard methods given by APHA(1995).

4.3.1 Most probable number (MPN) method

It includes three phases which are as follows:

I. Presumptive test

1. The water sample was collected from the source as described previously.
2. Three single strength lactose broth tubes were labelled as "0.1", another 3 tubes "1" and 3 double strength broths tube "10".
3. Each "10" tube was aseptically inoculated with 10ml of water sample using a 10ml sterile pipette.
4. Each "1" tube was aseptically inoculated with 1 ml of water sample using 1ml sterile pipette.
5. Each "0.1" tube was inoculated aseptically with 0.1ml of water sample using pipette.
6. All the nine-inoculated tubes were incubated at 37°C for 24-48 hrs.

II. Confirmed test

All primary tubes showing any amounts of gas or acid within 24-48 hrs. of incubation were submitted to the confirmed phase.

1. Primary tubes (positive) were gently shaken to resuspend the organism.
2. The EMB agar plate was inoculated with the positive culture with a sterile inoculating loop.
3. The plate was incubated for 24-48 hrs. at 37°C in the inverted position.

III. Completed test

To establish definitely the presence of coliform bacteria and to provide quality control data, the completed test was used on all positive confirmed cases.

1. The lactose-fermentation broth tube was inoculated with the isolated colony from agar plate using inoculated loop.
2. The nutrient agar slant was streaked with the colony from agar plate with inoculated loop.
3. The organism on slant was tested for gram stain and biochemical tests.

4.3.1.1 Faecal coliform test

In order to determine the fecal coliforms, samples were aseptically inoculated in lactose broth tubes and incubated for 24 hours at 44.5°C.

4.3.2. Standard plate count

Procedure

1. The water sample was thoroughly mixed and then appropriate serial dilution was made.
2. One ml from selected dilution was aseptically pipette into a sterile petri plate.
3. Sterile PCA (Plate Count Agar) was poured into the plate and thoroughly mixed.
4. The plates were allowed to solidify and than incubated at 37°C for 24-48 hrs.
5. The selected colonies were cultured on Nutrient Agar for purification.
6. From NA, Gram stain and biochemical tests were performed.

4.3.3 Detection of *Salmonella* spp. and *Shigella* spp.

This group of bacteria is supposed to be very low in water samples. Thus selenite F broth was used as an enrichment medium for the isolation of *Salmonella* spp. and *Shigella* spp. The selenite in this enrichment medium inhibits coliforms while permitting *Salmonella* spp. and *Shigella* spp. to grow.

Enrichment

1. 5 ml of water sample was inoculated into 45ml of selenite F broth and mixed thoroughly.
2. It was then incubated at 37 C for overnight.
3. A loopful of the upper part of broth was cultured on a selective media i.e. Salmonella- Shigella (SS) agar.
4. The plate was incubated at 37°C for 24 hours.

Isolation and identification

1. The pale colony with black center was further streaked onto NA for purification.
2. Isolated colonies were streaked onto NA slants and preserved at 4°C.
3. Bacteria isolated on respective media were identified on basis of their colony characters, morphological characters and biochemical properties.

4.3.4 Study of biochemical tests

Biochemical tests are based on the ability of microorganisms to produce enzymes thus utilizing different substrates. The isolated pure colonies were inoculated into different biochemical media for different tests are as follows:

Table4.1 Biochemical test performed for identification of Enterobacteriaceae isolates

S.N.	Tests	Biochemical Media
1.	Catalase	3% H ₂ O ₂
2.	Oxidaze	10% Tetramethyl - p- phenyl diamine dihydrochloride.
3.	Indole Production	Sulfide- Indole- Motility medium (SIM)
4.	Methyl Red test	Glucose phosphate peptone water or MR-VP medium.
5.	Voges- Proskaur test	Glucose phosphate peptone water or MR-VP medium.
6.	Citrate utilization test	Simmon's citrate agar.
7.	Fermentation of dextrose, lactose and sucrose, H ₂ S and gas production	Triple sugar Iron Agar (TSIA)
8.	Aerobic or anaerobic utilization of carbohydrate	Biochemical Media Hugh and Leifson Medium.
9.	Urease Production	Urea base agar.

4.4 Antibiotic susceptibility test

Antibiotic susceptibility of isolated enteric bacteria was assayed using a modified Kirby Bauer disk diffusion method (Bauer et al, 1966)

1. Bacterial culture was grown at 37°C in 5 ml of nutrient broth for about 4 hrs using pure cultures as inoculum.
2. The turbidity developed was compared with that of standard Barium sulphate.

3. A sterile cotton swab was taken and dipped into culture suspension of bacteria.
4. The entire agar surface of each plate was inoculated, first in a horizontal direction and then in vertical direction to ascertain the even distribution of the organism over the agar surface using a swab.
5. The agar surface was allowed to dry for 5 minutes.
6. A sterile antibiotic disc was picked up by the outer edge using a flamed, sterile forceps and placed the disc near the edge of agar surface of inoculated plate.
7. It was pressed gently with the sterile forceps to ascertain firm contact with the agar surface.
8. A second disc was placed at the opposite side of the former one.
9. The plate was allowed to stand at room temperature for 30 minutes for pre-diffusion and then incubated at 37°C for 16 to 18 hours.
10. The diameter of the zone of inhibition was measured at the completion of the incubation period.
11. Organisms were classified as sensitive or resistant to an antibiotic according to the diameter of the inhibition zone surrounding each antibiotic disc.

Preparation of barium sulphate standard:

- 99.5 ml of H₂SO₄ (0.36 N) was taken in a clean dry test tube.
- 0.5 ml of BaCl₂·2H₂O solution (1.175%) was added to it and tube was shaken to mix the contents well.

4.5 Study of physico-chemical parameters of water sample

Analytical methods manual of Nepal Water Supply Corporation reproduced by UNDP-NEP/91/027, 1994 was followed for the study of physico-chemical parameters of water.

a. Appearance

The unaided eye recorded the general physical appearance of water sample.

b. Temperature

Temperature was determined with the help of a standard mercury thermometer graduated up to 50°C.

c. p^H

Hydrogen ion concentration in the sample was measured with the help of the p^H meter by inserting the electrode into the water sample.

d. Conductivity

Conductivity was measured with the help of a conductivity meter. The electrode was first rinsed with the distilled water and then immersed into water sample and gently stirred and reading was noted.

e. Turbidity

It was measured with the help of turbid meter. The sample was kept in the clean, free nephelometer sample tube taking a reference with standard suspension and reading was noted on the scale.

f. Total alkalinity

- 100 ml sample was taken in conical flask and 2 drops of phenolphthalein indicator was added.
- If a pink color is produced, it was titrated with HCl until pink color was discharged. The volume of titrant was T, ml.
- 4 drops of methyl orange indicator was added.

- It was titrated with HCl solution until a pure gray color is obtained.

Let the volume of titrant used be T_2 .

$$\text{Total Alkalinity, mg/l} = \frac{A \times N \times 1000 \times 50}{\text{ml of sample}}$$

Where, A = Volume of HCl used with Phenolphthalein and methyl red indicator.

N = Normality of standard acid.

g. Total hardness

- 50 ml sample was taken in a clear, conical flask.
- 0.1-0.2 of indicator powder (solochrome black T) was added and mixed.
- 2 ml of ammonia buffer solution was added and mixed.
- It was titrated with EDTA solution until last reddish tinge disappears.
- Volume of EDTA consumed was noted.

$$\text{Total Hardness} = \frac{\text{ml EDTA used} \times 1000}{\text{ml of sample}}$$

h. Calcium hardness

- 50 ml sample was taken in a clean conical flask.
- 0.1-0.2 gm of murexide indicator was added and mixed.
- 2.0 ml of sodium hydroxide was added and mixed.
- It was titrated with EDTA solution until the pink color of the solutions changes to purple.
- The volume of titrant consumed was noted.

$$\text{Calcium hardness mg/l as CaCO}_3 = \frac{A \times 1000}{\text{ml of sample}}$$

Where, A = Volume of EDTA consumed.

i. Magnesium hardness

Magnesium hardness = Total hardness – Ca hardness mg/l

j. Calcium

$$\text{Calcium, mg/l} = \frac{A \times 400.8}{\text{ml sample}}$$

Where A = Volume of EDTA used.

k. Magnesium

Magnesium mg/l = [Total hardness – Ca hardness] × 0.243

l. Chloride

- 50 ml sample was taken in a conical flask.
- 3-4 drops of potassium chromate solution (K_2CrO_4) until there is the slight reddish coloration.
- The volume of $AgNO_3$ consumed was noted and chloride in water sample was estimated by following equation

$$\text{Chloride mg/l} = \frac{A \times N \times 1000 \times 35.5}{\text{ml of sample}}$$

m. Iron

- 50 ml sample was taken in a clean conical flask.
- 2 ml HCl solution was added and contents in flask was boiled.
- Then 2.0 ml thioglycollic acid was added and mixed.
- 3.0 ml of ammonia solution was added and mixed.
- The absorbance of the sample solution was measured on a spectrophotometer at 535 nm and concentration was found out with the help of standard curve.

n. Ammonia

Ammonia can be detected by Nessler's reagent method, the concentration of which can be measurement using calibrated ammonia disc.

- 50 ml of water sample was taken in a nesslerizer tube and 2 ml Nessler's reagent was added into it.
- The tube was inserted in the comparator and calibrated with the color of the ammonia disc.

Calculation: -

1 disc reading = 0.02 mg of ammonia (Ref: - NWSC laboratory, Bansbari)

CHAPTER-V

5. RESULTS

This study was carried out from March 2005 to January 2006. During the study period physico-chemical parameters and bacteriological assessment along with isolation and identification of the isolates, in addition, antibiotic susceptibility pattern of the isolates was determined. The effectiveness of the disinfections with chlorination against the coliform bacteria was also analyzed.

5.1 Bacteriological quality of the water

In order to find out the bacteriological quality of the water, a study was conducted with the hundred water samples collected from different sources as raw water, treated water in the treatment plant, well water, tap water from different locality and stone spouts of Kathmandu Valley.

Out of 100 samples 72% showed the presence of total coliforms, which exceed the WHO guideline for drinking water purpose. Moreover the percentage of fecal coliform present in the water was found out to be 62%. This data was beyond the standard limit of fecal coliform as certified by WHO guideline value. Table 5.8 shows the total coliform count of different water sources and table 5.7 shows the coliform count exceeding WHO guideline value. Table 5.9 shows the fecal coliform percentage.

Furthermore, the different types of bacteria included in family Enterobacteriaceae, the percentage of which are as follows: the majority of *Escherichia coli* (44%),

Enterobacter spp. (26%), *Klebsiella* spp. (22%), *Salmonella* spp. (5%) and *Shigella* spp. (3%).

The percentage of total coliforms isolated from different sources are as follows: Raw water 100%, Treated 0%, Well water 80%, Public tap and stone spout 90% and 85% respectively.

Table 5.7 Total coliform count exceeding WHO guideline

Total number of samples	No. of sample exceeding WHO guideline value	Percentage
100	72	72%

The table shows total number of samples exceeding WHO guideline value for total coliform are 72.

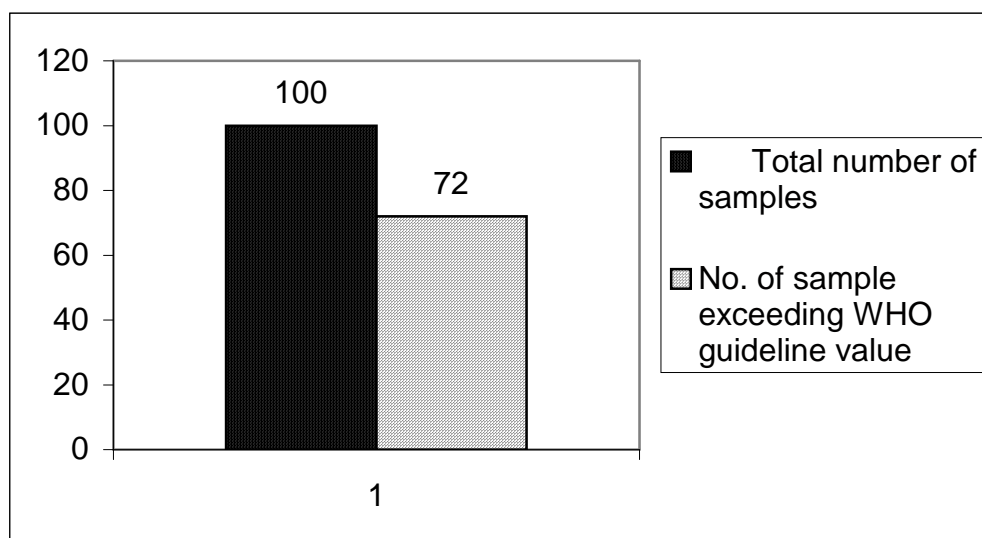


Figure1: Total coliform count exceeding WHO guideline

Table 5.8 Total coliform count from various sources exceeding WHO guideline

Sampling site	No. Sample	No. of sample exceeding WHO guideline	Percentage
raw water	20	20	100%
Treated water	20	0	0 %
Well-water	20	16	80%
Public tap water	30	26	85%
Stone spout	10	10	100%

The treated water supply is excellent while other sources exceed WHO guideline.

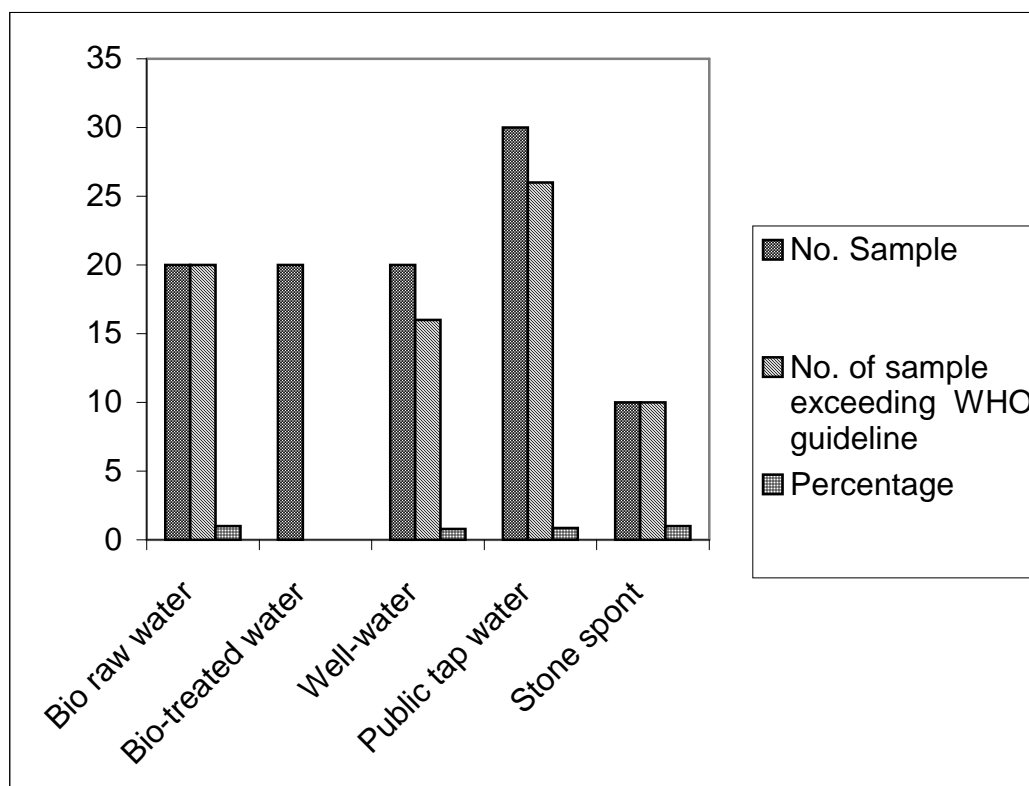


Figure 2: Total coliform count from various sources

Table 5.9 Faecal coliform count exceeding WHO guideline

Total Sample	No. of sample in which faecal coliforms are present	Percentage
100	62	62%

The table shows total number of samples exceeding WHO guideline for faecal coliform are 62.

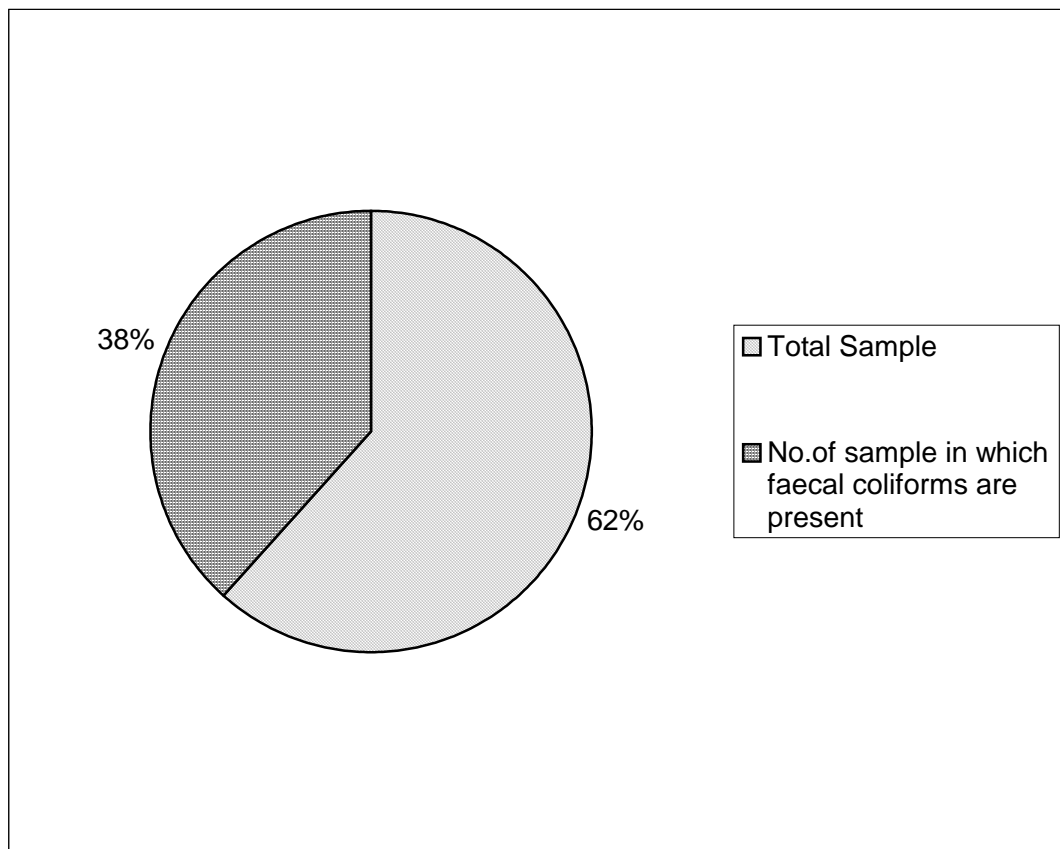


Figure 3: Faecal coliform count exceeding WHO guideline

Table 5.10 Types and percentage of bacteria of **Enterobacteriaceae** family

Types of organisms Isolated	Number of organisms	Percentage
<i>Escherichia coli</i>	60	44%
<i>Enterobacter</i> spp.	35	26%
<i>Klebsiella</i> spp.	30	22%
<i>Salmonella</i> spp.	7	5%
<i>Shigella</i> spp.	4	3%

E.coli were found in highest range while *Shigella* spp. count the lowest.

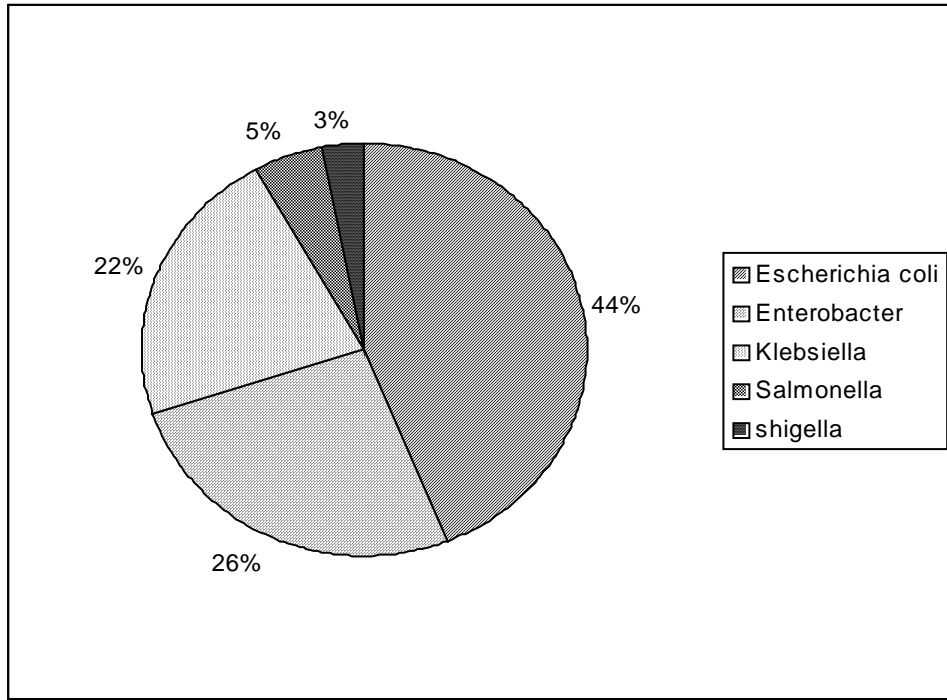


Figure 4:Types and percentage of bacteria of Enterobacteriaceae family.

5.2 Physico- chemical parameters of water

This study was conducted to analyze the different physico-chemical properties of the water samples of treatment plant, public tap water, well water and stone spout sample. A total of 100 samples were studied, out of which the values of color, temperature, conductivity, alkalinity, hardness and chloride were found to be within the WHO permissible level. While the parameters as pH, iron, ammonia were found to exceed WHO standard value by 3%, 20% and 25% respectively. The detail information of all physicochemical parameters is presented in appendix vi.

Table 5.11 Chemical parameters within and beyond WHO guideline value

S.N.	Parameters	Total No.of Samples	No.of sample within WHO guideline	No.of sample exceeding WHO guideline	% Exceeding WHO guideline
1.	Appearance		86 clear	10 unclear	14%
2.	Temperature		100	-	-
3.	pH	100	90	10	10%
4.	Conductivity		100	-	-
5.	Total alkalinity		100		
6.	Total hardness		95	5	5%
7.	Total Iron		80	20	20%
8.	Total ammonia		75	25	25%
9.	Chloride		100	-	-

5.3 Antibiotic susceptibility pattern of the isolated organism

Results regarding to antibiotic susceptibility pattern are presented in table 5.12 and 5.13. The antibiotics used were Tetracycline, Chloramphenicol, Ampicillin, Cephalexin, and Ofloxacin. The antibiotic susceptibility pattern is provided in the table as shown:

Table No.5.12 Susceptibility patterns of the isolates to different antibiotics

S.N.	Antibiotics	Sensitive %	Intermediate %	Resistant
1.	Tetracycline	90	10	-
2.	Chloramphenicol	100	-	-
3.	Ampicillin	-	-	100
4.	Ofloxacin	80	20	-
5.	Cephalexin	-	10	90

1.Susceptibility 2.Intermediate 3.Resistant

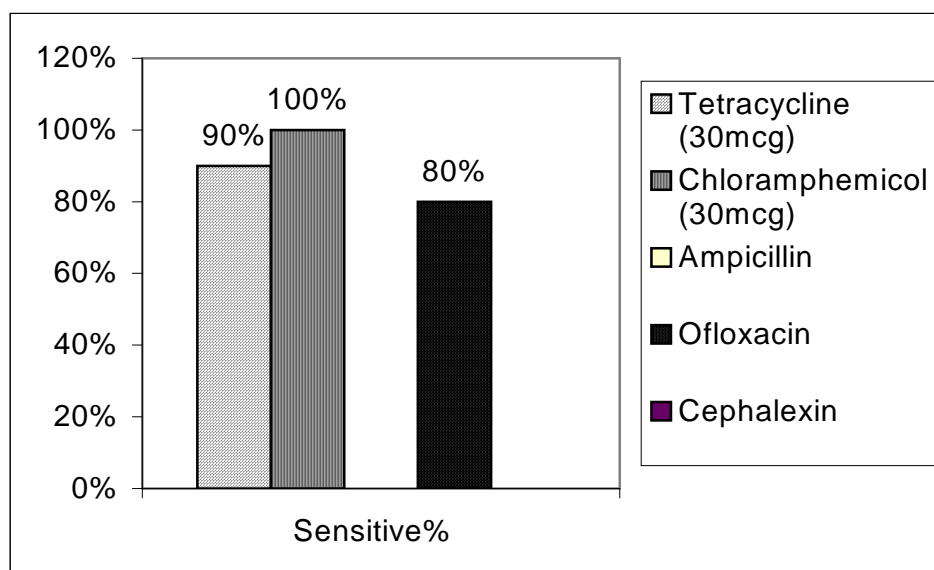


Fig.7 Bar diagram of antibiotics susceptibility pattern

Table 5.13 Frequency of antibiotic and multiple-antibiotic resistance among Enterobacteriaceae isolates.

Organisms	AR	MAR
<i>Escherichia coli</i>	70%	20%
<i>Enterobacter aerogenes</i>	20%	12%
<i>Citrobacter spp.</i>	5%	5%
<i>Klebsiella spp.</i>	80%	20%
<i>Salmonella spp.</i>	100%	25%

The high range of AR and MAR is shown by *Salmonella*..

AR → Resistant to at least one antibiotic

MAR → Resistant to at least two antibiotics.

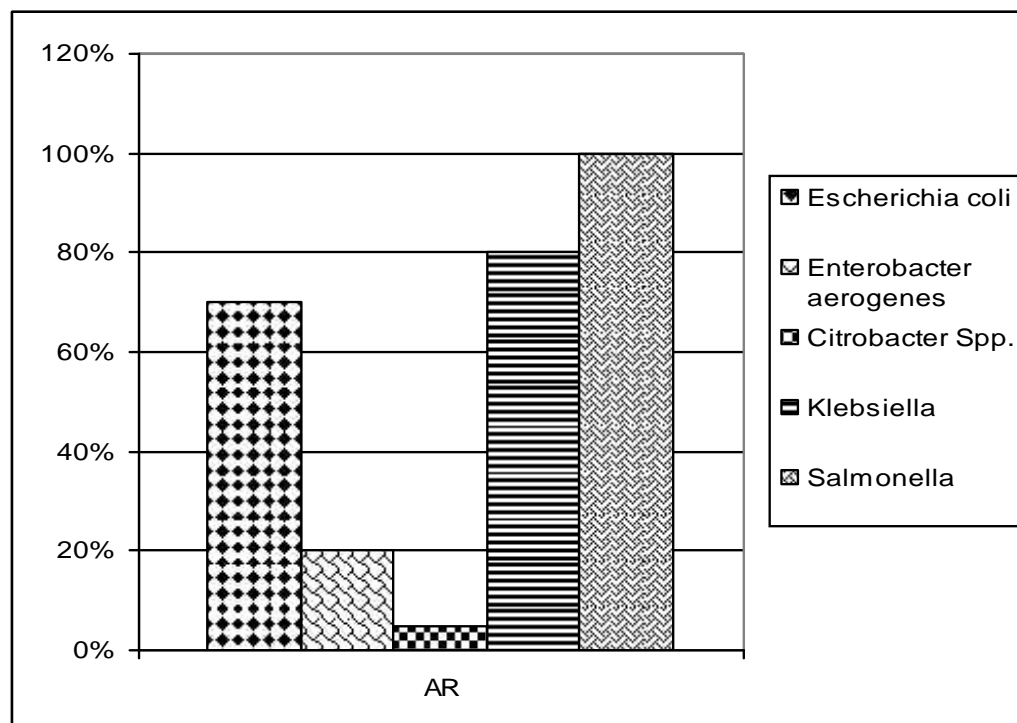


Fig10 Frequency of AR among isolates

CHAPTER-VI

6. DISCUSSION AND CONCLUSION

6.1 Discussion

This study was performed to examine the present condition of physical, chemical and bacteriological quality of drinking water from various sources in Kathmandu city. In addition to this, antibiotic susceptibility pattern of the isolates was also determined. Till the accomplishment of the study, a total of 100 water samples were analyzed bacteriologically and physico-chemically.

This lab analysis was accomplished in the laboratory of NWSC at Bansbari. The water treatment plant located at Bansbari supply the water to the areas as Maharajgunj, Lazimpat, Basundhara, Narayanthan and so on. NWSC has laid its every effort to fulfill the increasing demand of piped water to the people. It is distributing about 10 crore litres of water per day to the people(NWSC,2005); however, this amount isn't adequate to fulfill the thirst for water. Therefore, people search other ways as they use the water of stone spout, well, boring etc. Hence, the quality of other sources was also checked to bring into light hygienic condition of the water.

NWSC is also active in the testing of quality of water, but this study in more detailed form as it reveals the percentage of presence of indicator organism and the disease causing agents along with their precise identification and antibiotic susceptibility tests.

Every people in the world is well aware of the importance and significance of the water as it is indispensable for the survival of the cell as protoplasm of the cell comprises of about 80% of the mass; as well as its virtue is enlightened due to its necessity for drinking, washing, bathing, irrigation and as a universal solvent. The quality of water has its direct relevance to the public health as foul water due to anthropogenic activities as improper sewage disposal, solid waste disposal, contamination with industrial and agricultural effluents makes the water susceptible for many water borne diseases as typhoid, dysentery, diarrhoea and gastrointestinal disorders. Therefore, in order to lead a healthy life, provision of safe water is a must. WHO and UNICEF has launched several plans projects in order to provide pure water and improve sanitary condition so as to make people healthy and able to perform their duty efficiently thereby achieving the development of a nation. Hence, these organizations have made their goal as “water for all” and by doing so they are attempting to achieve its goal, in the near future.

6.1.1 Bacteriological quality

A total of 100 samples were studied during the study work. The efficiency of treatment system of water treatment plant was also analyzed. About 20 samples of raw water that is received from surface and ground water, which were found to be contaminated. All the raw water sample in the treatment system showed total coliform count beyond the WHO permissible level by 100%. Subsequently, the treatment water that was obtained after sedimentation, coagulation filtration and disinfection was absolutely pure that is free from fecal contaminations these were no coliforms neither fecal coliforms. This shows that treatment plant is being operated in an efficient manner. Filtration is followed by chlorination after which it is distributed to the public along the pipelines. About 30 samples from public supply were also analyzed, in which; the result is obtained as out of 30, 26 of the

samples showed total coliform present. The presence of coliform in about 85% sample imply that during the distribution course, the residual chlorine slowly depletes through the pipeline and the water gets contaminated due to leakage, rusty pipelines or sewage contamination or bad sanitary and hygienic practices.. Therefore, dosage of chlorination has to be made adequate and sanitary condition, as well as leakage, disrupts in the pipeline should be discouraged and improvisation in this aspect should be encouraged.

It is essential to make the people aware of the quality system and improvement of healthy and good sanitary practices have to be done. Likewise, people can use bleaching powder in appropriate amount, proper boiling and filtration is also an essential condition to drink safe water.

The well water constructed by NWSC also provide water to the people. Out of 20 samples from those wells Maharajgunj area about 16 sample showed coliform present. Lack of protection activity of well and improper sewage, solid waste disposal may also causes poor condition of water. 20% of the water samples were found in good quality within WHO permissible level.

The water of stone spouts was also analyzed which showed coliform and fecal coliform contamination. This condition is also due to lack of good hygienic and sanitary condition in and around the said area. As a whole out of 100 samples 72% and 62% of the samples showed total coliform and fecal coliform present.

It is worth mentioning that almost all of the studies carried out to date suggest water quality is in brink of good and hygienic condition and it is getting bad in course of time. All studies (Sharma, 1978, Sharma 1986, CEDA 1989, ENPHO 1992, DISVI 1995) indicate that the water quality is not satisfactory and needs improvement.

Sharma (1986) found that level of coliform contamination had increased upto 85%. In next study by Sharma in 1993 found 70 to 100% samples contaminated with coliforms in urban areas. Similarly, Adhikari et al, (1986) found 88% of samples unsatisfactory. CEDA in 1989 recorded almost all water samples contaminated. Results of this study and previous findings are quite in line to each other, however, in the course of 4 to 5 years, contamination of water resources has been found to be increased. Results of this data is also supported by findings of Thapa (1997). Similar study was performed by Maharjan (1998) in which 69% of the sample was found to exceed WHO permissible level of coliform. In a study conducted by DISVI (1989) it was found that bacteriological contamination increased as the water travelled from treatment plants to the distribution system. The study found that coliform bacteria count exceeded WHO standards.

In this study, bacteria included as indicator bacteria of Enterobacteriaceae family were isolated and identified by biochemical tests. The identified organisms include *E.coli*, *Enterobacter* spp., *Klebsiella* spp., *Citrobacter* spp., *Salmonella* spp. and *Shigella* spp. Recovery of *E.coli* was highest among isolates which constitutes about 44% of the isolates. *Enterobacter* spp. follows it, which comprise 26% of the isolates. Similarly, *Klebsiella* spp. were recovered from the water as 22%. This showed the presence and percentage of indicator organisms. These are the organisms commonly found in water and their detection method is also conventional. Their presence also facilitates the path for the pathogenic organisms to gain entry and prove ominous to the health of the people. *Salmonella* spp., the pathogenic ones were also isolated, which are the causative agents for typhoid, the lethal disease that cause mortality and morbidity in a large fraction of the people of Nepal and other under developed South Asian countries. These are found in the range of 5% in the water sample. Similarly, *Shigella* spp., the causative agent of

bacillary dysentery is also found in the 3% range. These are found in low numbers, however, are the most lethal ones.

All of the above studies clearly indicate that more than 60% of water resources are contaminated with coliform and fecal coliforms. The results of this study showed that about 72% of water sample are unacceptable for use. The pathogenic bacteria of medical significance were also detected from sources of water. Though this present condition show unsatisfactory coliforms level, people are bound to use the said sources. Therefore, serious attention and steps should be undertaken to provide high quality water.

6.1.2 Physico-chemical quality of water

The table in appendix vi shows the data regarding the Physico-chemical properties of the water, which were analyzed in the laboratory.

Temperature has its effect on the chemistry and biological reactions in the organisms in water. A rise in the temperature of the water leads to the speeding up of the chemical reactions in water, reduces the stability of gases and amplifies taste and odour. In the tests conducted in the lab the temperature of the water was found in the range of 19°C to 30°C. This shows the seasonal increment in the temperature in summer season.

pH is the measure of the intensity of acidity or alkalinity and measures the concentration of hydrogen ions in water. p^H of the water gets changed with time due to exposure to air, biological activity and change in temperature. In the lab analysis 90% of the samples lie in the p^H range recommended by WHO guideline, however the 10% of samples show deviation from normal range.

Turbidity of water is actually the expression of optical property and is caused by clay, silt, organic matter, phytoplankton etc. Majority of the samples show turbidity <5 (about 86%) while rest show values greater than 5 and make unsuitable for use.

Conductivity is a numerical expression of the ability of aqueous solutions to carry electric current. The entire sample exhibits conductivity level in the normal range.

Alkalinity of the water is its capacity to neutralize a strong acid. In the natural water there are many salts of weak acids as silicate, borate which cause alkalinity. Hardness is the property of water, which prevents the lather formation with soap and increases boiling point of water. Principal cations imparting hardness are Calcium and Magnesium. The anions responsible for hardness are bicarbonate and carbonate, sulphate, chloride, nitrates. 95% of the sample in the analysis showed hardness within the normal level while the rest showed greater value than normal. The major source of Calcium and Magnesium in the natural waters are sewage and wastes and due to the several salts in the deep layers of earth or over deposits of limestone, dolomite, and gypsum.

Iron is one of the most abundant elements of the rocks and soil. Iron has more solubility at acidic p^H , therefore large quantities of iron are leached out from the soil by acidic water. Iron has got a microbial significance since a few microorganisms are able to utilize dissolved iron. This gives a rusty appearance to the water. Iron in excess causes staining of clothes and utensils. The higher concentration of iron is also not suitable for food processing, beverage, dyeing, bleaching etc. In this study, 80% of the sample showed iron within WHO guideline while 20% exceeded the normal range.

Sewage has large quantities of nitrogenous matter, thus its disposal tends to increase the ammonia content of water. Occurrence of ammonia in the water can represent chemical evidence of organic pollution. It is toxic to men at higher concentration.

The toxicity of ammonia increases with p^H because at higher p^H most of ammonia remains in gaseous form. 75% of the samples showed NH_3 within normal range however 25% of the samples exceed WHO guideline value. Those with higher values of ammonia are unacceptable for use to human beings.

The most important source of chlorides in the waters is the discharge of domestic sewage. The higher chloride concentration serves as an indicator of pollution by sewage. It produces salty taste at 250-500 mg/l. All the samples exhibit chloride content upto the normal range as set by WHO guideline value.

The previous study showed that most of the Physico-chemical parameters of drinking water lie within WHO guideline value, and some parameter exceed the permissible value. ENPHO/DISVI (1991) found most of the parameters within the prescribed level except minor differences in rural areas of eastern Nepal. Similarly, Ghimire, (1996), Thapa (1997) found the values within safe range as set by WHO (1984) for most of the parameters. The concentration of iron, ammonia and hardness were found above recommended level in some cases.

6.1.3 Resistant of bacteria to antibiotics

Antibiotics are antimicrobial substances produced by living organisms, which inhibit other microorganisms. Antibiotics are generally described as bacteriostatic as they prevent the active multiplication of bacteria and bactericidal, those which kill the bacteria.

The resistant of bacteria to antibiotic has become a global problem. Most of the microbial resistance, which is now making it difficult to treat some infectious disease is of genetic origin and transferable between species and genera of bacteria. Acquired antibiotic resistance in developing countries is serious due to the following reasons:

- Antibiotics can be obtained outside of recognized treatment centers and taken without medical authorization. This leads to inappropriate use of antimicrobials and their being taken at wrong dosage and for insufficient length of time.
- Laboratory facilities and trained staffs are often not available to isolate pathogens and perform susceptibility tests.
- Guidelines regarding the selection of drugs and information about drug resistance are not communicated to those prescribing antimicrobial especially in rural areas.

Ways of limiting and controlling antimicrobial resistance include

- a) Restricting the general sale of antimicrobial drugs and making them available only on authorization of a medically qualified person.
- b) Antimicrobials need to be prescribed correctly and establish guidelines and regulations for appropriate use.

Studies regarding ground water of Patan by Maharjan and Shrestha (2000) showed that resistance was directed towards Ampicillin, Nitrofurantoin, Nalidixic acid. Chugh and Suheir (1983) studied drug resistance among *Salmonella* in which they found the bacteria resistance to Tetracycline, Kanamycin, Ampicillin. MAR was observed in 71% of the isolate. Similarly Antai (1987) studied antibiotic resistance of *E.coli* strains and found most strains resistant to antibiotics as Ampicillin, Tetracycline etc.

In this study susceptibility patterns of the isolate to different antibiotics as Tetracycline, Chloramphenicol, Ampicillin, Ofloxacin, Cephalexins were studied. The test bacteria against antibiotics are that of indicator bacteria and members of Enterobacteriaceae family. Tetracycline was found sensitive in 90% of the cases and 10% as intermediate; another bacteria were found resistant to 100% of

Ampicillin and 90% Cephalexin resistant while isolates were 80% sensitive to Ofloxacin and 20% as intermediate. It is found that, *E.coli*, *Enterobacter* spp., and *Citrobacter* spp., are 70%, 20% and 5% respectively resistant to at least one antibiotic, while multiple antibiotic resistance was expressed by 20%, 12%, 5% and 20% respectively.

6.2 Conclusion

From this study it is concluded that more than 50% of the water samples showed total coliforms while rest of the samples are satisfactory. The pathogenic bacteria are found in the least percentage. The isolates are sensitive to majority of the antibiotics however, isolates are resistant to Ampicillin and Cephalexin in most of the cases. Therefore it is essential to improve the quality of the water by proper treatment method and following hygienic steps.

CHAPTER-VII

7. SUMMARY AND RECOMMENDATIONS

7.1 SUMMARY

The examination of water quality is of great concern as it is directly linked with the healthy life. All the water borne diseases are caused by fecal contamination of water resources and unhygienic practices. Nevertheless, the incidence of disease can be minimized by safe disposal of waste, hygienic and good sanitary practices, additionally, by protecting water resources.

Water samples supplied by NWSC to localities as Maharajgunj, Balaju and raw source of water, treated water, well water and water from stone spouts were bacteriologically and chemically studied. Moreover, antibiotic susceptibility pattern of isolated bacteria was also performed.

The result of the study showed that about 72% and 62% of the sample exceed WHO guideline for total coliforms and fecal coliform. All the samples from raw water, none from treated water, 80% of the well, 85% tap water were found to contain total coliform. Coliform bacteria like *Escherichia coli*(44%) *Enterobacter* spp. (26%), *Klebsiella* spp. (22%), *Salmonella* spp. (5%) and *Shigella* spp. (3%) were also isolated.

In addition to the bacteriological study, the examination of chemical parameters was also performed which showed that appearance, p^H, iron, ammonia parameters exceed the WHO guideline by 10%, 10%, 20%, 25% respectively, while the rest of the samples were within WHO guideline.

Furthermore, the antibiotic susceptibility pattern of the isolated bacteria was also determined. The result showed that about 70% of *E.coli* were resistant to one antibiotic and 20% of the same bacteria were resistant to more than one antibiotic.

It is imperative for a nation to provide safe water to the public, however due to the several impedances it has become a biggest problem. The daily water supply essential for Kathmandu valley is 22 crore lit, but the supply is only 10 crore liters of water per day. Therefore people are forced to seek other sources as deep boring, dug wells and stone spouts. As well as maintenance of the safety and purity of the supplied water is also the essential task to ascertain the healthy life of the people.

7.2 RECOMMENDATION

On the basis of the findings of this study, following recommendation can be enunciated.

- Regular water quality testing and evaluation should be carried out to check up the degree of pollution and its causes.
- Periodic inspection of distribution system and treatment plant should be carried out.
- Basic education programmes regarding consciousness arousing on hygienic practice and sanitary practice; safe disposal of waste and prevention of waterborne disease should be carried out.
- Government, Non government agencies and local people should pay greater attention to the protection, preservation and maintenance of water resources.
- People should be educated about the simple and important techniques of water treatments as filtration, boiling and chlorination.
- Regular monitoring of antibiotic susceptibility pattern of the isolated organism should be done from public health point of view.

- The inappropriate and indiscriminate use of anti-microbials should be discouraged.
- The results ought to be useful for further planning, activities and policy formulation.

REFERENCES

- Asian Development Bank (1995) Nepal Water Supply and Sanitation Sector Profile.
Asian Development Bank, Manila.
- Adhikari R, Pokharel B M and Khadka JB (1986) Bacterial Study of Drinking Water Quality of Kathmandu Valley, Journal of Institute of Medicine vol8(3): 313-316.
- Allen MJ and Geldreich, EE (1975) Ground Water 13:1.
- Antai SP, (1987) Incidence of Antibiotic Resistant of Bacteria In Rural Water Supplies. J. Appl. Bacteriol. 62(4): 371-376.
- APHA (1989) Standard Method for the Examination of Water and Wastewater. American Public Health Association, Washington DC, 17th edition.
- APHA (1995) Standard Method for the Examination of Water and Wastewater. American Health Association, Washington DC, 18th edition.
- Atlas RM (1989) Microbiology Fundamentals and Application 2nd edition. Maxwell Macmillan, New York, London.
- Aneja KR (1996) Experiments in Microbiology Plant Pathology Tissue culture and Mushroom Cultivation. 2nd edition, Wishwa Prakashan.
- Bailey and Scoots (1990) Diagnostic Microbiology 8th edition, Ellen so Baron, Sydney M. Finegold Baltimore.
- Bauer AW Kirby, WMM Sherris JC and Truck T (1996) Antibiotic susceptibility testing by a single standardized method. American Journal of Clinical Pathology: 493-496.

- Benson HJ (1979) Microbiological Applications: A Laboratory Manual in Microbiological Applications: A Laboratory Manual in General Microbiology. 3rd edition, web Wm.C. Brown company publishers
- Bissonette GK, Mekron DM and calaprese, JP (1995) Antibiotic Resistant Gram-negative Bacteria in Rural Ground Water. Vol. 29(8):1902-1908.
- CBS (2005) Statistical Year Book of Nepal, HMG/NPC Central Bureau of Statistics, 2005: 211
- Cairncross S (1989) Water Supply And Sanitation An Agenda For Research, Tropical Medicine and Hygiene 92: 301-314.
- Chakraborty P (1995) A Text Book of Microbiology. New Central Book Agency (P) Ltd.
- Cheesbrough M (1993) Medical Laboratory Manual For Tropical Countries. Vol II ELBS edition.
- Clark JW, weismann WH and Hanner MJ (1977) Water Supply And Population Control, 3rd edition Harper and Raw Publisher, New york.
- Collee JG, Duguid JP, Fraser AG and Marion BP (1989) Mackie and McCartney: Practical Medical Microbiology. 13th edition, Churchill Living stone.
- DISVI (1989) Water Quality Monitoring of Kathmandu City Water Supply, Final Report. Health Education and service Project, Kathmandu.
- DISVI (1990); Water Quality Assessment in Terai Tubewell Project, Health Education and Service Project, DISVI, Kathmandu.
- Dubey RC and Maheshwari DK (2001) A Text Book of Microbiology, 1st edition, S. chand and company ltd.
- ENPHO/DISVI (1990) Water Quality of The Stone Taps In Kathmandu City : 1989-1990, ENPHO, Kathmandu.

- ENPHO/DISVI (1992) Monitoring of The Kathmandu City Water Supply With Reference to Chlorination and Microbiology Quality. 1991/1992 ENPHO and DISIVI, Kathmandu.
- ENPHO/DISVI (1995) Bacteriological Quality of Drinking Water in Kathmandu And Patan City. ENPHO, Kathmandu.
- Environment: A Journal of The Environment (2001) volume 6, No. 7 Ministry of Environment and Population, Nepal.
- Greenwood D, Slack RCB and Peutherer JF (1992) Medical Microbiology: A Guide To Microbial Infections: Pathogenesis, Immunity, Laboratory Diagnosis and Control 14th edition, ECBS, Churchill Livingston, UK.
- Hosny I, El Zanfaly and Fayez M (1988) Incidence of Antibiotic-Resistant Bacteria In Water-Environment Int. 14(5): 391-394.
- Hugo WB and Russell AD (1981) Pharmaceutical Microbiology 2nd edition, Blackwell Scientific Publication: 69.
- HMG Nepal (1980) International Drinking Water Supply and Sanitation Decade. Community water, Supply and Sanitation Country Report DWSS, Kathmandu.
- IUCN (1990) Environment Pollution In Nepal. A Review of Studies. HMG/NPC in collaboration with IUCN.
- Joshi J (1987) Sanitation Situation Analysis and SWMRMC's Activities in The Field of Sanitation. Journal of Solid waste Management and Resource Mobilization Centre.
- Krieg NR and Holt JG (1984) Bergey's Manual of Systematic Bacteriology Vol. I Williams and Williams and Wilkins, Baltimore, U.S.A.
- Mackie and McCartney (1989) Practical Medical Microbiology. 13th edition, Churchill Livingston, Melbourne, New York.

- Maha sneh IA (1992) Isolation and Characterization of Faecal Indicator Bacteria From Urban and Rural Natural Drinking Water Sources. Biomed, Lett (188)
- Maharjan Makhan (1998) Assessment of Groundwater Quality And Study of Antibiotic Resistance And Oligodynamic Action Against Some Isolated Enteric Bacteria. A thesis submitted to Central Department of Microbiology, T.U., Kirtipur
- Thapa M (1997) Bacteriological Study of Water And Its Treatment Using Plant Products.
- Miller GT (1988) Environment Science: An Introduction 2nd edition, words worth publication, California.
- Ministry of Health/WHO Nepal (1994) Implementation of Strategies For Health For All By Year 2000, Planning Foreign Aid Division, WHO Nepal.
- Narayan Parker D (1988) Low Cost Water And Sanitation: Tasks For All The People, World Health Forum 9: 356-360.
- Khatiwada N and Shrestha B(2000) Setting Drinking Water Standards In Nepal.
- Paniker CK and Ananthanarayan R (2000) Textbook of Microbiology, orient Longman Pvt. Ltd.
- Park K (1994) Textbook of Preventive and Social Medicine, 16th edition. M/S Banarasi das Bharat, India.
- Pandey S and Musarrat J (1993) Antibiotic Resistant Coliform Bacteria In Drinking Water. J. Environ. Biol. 14(4): 267-274.
- Pelczar MJ, Chan ECS and Krieg NR (1993) Microbiology. 5th edition, MC Graw Hill Book Company.
- Powar and Daginawala (2000) General Microbiology. 2nd edition; Himalayan Publishers.
- Pradhan BK and Pradhan PMS (1972) Water Pollution Control Journal of Nepal Engineering Association, 2nd edition:1.

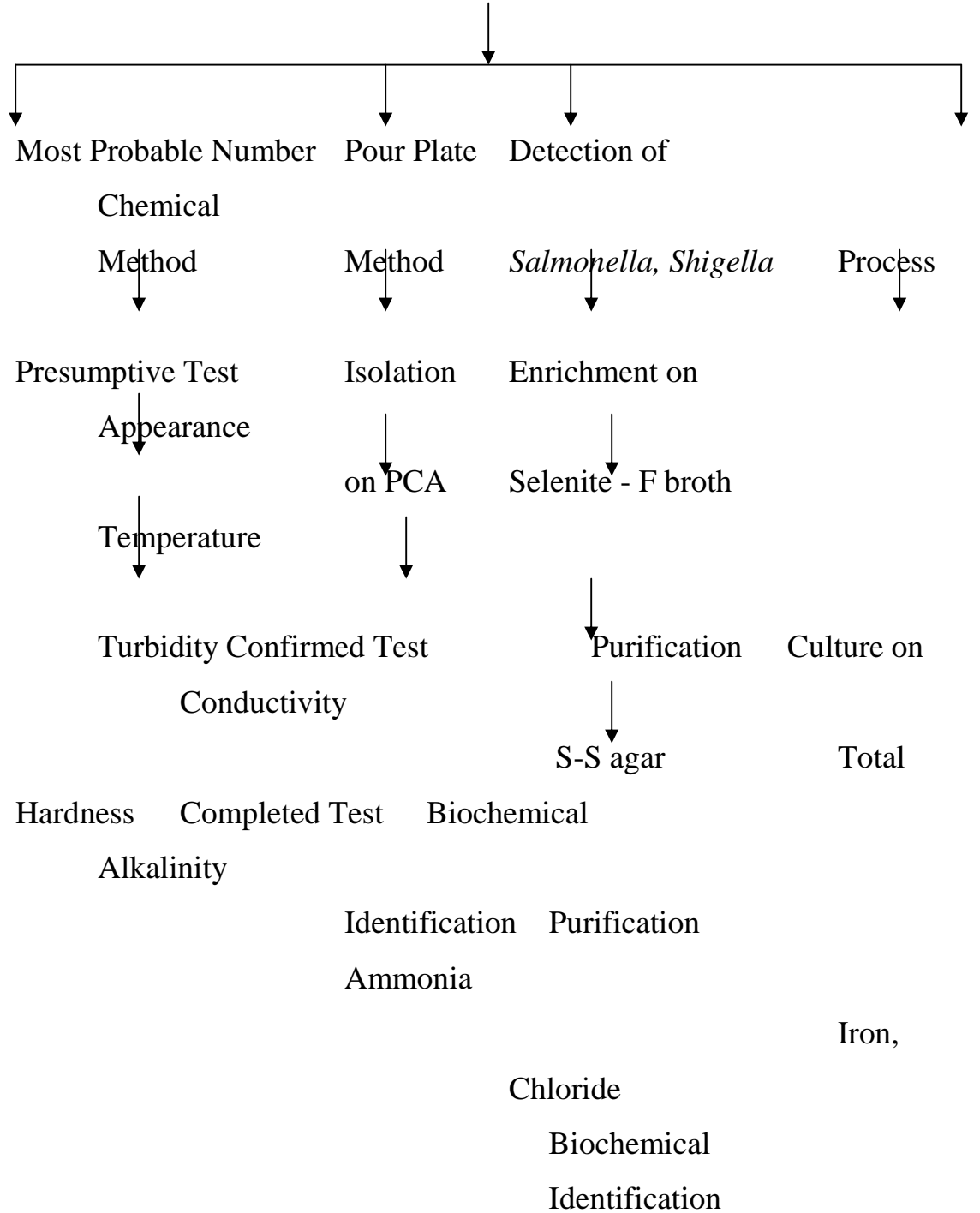
- Chitrakar RL (2002) Proposed Format For Nepal Drinking Water Standards.
- Rao CS(1999); Environmental Pollution Control Engineering. New Age Publishers,New Delhi .
- Sharma AP (1978) The Quality of Drinking Water In Kathmandu. J.Inst.Sc: 15-52.
- Sharma AP(1986) Study of Some Chemicals and Bacteriological Aspects of Drinking Water Quality In Kathmandu. A report submitted to National Committee for Man and Biosphere.
- Sharma AP (1993) Study of Bacteriological Standard of Drinking Water of Nepal, A report Submitted to National Committee for Man and Biosphere.
- Sharma AP (1994) Water Pollution in Nepal. Science Universal Vol4 (4), 1994:16-21.
- Thapa M (1997) Bacteriological Study of Water And Its Treatment Using Plant Products. A thesis submitted to Central Department of Microbiology,T.U.,Kirtipur
- Trivedy RK and Goel PK (1986) Chemical and Biological Methods for Water Pollution Studies, Environmental Publications, India.
- Topley and Wilson (1990) Principles of Bacteriology, virology, and Immunity, 8th edition Vol: 1,2,3,4.
- UNICEF(1992) Community Water Supply and Sanitation , Annual Progress Report.
- World Health Organisation (1982); Global Strategy Of Health For All By Year 2000, WHO, Geneva, Switzerland.
- World Health Organisation (1984) Guidelines for Drinking Water Quality. Vol. I WHO Geneva, 1984.
- World Health Organisation (1993) Guidelines for Drinking Water quality Vol. II WHO, 1993.
- World Health Organisation (1999) World Health Report, Making Difference, WHO, Geneva.

World Health Organisation (2001) Bulletin of world Health Organization.

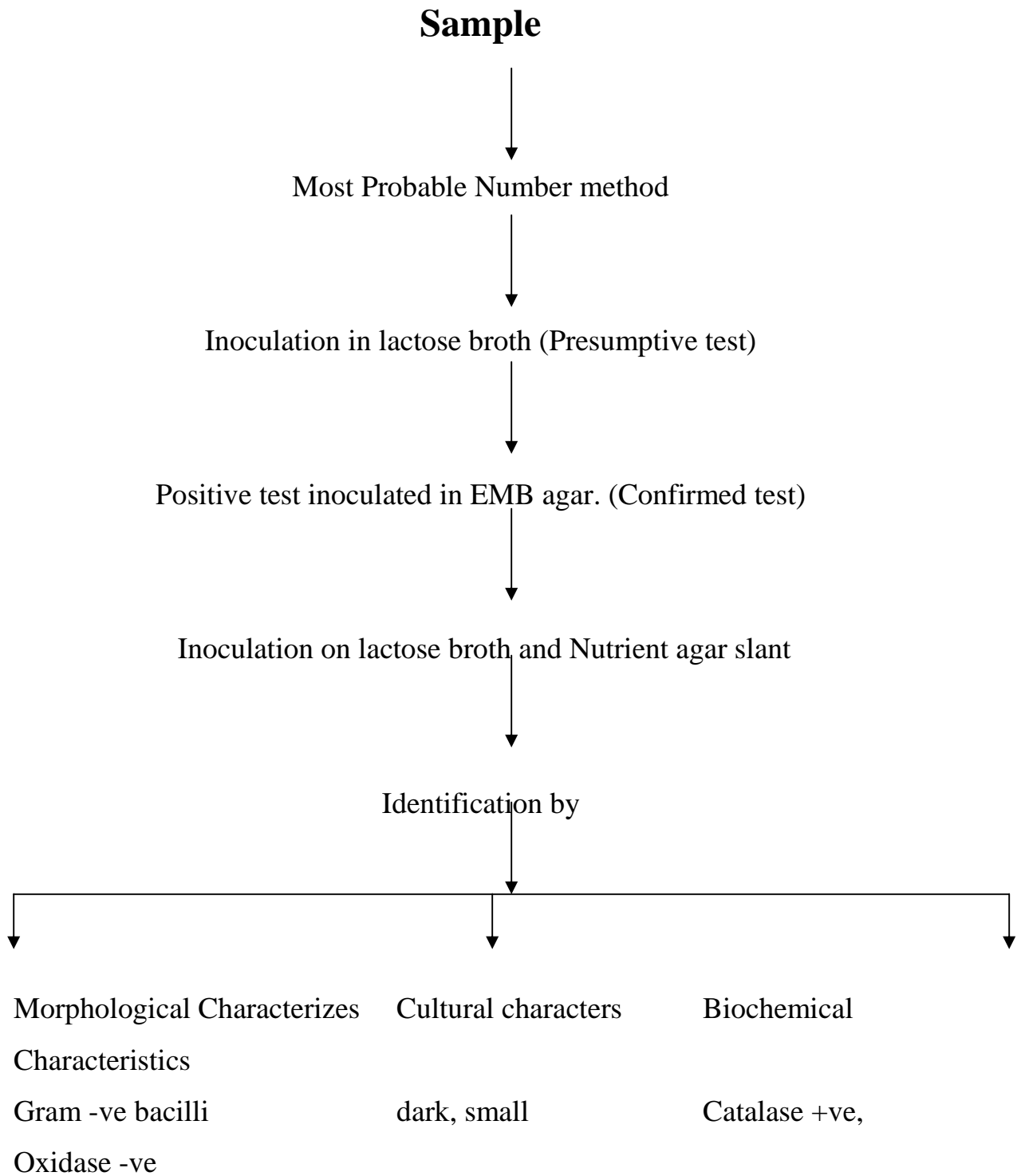
World Health Organisation (2001) Bulletin of World Health Organization.

Flow chart for general sample processing

Sample collection

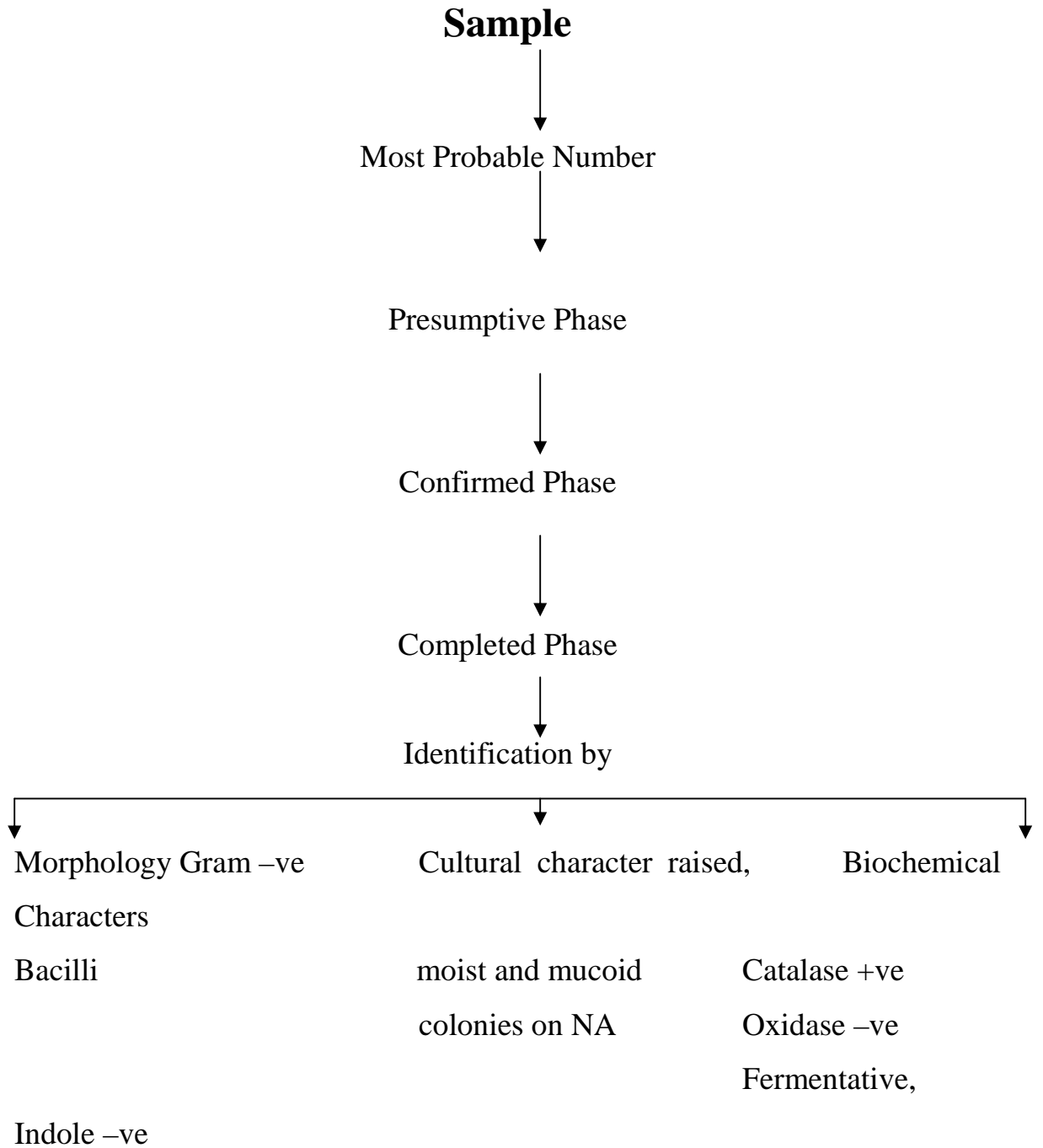


Flow chart for identification of *E. coli*



Indole +ve	colonies with	Fermentative,
+ve,	greenish metallic	Motility +ve, MR
TSIA	sheen	VP-Ve, Citrate-ve,
	Acid/Acid, gas+ve, H ₂ S-ve, Urease – ve	

Flow chart for identification of *Klebsiella* spp.



Voges

Methyl red -ve,

Proskauer +ve,

Citrate V

TSIA = Acid/Acid,

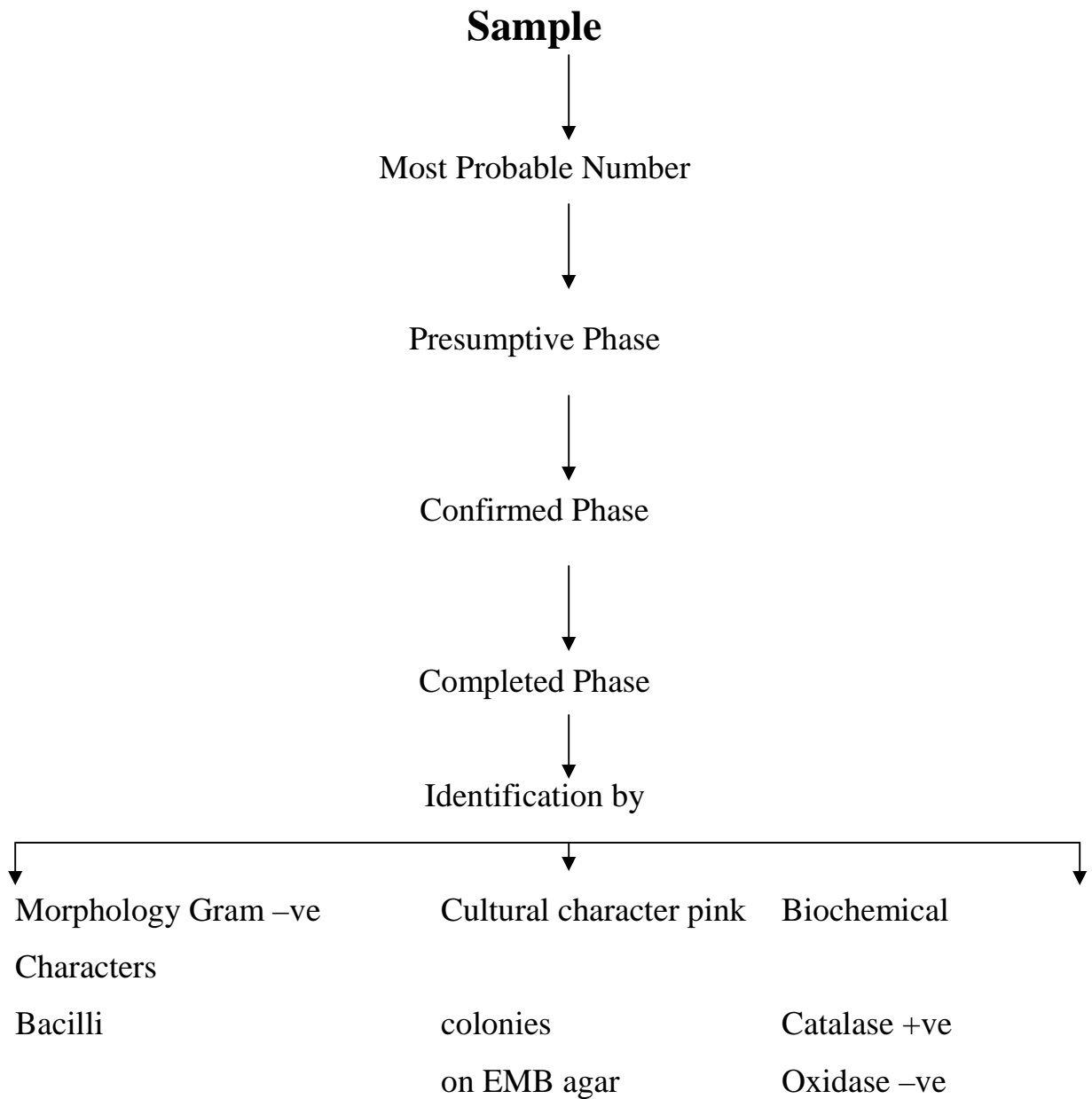
H₂S-ve, gas+ve,

urease V

V = Variable

Motility -ve

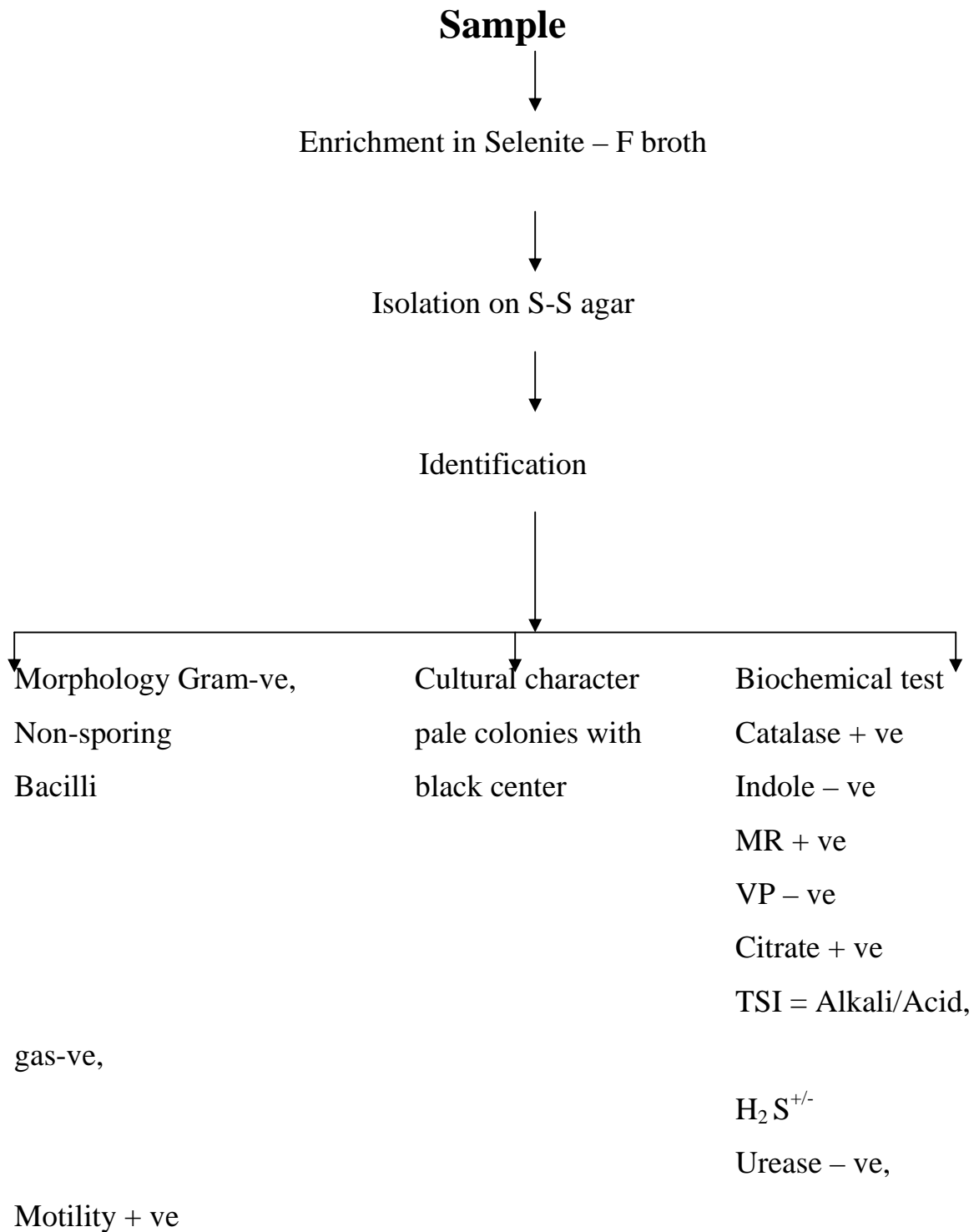
Flow chart for identification of *Enterobacter* spp.



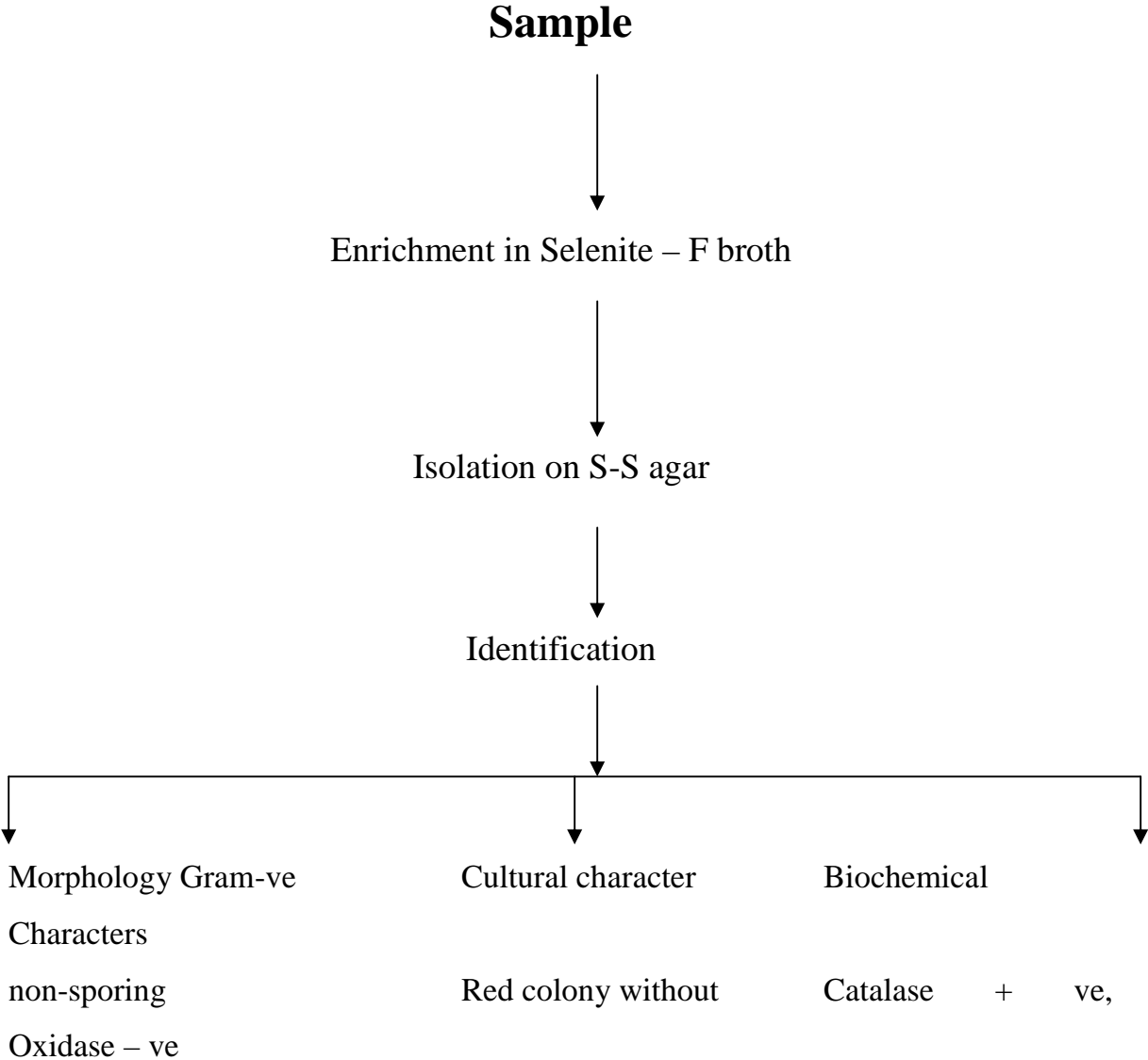
Indole -ve
ve
gas+ve,

Fermentative,
Motility +ve
MR - ve
VP + ve, Citrate -
TSI = Acid/Acid
Urease - ve

Flow chart for identification of *Salmonella* spp.



Flow chart for identification of *Shigella* spp.



Bacilli
-ve/+ve

black center

Fermentative, Indole

MR + ve, VP - ve

Citrate - ve, Motility

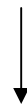
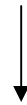
- ve

TSIA = Alkali/Acid,

Gas-ve,

H₂ S-ve

Urease - ve



Flow chart for antibiotic susceptibility test

Bacterial isolates were sub cultured on NA

Pure culture was obtained

Subculture in Nutrient broth

Incubated for 24 hrs at 37° C and
compared with half of Mc. Farland
Nephelometer Standard for turbidity

Swabbed on MHA agar plates

Antibiotic discs were placed aseptically

incubated at 37° C for 24 hrs

Result was noted by measuring zone of inhibition.

Table 5.1 Bacteriological analysis of bio-raw water samples

S.N.	Sample code source	Total coliform count CFU/100ml	WHO standard level
1	BR ₁	39	
2	BR ₂	50	Coliform organisms
3	BR ₃	160	4 CFU/100ml
4	BR ₄	180 ⁺	
5	BR ₅	90	
6	BR ₆	160 ⁺	
7.	BR ₇	50	
8.	BR ₈	180 ⁺	
9.	BR ₉	160	
10.	BR ₁₀	160	
11.	BR ₁₁	20	
12.	BR ₁₂	150	
13.	BR ₁₃	22	
14.	BR ₁₄	55	
15.	BR ₁₅	180 ⁺	
16.	BR ₁₆	50	
17.	BR ₁₇	90	
18.	BR ₁₈	22	

19.	BR_{19}	20	
20.	BR_{20}	160	

Table 5.2. Bacteriological analysis of bio-raw water supplies

S. No.	Sample code (Sources)	Total coliform count CFU/100ml.	WHO standard level
1.	BR 1	39	
2.	BR 2	50	Coliform Organisms
3.	BR 3	160	4 CFU/100ml.
4.	BR 4	180+	
5.	BR 5	90+	
6.	BR 6	180+	
7.	BR 7	50	
8.	BR 8	180+	
9.	BR 9	160	
10.	BR 10	160	
11.	BR 11	20	
12.	BR 12	150	
13.	BR 13	22	
14.	BR 14	50	
15.	BR 15	180+	
16.	BR 16	50	
17.	BR 17	90	
18.	BR 18	22	
19.	BR 19	20	
20.	BR 20	160	

BR=Bio Raw

Table 5.3 Bacteriological analysis of treated water supplies

No. of supplies	Total coliform count CFU/100ml.	WHO Standard Value
20	0	Coliform organisms 4 CFU/100ml.

Table 5.4 Bacteriological analysis of well water supplies

S. No.	Sample code (Sources)	Total coliform count CFU/100ml.	WHO standard level
1.	W.N 1	2	4 CFU/100ml.
2.	W.N 2	6	
3.	W.N 3	10	
4.	W.N 4	12	
5.	W.N 5	18	
6.	W.N 6	12	
7.	W.N 7	8	
8.	W.N 8	14	
9.	W.N 9	12	
10.	W.N 10	22	
11.	W.N 11	4	
12.	W.N 12	12	
13.	W.N 13	20	
14.	W.N 14	12	
15.	W.N 15	12	
16.	W.N 16	17	
17.	W.N 17	4	
18.	W.N 18	20	
19.	W.N 19	4	

20.	W.N 7	12	
-----	-------	----	--

W.N.=Well Number

Table 5.5 Bacteriological analysis of public tap water supplies

S. No.	Source	Total coliform count.	WHO guideline
1.	PT 1	9	
2.	PT 2	20	4 CFU/100ml.
3.	PT 3	11	
4.	PT 4	3	
5.	PT 5	11	
6.	PT 6	20	
7.	PT 7	43	
8.	PT 8	20	
9.	PT 9	4	
10.	PT 10	23	
11.	PT 11	20	
12.	PT 12	3	
13.	PT 13	43	
14.	PT 14	20	
15.	PT 15	9	
16.	PT 16	22	
17.	PT 17	15	
18.	PT 18	12	
19.	PT 19	14	
20.	PT 20	28	
22.	PT 21	20	
22.	PT 22	28	

23.	PT 23	40	
24.	PT 24	22	
25.	PT 25	60	
26.	PT 26	50	
27.	PT 27	40	
28.	PT 28	24	
29.	PT 29	22	
30.	PT 30	180+	

PT=Public Tap

Table 5.6 Bacteriological analysis of stone spout supplies

S. No.	Source	Total coliform	WHO guideline
1.	SS 1	11	
2.	SS 2	150	10 CFU/100ml.
3.	SS 3	7	
4.	SS 4	11	
5.	SS 5	14	
6.	SS 6	120	
7.	SS 7	13	
8.	SS 8	23	
9.	SS 9	20	
10.	SS 10	11	

SS=Stone Spout

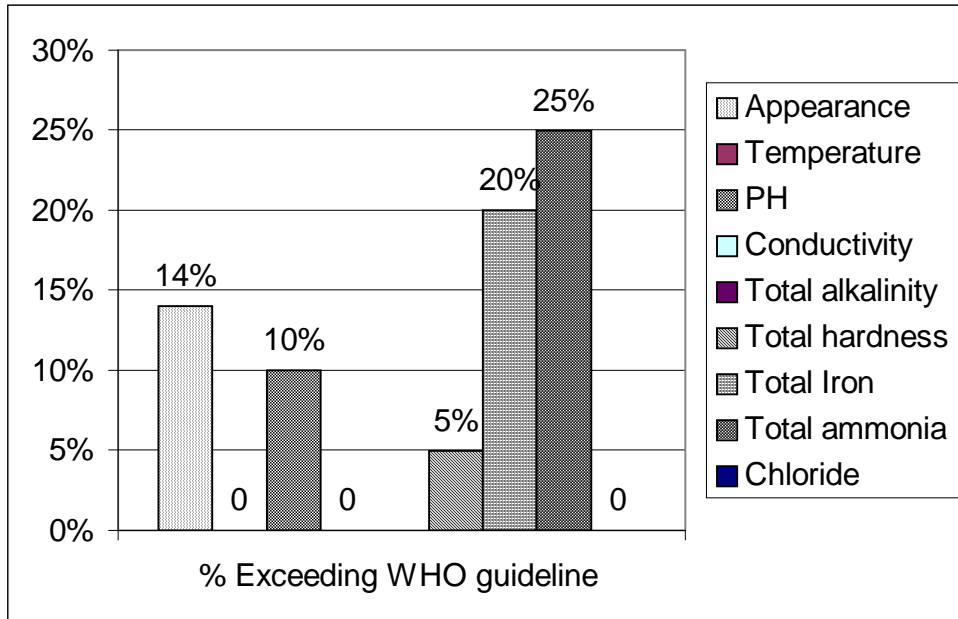


Figure 5: Chemical parameters exceeding WHO guidelines

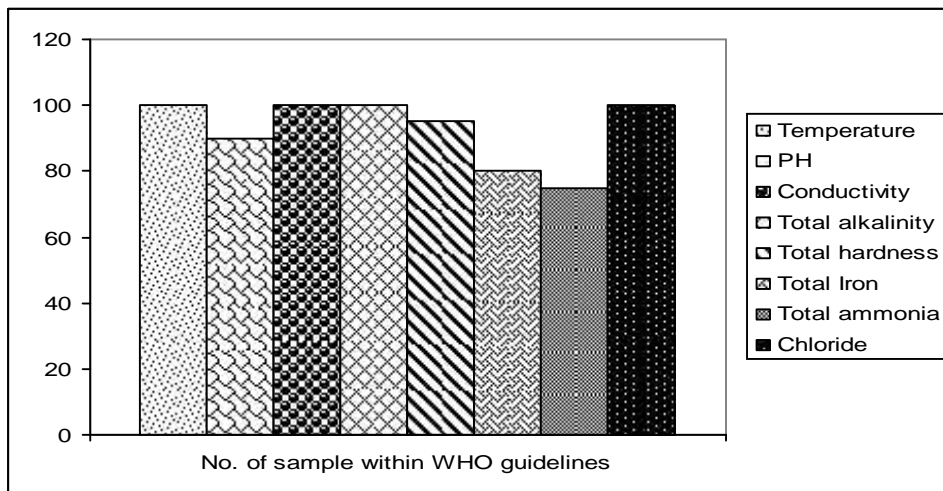
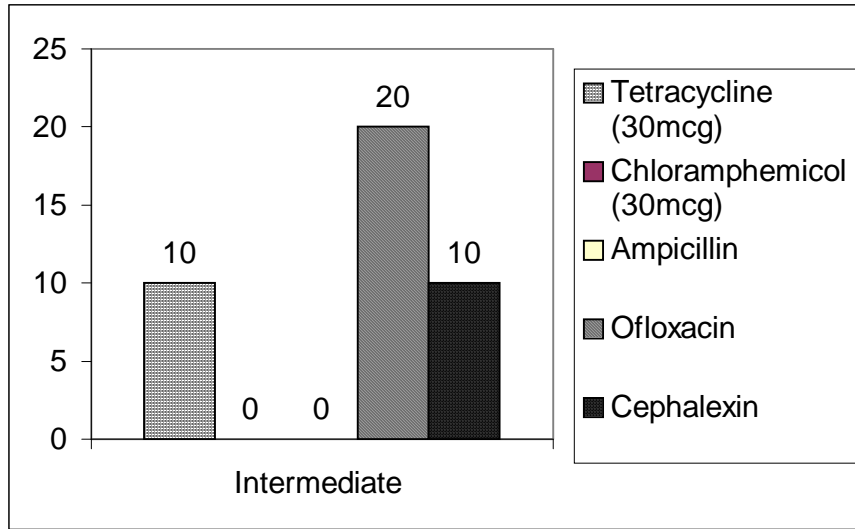


Figure 6: Chemical parameters within WHO guidelines



**Figure 8: Bar diagram of antibiotics susceptibility pattern
Intermediate**

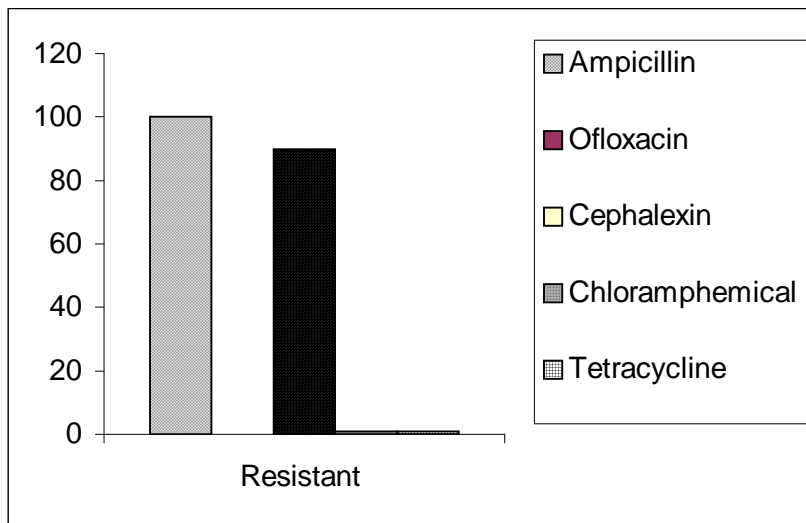


Figure 9: Antibiotic susceptibility pattern: resistant

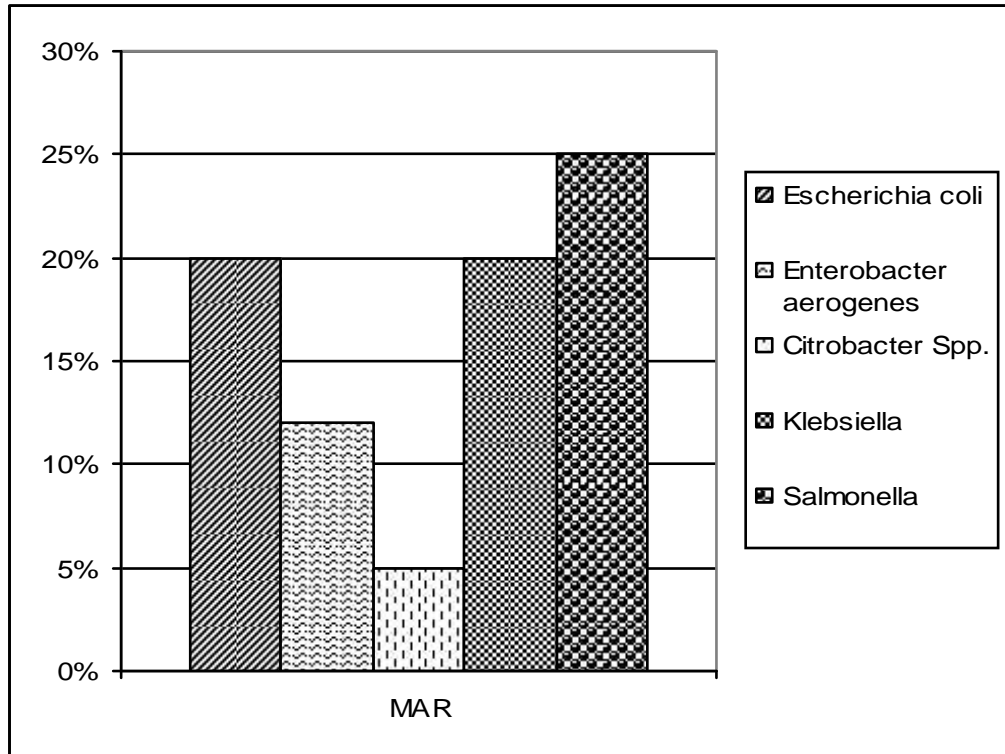


Figure 11: Frequency of MAR in isolates

APPENDIX – I

1. List of culture media, chemicals and equipments

1.1 Media

- a. Nutrient Agar
- b. Plate Count Agar
- c. Mac conkey broth
- d Eosin methylene agar
- d. Muller-Hinton Agar
- e. Selentite-F-broth
- f. Salmonella-Shigella Agar
- g. Hugh-Leifson Media
- h. Sulphide Indole Motility (SIM) Media
- i. MR-VP broth media
- j. Simmon's citrate Agar
- k. Triple Sugar Iron Agar Media
- l. Urease Agar

1.1 Chemical and reagents

- a. Acetic acid
- b. Alpha-naphthol
- c. Ampicillin
- d. Barium Chloride
- e. Chloramphenicol
- f. Ciprofloxacin
- g. Co-trimoxazole
- h. Crystal violet solution
- i. Erythromycin
- j. Ethanol
- k. Hydrogen Peroxide
- l. Kovac's Reagent
- m. Methyl Red indicator
- n. Oxidase reagent
- o. Phenol Red
- p. Safranin
- q. Tetracycline
- r. Urea

Equipments

- 1. Autoclave
- 2. Beakers
- 3. Conical flask
- 4. Culture tubes with Durham's tube
- 5. Balance
- 6. Hot air oven
- 7. Hot Plate
- 8. Incubator

APPENDIX – II

1. Composition and Preparation of Media used in isolation and identification of Bacteria.

I. Culture media

a. Nutrient agar (NA)

Composition	Gm/lit
Peptone	5.0
Sodium Chloride	5.0
Beef extract	1.5
Yeast extract	1.5
Agar	15.0
Final p ^H	7.4 ± 0.2

2.8 grams of media was dissolved in 100 ml of distilled water and heated to dissolve the media. The media was autoclaved at 15 lbs. Pressure at 121° C for 15 min.

b. Plate count agar (PCA)

Composition	gm/lit
Trypton	5.0
Yeast extract	2.5
Dextrose	1.0
Agar	9.0

Final p^H 7.4 ± 0.2

23.5 grams media was dissolved in 1000 ml of distilled water autoclaved at 15 lbs pressure at 121°C for 15 min.

c. Macconkey agar (MA)

Composition	gm/lit
Peptone	2.0
Lactose	20.0
Bile salt	5.0
Sodium chloride	5.0
Neutral Red	0.075
Agar	12.0
Final p ^H (at 25 ⁰ C)	7.4 ± 0.2

51.5 grams of media was dissolved in 1000 ml of distilled water and heated to dissolve the media. The media was autoclaved at 15 lbs pressure for 15 min.

d. Selenite F-broth

Composition	gm/lit
Trypton	5.0
Lactose	4.0
Sodium Phosphate	10.0

Sodium acid selenite	4.0
Final p ^H (at 25 ⁰ C)	7.4 ± 0.2

23 grams of media was dissolved in 1000 ml of distilled water and heated to dissolve the media and then sterilized in a boiling water bath for 10 min.

e. Salmonella-Shigella agar

Composition	gm/lit
Beef extract	5.0
Peptone	5.0
Lactose	10.0
Bile Salt	8.5
Sodium citrate	10.0
Sodium thiosulphate	8.5
Ferric citrate	1.0
Brilliant Green	0.00033
Neutral Red	0.025
Agar	15.0
Final p ^H	7.0 ± 0.2

63 grams of media was dissolved in 1000 ml of distilled water and heated to dissolve the media. No Autoclave.

f. M-Endo agar

Composition	gm/lit
Casein enzyme hydrolysate	3.7
Peptic digest of animal tissue	3.7
Tryptone	7.5
Yeast extract	1.2
Lactose	9.4
Dipotassium Phosphate	3.3
Monopotassium Phosphate	1.0
Sodium chloride	3.7
Sodium deoxycholate	0.1
Sodium Laurylsulphate	0.05
Sodium Sulphite	1.6
Basic Fuchsin	0.8
Agar	15.0
pH	7.2 ± 0.2

51 grams of media was dissolved in 1000 ml of distilled water it was boiled to distilled water it was boiled to dissolve the media and cooled to 45⁰ C. No Autoclaving.

g. Lactose broth

Composition	gm/lit
Beef Extract	3.0
Peptic digest	5.0
Lactose	5.0
Final p ^H (at 25° C)	6.9 ± 0.2

13 grams of broth was dissolved in 1 lit of distilled water. For double strength broth, 26 grams was dissolved in 1lit of distilled water. 10 ml broth was dispensed in tubes and sterilized by autoclaving at 15 lb at 121⁰ C for 15 min.

II. Composition and preparation of media used for antibiotic sensitivity test

Mueller-Hinton Agar (MHA)

Composition	gm/lit
Beef Infusion broth	300.0
Casein Acid hydro lysate	17.0
Starch	1.0
Agar	17.0
Final p ^H	7.0 ± 0.2

III. Composition of biochemical media

Peptonized Iron	0.2
Sodium thiosulphate	0.025
Agar	0.30
Final p ^H	7.3 ± 0.2

3.6 grams of media was dissolved in 100 ml of distilled water and heated to boil. Then the media was distributed in tubes and sterilized at 15 lbs pressure at 121⁰ C for 15 min.

Reagent: Kovac's reagent

Composition	gm/lit
P-Dim ethyl aminobenzaldehyde	5.0
ISO amyl alcohol	75.0 ml
Conc. Hydrochloric acid	25.0 ml

Procedure: The test organisms was inoculated in SIM media and incubated at 37⁰ C for 24 hrs. About 0.5 ml Kovac's reagent was added and shaken gently. Formation of pink colored ring on surface layer indicates positive test.

d. MR –VP media (Glucose – Phosphate broth)

Composition	gm/lit
Buffered peptone	7.0
Dextrose	5.0
Dipotassium Phosphate	5.0

Final p^H 6.9 ± 0.2

17 grams of media was dissolved in 1000ml of distilled water. It was distributed in test tubes in 10 ml and sterilized by autoclaving at 15 lbs pressure at 121⁰C for 15 min.

Reagent

Methyl Red

Composition gm/lit.

Methyl Red 0.04

Ethyl Alcohol 40.0 ml

Distilled Water 60.0 ml

Procedure: MR-VP broth was inoculated with culture to be tested and incubated at 37 C for 48 hrs. Methyl Red indicated was added to the culture. Development of red color indicates positive test.

Voges – Proskauer test.

Medium: MR-VP medium

Reagent: Baritt's reagent

Solution A

Composition gm/lit

Alpha-naphthol 5.0

Ethyl alcohol	100.0 ml
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Solution B

Composition	gm/lit.
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Potassium hydroxide	40.0
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Distilled water	100.0 ml
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Produce: MR-VP broth media was inoculated with fresh culture and incubated at 37⁰C for 30 min. after adding alpha-naphhtol and 40% KOH in 3% proportion is a positive test.

e. Citrate utilization test

Medium: Simmon's citrate agar

Composition	gm/lit.
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Magnesium sulphate	0.2
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Monoammonium Phosphate	1.0
------------------------	-----

Dipostassium Phosphate	1.0
------------------------	-----

Sodium Citrate	2.0
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Sodium Chloride	5.0
-----------------	-----

Bromothymol blue	0.08
------------------	------

Agar	15.0
------	------

pH	6.8 ± 0.2
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24.2 grams of media was dissolved in 100 ml distilled water. It was heated to boiling and then distributed in tubes and sterilized by autoclaving at 121°C for 15 lbs for 15 min.

Procedure: The media slants were inoculated with test organism by streaking with loop. Growth of organisms with an intense blue color on slant is indicative of positive test.

f. Triple sugar iron (TSI) agar

Composition	gm/lit
Peptone	10.0
Tryptone	10.0
Yeast extract	3.0
Beef extract	3.0
Lactose	10.0
Sucrose	10.0
Dextrose	1.0
Ferrous sulphate	0.2
Sodium Chloride	5.0
Sodium thiosulphate	0.3
Phenol Red	0.024
Agar	12.0
p ^H	7.4 ± 0.2

6.5 grams of media was dissolved in 100 ml of distilled water and boiled to dissolve completely. It was distributed in tubes and sterilized by autoclaving at 15 lbs at 121 C for 15 min. The medium was prepared in slant with a butt of about 1 inch.

TSI agar is used to determine the ability of an organism to utilize specific carbohydrate incorporated in media, with or without gas production along with determination of H₂ S production.

g. Urease test

Urea agar base

Composition	gm/lit
Peptone	1.0
Dextrose	1.0
Nacl	5.0
Mono Potassium Phosphate	0.8
Dipotassium Phosphate	1.2
Phenol Red	0.012
Agar	5.0
Final p ^H	6.8 ± 0.2

25 grams of media was dissolved in 950 ml distilled water. It was sterilized by autoclaving at 15 lbs at 15 lbs at 121 C for 15 min. It was then cooled to 55 C and 50 ml of sterile 40% urea solution was aseptically added. The contents were mixed well and distributed into tubes and allowed to set in slope position.

Procedure: The surface of slant was inoculated with test organism and incubated for 24 hrs. at 37 C. The positive test is indicated by the change of color of media to intense pink.

h. Oxidative/Fermentative test

Composition	gm/lit
Tryptone	2.0
Nacl	5.0
Dipotassium Phosphate	0.3
Agar	2.0
p ^H	7.1 ± 0.2
Glucose	10.0

1.93 grams of media was dissolved in 100 ml of distilled water. It was boiled to dissolve the medium completely and distributed into tubes. The media was then sterilized by autoclaving at 15 lbs at 121 C for 15 min.

APPENDIX – III

Composition of stain

Gram's staining

Heat fixed smear was flooded with crystal violet for one minute and excess stain was washed out. It was then treated with Gram's iodine for 1 minute and washed. Then smear was treated with alcohol for 15 sec. washed and stained with safranin for 1 minute and washed with water. It was dried and observed under microscope.

Preparation of stain

IX. I. Crystal violet

Solution A

Crystal violet	2.0 gms.
95% ethyl alcohol	20.0 ml

Solution B

Ammonium oxalate	0.8 gm
Distilled water	30.0 ml

Crystal violet was dissolved in ethyl alcohol, and ammonium oxalate in distilled water. Then solution A and B were mixed.

II. Gram's iodine

X.	Iodine	1.0 gm
XI.	Potassium iodide	2.0 ml
XII.	Distilled Water	300.0 ml

Iodine and potassium iodide were dissolved in distilled water.

III. Ethyl alcohol (95%)

XIII.	Absolute alcohol	95.0 ml
XIV.	Distilled water	5.0 ml

IV. Safranin

Safranin	10.0 ml
Distilled water	100.0 ml

APPENDIX – IV

Enterobacteriaceae

Biochemical characterization

S.N		Lac	Glue	Suc	Cit	Ind	Urea	Motile	H ₂ S	MR	VP
1.	Escherichia	+	+g	±	-	+	-	+	-	+	-
2.	<i>Klebsiella oxytoca</i>	+	+g	+	+	+	+	-	-	-	+
3.	<i>K.pneumoniae</i>	+	+g	+	+	-	+	-	-	-	+
4.	<i>Enterobacter aerogenes</i>	+	+g	+	+	-	-	+	-	-	+

5.	<i>Shigella dysenteriae</i>	-	+	-	-	±	-	-	-	+	-
6.	<i>Shigella sonnei</i>	±	+	-	-	-	-	-	-	+	-
7.	<i>Salmonella typhi</i>	-	+	-	-	-	-	+	+	+	-
8.	<i>S. paratyphi A</i>	-	+g	-	-	-	-	+	+	+	-
9.	<i>S. paratyphi B.</i>	-		-			-	+	-	+	-
10.	<i>Salmonella typhimurium</i>	-	+g	-	+	-	-	+	+	+	-
11.	<i>Citrobacter freundii</i>	-	+g	±	+	-	±	+	+	+	-
12.	<i>Serratia marcescens</i>	-	+g	+	+	-	-	+	-	-	+
13.	<i>Proteus mirabilis</i>	-	+g	±	±	-	+	+	+	+	±
14.	<i>Proteus Vulgaris</i>	-	+g	+	±	+	+	+	+	+	-
15.	<i>Hafnia alvei</i>	-	+g	-	-	-	-	+	-	±	+
16.	Providencia stuartii	-	+g	±	+	+	±	+	-	+	-

APPENDIX – V

WHO guideline value for bacteriological quality of drinking water

Organism	value	Unit	Guideline
A. Piped water supplied			
Treated water entering distribution system			
FEcal coliform		Number/100ml	0
Coliform		“	0
Untreated water entering Distribution system			
FEcal coliform		Number/100ml	0
Coliform organism		Number/100ml	3
Water in the distribution system			
FEcal coliform		Number/100ml	0
Coliform organism		Number/100ml	3
B. Unpiped water supplies			
FEcal coliform		Number/100ml	0
Coliform organism		Number/100ml	10

Appendix VI

Physico-chemical parameters of different samples of water

Date	Source	Appearance	Turbidity	colour (Hazen)	Temperature	pH	Ec	Total Hardness	Calcium Hardness	Mg Hardness	Total Fe	Total NH ₃	Chlo
1/12	Bio inlet	Clear	<5	<5	24° C	7.3	152	50	30	20	0.7	1.6	7.6
1/12	Out let	Clear	<5	<5	24° C	7.6	145.3	48	30	18	0.7	0.04	7.6
1/12	Treated	Clear	<5	<5	24° C	7.8	119.7	48	30	18	0.1	0.04	5.7
1/14	Inlet	Clear	<5	<5	25° C	7.5	178	38	20	18	0.9	1.2	7.6
1/14	Out let	Clear	<5	<5	25° C	7.6	153	42	22	20	0.6	0.04	7.6
1/14	R.S.F.	Clear	<5	<5	25° C	7.7	53.3	20	12	8	0.1	0.04	8.7
1/14	Treated	Clear	<5	<5	25° C	7.8	115.2	40	24	16	0.1	0.04	5.7
1/15	Inlet	Clear	<5	<5	22° C	7.4	172	40	24	16	0.8	1.2	7.6
1/15	Out let	Clear	<5	<5	22° C	7.6	160	40	24	16	0.5	0.04	7.6
1/15	Treated	Clear	<5	<5	22° C	7.2	39.1	20	12	8	0.1	0.04	5.7

Date	Source	Appearance	Turbidity	Colour	Temp	pH	Ec	Total Alkalinity	Total Hardness	C
062-3-1	Inlet	Clear	<5	<5	30° C	6.5	160	48	42	2
062-3-1	Outlet	Clear	<5	<5	30° C	6.8	180	54	50	3
062-3-1	Treat	Clear	<3	<5	30° C	7.6	80	24	20	1
062-3-3	Inlet	Clear	<5	<5	31° C	7.1	142	44	40	2
062-3-3	Outlet	Clear	<5	<5	31° C	7.5	134	40	36	2
062-3-3	Treat	Clear	<5	<5	31° C	8.4	40	12	10	0
062-3-10	Treat	Clear	<4	<5	22° C	7.1	61.3	20	32	2
062-3-10	Raw	Clear	<5	<5	22° C	6.85	35.6	12	18	1
062-3-10	Rapid Sand	Clear	<4	<5	24° C	7.05	50	16	24	1
						8.0	82	12	36	2

Date	Source	Appearance	Turbidity	Colour	Temp	pH	Ec	Total Alkalinity	Total Hardness	
062-4-5	M.Pes	Clear	3	<5	25° C	7.2	40.1	14	20	
062-4-5	Treat	Clear	5	<5	25° C	7.4	29.1	8	12	
062-4-5	Raw	Clear	3	<5	23° C	7.3	21	6	10	
062-4-5	R.S.F	Clear	2	<5	23° C	7.3	25	8	12	
062-4-10	Treat	Clear	3	<5	20° C	7.4	44	14	22	
062-4-10	Raw	Clear	3	<5	20° C	6.8	25	8	12	
062-4-10	M.Res.	Clear	6	<5	20° C	7.04	51.4	18	26	
062-4-16	M.Res.	Clear	4	<5	24° C	6.8	38	12	18	
062-4-16	Treat	Clear	4	<5	24° C	7.1	30	10	14	
062-4-16	Raw	Clear	5	<5	24° C	6.6	20.4	6	10	
062-4-17	Treat	Clear	5	<5	26° C	6.8	31.1	10	16	

Date	Source	Appearance.	Turbidity	Colour.	Temp.	pH	Ec	Total Alkalinity	Total Hardness	Ca	Mg	Tot Fe	N
062-1-2	Well 7	Clear	<5	<5	24° C	7	216	108	96	64	32	0.8	2
062-1-2	Maha	Clear	<5	<5	24° C	6.9	222	110	94	64	30	0.9	2
062-1-6	Chetrapati	Clear	<5	<5	23° C	7.65	227	116	108	68	40	0.1	0.
062-1-6	Balaju	Clear	<5	<5	23° C	7.62	238	116	110	68	42	0.1	0.
062-1-7	Ba sun	Clear	<5	<5	24° C	7.75	158	80	80	50	30	1	2
062-1-7	Dhapasi	Clear	<5	<5	24° C	7.8	172	82	86	56	30	0.1	0.

062-1-8	Soralchu	Clear	<5	<5	23° C	7.2	220	100	104	64	40	0.1	0
062-1-8	Balaju	Clear	<5	<5	23° C	7.5	205	100	102	64	38	0.1	0
062-1-11	Gongabu	Clear	<5	<5	25° C	7.34	157	46	48	30	18	0.8	0
062-1-11	Well 3	Clear	<5	<5	25° C	7.21	137	42	58	38	20	0.6	0
062-1-13	Ranibari	Clear	<5	<5	24° C	8.1	130	42	36	20	16	0.1	0
062-1-13	Samachusi	Clear	<5	<5	24° C	7.3	212	68	64	44	20	0.8	2
062-1-15	Balaju	Clear	<5	<5	22° C	7.2	280	100	110	72	38	1.2	4
062-1-16	Palcnap	Clear	<5	<5	24° C	7.7	216	110	106	66	40	0.1	0
062-1-16	Basund	Clear	<5	<5	24° C	7.3	172	80	76	46	30	0.7	2

Date	Source	Appearance.	Turbidity	Colour	Temp	p ^H	Ec	Total Alkalinity	Total Hardness	Ca	Mg	Fe	
062-2-2	Maharajgunj	Clear	<5	<5	23° C	7.2	140.7	40	40	24	16	0.6	0
062-2-2	Banasthali	Clear	<5	<5	23° C	7.24	186	54	50	30	20	0.1	0
062-2-4	Dhapasi	Clear	<5	<5	22° C	6.9	158.7	46	42	30	12	0.02	0
062-2-4	Basund	Clear	<5	<5	22° C	7.1	162	46	42	30	12	0.8	0
062-2-6	Sorakhu	Clear	<5	<5	23° C	7.5	160	50	46	30	16	0.1	0
062-2-6	Basundhara	Clear	<5	<5	23° C	7.2	168	52	50	30	20	0.8	0
062-2-10	Balaju Well	Sl.turbio	<5	<5	25° C	6.9	280	88	80	52	28	1.4	0
062-2-10	Banasthali	Clear	<5	<5	25° C	7.4	186	58	52	30	22	0.1	0
062-2-12	Balaju Well	Clear	<5	<5	27° C	6.9	287	90	82	52	30	1.3	0
062-1-12	Banasthali	Clear	<5	<5	27° C	7.3	195	62	56	32	24	0.1	0
062-2-16	Well 7	Clear	<5	<5	30° C	6.7	154	148	46	30	16	0.9	0
062-2-16	Mahadertha	Clear	<5	<5	30° C	7.2	142	44	40	24	16	1	0
062-2-18	Balaji (treat)	Clear	<5	<5	30° C	7.15	190	60	56	34	22	0.1	0

062-2-18	Dhapasi	Clear	<5	<5	30° C	6.9	155	48	42	24	18	0.1
062-2-20	Banasthali	Clear	<5	<5	30° C	7.8	190	60	56	36	20	0.1
Site	Source	Appearance.	Turbidity.	Colour	Temp	PH	Ec	Total alkalinity	Total Hardness	Ca	Mg	Fe
03-6	Balaju Well	Turbid	<5	<5	30° C		289	88	80	50	30	1.3
03-6	Banasthali	Clear	<5	<5	30° C		178	54	50	30	20	0.1
03-8	Basundhara	Clear	<5	<5	25° C		136.1	42	38	22	16	0.8
03-8	Well 7	Clear	<5	<5	25° C		138	42	38	22	16	0.6
03-10	Sarakhuth	Clear	<5	<5	22° C		146	60	70	46	24	0.1
03-10	Pokahjol	Clear	<4	<5	22° C		151	62	72	48	24	0.1
03-19	Balaju Chowk	Clear	<4	<5	26° C	6.8	100	44	50	36	24	0.1
03-19	Dhapasi	Clear	<4	<5	26° C	6.9	140	60	66	46	20	0.1
03-16	Sarakhuth	Clear	<4	<5	25° C	6.7	128	56	60	40	20	0.01
03-16	Naya Bazar	Clear	<4	<5	25° C	6.5	187	82	90	60	30	0.1
03-20	Naya Bazar	Clear	<4	<5	28° C	6.8	133	58	66	40	26	0.1
03-20	Balaju Chowk	Clear	<4	<5	28° C	7	97	42	46	30	16	0.1
03-22	Naya Bazar	Clear	<4	<5	25° C	7	125.5	54	62	40	22	0.1
03-22	Sarakhuth	Clear	<8	<5	25° C	6.8	130	56	64	40	24	0.1
03-24	Pokahjol	Clear	<6	<5	28° C	6.8	97	42	46	30	16	0.1

Date	Source	Appearance	Turbidity	colour	Temp	Ec	Total Alkalinity	Total Hardness	Ca	Mg	Fe
062-4-2	Balaju (treat)	Clear	<5	<5	24° C	170	74	84	30	34	0.1
062-4-2	Balaju Chowk	Clear	<5	<5	24° C	167	72	82	50	32	0.1
062-4-4	Balaju Well	Clear	<10	<5	24° C	180	78	88	50	38	1.3
062-4-4	Banasthli	Clear	<6	<5	24° C	180	74	90	60	30	0.1
062-4-7	Basundhara	Clear	<6	<5	26° C	160	70	80	50	30	0.8
062-4-7	Dhapasi	Clear	<6	<5	26° C	168	72	84	50	34	0.1
062-4-9	Sarakhote	Clear	<6	<5	19° C	55	22	26	16	10	0.1
062-4-9	Nayabazar	Clear	<6	<5	19° C	98	42	48	30	18	0.1
062-4-12	Balaju Chowk	Clear	<6	<5	22° C	112	48	56	34	22	0.1
062-4-16	Bus Park	Clear	<8	<5	28° C	145	62	72	50	22	0.8
062-4-16	Shobabhog	Clear	<8	<5	28° C	97	40	48	30	18	0.1
062-4-18	Banasthli	Clear	<6	<5	28° C	115	50	58	34	24	0.1
062-4-18	Dhapasi	Clear	<8	<5	28° C	160	70	80	50	30	0.1
062-4-21	Sorakhutte	Clear	<6	<5	26° C	102	44	50	30	20	0.1