Isolation and Characterization of *Salmonella* from Drinking Water Samples of Urban Water Supply System of Kathmandu

A Dissertation

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by

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RECOMMENDATION

This is to certify that **Ms. Esha Shrestha** has completed this dissertation work entitled "**Isolation and characterization of *Salmonella* from drinking water samples of urban water supply system of Kathmandu**" as a partial fulfillment of M. Sc. Degree in Microbiology under our supervision. To our knowledge this thesis work has not been submitted for any other degree.

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ABSTRACT

Drinking water pollution has become a crucial issue throughout the world. In developing countries water-borne disease account for a large scale of morbidity and mortality. This study was carried out with an aim to isolate and characterize salmonellae from Urban Water Supply System (UWSS) of Kathmandu district. The study was conducted from August 2008 to March 2009 in the laboratory of Central Department of Microbiology, T.U. During the study period a total of 86 water samples were randomly collected from taps of different localities. The samples were analysed by the standard method for physico-chemical and microbiological parameters to assess the drinking water quality.

Distinct variation in physico-chemical parameters were not observed. Analysis of pH revealed that a total of 11(12.79%) samples were not found in accordance WHO and national standard. Total coliform count showed 100% of the samples crossed the WHO guideline value.

Out of 86 water samples 4(4.65%) samples were positive for *Salmonella.* A total of 10 salmonellae were isolated. The isolates were subjected to antibiotic susceptibility test by Kirby-Bauer method. Antibiotic susceptibility pattern showed that all the isolates were 100% susceptible to tetracycline, chloramphenicol, cotrimoxazole, nalidixic acid and ciprofloxacin and 70% were restitant to amoxicillin, 20% to cephalexin and 10% ceftizoxime.

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LIST OFABBREVIATION

ADB	-	Asian Development Bank
APHA	-	American Public Health Association
CDM	-	Central Department of Microbiology
CEDA	-	Centre for Economic Development and Administration
Cfu	-	Colony Forming Unit
DWSS	-	Department of water supply and sewerage
DoHS	-	Department of Health Services
ENPHO	-	Environment and Public Health Organization
HMG	-	His Majesty's Government
ISRSC	-	Informal Sector Research and Study Centre
IUCN	-	International Union for Conservation of Nature
KUKL	-	Kathmandu Upatakya Khanapani Limited
MF	-	Membrane Filter
MA	-	Mac-Conkey Agar
MDR	-	Multi-Drug Resistant
MHA	-	Mueller Hinton Agar
NA	-	Nutrient Agar
NCCLS	-	National Committee for Clinical Laboratory Standards
S.S	-	*Salmonella Shigella*
TSI	-	Triple Sugar Iron
UNEP	-	United Nations Environment Protection

UNICEF	-	United Nations Children Fund
UWSS	-	Urban Water Supply System
VP	-	Voges Proskauer
WHO	-	World Health Organization
WSSB	-	Water Supply and Sewerage Board

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CHAPTER-I

1 INTRODUCTION

Water is the most vital resources for all kind of life on planet. Water intended for human consumption should be both safe and wholesome. Much of the ill-health which affects humanity, especially in developing countries can be traced to lack of safe and wholesome water supply. There can be no state of positive health and well-being without safe water (Park, 2000).

Though water is necessary for human survival, many are denied access to sufficient potable water supply and sufficient water to maintain basic hygiene. A large fraction of world's population around 1.1 billion (one-sixth) do not have access to safe drinking water. The majority of these are in Asia and Sub-Saharan Africa. Furthermore, 2.6 billion people are living with no proper means of sanitation (WHO/UNICEF, 2000).

The growing imbalance between supply and demand have resulted in pollution and environmental degradation. The quality of water for drinking has deterioted because of the inadequacy of treatment plants, direct discharge of untreated sewage into rivers, and inefficient management of piped water distribution system (UNEP, 2001). Despite major effort to deliver safe piped, community water to the world's population, the reality is that water supplies delivering safe water will not be available to all people in near term (Agarawal, 1981) As a consequence of such water quality condition water borne disease such as diarrhea, dysentery and gastroenteritis occur often. These diseases are prevalent in both urban and rural areas throughout the nation. Diseases caused by contaminated water are among the ten most prevalent water borne disease in Nepal (DoHS, 1998). Thousands of people die or suffer from water and sanitation related diseases. Drinking water has direct impact on human health and can be extremely dangerous when it becomes the vehicle of transmission of disease (Sharma, 2000).

The drinking water supply in most of the rural areas and municipalities of Nepal are usually inadequate in terms of overall coverage, quantity of water and of course poor

water quality. Kathmandu is the best example of this where people are struggling to get adequate water to meet the daily requirements and at the same time facing problem of unsafe water. In Kathmandu Valley, most of the sources of water can't be regarded safe and measured up to the guidelines recommended by WHO (Bottino *et al*., 1999; Prasai, 2002).

Water, although an absolute necessity for life can be a carrier of many water borne diseases such as typhoid, cholera, hepatitis, dysentery and other diarrhoeal related diseases. UNICEF estimates that over 80% of the world diseases are water borne. Children bear the greatest health burden associated with poor water and sanitation. Diarrhoeal diseases attributed to poor water supply, sanitation and hygiene account for 1.73 million deaths each year and contribute over 54 million disability adjusted life years, a total equivalent to 3.7% of the global burden of disease. This place diarrhoeal disease due to unsafe water, sanitation and hygiene as the 6^{th} highest burden of disease on a global scale, a health burden that is largely preventable (WHO, 2002).

Approximately 4 billion cases of diarrhoea each year cause 2.2 million deaths, mostly among children under the age of five which is equivalent to one child dying every 15 seconds. These deaths represent approximately 15% of all child deaths under the age of five in developing countries. Water, sanitation, and hygiene interventions reduce diarrhoeal disease on average by between one-quarter and one-third (Esrey, 1999; WHO, 2000).

The potential of drinking water to transport microbial pathogens to great numbers of people, causing subsequent illness, is well documented in countries at all levels of economic development. The number of outbreaks that have been reported throughout the world demonstrates that transmission of pathogens by drinking water remains a significant cause of illness.

Outbreaks of water borne epidemic in Nepal is not uncommon. The incidence of such epidemics is considerably high in comparison with that of other diseases. Each and every summer water borne epidemics hit different parts of the country including Kathmandu City, the capital. Contaminated drinking water is one of the major routes

for spreading such diseases. Annual report from DoHS (2004/2005) showed that there were 2,332 cases of typhoid, 18,611 cases of diarrhoeal diseases, 9,322 cases of intestinal worms, 543 cases of jaundice and infectious hepatitis in Kathmandu City.

Nepal is now becoming a new challenge for the nation's water supply sector (ENPHO, 2003). Therefore, drinking water quality assessment has always been crucial with reference to public health importance in Kathmandu Valley.

Even though pharmacological industries have produced a number of new antibiotics in the last three decades, resistance to these drugs by microorganisms has increased (Nascimento *et al.*, 2000). The problem of microbial resistance is growing and the outlook for the use of antimicrobial drugs in the future is still uncertain. Therefore, actions must be taken to reduce this problem, for example, to control the use of antibiotic, develop research to better understand the genetic mechanisms of resistance, and to continue studies to develop new drugs, either synthetic or natural. The ultimate goal is to offer appropriate and efficient antimicrobial drugs to the patient.

The drinking water quality is a current and happening problem in Kathmandu, getting intensified with the rapid rate of urbanization and industrialization. Water borne epidemics are regular phenomenon due to poor water and sanitation facilities where the poor and marginalized people are most affected. The lack of studies in these sectors may have serious consequences in terms of morbidity and mortality. The bacterium *Salmonella* contains more than 2300 serovars of which more than 1000 serovars can cause human infection (typhoid and non-typhoidal disease). Hence this study aims to isolate, identify and characterize salmonellae in drinking water and their risk associated with exposed population. This study is thus conducted to reveal the status of the drinking water and to determine the antibiotics susceptibility pattern of salmonellae isolates to provide implication in infection control.

CHAPTER-II

2 OBJECTIVES OF THE STUDY

2.1 General Objective

To isolate and identify *Salmonella* from drinking water samples of UWSS of Kathmandu district and to determine the antibiotic susceptibility pattern of isolates

2.2 Specific objectives

1. To study physico-chemical parameters of drinking water from different localities of Kathmandu
2. To study the indicator organisms (total coliform and thermotolerant coliform)
3. To isolate and identify *Salmonella* from water sampels
4. To study the antibiotics susceptibility pattern of the isolates

CHAPTER - III

3 LITERATURE REVIEW

3.1 Water Quality

Nepal though rich in water resources, still suffers from water related problems. National water supply coverage is said to have increased substantially in the past decade yet people are still spending hours to get a bucket of water in both rural and urban communities including the capital city. Increasing water demand, shortage of clean drinking water and pollution of water resources are common phenomena of urban development (ENPHO, 2003).

Although almost 90% Kathmandu cities have access to municipality piped water supply, people are still suffering from diarrhoeal diseases. Several researches have already pointed out that piped water supply in Kathmandu city is unsafe for drinking. Diarrhoea and gastroenteritis are the major problems in developing countries like Nepal due to unsafe and inadequate water supplies and sanitation, little or no health education, illiteracy, under nutrition, wide spread faecal contamination of environment, dense population etc (Chand *et al.*, 2001). In Nepal, the reported cases of water borne communicable diseases were typhoid (2,15,191), diarrhoeal diseases (9,21,901), intestinal worms (6,11,072) and jaundice and infectious hepatitis (25,686) in 2004/2005 (DoHS, 2004/2005).

Access to safe drinking water supply and sanitation services is fundamental to improving public health and meeting national poverty reduction objectives. As it is now widely recognised, lack of access to these essential basic services contributes substantially to the high burden of disease that needlessly foreshortens and impairs the lives of far too many of Nepal's citizens. Around 80% of all diseases may be attributed to water and sanitation related causes and account for around 13,000 child deaths each year from diarrhoeal diseases such as dysentery, jaundice, typhoid and cholera (Nepal Urban Water supply and Sanitation Sector Policy).

Only 76 percent of the Nepali have ascess to drinking water as against the global average of 87 percent. Most of the pipe network is old and leaks, while water sources are drying up. According to KUKL (Kathmandu Upatakya Khanapani Limited) it had also been supplying untreated water in part of the city, as 23 out of 27 treatment plants were not functional. Last August that chlorine content in 47 percent of piped water samples from 120 different places in Kathmandu was nil (The Himalayan Times, 2009).

3.2 Physicochemical parameters of water

The ordinary consumer judges the water quality by its physical characteristics. The provision of drinking water that is not only safe but also pleasing in appearance, taste and odor is a matter of high priority (Park, 2005). Chemical tests identify impurities and other dissolved substances that affect water used for domestic purposes.

3.2.1 Temperature

Water bodies undergo temperature variation along with normal climatic fluctuation. These variations occur seasonally and, in some water bodies, over periods of 24 hours.The 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 reduce solubility of gases and amplify tastes and odours (Trivedi and Goel, 1986). High water temperature enhances the growth of microorganisms and may increase taste, odour, colour, and corrosion problem. Water in the temperature range of 7°C to 11°C has pleasant taste and is refreshing. Thus, cool water is generally more palatable than warm water (WHO, 1993). Temperature of water bodies undergoes variation along with normal climatic fluctuations. Temperature of surface water is influenced by latitude, altitude, season, time of day, air circulation, cloud cover and the flow and depth of water body (Chapman, 1999).

3.2.2 pH

pH 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. pH of water gets drastically changed with time due to exposure to air, biological activity and temperature changes. Significant changes in pH occur due to disposal of industrial wastes, acid mine drainage etc. pH of water also change diurnally and seasonally due to variation in photosynthetic activity which increase pH due to consumption of CO_2 in the process. Determination of pH is one of the important objectives in treatment of wastes. pH 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 pH value hasten the scale formation in water heating apparatus and also reduce the germicidal properties of chlorine. In the water supplies, pH is also an important factor in fixing alum dose in drinking water treatment.

Measurement of pH is the most important and frequently used tests in water chemistry (APHA, 1995). Although pH usually has no direct impact on consumers, it is one of the most important operational water quality parameters. Careful attention to pH control is necessary at all stages of water treatment to ensure satisfactory water clarification and disinfection. For effective disinfection with chlorine, the pH should preferably be less than 8, however, lower pH water is likely to be corrosive. The pH value of drinking water from any sources should be within range, 6.5-8.5 (Trivedi and Goel, 1986). The pH of the water entering the distribution system must be controlled to minimize the corrosion of water mains and pipes in house hold water systems. Alkalinity and calcium management also contribute to the stability of water and control its aggressiveness to pipe and appliance (WHO, 2004).

3.3 Microbiological characteristics of water

Health effects assessments for waterborne pathogens can be based on number of approaches. The ultimate objective for determining the microbiological water is to identify and then minimize the public health risk from consuming water intended for drinking and from exposure to recreational water (National Research Council, 2004).

3.3.1 Coliform bacteria

Coliform bacteria have been recognized as a suitable microbial indicator of drinking water quality. They make up around 10 percent of the intestinal microflora of the human and animal intestine. The term coliform organism refers to gram negative, catalase positive, oxidase negative, non sporing rods capable of growing aerobically on agar medium containing bile salts and able to ferment lactose within 48 hours at 35-37^{0}C with the production of both acid and gas (Cheesebrough, 1993 and Anderson and Davidson, 2002).

The most indicators to date have been "total coliform" bacteria, containing a subset of fecal coli common form. Using total coliform includes counting fecal and non-fecal coliform, which may result in data that are misleading and do not relate to the risk of water-borne illness (National Research Council, 2000). Fecal coliform is thought to be a better indicator of fecal contamination because fecal bacteria tolerate higher environmental temperatures; hence, they are more similar to the fecal-oral pathogens (Griffin *et al*., 2001). The indicator analyses used today require culturing the bacteria until numbers of "colony forming units" can be enumerated (Draft, 2002). Fecal-indicator organisms are excreted in large numbers by humans and warm blooded animals; therefore, the concentrations of indicator organisms in fecally contaminated water generally are much higher and more consistent than those of pathogens (Moe, 2002).

3.3.2 Thermotolerant Coliform

These are defined as the group of coliform organisms that are able to ferment lactose at 44-45ºC , they comprises the genus *Escherichia,* to at lesser extent, species of *Klebsiella, Enterobacter* and *Citrobacter.* Thermotolerant coliforms other than *E. coli*

may also originate originally from organically enriched water such industrail effluents or from decaying plant materials and soils. For this reason, the often-used term ‘fecal’ coliform is incorrect and its use should be discontinued.

Regrowth of thermotolerant coliform in the distribution system is unlikely unless suffient bacterial nutrients are present or unsuitable materials are in contact with the treated water, water temperature is above 13ºC and there is no free residual chlorine (WHO, 1993).

3.3.3 Utility of indicators

Indicator microbes are used to predict and/or minimize the potential risk from pathogenic microbes. Indicator organisms are useful in that numerous pathogens may be transmitted via a water route. Indicators circumvent the need to assay for each and every pathogen. Ideally, indicators are rapidly detected, easily enumerated, and have survival characteristics that are similar to those of the pathogens of concern surface, recreational, and shellfish growing water (Sharma *et al*., 2005).

One approach to monitoring drinking water for pathogens is direct detection of the pathogen itself. However, it would be practically impossible to test for each of the wide variety of pathogens that may be present in polluted water. Furthermore, these methods are often difficult, relatively expensive, and time-consuming. Instead, water monitoring for microbiological quality is primarily based on a second approach, which is to test for indicator organisms. The rationale for using indicator organisms can be crudely illustrated below: [indicator organism] a fecal contamination [pathogen] disease occurrence. This shows the indirect relationship between the concentration of indicator organisms and pathogen population. It has been established that when a certain population of pathogens is present in humans, they can cause diseases. Therefore, when indicator organisms are present, that would indicate the likely presence of pathogens too.

The indicator organism should fulfill the following criteria:

1. An indicator should always be present when pathogens are present.
2. Indicators and pathogens should have similar persistence and growth characteristics.
3. Indicators and pathogens should occur in a constant ratio so that counts of the indicators give good estimate of the numbers of pathogens present.
4. Tests for the indicator should be easy to carry out and applicable to all types of water.
5. The test should detect only the indicator organisms thus not giving false-positive reactions.

3.3.4 *Salmonella*

3.3.4.1 General description

Salmonella spp. belong to the family Enterobacteriaceae. They are motile, Gram negative bacilli that do not ferment lactose, but most produce hydrogen sulfide or gas from carbohydrate fermentation. Originally, they were grouped into more than 2000 species (serotypes) according to their somatic (O) and flagellar (H) antigens (Kauffmann-White classification). It is now considered that this classification is below species level and that there are actually no more than 2–3 species (*Salmonella* enterica or *Salmonella choleraesuis, Salmonella bongori* and *Salmonella* Typhi), with the serovars being subspecies. All of the enteric pathogens except *S.* Typhi are members of the species *S. enterica.* Convention has dictated that subspecies are abbreviated, so that *S. enterica* serovar Paratyphi A becomes *S.* Paratyphi A. Human health effects *Salmonella* infections typically cause four clinical manifestations: gastroenteritis (ranging from mild to fulminant diarrhoea, nausea and vomiting), bacteraemia or septicaemia (high spiking fever with positive blood cultures), typhoid fever / enteric fever (sustained fever with or without diarrhoea) and a carrier state in persons with previous infections. In regard to enteric illness, *Salmonella* spp. can be divided into two fairly distinct groups: the typhoidal species/serovars (*Salmonella* Typhi and *S.* Paratyphi) and the remaining non-typhoidal species/serovars. Symptoms of nontyphoidal gastroenteritis appear from 6 to 72 hrs after ingestion of contaminated

food or water. Diarrhoea lasts 3–5 days and is accompanied by fever and abdominal pain. Usually the disease is self-limiting. The incubation period for typhoid fever can be 1–14 days but is usually 3–5 days. Typhoid fever is a more severe illness and can be fatal. Although typhoid is uncommon in areas with good sanitary systems, it is still prevalent elsewhere, and there are many millions of cases each year. Source and occurrence *Salmonella* spp. are widely distributed in the environment, but some species or serovars show host specificity. Notably, *S.* Typhi and generally *S.* Paratyphi are restricted to humans, although livestock can occasionally be a source of *S.* Paratyphi. A large number of serovars, including *S.* Typhimurium and *S.* Enteritidis, infect humans and also a wide range of animals, including poultry, cows, pigs, sheep, birds and even reptiles. The pathogens typically gain entry into water systems through faecal contamination from sewage discharges, livestock and wild animals.

3.3.4.2 Routes of exposure

Salmonella is spread by the faecal–oral route. Infections with non-typhoidal serovars are primarily associated with person-to-person contact, the consumption of a variety of contaminated foods or water and exposure to animals. Infection by typhoid species is associated with the consumption of contaminated water or food, with direct person-to person spread being uncommon.

3.3.4.3 Significance in drinking-water

Waterborne typhoid fever outbreaks have devastating public health implications. However, despite their widespread occurrence, non-typhoidal *Salmonella* spp. rarely cause drinking-water-borne outbreaks.. Transmission, most commonly involving *S.* Typhimurium, has been associated with the consumption of contaminated groundwater and surface water supplies. In an outbreak of illness associated with a communal rainwater supply, bird faeces were implicated as a source of contamination. *Salmonella* spp. are relatively sensitive to disinfection. Within a water spread pathogen, control measures that can be applied to manage risk include protection of raw water supplies from animal and human waste, adequate treatment and protection of water during distribution. *Escherichia coli* (or, alternatively,

thermotolerant coliforms) is a generally reliable index for *Salmonella* spp. in drinking-water supplies.

3.3.4.4 Diseases and Symptoms

In general, *Salmonella* symptoms begin with nausea and vomiting and progress to abdominal pains and diarrhea. Additional signs and symptoms include fever, chills and muscle pains, and can last anywhere from several days to two weeks.

There are more than 2,300 types of *Salmonella* bacteria, although fewer than a dozen types are responsible for most illness in humans. Other symptoms may be present depending on the type of *Salmonella* germ causing infection. The most prevalent *Salmonella* related illnesses are:

Gastroenteritis: This increasingly common *Salmonella* induced illness is caused by the *S.* enteritidis bacterium, which is most often ingested through raw or undercooked meat, poultry, eggs or egg products.. The incubation period ranges from several hours to two days, and additional signs include blood in the stool.

Enteric fever: Also known as typhoid fever, this illness is caused by the *S.* Typhi bacterium and is most commonly contracted by drinking *Salmonella* contaminated water. The incubation period ranges from five to 21 days following infection. Additional signs and symptoms may include constipation, cough, sore throat, headache and mental confusion. Slightly raised, rose-colored spots on upper chest also may appear. In addition, a slowing of heartbeat (bradycardia) and enlargement of liver and spleen (hepatosplenomegaly) may be present.

Bacteremia: This condition results when *Salmonella* bacteria enter and circulate throughout the bloodstream. Infants and people with compromised immune systems are at special risk of developing serious complications, including infection of tissues surrounding the brain and spinal cord (meningitis) and infection within the bloodstream (sepsis). People with *Salmonella*-induced bacteremia may show few symptoms; however, fever can be present.

3.3.4.5 *Salmonella* serotypes

All of the *Salmonella* serovars belong to two species: *Salmonella bongari* containing eight serovars and *Salmonella enterica* containing the remaining 2300 serovars divided among six subspecies. The six subspecies of *S. enterica* are:

S. enterica subsp. *enterica* (I or 1)
S. enterica subsp. *salamae* (II or 2)
S. enterica subsp. *arizonae* (IIIa or 3a)
S. enterica subsp. *diarizonae* (IIIb or 3b)
S. enterica subsp. *houtenae* (IV or 4)
S. enterica subsp. *indica* (VI or 6)

Nomenclature and classification of these bacteria have changed a lot in recent years. Serovar names of *S. enterica* are not italicized and begin with a capital letter, e.g the strain formerly known as *S. typhimurium* is now known as *Salmonella enterica* serovar Typhimurium. This can be shorterned to *Salmonella* Typhimurium. Other subspecies of *S. enterica*(except in some subspecies of salame and houtenae) and those of *S. bongori* are not named, and are designated by their antigenic formula (Knutson, 2001).

3.4 Microbiological test

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

3.4.1 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 37ºC for 24 hours.

Advantages to membrane filter technique

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

3.5 Water borne diseases

Water-borne diseases are “dirty-water” diseases; mainly attributed to water that has been contaminated by human, animals or chemical wastes (Chabala and Mamo, 2001). Deteriorating water quality impacts health and economy of a country. A range of health and environmental health sector interventions, environmental pollution, unsafe water, poor sanitation practices, malnutrition, behavioral attitudes, illiteracy, climatic condition, and poverty continue to be responsible for the persistence of pre-transition diseases (Misra *et al*., 2003).

"Water-borne diseases are the major contributing factors of morbidity and mortality in the developing world. Despite technological innovations with regard to development of new drugs, diagnostic techniques, vaccines and several policy formulations, there is little evidence of reduction of disease burden (Shankar *et al.,* 2007). Water-borne diseases are among the most recent emerging and re-emerging infectious diseases throughout the world. The emerging and re-emerging infectious diseases have recently proven to be the biggest health threat worldwide and they contribute between 70-80% of health problems in developing countries. The most well known water-borne diseases such as cholera, dysentery, and typhoid are the leading causes of morbidity and mortality. The causative agents of water-borne diseases may be bacterial, viral and protozoal in nature, and this is true during both epidemic and endemic periods.

Table 1 Survival time of some excreted pathogens in sewage contaminated water

Pathogens	Survival Times
Giardia lamblia	20 days
Entamoeba	15 days
Ascaris egg	1 days
Escherichia coli	20 days
Salmonella Typhi	30 days
Salmonella Paratyphi	30 days
Vibrio cholerae	20 days
Hepatitis virus	60 days

Source: World Bank Technical Paper No. 51, quoted by Sharma, 1993

3.5.1 Sources of pathogens

The primary sources of water-borne pathogens are thought to be fecal pollution from humans and other animals (National Research Council, 2000) with transmission of disease determined by the concentration of the pathogen and an individual's natural resistance. Common sources of water-borne pathogens include outfalls from sewage treatment plants, septic tanks, meat packing plants, and waste lagoons from dairy operations. Other more diffuse sources of fecal contamination include storm runoff from streets (cat and dog feces) and farm fields (cow, sheep, horse and other farm animal feces), wildlife, and boat waste-discharge. Given the range of possible sources, it is clear that the sources and processes that transfer contamination into the receiving waters will be watershed specific. An important part of investigations in the TMDL listed watersheds of the Bay Area will be to determine what combination of sources and processes are causing impairment. This information will increase the likelihood of implementation of sound management solutions. Water-borne pathogens may be found in the water column and in bed sediment. Pathogens present in bed sediment can be reintroduced to the water upon disturbance during periods of high water flow in the winter season, or may be a direct cause of a loss of a beneficial use if these pathogens enter the tissue of animals such as bivalves.

Public health studies and regulatory monitoring tend to focus on human waste contamination of waters, though there are known human pathogens whose origins are not fecal contamination. *Vibrio vulnificus, V. parahaemolyticus, V. cholerae* (pathogen of the cholera bacteria that continues to cause large pandemics) occur naturally in the environment (National Research Council, 2000; Griffin et al., 2001) and cause illness through contaminated food or water. In recent years, the bilges of international ships have been recognized as possible sources for human pathogens, as well as other exotic organisms.

3.5.2 Outbreaks of water-borne diseases in Nepal

The WHO has estimated that up to 80% of all sickness and disease in the developing countries is caused by inadequate sanitation, polluted water, or unavailability of water (Cheesebrough, 1993). The number of outbreaks that has been throughout the world demonstrates that transmission of pathogens by drinking water remains a significant cause of illness. However, estimates of illness based solely on detected outbreaks are likely to be underestimating the problem. Out breaks of waterborne epidemic is rampant in Nepal as in most of the third world countries. Mortality and morbidity due to such disease still top the list. Every year the onset of the epidemics comes also with the monsoon (Shrestha, 2002). Many outbreaks of waterborne diseases probably are not recognized; therefore, their incidences are not reported. But there are real incidents of waterborne disease, in which improvements in drinking water quality could have saved many lives. As mentioned in the UNICEF situation analysis (UNICEF, 1987), in Nepal water and hygienic related diseases, are responsible for 15% of all cases and 8%of all deaths in the general population. In 1985, over 50% of hospital patients in Nepal were found to be suffering from gastro-intestinal disorder normally caused by waterborne pathogens. In 1990, cholera outbreak during summer hit different parts of the country including the capital city and caused an enormous loss of lives (DISVI, 1990) In 1990, Public Health Division recorded 23,888 gastro-enteritis cases in 39 districts with maximum 8,437 in the Kathmandu valley. In 1991 as well, the disease started to spring up at the beginning of the summer, striking badly the western, mid-western and central region of the country, which the eastern region was less affected. In between April to August, Public Health Division reported 43,520 gastro-enteritis cases with 1,252 deaths (ENPHO/DISVI, 1991).

There was outbreak of gastro-enteritis in Bhaktapur district during summer in 1995 (ENPHO/DISVI, 1995). The outbreak of gastro-enteritis in eleven districts of the Kingdom was recorded 2,300 cases with 69 deaths in between March-April, 1998 (Gorkhapatra Daily, 1998). Kathmandu Valley is affected by the severe water crises. The shortage of water is a problem in itself, but besides the quantitative shortage, the risk of waterborne diseases has become an even serious problem. People are not having access to hygienic drinking water. Outbreak of diseases like typhoid fever, cholera, dysentery, worm infestation, hepatitis and many other are spread by contaminated water, and are prevalent in urban and rural areas. A report from HMG Ministry of Health, 0.27% of patients had typhoid, 1.63% from Diarrhoea, and 0.07% from Jaundice and Hepatitis in Kathmandu in 2002/2003. In Lalitpur: typhoid cases were 0.58%, diarrhea 2.60%, and jaundice and infective hepatitis 0.09% and in Bhaktapur: typhoid 0.37%, diarrhea 1.53%, and jaundice and infective hepatitis 0.08%.

3.6 Study of Physico-Chemical Parameters of Drinking Water

Sharma, 1986 studies chemical parameters of tap water samples from 51 localities in Kathmandu. He found that little variation was observed in the chemical content of drinking water supplied to different localities in Kathmandu. The pH content ranged from 6.5 to 7.5, while $Caco_3$ content varied from 26 to 30 mg/l. The chemical constituents tested were found to be within the standards prescribed by (WHO, 1984).

CEDA, 1989 carried out similar studies from 13 sites including three reservoirs-Balaju, Maharajgunj, Mahankal Chour. The chemical parameters tested including pH, Dissolved Oxygen (DO) Biochemical Oxygen Demand (BOD), chemical Oxygen Demand (COD), Ammonia (NH_3), Nitrate (NO_3), Phosphate (PO_4) and chloride (Cl).

DISVI, 1990a detail chemical analysis of drinking water which corrode pipelines, affect the aesthetic quality of water, and indicate contamination by domestic sewage. The DISVI study covered treatment plants and reservoirs, hospital storage tanks and tape in the public water supply systems.

DISVI, 1990b studied in seven rural areas of Illam in Eastern Nepal found that water samples from six sources were acidic with pH values outside the acceptable limit set by WHO. Total hardness and chlorides were also not within the desirable level

DISVI, 1990c studied in seven rural areas in the Eastern Development Region of Morang, Sunsari, Jhapa, Siraha and Saptari and found that chemical parameters of most of the water samples within WHO standards for drinking water quality study found that only 3 percent of the samples collected from project tubewells and none of the samples collected from project other sources were above WHO guidelines.

Bottino *et al*., 1991 collected water samples of public taps, reservoirs and treatment plants from the different parts of the Kathmandu city during January 1988 to June 1988 and conducted chemical and microbiological analysis. Most of the chemical parameters of the measurable samples were found to be less than the permissible value whereas $N\text{-}NH_3$ and $N\text{-}NO_2$ ions concentration were higher than the limit in some samples. The iron concentration in most of the samples of the tap water, reservoirs and treatment plants were higher than the permissible values.

Ghimire, 1996 assessed 11 groundwater samples from Patan areas in two seasons. In rainy season, pH and temperature ranged from 5.6-6.3 and 19.7-22.5^{0}C respectively. Conductivity, hardness and N-ammonia for all samples were found to be within WHO permissible level. Chloride and ammonia ranged from 60-288 mg/l, and > 1.5 mg/l respectively. Similarly, in summer season, pH and temperature ranged from 5.9-6.7 and 21^{0}C – 22^{0}C.

Thapa, 1997 recorded most of the parameters analysed within WHO standard for drinking water except BOD value of some drinking water samples. Temperature, pH, total hardness, chloride were found within safe limits set by WHO, 1993

3.7 Microbial Examination of Drinking Water in Nepal

Most water pollution studies have examined the quality of drinking water supplied to the Kathmandu valley, particularly to its urban areas. Very few places have been survived elsewhere in the country. However, the extent of drinking water contamination in the areas covered by pollution studies suggests that the problem is nation wide.

Sharma, 1978 studied the quality of drinking water supplied to the household of the Kathmandu valley. He performed coliform tests on water samples from 39 localities. He found that the number of coliform cells per 100ml of water ranged from 4 to 460.

Sharma, 1986 in a same follow-up study examined water samples from different sources from Kathmandu and Lalitpur area. He found contamination level reached 4800 coliform cells per 100 ml. Water samples were taken in the dry summer season, the rainy season and winter season. The coliform bacteria count ranged from 0 to 4800 during the rainy season, 0 to 75 in winter and 0 to 460 per 100 ml in the summer months.

Sharma, 1993 carried out microbiological examination of water samples from different major cities of Nepal. He found that the highest coli form count was 2400 cells per 100 ml of water in Kathmandu, 4800 cells per 100 ml in Pokhara. He isolated enteric pathogenic bacteria such as *Salmonella* Typhi, *Proteus* spp. *Klebsiella* spp. and *Shigella* spp. from the water samples.

Ghimire, 1996 assessed 11 groundwater samples from Patan areas in two seasons. All the spouts and well were found to be heavily contaminated during rainy season.

Adhikari *et al.,* 1986 carried out coliform tests of 100 samples of drinking water (taps water, natural springs and ponds) from different areas in the Kathmandu valley and

found unsatisfactory results with more than 1800 coliforms per 100 ml of water. CEDA in 1989 tested water samples from different localities in Kathmandu and reported that all samples were contaminated with faecal matter indicating that tap and ground water sources were unsafe for drinking. Similarly, ENPHO/DISVI in 1990 conducted a study on water quality of 21 different stone spouts of the Kathmandu valley and found all faecally contaminated. The coliforms densities reported were in the range of 1 to 37,602 col/100 ml of water. Out of total, 81 % of the stone spout showed very high contamination (>100col/100ml) and 19% exhibited less than 100 col/100ml.

Bottino *et al*., 1991 collected water samples of public taps, reservoirs and treatment plants from the different parts of the Kathmandu city during January 1988 to June 1988 and conducted chemical and microbiological analysis. 70% of the samples did not contain coliforms from Sundarighat and Balaju treatment plants. In case of tap waters, total coliform counts in most of the samples are high, which indicates the contamination in the distribution system.

EHPHO/DISVI in 1992 conducted a one year monitoring on microbial quality of water supply in the Kathmandu valley. 39 samples from 5 treatment plants and 172 samples from 37 public taps were examined from different localities, 7 samples that is, 18% from treatment plants were found contaminated with an average faecal coliform of 4 col/100ml. Similarly, 50% samples from public taps were found contaminated. The bacterial densities in contaminated samples ranged from 1 to TMTC col/100ml.

DISVI, 1990 b carried out a bacteriological tests of drinking water quality assessment in seven rural areas of Ilam in Eastern Nepal. The seven villages were surveyed and samples were collected from 36 households and water sources including springs, spring well, aquifers (kuwa), rivers and river water reservoir. Bacteriological, physical and chemical parameters were tested. Study found that unacceptable levels of faecal coliform bacteria ranges from 2 to 2,400 cells per 100ml.

CEDA, 1989 tested water samples from different localities in Kathmandu. CEDA study found that all samples were contaminated with faecal materials. None of the tap and ground water sources were safe for drinking.

Joshi, 1987 carried out bacteriological tests of drinking water sources of two villages central Nepal nearer to the capital: chaubas (shivpuri) and syabru (Langtang). The coli form count ranged from 5-100 cells per 100ml of water. In chaubas, water from uncovered springs showed contamination within the range of 20-100 cells/ml.

Joshi et al., 1999 analyzed chemical parameters such as pH, total dissolved solid, total hardness, total alkalinity, Chloride, Sulphate, ammonia and minerals (Ca, Mg,Fe,Cd, Pb and Zn) in twenty different brands of mineral water for hygienic quality and chemical constituents. Total mesophilic count, coliforms, *Salmonella*, *Shigella* spp. were also analysed. Coliforms were detected in three samples, while faecal coliforms were detected in one sample and *Salmonella* spp. in two samplels. *Shigella* spp. was detected in two samplels. Total mesophilic count varied from 4 $x10^1$-7 $x10^3$ c.f.u /ml and was found in two samples.

Joshi, *et al.*, 2004 analyzed 160 samples, randomly collected from 86 tube wells and 77 open wells in urban areas and reported that more than 87% of analyzed ground water samples of tube wells and open wells were contaminated. Temperature ranged from 14.7^0C to 27.4^0C and pH ranged from 6.5 to 7.5. More than 87% of analysed ground water samples of tube wells and open wells were found to be contaminated with coliform bacteria.

Maharjan, 1998 examined 70 water samples randomly collected from shallow pumps, shallow wells, stone spout and dug well in urban area of Patan city and recorded 85.6% of samples contained total coliforms and 68.6% contained faecal coliforms.

Recovery of *Enterobacter* Spp was found maximum followed by *Escherichia coli* > *Citrobacter* spp > *Salmonella* spp and others.

Prasai, 2002 also reported the same report. Total bacterial and coliform count was 82.6% and 92.4% of total samples collected. Percentage recovery of *E. coli* (26.4%) was found to be maximum followed by *Enterobacter* spp (25.6%), *Citrobacter* spp (22.6%), *P. aeruginosa* (6.3%), *Serrattia* spp (2.52) and *Vibrio chlorae* (0.84%). 8.3% of water sample have pH value crossed the WHO guideline value. For conductivity, turbidity, and iron content 43.2%, 81.1% and 41.7% of water samples have crossed the permissible value. The hardness of all samples was within the guideline values and arsenic was not detected. ENPHO (2002) also carried out special test of 24 stone spouts around the city and found most water samples are chemically as well as bacteriologically contaminated.

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.

3.8 Studies on *Salmonella* serovars from different samples

Bhatta *et al.,* 2006 studied the occurrence and diversity of *Salmonella* serovars in urban water supply system of Nepal and detected *Salmonella* in 42 out of 300 water

samples. A total of 54 isolates were identified to genus level by standard tests and were subsequently confirmed by serotyping, phage typing and PCR detection of virulence genes (inv A and spv C). The predominant serotype was *Salmonella* Typhimurium, followed by *Salmonella* Typhi, *Salmonella* Paratyphi A and *Salmonella* Enteritidis.

Banani *et al.*, 2006 serotyped *Salmonella* isolates from commercial chickens and domestic pegions. *Salmonella* serotypes from chicken were identified as *S.* Enteritidis, *S.* Typhimurium, *S.* Nigeria, *S.* Rostock, *S.* Montevideo, *S.* Eko, *S.* Naestved, *S.* Moscow and *S.* Belgdam.The two isolates from pegions were *S.* Enteritids and *S.* II.

Bangtrakulnonth *et al.*, 2003 serotyped 44,087 *Salmonella* isolates from humans and 26148 from other sources through 1993 through 2002. The most common serovar causing human Salmonellosis in Thiland was *S. enterica* Weltevreden. Among the 984 isolates from water, they were *S.* Weltevreden (14.5%), *S.* Anatum (11.5%), *S.* Rissen (9.5%) and *S.* Derby (7.2%).

White *et al.*, 2003 isolated *Salmonella* from dog treats and found 41% (65/158) samples were positive for *Salmonella*. Eighty-four *Salmonella* isolates comprising 24 serotypes were recovered from the 65 positive samples Fourteen samples were contaminated with more than one *Salmonella* serotypes.

3.9 Status of Water Supplies in Nepal

The history of a piped water supply system in Nepal dates as far back at 1898, “Bir Dhara” was commissioned to supply water to the people of Kathmandu. A few water supply systems were installed in other towns of the country but practically no action was taken for the improvement of community water supplies up to the end of the nineteen sixties.

Till 1950, drinking water supply was limited to the urban areas of Kathmandu. Now most of the 33 urban centres in the country have piped water (CBS 1989). However, many supply systems provide water for only a few hours each day (ADB 1985) and despite receiving level of treatment bacteriological contamination remains high.

The total population having access to the piped water at the end of the decades of 70's, 80's and 90's were noticed to be 6%, 11% and 37% respectively. At present, the population having access to such piped water facility is estimated to be 70%. Although some rural water supply schemes exist, most of the rural population was traditional source of water, irrespective of quality (ADB, 1985). In rural areas as well, coliform contamination of drinking water is a major concern.

In early 1970, HMG of Nepal with the purpose to provide a water supply to the people established three active agencies which are:

a) Department of water supply and sewerage (DWSS)-responsible for providing drinking water supplies to all rural communities with more than 1500 population.

b) Ministry of Local Development (MLD)-responsible for providing drinking water supplies to all rural communities with more than 1500 population.

c) Water supply and sewerage Board (WSSB)-responsible for providing water supplies to tall the urban centers.

In 1980, only 6.6% (861,000) of the rural and 83% (687,000) of the urban population of the total population had house connections or easy access to a piped water supply.

The population in the hills and mid lands obtain water for their domestic and other uses from springs, ponds, canals, streams, river where as people in the Terai get water from privately owned dug wells and tube wells. Generally, the wells are polluted due to poor construction improper usage. In the urban areas nearly 20% of the population has house connections, while 55% get water from stand posts.. Since water is supplied only two times a day for about 4 to 8 hours, those who have connections provide a tank in their house to stone water during the flow hours.

At present, Nepal and many other developing countries is launching the international drinking water supply and sanitation. Decade plant as proclaimed by the UNO. By 1990, the government of Nepal planned to provide potable water to 67% of the rural and 94% of the urban population.

Those urban centers where piped water supply exists receive treatment with bleaching powder or lime used for disinfection. But most of our rural communities, there is no such water disinfection system.

3.9.1 Treatment of Drinking Water in Rural Community of Nepal

Most of our rural communities are depending on piped water supply disinfection or treatment of Drinking water has never been done for moral of contamination in rural communities. Some localities even do not have water sources for drinking water that people collect water from sources away from point of use.

The fundamental purpose of water treatment is to protect the consumers from pathogens and impurities in the water that may be offensive or injurious to human health. The purpose of water treatment is to provide water with most suitable composition. The major water treatment processes employed in practices are: filtration, ultra filtration and reverse osmosis, sedimentation, flocculation, adsorption, ion-exchange, addition of conditioning agents or additives, degasification, oxidation and disinfection. The selection of the appropriate system for water treatment includes different factors including requirements and preferences of the users, laws and regulations treated water availability requirement and finally minimum total cost comprising capital investment, operating costs, space, requirements, operating material usage, water and energy consumption (WHO, 1993.)

Urban treatment of water is similar globally and consists of steps pre disinfection, coagulation, sedimentation, filtration and disinfection etc. Those rural consumers who do not have piped water supply and disinfection treatment consume water directly

from natural sources and need conventional method to rid water of disease carrying germs, can take a long time and use up of a lot of fuel (IWTC,1990).

Rapid and slow sand filtration methods are also simple techniques which can be used in rural communities but these are not in common practice. Before selecting any treatment process for disinfection of drinking water, economic status of community people should be revalued. Most of our communities are below poverty line thus people even do not take interest to afford money for water treatment because they are happy to consume contaminated and polluted water unknowingly, thus, community people need a cheap reliable and simple treatment method for drinking water.

3.10 Antibiotics and bacterial resistance to antibiotics

In 1929 Alexander Fleming discovered the first antibiotic, penicillin, a metabolic product of *Penicillium* spp (*Penicillium notatum*). This discovery opened the era of antibiotics (Pelczar *et al*., 1986). An antibiotic refers to a substance produced by a microorganism or to a similar substance produced wholly or partly by chemical synthesis which in low concentrations, inhibits the growth of other microorganisms (Hugo and Russell, 1981).

The term broad spectrum is applied to antibacterial with activity against a wide range of Gram positive and Gram negative organism*S.* They include tetracyclines, aminoglycosides, sulphonamides, chloramphenicol, etc. Narrow spectrum antibiotics are those with activity against one or few types of bacteria, e.g. vancomycin against *Staphylococci* and *Enterococci*.

Antibiotic susceptibility tests measure the ability of an antibiotic or other antimicrobial agent to inhibit bacterial growth in vitro (WHO, 2002). In the treatment and control of infectious diseases, especially when caused by pathogens that are often drug resistant, sensitivity (susceptibility) testing is used to select effective antimicrobial drugs.. Sensitivity testing is not usually indicated when the sensitivity reaction of a pathogen can be predicted (Cheesebrough, 2000).

Most of the antimicrobial resistance which is now making it difficult to treat some infectious diseases is due to the extensive use and misuse of antimicrobial drugs which have favoured the emergence and survival of resistant strains of microorganisms. Bacteria become resistant to antimicrobial agents by a number of mechanisms, the commonest being.

- production of enzymes which inactivate or modify antibiotics
- changes in the bacterial cell membrane, preventing the uptake of an antimicrobial.
- modification of the target so that it no longer interacts with the antimicrobial.
- development of metabolic pathway by bacteria which enable the site of antimicrobial action to be by passed.

Bacteria can develop a large variety of mechanisms for antibiotic resistance and they also possess genetic mechanism for the spread of this resistance. Antibiotic resistant plasmids (R-Plasmids) are found in several bacterial genera both gram positive and gram negative. Repeated exposure of bacteria to antibiotics may lead to the development of resistant bacterial strains. Some bacterial cells under go genetic mutations or produce enzymes that destroy or inactivate antimicrobials, as a consequence, make them resistant to the antibiotics. In this way, they live and multiply in the presence of the drugs giving rise to a resistant strain (Crumplin and Odell, 1987).

Antimicrobial substances have proven an effective weapon against bacterial contamination and infection. But, the resistance, activity, stability and selective toxicity of some of these substances against certain microorganisms have noted to be lost. Most of the microbial resistance which is now making it difficult to treat some infectious diseases is of genetic origin and transferable between species and genera of bacteria. Acquired antimicrobial resistance is a worldwide problem (Cheesebrough, 1993).

3.10.1 Studies on antibiotic resistance among water borne bacterial isolates

Maharjan, 1998 isolated 120 enteric bacteria from 70 ground water samples randomly collected from Patan area He reported that resistant was more commonly directed toward nitrofurantoin (68.3%) followed by ampicillin (44.2%) and tetracycline (28.3%). Out of total 82.5% isolates were resistant to at least one antibiotic, 2.5% were resistant to five or more antibiotics.

Shrestha, 2002 investigated 95 water samples from five different sources. The organisms isolated were *Escherichea coli* (25.65%), *Enterobacter* spp. (68.4%), *Citrobacter* spp. (56.05%), *Shigella* spp. (27.55%), *Pseudomonas* spp. (24.7%), *Providomonas* spp. (24.7%) and *Serratia* spp. (10.45%). Isolated *E. coli* were analysed for antibiotic sensitivity pattern and 74.07% was found to be resistant to at least one antibiotic and MAR was found in 3.07% of the total isolates.

The study was also conducted in other countries like Chugh and Suheir, 1983 assessed drug resistance among *Salmonella* spp. prevalent in Kuwait. Out of 345 isolates, only 9.6% were sensitive to all the 14 drugs tested. There was resistance to tetracycline (69%), kanamycin (61%), ampicillin (56%) and chloramphenicol (38%). Multiple drugs resistance was observed in 71% of isolates and many of them were resistant to five or more drugs.

Antai, 1987 found 17.5-27.2 % of *E. coli* strains isolated from rural water supplies in Port Harcount, Nigeria resistant to three or more antibiotics. *E. coli* recovered from wells exhibited the greatest degree of multiple resistance. Some strains appeared to be ampicillin resistant (889.7%). Out of isolates 62.4-98% exhibited resistance to two or more antibiotics, and a total of 363 multiple-antibiotic resistant strains were identified.

Hosny *et al.,* 1988 examined 101 isolates from underground water in Cairo (Egypt) for their resistance towards four antibiotics. He found 32 and 18 isolates resistant to tetracycline and chloramphenicol, respectively.

Ramtete *et al.*, 1991 studied coliform isolates from different drinking water sources for antibiotic resistance. They found that resistance to ampicillin was found more prevalent among the coliform isolates.

Pandey and Musarrat, 1993 studies antibiotic resistance of coliform bacteria isolated from drinking water in the urban area of Aligarh city, India. Multiple-drug resistant. *E. coli* was isolated, and the frequency of drug resistance was determined to be significantly high to ampicillin and tetracycline.

Pathak, 1994 studied antibiotic resistance among coliforms isolated from about 49.4% of 89 drinking water samples in India. Out of coliform isolates 36.4% exhibited resistance for varying number of antibiotics (12nos). Resistance for ampicillin (56.8%) was found maximum. Half isolates exhibited multiple- antibiotic resistance.

Bissonnette *et al.,* 1995 examined 265 isolates from rural ground warer supplies in West Virginia (USA) for resistance to 16 antibiotics. All of the non-coliforms and 87% of the coliforms were resistant to at least one antibiotic. Percentage resistant to ampicillin, nitrofurantoin, tetracycline, chloramphenicol and nalidixic acid were observed as 69.4, 47.7, 32.3, 16.9 and 12.0 respectively, while below 10.0 for amikacin and gentamycin.

3.10.2 Studies on antibiotic resistance among *Salmonella* isolates

Asghar *et al*., 2002 isolated 6 *Salmonella* from a total of 150 samples.The isolates were found to be susceptible to gentamicin, ofloxacin, cefotaxime, amikacin, tobramycin, cefaclor, while resistant to ampicillin, cefamendole, chloramphenicol, gentamicin and cefuroxime.

Banani *et al.*, 2006 isolated *Salmonella* from commercial chickena and domestic pegions. The isolates were subjected to antibiotic sensitivity test. All of the tested isolates were susceptible to ciprofloxacin, ceftriaxone, ceftiofur, ceftizoxime and florfenicol. The percentage of *Salmonella* isolates susceptible to amikacin, chloramphenicol, gentamicin, lincospectin, sulphamethoxazole-trimethoprime, fluoquine, streptomycin, neomycin, enrofloxacin, furadolidone, cephalexin, nalidixic acid, doxycycline, oxyletracycline, tetracycline, nitrofurantoin, amoxicillin, ampicillin, furaltodone and colistin were 97.6, 92.3, 92.1, 84.7, 83.3, 65.6, 64.9, 63.6, 61.8, 46.0, 44.9, 25.4, 20.5, 20.0, 19.8, 16.4, 12.4, 11.1, 4.7 and 1.5% respectively.

White *et al.,* 2003 isolated *Salmonella* from animal derived dog treats and subjected them to antibiotic susceptibility test. The majority of the *Salmonella* isolates were susceptible to antimicrobials tested however resistance was observed to tetracycline (26%), streptomycin (23%), sulfamethoxazole (19%), chloramphenicol (8%) and ampicillin (8%). Twenty-eight (36%) *Salmonella* isolates were resistant to at least ane antimicrobial and 10 (13%) isolates displayed resistance to four or more antimicrobials.

Bhatta *et al.,* 2006 examined 54 isolates of *Salmonella* spp. for resistance to different antibiotics. Many isolates of *Salmonella* Typhi, *Salmonella* Paratyphi A, *Salmonella* Typhimurium and *Salmonella* Enteritidis were MDR as they were found resistant to at least four antibiotics. All the *Salmonella* Typhi and *Salmonella* Paratyphi A, *Salmonella* Typhimurium and *Salmonella* Enteritidis isolates were found to be sensitive to ciprofloxacin and ofloxacin. All the isolates of *Salmonella* Enteritidis and four isolates of *Salmonella* Typhimurium were resistant to ceftriaxone. No strains of *Salmonella* Typhi and *Salmonella* Paratyphi A were resistant to ceftriaxone.

Shahane *et al*., 2007 isolated 85 salmonellae (65.9% S. Typhi and 34.1% non-typhoidal salmonellae) from clinical samples of patients of Sassoon General Hospital. 86% non-typhoidal salmonellae were resistant to ampicillin; and 72.4% resistance to cefotaxime, chloramphenicol and gentamicin was observed. No resistance was seen with amikacin and ciprofloxacin.

CHAPTER - IV

4 MATERIALS AND METHODS

4.1 Materials

A list of materials, chemicals, equipments, media and reagents required for the study is listed in appendix I.

4.2 Methods

For this study, tap water samples were collected randomly from different localities of Kathmandu. The study period was from August 2008 to March 2009.

4.2.1 Study area

This study was conducted in 8 different localities of Kathmandu district viz kuleshwor, madannagar, kirtipur, teku, bagbaazar, maitidevi, balkhu and gaushala.

4.2.2 Collection of samples for water analysis

Sample collection was done according to standard method described by Cheesbrough, 2000. For bacteriological testing sterilized bottles having capacity 500ml containing 1 ml of 0.2N sodium thiosulphate was used. Bottles were tightly capped and great care was taken to prevent contamination. The sterile bottle was held by the base in one hand and the other hand was used to remove the stopper and cover the bottle. This way a total of 86 samples were randomly collected from tap of 8 different localities of Kathmandu district.

4.2.2.1 Sample collection from tap

1. Any external fittings from the tap were removed, such as an antisplash nozzle or rubber tube.
2. Outside nozzle of the tap was cleaned carefully, especially any grease, which has been collected.
3. The tap was turned on full, and the water was allowed to run to waste for 1 minute in order to flush the tap and discharge any stagnant water.
4. The tap was sterilized by igniting a piece of cotton-wool soaked in methylated spirit and holding it with a pair of tongs closed to the nozzle until the whole tap was unbearably hot to touch.
5. The tap was allowed to cool by running the water to waste for a few seconds.
6. The sample bottle was filled from a gentle flow of water, and replaced cap of the bottle.
7. The sample bottle was labelled with the sample code number immediately after filling it with the water sample.

4.2.2.2 Transportation and preservation of sample

The collected water samples were kept within an ice-box at 4ºC during transportation and analyzed in Central Department of Microbiology, Laboratory Kirtipur on the same day immediately after its delivery and always within 6 hours of collection. In some cases when immediate analysis was not possible, the samples were preserved at 4^0C

4.3 Processing of water samples

Basically two types of parameters viz. physicochemical and microbial were analyzed for assessment of drinking water quality.

4.3.1 Study of physico-chemical parameters of water samples

Analysis of the physico-chemical parameters of water were done by following "Standard Methods for the Examination of Water and Wastewater" (APHA, 1995). The temperature and pH of water samples were recorded at the site during sampling period. All other parameters were analyzed in the laboratory of Central Department of Microbiology.

A. Temperature

Temperature was determined with the help of a standard mercury thermometer graduated up to 50^0C. Soon after collection of the sample, thermometer bulb was immersed into the water and noted the reading.

B. pH

Hydrogen ion concentration in the sample was measured with the help of a pH meter by inserting the electrode into the water sample.

4.3.2 Microbial examination of water sample

4.3.2.1 Total coliform count

In this study, total coliforms were enumerated by the membrane filtration (MF) technique as described by APHA (1995).

Standard total coliform and thermotolerant coliform membrane filter procedure

1. First of all, sterile filter holder with stopper was assembled on the filter flask.
2. Using sterile blunt-edged forceps, a sterile membrane filter of pore size 0.45 µm (grid side up) was placed over the porous disc in such a way that it overlapped the entire circumference of sintered filterable area.
3. The sterile funnel was securely placed on the filter base.

4. The sample of water was well mixed by inverting the bottle several times, and then 100 ml of the water sample was poured into the funnel.

5. The sample was slowly filtered under partial vacuum by using electric vacuum pump.

6. The funnel was removed and the membrane was directly transferred, keeping its upper side upwards, onto a plate of M-Endo agar with the help of sterile forceps. Care was taken not to entrap air bubbles between the membrane and the medium.

   a. Then it was incubated for 24 hrs at 37^0C for total coliform and at 44.5^0C for thermotolerant coliform in an inverted position.

7. After proper incubation total colony forming unit (CFU) were counted.

4.3.2.2 Isolation of Salmonella

4.3.2.2.1 Concentration

Concentration is attained by filtration through 0.45 membrane filter in a membrane filter apparatus as follow:

a) 100ml of water sample was aseptically filtered through a milipore membrane filter of pore size 0.45 µm.

4.3.2.2.2 Enrichment of water sample for isolation

a) The membrane filters were enriched in tube containing 10 ml Selenite F Broth.
b) Then media was incubated at 37°C for 24hrs.
c) A loopful of suspension was streaked on S-S agar media and plate was incubated at 37°c for 24 hours.
d) Suspected colonies (black centered colonies and all other typical colonies) were sub cultured on nutrient agar.

4.3.2.3 Identification

Black centered colonies and all other typical colonies from SS agar were sub-cultured onto NA agar for pure culture. Bacteria isolated selective media were identified on the basis of their colonial characteristic, morphological characteristics and biochemical properties according to Bergey's Manual of Determinative Bacteriology, 1994.

A) Cell Morphology

Cell Morphology was studied using gram's reaction. The morphology was studied under oil immersion

B) Biochemical tests of bacterial isolates

Different biochemical tests like TSI, Urease, O/F Catalase, Oxidase, SIM, MR-VP and Citrate tests were performed based on the ability of microorganisms to produce enzymes thus utilizing different substrates of the environment as described by Cheesbrough, 1993 (Appendix VI).The isolated pure colonies were inoculated on to different bio chemical medium for the test as shown in table 1.

Table 1. Biochemical Tests performed for Identification of Enteric Bacteria

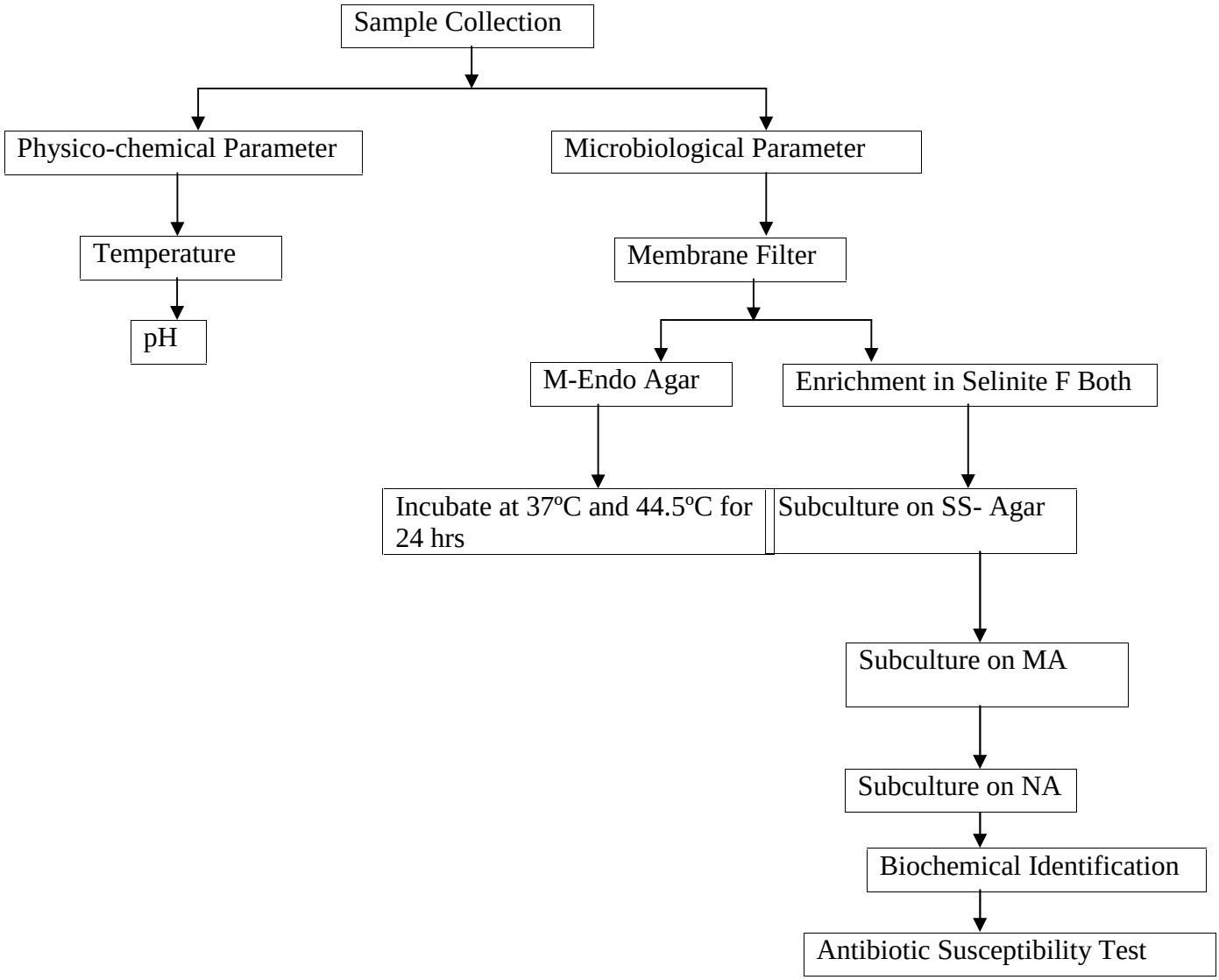
S.N.	Biochemical Medium	Tests
1.	3% Hydrogen Peroxide	Catalase
2.	1% Trimethyle paraphenylelediamine dihydrochloride	Oxidase
3.	Glucose Phosphate broth	Mixed acid Fermentation
4.	Peptone water	Indole
5.	Simmon's Citrate	Utilization of citrate
6.	Triple Sugar Iron agar	Acid Fermentation
7.	Urea Agar	Urease

4.4 Study of antibiotic susceptibility test

Antibiotic susceptibility test of the isolates were assayed using a modified Kirby-Bauer disk diffusion method (Bauer et al., 1966). Cells were grown at 37^0C in 5ml of nutrient broth for about 4 hrs using pure cultures as inoculum. The turbidity developed was compared with that of standard barium sulphate. A sterile cotton swab was dipped into the properly prepared inoculum and firmly rotated against the upper inside wall of the tube to expel excess fluid, and then swabbed onto Mueller-Hinton agar. During swabbing the plate was streaked with the swab three times turning the plate 60^0C between each streaking to achieve a lawn of confluent bacterial growth. The plate was kept at room temperature for 5 to 10 minutes, but no longer than 15 minutes to dry the inoculum. Sensitivity discs from their respective vials were carefully placed in the plate with the help of a flamed forceps, at equal distance and sufficiently separated from each other to avoid the overlapping of the inhibition. The discs were lightly pressed with the forceps to make complete contact with the surface of the medium. The plate was allowed to stand at room temperature for 30 minutes for prediffusion and then incubation periods at 37^0C for 24hrs. The diameter of the zone of inhibition was measured at the end of the incubation period. Organisms were classified as sensitive or resistant to an antibiotic according to the diameter of the inhibition zone surrounding each antibiotic disk. Organisms considered to be intermediate resistance were scored as sensitive.

4.5 Quality Control

In all the test performed *Escherichia coli* ATCC 25922 was used as reference strain for quality control (NCCLS, 1997, 2002).



Flow chart 1: Diagrammatic representation of overall procedure

CHAPTER - V

5. RESULTS

5.1 Water quality assessment

A total of 86 samples of drinking water randomly collected from different localities of Kathmandu were assessed for the microbiological (total coliform, thermotolerant coliform and salmonellae) and physico-chemical parameters (temperature and pH).

5.1.1 Physicochemical parameter of water

Physicochemical parameters such as temperature and pH of collected water samples were analyzed.

5.1.1.1 Temperature

A distinct variation in temperature of tested water samples were recorded ranging from 11.8^0C to 26.7^0C with an average of 19.25ºC as shown in table 2.

5.1.1.2 pH value

pH value of water samples showed no much variation. The pH value ranged from 6.2 to 8.8 as shown in table 3. A comparison of pH values with the WHO guideline and national standard revealed that 75(87.21%) samples were within the permissible limit while 5.0 (5.81%) samples showed below the limit and 6(6.98%) samples crossed the upper limit of guideline value (Table 3).

Table 2: Results of Chemical Analysis of Water Samples.

S.N	Parameters	Minimum	Maximum	Average
1.	Temperature	11.8	26.7	19.25
2.	pH	6.2	8.8	7.5

Table 3: Comparisons of temperature and pH values of water samples with WHO guideline

S.N	Parameters	Within WHO Guideline value(%)	Below WHO Guideline value(%)	Above WHO Guideline value(%)
1.	Temperature	-	-	-
2.	pH	75 (87.21)	5(5.81)	6(6.98)

5.2 Bacteriological analysis of water

Bacteriological analysis of the collected water samples were performed by membrane filter method.

5.2.1 Coliform count

Of 86 water samples collected, 86(100%) of the samples showed the presence of coliforms. The coliform count ranged from 44 - >300 c.f.u/100ml of water samples hence crossing the WHO guideline value of 0 c.f.u/100ml water sample. (Table 5).

Table 4: Comparison of coliform count with WHO guideline

S.N	Source	No. of sample within WHO Guide line value	No. of sample above WHO guide line value(%)	Total
1.	Tap water	0	86(100)	86

5.2.2 Thermotolerant coliform count

Of 86 samples collected, 20(23.3%) of the samples showed the presence of thermotolerant coliform crossing the WHO guideline value of 0 c.f.u/100ml water sample as given in table 6. Thermotolerant coliform count ranged from 0-52 c.f.u/100ml sample.

Table 5: Comparison of coliform count with WHO guideline

S.N	Source	No. of sample within WHO Guide line value(%)	No. of sample above WHO guide line value(%)	Total
1.	Tap water	66(76.7)	20(23.3)	86

5.2.3 Isolation and identification of *Salmonella* spp.

Of 86 drinking water samples collected from 8 different localities 4(4.7%) samples form three localities were positive for *Salmonella* (as given in table 7) A total of ten organisms were identified as *Salmonella* by conventional biochemical method; of which 1(10%) was identified as *S.* Paratyphi A and 9(90%) as non-typhi.

Table 6: *Salmonella* isolates from water samples

S.No	*Salmonella* isolates	Positive(%)
1.	*S.*Paratyphi A	1(10)
2.	Non-typhi	9(90)

Table 7: Area wise distribution of *Salmonella*

Code no.	**Positive(%)**	**Total(%)**
kw1-kw10	0(0.0)	10(11.6)
bm1-bm10	0(0.0)	10(11.6)
kp1-kp10	0(0.0)	10(11.6)
t1-t10	0(0.0)	10(11.6)
md1-md10	1(25.0)	10(11.6)
bb1-bb16	2(50.0)	16(18.6)
b1-b10	1(25.0)	10(11.6)
g1-g10	0(0.0)	10(11.6)
Total	4(100.0)	86(100.0)

NB: kw - kuleshwor, bm - madannagar, kp - kirtipur, t - teku, md - maitidevi, bb - bagbazar, b - balkhu and g - gaushala.

5.2.3 Antibiotic Susceptibility Pattern of *Salmonella*

Ten isolates were subjected to antibiotic susceptibility pattern using 8 different antibiotics. Antibiotic susceptibility pattern showed that all the isolates were 100% susceptible to tetracycline, chloramphenicol, cotrimoxazole, nalidixic acid and ciprofloxacin and 70% were restitant to amoxicillin, 20% to cephalexin and 10% ceftizoxime.

Table 8: Antibiotic Susceptibility Pattern of *S*. Paratyphi A

Code no.	Organism	Antibiotics							
		Am	C	T	Co	Cp	NA	CF	CK
S2	S. Paratyphi A	R	S	S	S	S	S	S	S

Table 9: **Antibiotic Susceptibility Pattern of non-Typhi**

Code no.	Organism	Antibiotics							
		Am	C	T	Co	NA	Cp	CF	CK
S1	Non-Typhi	R	S	S	S	S	R	S	R
S3		R	S	S	S	S	S	S	S
S4		R	S	S	S	S	S	S	S
S5		I	S	S	S	S	S	S	S
S6		I	S	S	S	S	S	S	S
S7		I	S	S	S	S	S	S	S
S8		R	S	S	S	S	S	S	S
S9		R	S	S	S	S	S	S	S
S10		R	S	S	S	S	R	S	S

NB: Am-Amoxicillin (10 µg); C-Chloramphenicol (30 µg); T-Tetracycline(30 µg); Co-Co-Trimoxazole (25 µg); Cp- Cephalexin(30 µg); NA- Nalidixic acid (30 µg); CF-Ciprofloxacin (5 µg); CK- Ceftizoxime(30 µg)

Table 10: Percentage of Antibiotic susceptibility pattern of isolates

S.N	Antibiotics	% Resistant	**%** Intermidiate	% Sensitive	Total isolates
1.	Amoxycillin	70	30	0	10
2.	Ceftizoxime	10	0	90	10
3.	Cephalexin	20	0	80	10
4.	Cotrimoxazole	0	0	100	10
5.	Nalidixic acid	0	0	100	10
6.	Tetracyclin	0	0	100	10
7.	Ciprofloxacin	0	0	100	10
8.	Chloramphenical	0	0	100	10

CHAPTER - VI

6. DISCUSSION AND CONCLUSION

6.1 DISCUSSION

Kathmandu being the capital is a very crowded and polluted city. The growing imbalance between supply and demand have resulted in pollution and environmental degradation. Also the old and leaky pipes and the supply of untreated water in parts of city is the major cause of bacterial contamination (The Himalayan Times, 2009). Hence this study was undertaken to assess the occurrence of *Salmonella*e in piped water supply of Kathmandu. Antibiotic susceptibility pattern of the isolates were studied and physico-chemical parameters like temperature and pH of collected water samples were also assessed.

Physico-chemical Parameters were analyzed to identify the physical status of water. Distinct variations in temperature of tested water samples were recorded. The temperature ranged from 11.8 ^{0}C to 26.7^{0}C with an average of 19.25ºC. In the previous studies most of the physico-chemical parameters of drinking water lied within the WHO guideline value (Bottino *et al.*, 1991; Ghimire, 1996; Thapa, 1997 and Pradhananga *et al.* 1993). In similar studies done by Joshi *et al.*, 2004 and Ghimire, 1996 the temperature ranged from 14.7ºC-27.4ºC and 19.7ºC-22.5ºC respectively. Water bodies undergo temperature variation along with normal climatic fluctuations and seasonal variation. Temperature of water is influence by latitude, altitude, season, time of day, air circulation, cloud cover and the flow and depth of water body. A rise in temperature of the water leads to the speeding up of the chemical reaction in water, reduces the solubility of gases, enhanced growth of microorganisms and amplifies the tastes, odor and corrosion problem however there is no standard guideline value regarding the temperature. Also temperature increase in the water bodies is an indicative of microbial activity.

Of 86 samples pH of 87.21% of water samples were recorded within the permissible limit while 5.81% samples showed below the limit and 6.98% samples crossed the

upper limit of guideline value. The pH value range from 6.2 to 8.8. Water samples from six different sources were acidic with pH value outside the limit as set by WHO (DISVI 1990 b). In drinking water, acidic pH may cause corrosion of metal pipes in the distribution system and alkaline pH adversely affect the disinfection process.

In this study, of 86 samples 100% samples contained coliforms. The previous studies carried out to-date showed that the water quality is deteriorating with time in all types of sources. All studies carried out by Sharma, 1978; Sharma 1986; Adhikari *et al.*, 1986; CEDA 1989; Bottino,1991 and ENPHO/DISVI, 1992 indicates that the public water supply is far from satisfactory in almost all localities. Sharma, 1978 found only 50% samples contaminated with faecal material in Kathmandu but in a follow up study in 1986 Sharma found that the level of coliform contamination had significantly increased upto 85% in between nine years. Adhikari *et al.*, 1986 found 88% of the samples unsatisfactory. Likewise, CEDA, 1989 recorded that all samples were contaminated with faecal matter and none of the tap and ground water sources were safe for drinking. Bottino, 1991 found total coliforms in most of the water samples were high indicating contamination in distribution system. Sharma, 1993 examined bacteriological quality of the potable water of different urban and rural areas of Nepal and reported that maximum densities of coliforms and faecal coliforms were detected in Kathmandu, then in Hetauda, followed by Birgunj, Pokhara and Biratnagar. This study indicates urban area is heavily contaminated by coliform bacteria than in rural area. There is high chances of microbial contamination in water due to improper treatment (chlorine demand is not calculated before chlorination), during storage in resorvoir or due to leaky pipes during distribution. The presence of coliform indicates the likely presence of pathogens too.

Water-borne diseases are among the most emerging and re-emerging disease. It is therefore imperative that safe drinking water is provided. Untreated water is being supplied to most part of the city. The waste water drainage pipelines and sewer lines are laid close to the drinking water distribution lines and chances of cross

contamination are higher (Chitrakar and Jackson, 2002; Nepal Water Supply Corporation 2004/2005). *Salmonella* spp. was detected in most of the water samples (Chitrakar and Jackson,2002; Bhatta *et al.*,2006). Of 300 samples collected 14% were positive for *Salmonella* and the organisms were identified as S. Typhi, S. Paratyphi A, S. Typhimurium and S. Enteritidis by conventional biochemical method. In this study 4.65% samples were positive for *Salmonella*. Ten organisms were identified as *Salmonella* by the conventional biochemical method, of which 10% was S. Paratyphi A and 90% were non-typhi. *Salmonella* was detected only in those samples that were positive both for total coliform and thermotolerant coliform but not all the samples containing the indicator organisms were positive for *Salmonella*. *Salmonella* enterica Paratyphi A has emerged as a major cause of enteric fever in Asia. Remarkably, studies in some geographic localities have shown that up to 64% of the patients with culture-proven enteric fever are infected with S. Paratyphi. A rather then S.Typhi strains. From a clinical, epidemiological and infection control prospective, it is essential to gather accurate data on all the precise etiological agent of enteric fever because present day vaccines are effective against S. Typhi only and antibiotic susceptibility pattern between serovars potentially differ markedly from one locality to the next (Hong-Yu Qu *et al.* 2007). Salmonellosis in human caused by non-typhoid *Salmonella* strains usually result in a self-limiting diarrhea that does not warrant antimicrobial therapy, however there are occasions when these infection can lead to life-threatining systemic infection that require effective chemotherapy. Of increasing concern is the worldwide emergence of MDR phenotypes among *Salmonella* serotypes, in particular *S*. Typhimurium and more recently S. Newport (White *et al.* 2003). *The* presence of *Salmonella* spp. in water samples indicated that the microbiological quality of public water supplying Kathmandu is of poor quality and chances of water borne *Salmonella* infection is higher in people consuming the water without proper disinfection.

Occurrence of *Salmonella* in UWSS of Nepal is due to old leaky pipes, improper disinfection and cross-contamination (Jackson 2002; Lewis *et al.* 2005). The presence of bacteria in WSS (Water Supply System) indicates that they might have adhered and formed biofilms in the interior of distribution pipelines. Water distribution systems have been reported to provide unique condition for the development of

biofilm community(Ford 1999; Scher *et al.* 2005). Chances of biofilm formation in Nepalese water distribution system is due to intermittent water supply (Jackson 2002; Nepal Water Supply Corporation 2004/2005).

Areawise distribution of *Salmonella* showed that higher percentage (50%) of *Salmonella* was isolated from bagbazaar area. The pipelines of bagbazaar is old and leaky at most places and the piped water in the area is turbid.

Ten Salmonellae isolates were assayed against 8 different antibiotics. The majority of isolates were susceptible to most of the antimicrobials tested; however resistance was found most commonly directed towards amoxicillin (70%), chephalexin (20%) and ceftizoxime (10%). Similarly, Bhatta *et al.*, 2006 examined 54 isolates of *Salmonella* spp. for resistance to different antibiotics. Many isolates of *S.* Typhi, *S.* Paratyphi A, *S.* Typhimurium and *S.* Enteritidis were MDR as they were found resistant to at least four antibiotics. All the *S.* Typhi and *S.* Paratyphi A, *S.* Typhimurium and *S.* Enteritidis isolates were found to be sensitive to ciprofloxacin and ofloxacin. All the isolates of *S.* Enteritidis and four isolates of *S.* Typhimurium were resistant to ceftriaxone. No strains of *S.* Typhi and *S.* Paratyphi A were resistant to ceftriaxone. White *et al.*, 2003 found majority of the *Salmonella* isolates were susceptible to antimicrobials tested however resistance was observed to tetracycline (26%), streptomycin (23%), sulfamethoxazole (19%), chloramphenicol (8%) and ampicillin (8%). Twenty-eight (36%) *Salmonella* isolates were resistant to at least one antimicrobial and 10 (13%) isolates displayed resistance to four or more antimicrobials and Banani *et al.*, 2006 subjected the *Salmonella* isolates to antibiotic sensitivity test. All of the tested isolates were susceptible to ciprofloxacin, ceftriaxone, ceftiofur, ceftizoxime and florfenicol. The percentage of *Salmonella* isolates susceptible to chloramphenicol, sulphamethoxazole-trimethoprime, cephalexin, nalidixic acid, tetracycline, nitrofurantoin, amoxicillin and ampicillin were 92.3, 83.3, 44.9, 25.4, 19.8, 12.4 and 11.1 respectively.

6.2 Conclusion

Distinct variation in physicochemical parameters was not observed. Presence of total coliform in all the samples indicated the likely presence of pathogens too. *Salmonella* was present in 4(4.7%) samples and the isolates were identified as *S*. Paratyphi A and non-typhi. Isolation of *Salmonella* from drinking water and it's resistance to antibiotics indicates the risk associated to the exposed population. This study indicates that quality of piped water of Kathmandu is very poor hence strict monitoring in water treatment is needed. Systematic and regular mechanism for surveillance and monitoring of waterborne pathogens in water supply system is a must.

CHAPTER - VII

7 SUMMARY AND RECOMMENDATIONS

7.1 SUMMARY

"Water-borne diseases are the major contributing factors of morbidity and mortality in the developing world. Despite technological innovations with regard to development of new drugs, diagnostic techniques, vaccines and several policy formulations, there is little evidence of reduction of disease burden (Sarkar *et al.*, 2007). This study was conducted to isolate and characterize *Salmonella* from UWSS of Kathmandu.

1. A total of 86 water samples were randomly collected from different localities of Kathmandu. All water samples were analyzed for physicochemical and microbiological parameters to assess the drinking water quality.
2. Distinct variation in physicochemical parameter was not observed. 5.0(5.81%%) samples showed below the limit and 6(6.98%) samples crossed the upper limit of guideline value.
3. Of 86 water samples 100% samples showed the presence of coliform while thermotolerant coliform was present in 23.3% samples.
4. All water samples were analysed for the presence of *Salmonellae*. 4 (4.7%) samples were positive for *Salmonella*. A total of 10 Salmonellae were isolated and identified as 1(10%) S. Paratyphi A and 9(90%) non-typhi.
5. Isolates were subjected to antibiotic susceptibility test using 8 different antibiotics. Among the total isolates resistance was directed towards 70% Amoxicilin, 20% Cephalexin ,14.28% Ceftizoxime respectively.

7.2 RECOMMENDATION

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

- This study is limited to Kathmandu. Thus the findings may not generalize the whole Kathmandu valley. Water quality testing should also be done in other parts of Kathmandu Valley, which are not covered by the present study.

- Awareness building and motivational programs regarding water pollution and its possible health impacts should be organized by NWSC and other concerned agencies to make people aware and conscious about the quality of drinking water.

- People should be trained regarding the simple and cheap techniques of water treatments such as boiling, filtering and chlorinating.

- Isolated organisms were identified by the conventional biochemical method. Serotyping of the isolates must be done for confirmation.

- Regular monitoring and 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.

REFERENCE - VIII

ADB (1985): Nepal water supply and sanitation sector profile, Asian Development Bank, Manila.

Adhikari R, Rai SK, Pokhrel BM and Khadka JB (1986) Bacteriological Study of Drinking Water of Kathmandu Valley. Journal of Institute of Medicine 8 (3): 313-376

Agarwal A (1981) Water, Sanitation, Health - for all: Prospects for the International Drinking Water Supply and Sanitation Decade, 1981-90. International Institute for environment and Development, Earthscan Publication, London, pp 2

Anderson S and Davidson P (2002) Drinking Water and Recreational Water Quality: Microbiological Criteria, Food Science and Technology at University of Idaho CIS 1069, pp 7-9

Antai SP (1987) Incidence of *S. aureus*, Coliforms and Antibiotic-Resistant Strains of *E. coli* in Rural Water Supplies in Port Harcourt (Nigeria), Journal of Applied Bacteriology 62(4): 371-376

APHA manual (1995) Standard Methods for the Examination of Water and Wastewater, 20th edn. Washington, DC: American Public Health Association (APHA), the American Water Works Association (AWWA) and the Water Pollution Control Federation (WPC).

Asghar U, Saba U.S, Samad A and Qazibash A.A (2002) Identification, characterization and antibiotic susceptibility of *Salmonella* and *Shigella* species isolated from blood and stool samples of patients visiting N.I.H, Islamabad J.med. sci., 212:85-88, 2002.

Banani M, Pourbakhsh S.A, Khaki P, Nikookhesal G.H (2006) Serotyping and drug sensitivity of Salmonella isolates from commercial chickens and domestic pegions. Razi institute, Karaj, Iran

Bangtrakulnonth A, Pornreongwong S, Pulsrikaran C, Sawanpanyalert P, Hendrriken R.S, Lo Fo Wong D.M.A and Areslrup F.A (2003) Salmonella serovars from humans and other sources in Thiland, 1993-2002. Emerging Infectious Diseases

Bhatta DR, Bangtrakulnonth A, Tishyadhigama P, Saroj SD, Bandekar JR, Hendriksen RS, Kapadnis BP (2007) Serotyping, PCR, phage-typing and antibiotic sensitivity testing of Salmonella serovars isolated from urban drinking water supply systems of Nepal. Letters in Applied Microbiology

Bergey's Manual of systematic Bacteriology Vol. (1986) : Sneath PHA, Mair NS, Sharpe ME, Holt J.G, Williams and Wilkins, Baltimore , London, Los Angeles, Sydney, Tokyo

Bissonnette GK, Mekeon DM and Calabrese JP (1995) Antibiotic Resistant Gram-Negative Bacteria in Rular Groundwater Supplies, Water Resources 29(8): 1902-1908

Bottino A, Thapa A, Scatoline A, Ferino B, Sharma S and Pradhananga MT (1991) Pollution in the Water Supply System of Kathamndu City. J. Nep. Chem. Soc 10: 33-44

CBS (1989): Statistical Year Book of Nepal 1989. HMG/NPC Central Bureau of Statistics, Kathmandu.

CEDA(1989): A study on the Environmental Problems due to urbanization in some selected Nagar Panchayats of Nepal. Report Submitted to UNDP/Kathmandu. Centre for economic development and Administration, Tribhuvan University, Kathmandu.

Chabalala H and Mamo H (2001) Prevalence of Water-borne Diseases within the health facilities in Nakuru District, Applied Epidemiology University of Nairobi, Kenya, pp 34

Chapman D (1993) Water Quality Assessment. A guide to the Use of Biota, Sediments and Water in Environmental monitoring. UNESCO, WHO, UNEP and Chapman and Hill

Chand AB, Sherchand JB and Basnet SR (2001) Study of aetiology of children diarrhoeal cases in relation to parasites to determine antibiotic sensitivity pattern. Journal of Nepal Association Medical Laboratory Sciences 3:17-20

Cheesbrough M (1993) Bacteriological testing of water supplies In: Medical Laboratory

Cheesbrough M (1993) Microbiology tests In: District Laboratory Practice in Tropical countries. Low Price Edition, Cambridge University Press, UK, pp 1-266

Cheesebrough M (2000) Water Related Diseases and Testing of Water Supplies. In: District Laboratory Practice in Tropical Countries Part (2). Low Price Edition 2000, Cambridge University Press, pp143-157

Chitrakar R.L and Jackson D (2002) Proposed format for Nepal's drinking water standards/guidelines. National drinking water quality standard task force report, Government of Nepal.

Crumplin GC and Odell M (1987) Development of Resistance to Ofloxacin. Drug 34 (1): 1-8

Department of Health Services (1990) Annual Report (1997/98) DoHS, Kathmandu

DISVI (1990) Water Quality Monitaring of Kathmandu City Water Supply. Environment and Public Health Organization and DISVI International Co-operation, Kathmandu

Draft (2002) Pathogens Occurance and Analysis in Relation to Water Quality Attaininment in San Francisco Bay Area Watersheds, Estuary Institute, pp 3

DoHS (2004/2005) Annual report, Ministry of Health and Population, Department of Health Services, Kathmandu, Nepal

Environment and Public Health Organization / Italian International Co- operation (1990) Water quality of the Stone Taps (Kathmandu City) 1989-1990. ENPHO/DISVI International Cooperation, Kathmandu

Environment and Public Health Organization / Italian International Co- operation (1991) A case Study on Gastroenteritis Epidemic in the Mid-Western Region of Nepal (Rukum and Dang). ENPHO/DISVI International Co- operation, Kathmandu

Environment and Public Health Organization / Italian International Co- operation (1995) Bacteriological Quality of Drinking Water in Kathmandu and Patan City: 1988-1995. ENPHO/DISVI International Co- operation, Kathmandu

ENPHO (2003) An overview of arsenic contamination and its mitigation in Nepal Red Cross Society Program Areas. Environment and Public Health Organization, Kathmandu

Environment and Public Health Organization (2005) Arsenic Vulnerability in Ground Water Resources in Kathmandu Valley (Final Report). A joint study conducted by JiCA Exper Office at MPPW and ENPHO, Kathmandu, pp 24

A case Study on Gastroenteritis Epidemic in the Mid-Western Region of Nepal (Rukum and Dang). ENPHO/DISVI International Co- operation, Kathmandu

Esrey SA (1999) Effects of improved water supply and sanitation on ascariasis, diarrhoea, dracunculiasis, hookworm infection, schistosomiasis and trachoma. Bulletin of the World Health Organization 69(5):609-21

Ford, T.E. (1999) Microbiological safety of drinking water, United States and global perspectives. Environ Health Perspect supplements 107(S1), 1–33.

Ghimere MK (1996) Ground Water Quality Analysis in Patan Area. M. Sc. Dessertation Submitted to the Central Department of Meterology, Tribhuvan University, Kathmandu

Gorkhapatra (1998) Outbreaks of Gastero-enteritis and Fever. Gorkhapatra Daily, May 18th 1998

Griffin DW, Lipp EK, Mclaughlin MR and Rose JB (2001) Marine Recreation and Public Health Microbiology: Quest for the Ideal Indicator. BioScience 51(10): 817 – 825

Hugo WB and Russell AD (1981) Antimicrobial Agents. In: Pharmaceutical Microbiology, 2nd edition. Black Well Scientific Publication : 69, pp 99-134

Hong-Yu Qu, Cindy The Shurn Ju, Kwai-Lin Tong, Ahmad N, Deng Z, Barer M.R and Rajkumar K (2007) Translational Genomics to develop a *Salmonella enterica* serovar Paratyphi A Multiplex PCR Assay. Journal of Molecular Diagonosis 2007, vol 9, no.5.

Hosny I., EI-Zanfaly, HT, Fayez M and Shaban A (1988) Incidence of Antibiotic-Resistant Bacteria in Underground Water. Environ. Int. 14(5): 391-394

Indication for Water Borne Pathogens (2004) Commitee on Indicators for Water-borne Pathogens. National Research Council, Paperback 2004, pp 7

ISRSC (2002) District Demographic Profile of Nepal, Informal Sector Research and Study Centre, Kathmandu, Nepal, pp 246-56

IWTC (1990) : Woman and water. International Women's Tribune Centre (IWTC) 777 United Nations Plaza, New York, NY 10017, July 1990.

Jackson D (2002) Chemical safety of drinking water: Nepal's trial of WHO publication for prioritizing chemicals, project report. Nepal: WHO country office.

Joshi DR and Baral MP (2004) Chemical and Microbial Quality of Groundwater of Kathmandu Valley. Abstracts of 4th National Conference on Science and Technology. March 23-26, pp 252

Joshi R, Shrestha S, Manita J and Karki T (1999) Microbial Quality and Chemical Constituents of Different Brands of Mineral Water Available in Kathmandu Valley. Proceedings of 3rd National Conference on Science and Technology, March 8-11, Vol. 3: 638-641

Maharjan M (1998) Assessment of groundwater quality and study of antibiotic resistance and oligodynamic action against some isolated enteric bacteria. M.Sc Thesis, Central Department of Microbiology, Tribhuvan University, Kirtipur, Kathmandu

Manual in Tropical Countries. ESBL Reprinted Edition,pp 206-24

Misra R, Chattterjee R and Rao S (2003) India Health Report, Oxfort University Press, New Delhi, pp 9

Moe C (2002) Waterborne Transmission of Infectious agents, in Hurst CJ, Manual of Environmental Microbiology (2d ed.) Washington DC, ASM Press, pp 184–204

NCCLS (1997) National Committee for Clinical Laboratory Standards (M 2- A6) Performance Standards for Antimicrobial Disc Susceptibility Tests, 6th edn. PA, USA: Wayne.

NCCLS (2002) Performance Standards for Antimicrobial SusceptibilityTesting; Twelfth Informational Supplement 2002. NCCLS Document M100-S12. PA, USA: Wayne.

Nepal Water Supply Corporation. (2004/2005) Annual Report, 2004/2005, Published by 16th Anniversary Committee. Tripureswor, Kathmandu, Nepal: Nepal Water Supply Corporation, pp. 24.

Pandey S and Musarrat J (1993) Antibiotic Resistant Coliform Bacteria in Drinking Water. Journal of Environmental Biology 14(4): 267-274

Park K (2005) Park's Text book of Preventive and Social Medicine. M/s Bamarisidas Bhanot Publisher, 18th Edition, Prem nagar, India, pp 519-540

Pathak S and Gopal K (1994) Antibiotic Resistance and Metal Tolerance Among Coliform sp. from Drinking Water in a Hilly Area. J. Environ. Biol. 15(2): 139-147

Pelczar MJ, Chan ECS and Krieg NR (1986) Microbiology, 5th edition, McGraw-Hill Book Company, pp 339

Pradhananga TM, Thapa A, Sharma U, Shai RR and Shrestha S (1993) Water Quality Monitaring of Stone Taps of Pashupati Area. J. Nepal Chemical Society 12: 55-62

Prasai T (2002) Drinking Water quality Assessment of Kathmandu Valley and Antibacterial Properties of Medicinal Plants against Enteric Bacteria isolated from water. A Dessertation Submitted to Central Department of Microbiology, Tribhuvan University, Kirtipur, Kathmandu

Ramete PW, Pathak, SP and Bhattaecherjee JW (1991) Isolation of Antibiotic Resistant Coliform sp. from Rural Drinking Water. Journal of Environmental biology 12: 153-158

Salmonella Serotyping in Nebraska, 2001. Beth Knutson, MT (ASCP)

Scher, K., Romling, U. and Yaron, S. (2005) Effect of heat, acidification, and chlorination on Salmonella enterica Serovar Typhimurium cells in a biofilm

formed at the air-liquid interface. Applied Environmental Microbiology 71, 1163–1168.

Shankar A and Panigrahi SK (2007) Water Borne Diseases in India: Environmental Health and Policy Perspectives, New Delhi, Manak, pp230

Sharma, A.P. (1986): Kathmandu Drinking Water Monitoring. Report Submitted to Nepal National Committee for Man and the Biosphere, Kathmandu

Sharma, A.P. (1993): Study of Bacteriological Standard of Drinking Water of Nepal. A report submitted to National Committee for Man and Biosphere Nepal, 1993

Sharma A (2000) Water Pollution and its impact on Health: Nepalese Context. Central Department of Microbiology, Tribhuvan University, Kirtipur, pp 1-3

Sharma S, Bajracharya R, Sitaula BK and Merg J (2005) Water quality in the Central Himilaya, Asian Institute of Technology. Current Science, Thailand 89(5): 782

Shrestha S. (2002) Assessment of Drinking water quality supplied by Nepal supply corporation Sundarighat and identification, Antibiotic sensitivity pattern and serotyping of isolated *Escherichia coli*. A Dessertation Submitted to Central Department of Microbiology, Tribhuvan University, Kirtipur, Kathmandu

Thapa M. (1997) Bacteriological study of water and its treatment using plant products. M.Sc Thesis, Central Department of Microbiology, Tribhuvan University, Kathmandu

The Himalayan Time (2009). Parched throats, dry taps mark World Water Day. The Himalayan Times, March 23, 2009.

Threlfall, E.J. and Ward, L.R. (2001) Decreased susceptibility to ciprofloxacin in Salmonella enterica serotype Typhi, United Kingdom. Emerg Infect Dis 7, 448–450.

Trivedi RK and Goel PK (1986) Chemical and Biological Methods for Water Pollution Studies, Environmental publication, Post Box 60, Karad , pp 13- 42

United Nations International Children Emergency Fund (1987) Children and Women of Nepal, A Situation Analysis, UNICEF, Nepal

United Nations Environment Programs (2001) Nepal: State of the Environment Nepal (UNEP), in collaboration with MoPE/HMGN, SACEP, ICIMOD and NORAD

Shahane V, Muley V, Kagal A, Bharadwaj R (2007) Non-typhoid Salmonellosis: Emerging infection in Pune Department of Microbiology, B. J. Medical College, Pune - 411 001, Maharashtra. Indian Journal of Medical Microbiology, Vol. 25, No. 2, April-June, 2007, pp. 173-174

White D.G, Datta A, Mc Dermott P, Friedman S, Qaiyumi S, Ayers S, English L, Mc Dermott S, Wagner D.D and Zhao S (2003) Antimicrobial susceptiblity and genetic relatedness of Salmonella serovars isolated from animal- derived dog treats in USA

WHO (1993): Guidelines for Drinking water quality, Volume II World Health Organization, Geneva, 1993.

WHO (2000) The world health report: making a difference. World Health Organization, Geneva

WHO/UNICEF (2000) Global Water Supply and Sanitation Assessment Report. World Health Organization, Geneva

World Health Organization (2002) Procedure for Clinical Bacteriology. WHO,Geneva

WHO (2003) Background Document: The Diagnosis, Treatment And Prevention Of Typhoid Fever. Geneva Switzerland: Publication of World Health Organization, The Department of Vaccines and Biologicals.

World Health Organization (2004) Guidelines for Drinking Water Quality. Volume III, WHO, Geneva

APPENDIX – I

4.1.1List of materials

A) Equipments

1. Autoclave- Life steriware, India
3. Electric balanceOHAUS GA 2000
4. Hot air oven-Universal, India
5. Incubators-Universal, India
7. Membrane filter apparatus-Millipore
8. Microscope-Olympus, Japan
9. Nephelometer- Elico, India
10. pH meter-Quikchek Orion
11. Refrigerator- Gold star

B)Glassware

Beakers	Glass rods	Pipette
Burette	Graduated cylinders	Reagent bottles
Conical flasks	Microscopic-slides	Screw capped test tubes
Petridishes	Micropipettes	

C) Miscellaneous

Aluminum foil Forceps
Immersion oil
Lens paper
Inoculating loop
Membrane Filter Paper Measuring scale
Blotting paper Sampler (sample collecting bottles)
Detergent
Labelling tape
Transport tray
Cotton role
Cotton-swab
Dropper

D) Chemicals/Reagents

Acetic acid
ℑ-Naphthol
Methyl red
Barium Chloride
Conc. Hydrochloric acid
Crystal violet
Ethanol
Sodium hydroxide
Saffranin
Kovac's reagent
Paraffin
Potassium iodide
Potassium hydroxide
Sulphuric acid
Hydrogen peroxide

E) Microbiological media

M-endo Agar (Hi-media)
Muller Hinton Agar (Hi-media)
Nutrient Agar (Hi-media)
Nutrient Broth (Hi-media)
Peptone (Hi-media)
MR-VP Broth (Hi-media)
Salmonella-Shigella Agar (Hi-media)
Selenite F Broth (Hi-media)

F) Biochemical media

Sulphide Indole Motility Medium (Hi-media)
Triple Sugar Iron Agar (Hi-media)
Urea Agar Base Broth (Hi-media)
Hugh-Leifson's Agar (Hi-media)
Simmon Citrate Agar (Hi-media)

Composition and preparation of bacteriological media

I. Culture media

1.Nutrient Agar (NA)

Composition	(Gram/Litre)
Peptone	5.0
Sodium Chloride	5.0
Beef extract	1.5
Yeast extract	1.5
Agar	15.0
Final pH (at 25^0C)	7.4±0.2

Procedure

Twenty-eight gm of media was dissolved in 1000 ml of distilled water and heated to dissolve the media. The media was autoclaved at 15 lbs pressure at 121^0C for 15 minutes.

2. Nutrient Broth (NB)

Composition	(Gram/litre)
Peptone	5.0
Sodium Chloride	5.0
Beef extract	1.5
Yeast extract	1.5
Final pH (at 25^0C)	7.4±0.2

Sterilized by autoclaving at 15 lbs pressure (121^0C) for 15 minutes.

3.Selenite –F- Broth

Composition	(Gram/Litre)
Tryptone	5.0
Lactose	4.0
Sodium phosphate	10.0
Sodium Acid Selenite	4.0
Final pH at (25^0C)	7.4±0.2

Preparation
Warmed to dissolve the medium and mixed well then sterilized in a boiling water bath for 10 minutes. The media was not autoclaved and excessive heating was avoided.

4. M-Endo Agar

Composition	(Gram/Litre)
Yeast extract	6.0
Peptone	20.6
Lactose	25.0
Dipotassium phosphate	7.0
Basic fuchsin	1.0
Sodium sulphite	2.5
Agar	15.0
Final pH (at 25^0C)	7.5±0.2

Preparation
As directed by the manufacturing company, 5.1 grams of media was dissolved water containing 2ml of 95% ethanol. It was boied to dissolve the medium completely. It was cooled to 45^0C and poured in petriplates.
Note: The medium was not autoclaved.

5. Salmonella - Shigella (SS) Agar

Composition	(Gram/Litre)
Peptic digest to animal tissue	5.0
Beef extract	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 pH (at 25^0C)	7.0 ±0.2

Preparation
As directed by the manufacturing company, 6.3gms of media was dissolved in 100 ml distilled water and heated with frequent agitation to dissolve the media completely. The media was not autoclaved and over heating was avoided.

6. Xylose Lysine Deoxlcholate (XLD) Agar

Composition	(Gram/Litre)
Xylose	3.5
L-Lysine	5.0
Lactose	7.5
Sucrose	7. 5
Sodium Chloride	5.0
Yeast Extract	3.0
Sodium Deoxycholate	2.5
Ferric Ammonium Citrate	0.8
Phenol Red	0.08
Agar	15.0
Final pH	7.4±0.2

Preparation

As directed by the manufacturing company, 6.3 gms of media was dissolved in 100ml of distilled water and heated with frequent agitation to dissolve the media completely. The media was not autoclaved and overheating was avoided..

7. Mueller Hinton Agar (MHA)

Composition	(Gram/Litre)
Beef Infusion Broth	300.0
Casein Acid Hydrolysate	17.0
Starch	1.0
Agar	17.0
Final pH	7.0±0.2

Preparation:

As directed by the manufacturing company, 3.8 gm of media was suspended in 100 ml distilled water, boiled to dissolve and sterilized by autoclaving at 121^0C for 15 minutes. It was poured while at 45-55^0C in sterile 9 cm diameter plates in 25 ml quantities. To ensure the uniformity in depth of medium, the plates were placed over level surface and the medium was poured into it.

II. Composition of stains and Reagents

1. Gram's staining

i. Crystal violet

Solution A

Crystal Violet	2.0gm
95% ethyl alcohol	20.0ml

Solution B

Ammonium oxalate	0.8gm
Distilled water	30.0ml

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

ii. Gram's Iodine

Iodine	1.0gm
Potassium iodide	2.0ml
Distilled water	300.0ml

Iodine and potassium iodide were dissolved in distilled water.

iii. Ethyl Alcohol (95%)

Absolute alcohol	95.0ml
Distilled water	5.0ml

iv. Safranin

Safranin	10.0ml
(2.5 % solution in 95% ethyl alcohol)	
Distilled water	100.0ml

Procedure

Heat fixed smear of bacterial culture was flooded with crystal violet for one minute and excess stain was washed out. The slide was treated with Gram's Iodine for 1 minute and washed. It was flooded with decolorize alcohol and immediately washed with water. Then smear was treated with safranin for 1 minute and washed with water. It was dried and observed under microscope.

Catalase Test

Catalase test is done to test the presence of enzyme catalase. The enzyme catalase splits hydrogen peroxide to water and oxygen.

Reagents: (3 % Hydrogen peroxides).

Composition

Concentration hydrogen peroxide	3ml
Distilled water	97ml

Procedure

Three ml of 3 % hydrogen peroxide was taken in a test tube and colony of bacteria to be tested was picked up from nutrient agar with the help of glass rod and inserted into the tube-containing reagent. The production of gas bubbles immediately indicates positive catalase test.

Oxidase Test

Oxidase test is done to determine the presence of the oxidase enzyme. Oxidase reaction is due to the presence of a cytochrome oxidase system.

Oxidase reagent

Whatman No. 1 filter paper was cut into strips of 6-8 cm in diameter. It was soaked in the reagent till saturation. The paper strips were drained and freeze dried and stored in a dark tightly sealed bottle.

Procedure

The Oxidase test paper was moistened with distilled water. A colony was picked using glass rod and rubbed to the paper. Development of violet colour within 10 seconds is an indicative of positive test.

Sulfide-Indole-Motility Medium (SIM)

Sulfide-Indole-Motility is a semi solid medium used for the determination of sulfide production, Indole formation and motility of enteric bacteria.

Composition	(gm/litre)
Beef extract	3.0
Peptone	30.0

Peptonized iron	0.2
Sodium thiosulfate	0.025
Agar	3.0
Final pH (at 25^0C)	7.3±0.2

Preparation
Thirty-six grams was suspended in 100ml-distilled water. It was heated to boil to dissolve the medium completely. It was dispense in tubes and sterilized by autoclaving for 15 minutes at 15 lbs pressure (121^0C). The medium was allowed to solidify in vertical position.

Procedure
The test organisms was stabbed into the medium and incubated at 37^0C for 24 hours. Motile organism show diffuse growth or turbidity away from the line of inoculation and non-motile grows only along the line of inoculation. Blackening along the line of inoculation indicates H2S positive test.
0.2ml of Kovac's reagent was added to the tube and allowed to stand for 10 minutes. A dark red colour in the reagent indicates a positive indole test.

Indole Test
Indole test is done to determine the ability of an organism to split Indole from tryptophan molecule.
Medium peptone water.

Composition	(gm/litre)
Peptone containing tryptophan	10.0gm
Sodium Chloride	5.0gm
Distilled water	1000.0ml

Preparation
A Fiften gram was dissolved in 1000ml-distilled water. It was boiled to dissolve completely. It was dispensed in test tubes and sterilized at 15lbs pressure (121^0C) for 15minutes.
Reagent: Kovac's reagent

Composition	(gm/litre)
P-dimethyl aminobenzaldehyde	5.0gm
Isoamyl alcohol	75.0ml
Concentrated hydrochloric acid	25.0ml

Preparation
Aldehyde was added to flask containing alcohol and it was dissolved by gentle warming to 55^0C in a water bath. It was cooled and HCl was added. It was stored in a dark glass bottle in a refrigerator.

Procedure
The test organisms was inoculated in peptone water and incubated at 37^0C for 48 hours. About 0.5 ml Kovac's reagent was added and shaken gently. Formation of pink coloured ring over surface layer indicated positive test.

Methyl Red Test

The methyl red test is done to test the ability of an organism to produce and maintain stable acid products from glucose fermentation and to overcome the buffering capacity of the system.

MR-VP medium (glucose-phosphate broth).

Composition	(gm/litre)
Buffered peptone	7.0
Dextrose	5.0
Tripotassim phosphate	5.0
Final pH (at 25^0C)	6.9±0.2

Preparation

Seventeen grams was dissolved in 1000ml-distilled water. It was distributed in test tubes in 10 ml amount and sterilized by autoclaving at 15 lbs pressure for 15 minutes.

Reagent – Methyl Red

Composition	(gm/litre)
Methyl red	0.04gm
Ethyl alcohol	40.0ml
Distilled water	60.0ml

Preparation

Methyl red was dissolved in ethyl alcohol and water was added.

Procedure

The glucose phosphate broth was inoculated with culture to be tested and incubated at 37^0C for 48 hours. Methyl red indicator was added to the culture and development of red colour indicates positive test while yellow colour indicates negative test.

Voges – Proskauer Test

Voges – Proskauer test determine the ability of organism to produce a neutral end product, acetylmethylcarbinol from glucose formation.

Medium -MR-VP medium (Glucose – phosphate broth)

Solution A	
ά-naphthol	5.0gm
Ethyl alcohol (95%)	100.0ml
Solution B	
Potassium hydroxide	40.0gm
Distilled water	100.0ml

Procedure

Sterile broth was inoculated with fresh culture medium and inoculated with fresh culture medium and incubated at 37^0C for 48 hours. Development of pink-red colour within 30 minutes after adding of ά-naphthol and 40% potassium hydroxide in 1:3 proportions was recorded as positive test.

Citrate Utilization Test

Citrate utilization test is performed to determine if an organism is capable of utilizing citrate as the sole source of Carbon for metabolism with resulting alkalinity.

Medium – Simmon's Citrate Agar

Composition	(Gram/Litre)
Monoammonium phosphate	1.0
Dipotassium phosphate	1.0
Sodium Chloride	5.0
Sodium Citrate	2.0
Magnesium Sulphate	0.2
Bromothymol blue	0.08
Agar	15.0
Final pH (at 25^0C)	6.8±0.2

Preparation

As directed by the manufacturing company, 24.2 grams was suspended in 1000ml-distilled water. It was heated to boil to dissolve the medium completely. It was distributed in tubes and sterilized by autoclaving at 15 lbs pressure (121^0C) for 15 minutes. The mediums in tubes were solidified in slanted position.

Procedure

The slant was streaked with test organism and incubated at 37^0C for 48 hours. Growth of organism with an intense blue colour on slant is the indicative of positive test. No growth no change in colour (green) is the negative test.

Hydrogen Sulfate Test (Triple Sugar Iron Agar Test)

The test is done to determine the ability of an organism to utilize specific Carbohydrate incorporated in the medium, with or without the production of gas, along with determination of possible hydrogen sulfide production

Composition	(gm/litre)
Peptone	10.0
Tryptone	10.0
Yeast extract	3.0
Beef extract	3.0
Lactose	10.0
Sucrose	10.0
Dextrose	1.0
Ferrous sulfate	0.2
Sodium Chloride	5.0
Sodium Thiosulfate	0.3
Phenol- red	0.024
Agar	12
Final pH (at 25^0C)	7.4±0.2

Preparation

As directed by the manufacturing company, 6.5 grams was suspended in 1000ml-distilled water. It was boiled to dissolve completely. It was distributed in tubes and sterilized by autoclaving at 15 lbs pressure (121^0C) for 15 minutes. The medium was allowed to set in slopped from with a butt about 1 inch long.

Procedure

The test organism was stabbed in the butt and streaked on the slant. The tubes were incubated at 37^0C for 24 hours. Black colouration of butt was indicative of H_2S formation. The change in colour of butt, slant and gas formation was also noted and recorded as alkali/alkali, alkali/acid and acid/acid for the growth of fermenters and all sugar fermenters.

Urease Test

Urease test demonstrates the ability of an organism to split forming two molecules of ammonia by the action of the enzyme urease.

Medium – Urea agar base.

Composition	(gm/Litre)
Peptone	1.0
Dextrose	1.0
Sodium Chloride	5.0
Disodium phosphate	1.2
Monopotassium phosphate	0.8
Phenol red	0.012
Agar	15.0
Final pH (at 25^0C)	6.8±0.2.

Preparation

Twenty-four grams urea agar base was suspended in 950ml of distilled water. It was boiled to dissolve completely and sterilized by autoclaving at 10 Lbs pressure (115^0C) for 20 minutes. It was cooled down to 55^0C and aseptically introduced 50ml of sterile 40% area solution and mixed well. It was distributed in sterile test tubes and allowed to solidify in slanted position.

Procedure

Fresh culture of test organism was streaked heavily on the slant and incubated at 37^0C for overnight. Change in colour of medium to pink indicates positive test and no change in colour indicate negative test.

Hugh and Leifson Medium

Composition	(Gram/Litre)
Peptone	20.0
Sodium Chloride	50.0
Dipotassium Phosphate	3.0
Agar	20.0
Bromo Thymol Blue	0.5
Glucose	100.0
Final pH	7.1±0.2

Preparation

As directed by the manufacturing company, 193 grams of media was dispensed in 1000ml of distilled water. It was boiled to dissolve the medium completely and distributed into tubes in duplicate. The medium was then sterilized by autoclaving at 15 lbs at 121^0C for 15 minutes.

APPENDIX-II

Sampling sites and their codes

S.N	Location	Code No.
1	Shridishi galli, House no. 66/6	KW1
2	Gaurishankar Marga, House no.371	KW2
3	Gaurishankar Marga, House no.451	KW3
4	Gaurishankar Marga, House no.666	KW4
5	Lik Marga, House no. 417	KW5
6	Lik Marga, House no. 500	KW6
7	Lik Marga, House no. 605	KW7
8	Lik Marga, House no. 725	KW8
9	25 Maharjan House no. 4	KW9
10	Parijat margha House no.129	KW10
11	Madannagar House no.284	BM1
12	Madannagar House no. 317	BM2
13	Madannagar House no.202/20	BM3
14	Madannagar House no.318/85	BM4
15	Madannagar House no.271	BM5
16	Madannagar House no.402	BM6
17	Madannagar House no.318/44	BM7
18	Madannagar House no.443	BM8
19	Madannagar House no. 318/66	BM9
20	Madannagar House no. 382	BM10
21	Bhajangal ward no. 18	KP1
22	Bhajangal ward no. 17(242-08-053/1)	KP2
23	Bhajangal ward no. 17(241-18-401)	KP3
24	Bhajangal ward no. 17(242-08-065)	KP4
25	Bhajangal ward no. 17(242-08-045)	KP5
26	Bhajangal ward no.17(242-08-36)	KP6
27	Bhajangal ward no. 17(242-08-47)	KP7
28	Bhajangal ward no.17(244-20-027)	KP8
29	Bhajangal ward no.17(244-19-1)	KP9
30	TU quarter house no.1	KP10
31	40- Ujamo galli, Jyabahal	T1

32	132/37 Jamana, Gubhajub Marg	T2
33	132/73 Jamana, Gubhajub Marg	T3
34	21/17 - Ujamo galli, Jyabahal	T4
35	Public tap, Jyabahal	T5
36	107 Jamana, Gubhajub Marg	T6
37	132/42 Jamana, Gubhajub Marg	T7
38	134 Jamana, Gubhajub Marg	T8
39	131 Jamana, Gubhajub Marg	T9
40	44 Jyabaha Marg	T10
41	Maitidevi panchakumarimarg	MD1
42	Shantigalli house no. 4	MD2
43	Mahakabimarg House no. 463	MD3
44	Janata marg House no. 251	MD4
45	Iswari marg House no. 69	MD5
46	Maitidevi marg House no. 360	MD6
47	Panchakumarimarg House no.22	MD7
48	Panchakumarimarg House no 84	MD8
49	Panchakumarimarg House no. 66	MD9
50	Panchakumarimarg House no. 204	MD10
51	Ghumti galli House no. 103	BB1
52	Ghumti galli House no. 110	BB2
53	Ghumti galli House no. 94	BB3
54	Ghumti galli House no. 98	BB4
55	Ghumti galli House no. 88	BB5
56	Ghumti galli House no. 64	BB6
57	Ghumti galli House no. 71	BB7
58	Ghumti galli House no. 13	BB8
59	Adwait marga House no. 12	BB9
60	Adwait marga House no. 120	BB10
61	Adwait marga House no. 114	BB11
62	Adwait marga House no. 102	BB12
63	Tukucha galli 116	BB13
64	Tukucha galli 111	BB14
65	Baagbazar galli 114	BB15
66	Baagbazar galli 102	BB16
67	Bishobidhalaya path 277/19	B1
68	Bishobidhalaya path 219	B2
69	Bishobidhalaya path 345	B3
70	Bishobidhalaya path 290/9	B4
71	Khadya kirana bazaar marg 127	B5
72	Khadya kirana bazaar marg 108	B6
73	Khadya kirana bazaar marg 75/30	B7

74	Bishobidhalaya path 315	B8
75	Bishobidhalaya path 347/20	B9
76	Hanagul marga 369	B10
77	Abhibyakti marg House no. 10	G1
78	Abhibyakti marg House no.33	G2
79	Abhibyakti marg House no. 90	G3
80	Abhibyakti marg House no. 131	G4
81	Abhibyakti marg House no.186	G5
82	Dharma marg House no. 253	G6
83	Dharma marg House no. 123/48	G7
84	Dharma marg House no. 585	G8
85	Dharma marg House no. 731	G9
86	Dharma marg House no. 650	G10

APPENDIX- III

1. Water quality analysis of collected water samples

Physical analysis of well water samples

S.N	Code No.	Temperature (^{0}C)	pH
1	KW1	20.6	7.6
2	KW2	20.8	7.4
3	KW3	20.6	7
4	KW4	20.4	8
5	KW5	20.5	7
6	KW6	20.0	6.2
7	KW7	21.0	8.7
8	KW8	21.0	6.7
9	KW9	20.7	6.8
10	KW10	20.8	6.5
11	BM1	24.0	6.8
12	BM2	25.1	8.8
13	BM3	25.1	8.6
14	BM4	25.7	7.8
15	BM5	26.0	6.6
16	BM6	25.7	7.3
17	BM7	24.1	7.0
18	BM8	25.6	7.3
19	BM9	25.5	7.2
20	BM10	26.7	7.0
21	KP1	12.2	7.2
22	KP2	12.0	6.9
23	KP3	13.2	7.2
24	KP4	12.4	6.7
25	KP5	12.2	6.7
26	KP6	13.0	6.7
27	KP7	13.0	7.3
28	KP8	11.8	6.7
29	KP9	12.2	6.7
30	KP10	12.0	6.6
31	T1	16.6	6.7
32	T2	16.6	7.2
33	T3	15.0	7.4
34	T4	16.1	6.6
35	T5	16.0	7.2
36	T6	16.0	6.8
37	T7	15.6	6.6
38	T8	16.2	6.9
39	T9	15.0	6.8
40	T10	15.6	6.6
41	MD1	14.4	6.9
42	MD2	15.0	6.7

43	MD3	14.1	7
44	MD4	14.0	6.8
45	MD5	13.8	6.2
46	MD6	13.6	8.4
47	MD7	13.0	6.9
48	MD8	14.2	7
49	MD9	14.2	6.8
50	MD10	19.0	6.8
51	BB1	20.6	7
52	BB2	20.8	7
53	BB3	20.6	7
54	BB4	20.4	8
55	BB5	20.5	7
56	BB6	18.8	6.2
57	BB7	18.1	8.8
58	BB8	18.1	6.7
59	BB9	18.0	6.8
60	BB10	18.2	6.5
61	BB11	19.0	6.8
62	BB12	19.2	8.8
63	BB13	17.9	8.6
64	BB14	18.2	6.8
65	BB15	18.9	6.6
66	BB16	25.7	7.8
67	B1	24.1	6.8
68	B2	25.6	6.6
69	B3	25.5	7.2
70	B4	26.7	7.0
71	B5	26.2	6.2
72	B6	26.5	6.9
73	B7	26.4	6.2
74	B8	12.4	6.7
75	B9	12.2	6.7
76	B10	16.5	6.7
77	G1	17.1	7.3
78	G2	17.0	6.7
79	G3	16.8	6.7
80	G4	16.2	6.6
81	G5	16.5	6.7
82	G6	17.4	7.2
83	G7	17.0	7.4
84	G8	16.8	6.6
85	G9	17.0	7.2
86	G10	17.2	6.8

APPENDIX IV

Bacteriological analysis of tap water samples

Sample Code No.	Total Coliform Count/100	Thermotolerant coliform	WHO Permissible Value
KW_1	>300	-	
KW_2	80	-	
KW_3	>300	-	
KW_4	>300	-	
KW_5	>300	2	
KW_6	>300	-	
KW_7	>300	-	
KW_8	46	-	
KW_9	208	-	
KW_{10}	>300	2	
BM_1	>300	-	
BM_2	>300	-	
BM_3	>300	16	
BM_4	>300	4	
BM_5	>300	-	
BM_6	>300	-	
BM_7	>300	-	
BM8	>300	-	
BM_9	>300	8	
BM_{10}	>300	-	
KP_1	30	-	
KP_2	>300	-	
KP_3	80	-	
KP_4	>300	-	
KP_5	214	-	0 C.F.U./ 100ml
KP_6	>300	-	
KP_7	>300	-	
KP_8	>300	-	
KP_9	208	-	
KP_{10}	>300	-	
T1	>300	-	
T2	>300	-	
T3	>300	-	
T4	>300	-	
T5	>300	-	
T6	>300	-	
T7	>300	-	
T8	44	-	
T9	97	-	
T10	>300	-	
MD1	>300	25	
MD2	>300	-	
MD3	>300*	52	
MD4	>300	-	
MD5	>300	-	
MD6	>300	-	
MD7	>300	-	
MD8	>300	4	

MD9	>300	2	
MD10	>300	2	
BB1	>300	18	
BB2	>300*	28	
BB3	>300	-	
BB4	>300	25	
BB5	>300	4	
BB6	>300	-	
BB7	>300	-	
BB8	>300	-	
BB9	>300	28	
BB10	>300*	12	
BB11	206	-	
BB12	>300	-	
BB13	>300	-	
BB14	>300	-	
BB15	>300	4	
BB16	>300	-	
B1	>300	-	
B2	80	-	
B3	>300	2	
B4	>300*	26	
B5	97	-	
B6	>300	-	
B7	>300	2	
B8	>300	-	
B9	208	-	
B10	>300	-	
G1	>300	-	
G2	>300	-	
G3	208	-	
G4	>300	-	
G5	>300	-	
G6	>300	-	
G7	>300	-	
G8	>300	-	
G9	>300	-	
G10	>300	-	

APPENDIX - V

Chart for identification of bacterial isolates

Organisms	Biochemical Tests										
						SIM			TSI		
	MR	VP	Urease	Citrate	OF	H_2S	Indole	Motility	Slant	Butt	Gas
S1	+ve	-ve	-ve	+ve	F	+	-ve	+ve	Alkaline	Acid	+ve
S2	+ve	-ve	-ve	-ve	F	-ve	-ve	+ve	Alkaline	Acid	+ve
S3	+ve	-ve	-ve	+ve	F	+	-ve	+ve	Alkaline	Acid	+ve
S4	+ve	-ve	-ve	+ve	F	+	-ve	+ve	Alkaline	Acid	+ve
S5	+ve	-ve	-ve	+ve	F	+	-ve	+ve	Alkaline	Acid	+ve
S6	+ve	-ve	-ve	+ve	F	+	-ve	+ve	Alkaline	Acid	+ve
S7	+ve	-ve	-ve	+ve	F	+	-ve	+ve	Alkaline	Acid	+ve
S8	+ve	-ve	-ve	+ve	F	+	-ve	+ve	Alkaline	Acid	+ve
S9	+ve	-ve	-ve	+ve	F	+	-ve	+ve	Alkaline	Acid	+ve
S10	+ve	-ve	-ve	+ve	F	+	-ve	+ve	Alkaline	Acid	+ve

+ve : Positive

-ve : Negative

APPENDIX-VI

Antibiotic sensitivity pattern of isolates

S.No	Code No.	Antibiotics							
		Am	C	T	Co	Cp	NA	CF	CK
1.	S1	R	S	S	S	R	S	S	R
2.	S2	R	S	S	S	S	S	S	S
3.	S3	R	S	S	S	S	S	-	S
4.	S4	R	S	S	S	S	S	S	S
5.	S5	I	S	S	S	S	S	S	S
6.	S6	I	S	S	S	S	S	S	-
7.	S7	I	S	S	S	S	S	S	-
8.	S8	R	S	S	S	S	S	S	S
9.	S9	R	S	S	S	S	S	S	S
10.	S10	R	S	S	S	R	S	S	-

NB: Am-Amoxicillin (10 µg); C-Chloramphenicol (30 µg); T-Tetracycline(30 µg); Co-Co-Trimoxazole (25 µg); Cp- Cephalexin(30 µg); NA- Nalidixic acid (30 µg); CF-Ciprofloxacin (5 µg); CK- Ceftizoxime(30 µg)

Inhibition zone diameter size interpretive chart (CLSI, 2006)

Antimicrobial agent (Disc potency)	Diameter of zone of inihibition in mm			NCCLS QC strain agent potency *E. coli* ATCC 25922
	resistant	intermediate	sensitive	
Amoxycillin (10 µg)	13	14-17	18	18-24
Chloramphenicol(30µg)	12	13-17	18	21-27
Cotrimoxazole(25µg)	16	11-15	10	23-29
Nalidixic acid(30µg)	13	14-18	19	22-28
Ciprofloxacin(5µg)	15	16-20	21	30-40
Ceftizoxime (30µg)	14	15-19	20	30-36
Tetracycline(30µg)	14	15-18	19	18-25

APPENDIX- VII

International standard for drinking water

Parameter	Maximum permissible level (Guidelines values)	
	WHO Standard	Nepal Standard
Colour (TCU)	15	15
Taste	Inoffensive	-
Odour	Inoffensive	-
Temperature	-	-
pH	6.5-8.5	6.5-8.5
Conductivity (µS/cm)	50-500	1500
Turbidity (NTU)	5	5
Hardness as $CaCO_3$ (mg/l)	500	500
Iron (mg/l)	0.3	0.3
Ammonia (mg/l)	1.5	1.5
Nitrate (mg/l)	50	50
Arsenic (mg/l)	0.01	0.05
Coliform Count	0/100ml	0/100ml