CHAPTER - I

1. INTRODUCTION

The vital importance of water for the sustenance of life on the earth cannot be estimated. It is supported by the fact that 80% of the protoplast constitutes water. This finite resource can get scarce in the future due to the incessantly growing demand for it (Water Resource, 2006). The safe water quality bears the utmost public health importance as health and well being of the human race is closely tied up with the quality of water used (Sharma *et al.*, 2005). WHO has stressed health as being one of the fundamental rights of every human and even article 16 of the interim constitution of Nepal secures health as a constitutional right. Improved health is possible by means of adequate, safe drinking water and good sanitation provision (Park, 2005).

Water pollution is any physical, chemical or biological changes in water that can adversely affect living organisms. About 80 percent of all diseases in the developing world are attributed to unsafe water supply and inadequate sanitation. Waterborne diseases are among the leading causes of morbidity and mortality in developing countries and every year around 2.2 million people die due to basic hygiene related diseases. Interventions in hygiene, sanitation and water supply proved to control these diseases. Universal access to safe drinking water and sanitation has been promoted as an essential step in reducing these preventable diseases (Tambekar and Banginwar, 2005).

Only safe and wholesome water is liable for human consumption. Safe drinking water is defined as water free from microorganisms and chemicals in concentration, which could have detrimental effect on human health. Such water may be termed potable water. Only 3% of the total available water on the earth's surface is potable. The WHO estimates that over 1.1 billion population in developing world lack access to improved water supplies (WHO, 2006).The lack of proper purification and sanitation of drinking water in developing countries leads to the scarcity of safe drinking water among one

third of the population along with the increased prevalence of water borne diseases, diarrhoea being the major cause for death, mostly among children under the age of five years. Diarrhoea kills an estimated 1.8 million people each year and is the third leading cause of death among infectious diseases after respiratory infections and HIV/AIDS. It ranks ahead of tuberculosis and malaria (WHO, 2005). Nepal faces enormous challenge to provide potable drinking water to all its population. At present, the population having access to pipe water facility is estimated to be 47% (WHO, 2004).

In Kathmandu valley most of the sources of water cannot be regarded safe and measured up to the guidelines recommended by WHO (Bottino, 1999; Prasai, 2002). The factors responsible for contaminating drinking water at source points included the lack of protection and proper treatment of water, leakage in pipe distribution system, intermittent supply of water, poor drainage system and poor environmental surroundings of water sources (NHRC/WHO, 2002). Majority of the households in the country, i.e. 57.7 percent had no toilets and therefore they had to use open spaces including public places, roadside, riverbank, field, etc. for defecation (CBS/HMG 2001). Hence, the open defecation is still a serious problem that could directly affect the quality of water and sanitation in the country (NHRC/WHO, 2002).

Water though is an absolute necessity for life, can also be a carrier of many water borne diseases such as typhoid, cholera, hepatitis, dysentery and other diarrhoea related diseases. It has been estimated that 4% of the global burden of disease is attributed to unsafe water supply (Magali *et al.*, 2007). In Nepal, unsafe and inadequate water sanitation, little or no health education, illiteracy, under nutrition, wide spread faecal contamination of environment, dense population, etc are considered as the major causes of disease relating to the water sanitation (Chand *et al.*, 2001).The disease caused by contaminated water is among top ten most prevalent diseases in Nepal. The incidence of water borne disease outbreaks is considerably high as compared to other diseases. The reported cases of water borne communicable diseases in Nepal were typhoid (2,15,191), diarrhoeal diseases (9,21,901), intestinal worms (6,11,072) and jaundice and infectious

hepatitis (25,686) (DoHS, 2004/2005). The recent outbreak of cholera in Jajarkot and neighbouring districts claimed enormous number of lives.

The most sensitive and specific way of assessing water quality has been the detection of faecal indicator organisms. The presence of these indicator organisms in drinking water suggests the possible infiltration of the pathogens. *Escherichia coli*, the predominant thermotolerant bacteria, is an ideal indicator of faecal pollution as it is exclusively of faecal origin and fulfils all the criteria of a faecal indicator bacterium. In poor country like Nepal, drinking water quality often remains unevaluated because of high cost of specialized water quality testing (Gyewali, 2007).

Antibiotic resistance in bacteria is a contemporary global public health problem (Wright, 2007). Humans may be affected either directly through ingestion of water contaminated with antibiotic resistant bacteria or indirectly through exposure to an environment or food that has been contaminated by the water (Lee *et al.,* 2002). Especially heavy metals show a lethal effect upon microbial cells, even in relatively low concentration. This characteristic of heavy metals has been applied to water purification, ointment manufacture and the treatment of bandages and fabrics (Benson, 1979). From ancient time, metallic water pots have been in common use in most parts of Nepal.

The deteriorating drinking water quality in Kathmandu has been implicated for epidemics of water borne diseases affecting especially the poor and marginalized people. The principle factors behind it being the ever increasing population, faecal contamination of drinking water sources, urbanization and industrialization. This study has been designed with an aim to analyze the situation of microbiological quality of urban drinking water supply of Kathmandu. The study also evaluates the antibiotic resistance and oligodynamic action against *E. coli* isolates.

CHAPTER - II

2. OBJECTIVES

2.1 General Objective

To assess drinking water quality of urban Kathmandu.

2.2 Specific Objectives

- To enumerate indicator organisms (total coliform and thermotolerant coliform) in drinking water.
- To determine physico-chemical parameters (temperature and pH) of drinking water.
- To isolate and identify *E. coli* from drinking water.
- To study antibiotic susceptibility pattern of the isolates.
- To study oligodynamic action against the isolates.

CHAPTER - III

3 LITERATURE REVIEW

3.1 Water pollution

Water pollution is defined as any physical, chemical or biological alteration in composition or condition of water, directly or indirectly as a result of anthropogenic activities, so that it becomes unsuitable for any of the functions and purposes for which it would be suitable in its natural state (WHO, 2006). Water pollution is one of the most important problems being faced by both developed and developing world together. It is responsible for a very large number of mortalities and incapacitations throughout the world. Water pollution is the most serious environmental quality issue in Nepal. It is caused by the disposal of solid and liquid wastes on land or into surface water (Chand *et al.,* 2001).

3.2 Sources of water pollution in Kathmandu city

3.2.1 Domestic waste

Domestic waste includes both grey and black water. In Nepal, only the Kathmandu Valley towns have a sewerage network system and the sewerage facility is provided to 15% of the houses only (NWSC, 2001). Some households have septic tanks, but the majority of domestic sewers discharge directly into the rivers without treatment. Even so, much domestic wastewater percolates directly into the groundwater or flows as runoff into local streams. It is also seen even in the valley that people defaecate and urinate on open ground, often along the banks of rivers and streams. On the basis of per person per day water use, the wastewater generated per person is estimated to be about 60 liters for the urban area. About 85% of the total water used ends up as domestic waste water (NPC, 1997).

3.2.2 Industrial waste

The Kathmandu Valley hosts more than 72% of the country's water-polluting industries. Many of these industries discharge effluents into local rivers without treatment, spoiling the quality of river water. The contribution of industrial effluents to the rivers is about seven per cent of the total effluents (domestic and industrial) in the Kathmandu Valley. In terms of relative contribution of effluent, the major polluting industries are the vegetable oil, distillery, and leather industries (Devkota and Neupane, 1994).

3.2.3 Increase in the use of agro-chemicals

Vegetable farming intensified in the valley due to an increased use of agro-chemicals. Altogether 250 types of pesticides were used in Nepal and these pesticides are organochlorides, organophosphates, carbamates, herbicides and pest repellents, and disinfectants. Organochlorides are persistent organic pesticides which pass through the food chain through the processes of bioaccumulation and biomagnification, and thus are hazardous to health (Palikhe, 1999).

3.3 Water supply in Kathmandu City

In KMC, water is supplied by Nepal Water and Sewerage Corporation (NWSC) and private tanker services. In addition to that, many industries, hotels, hospitals and a large number of households use a huge amount of ground water extracted by pumping. Water from stone spouts, ponds and wells are also being used for domestic purposes in many places. Water has become a serious problem for the people of Kathmandu both in terms of quantity and quality. Current water demand of the city has increased tremendously because of the rapid growth of population and urbanization. Almost all major rivers have been tapped at source for drinking water supplies. This supply is only about 120 million litres per day (MLD) during the rainy season, 80 MLD during dry season of the estimated daily demand of 170 MLD (NWSC, 2001). In dry season, 60-70% of the water supply comes from groundwater. Only 79% of the total demand for water of the urban population has been met.

NWSC supplies water to Kathmandu Metropolitan City through six water supply systems. They are Mahankalchour, Bansbari, Balaju, Sundarijal, Sundarighat and Maharajgunj water supply systems. Out of the total water supplied, 30% to 40% loss due to leakage and wastage was reported by NWSC itself. Tremendous socio-economic, political, commercial, educational and other activities are going on every moment. For all such activities, water is essential. But the rate of increase of supply of water is very low as compared to the rate of population growth and urban expansion coupled with hectic social, industrial, commercial and other activities. More than that, it is heart breaking to know that the total amount of water that can be harnessed inside the Kathmandu valley in no way will be able to meet the future demand of increasing population. Insufficiency in water supply meant further deterioration of the problem of wastewater. So, any delay in finding a solution for the water supply and waste water treatment could lead to a serious environmental disaster in Kathmandu.

3.4 Methods of purification of water

The primary risk of consuming untreated water is the transmission of communicable diseases by pathogenic organisms. Securing the microbial safety of drinking water supplies is based on the use of multiple barriers, from catchment to consumer, to prevent the contamination of drinking water and to reduce contamination to levels not injurious to health. Safety is increased if multiple barriers are in place, including protection of water resources, proper selection and operation of a series of treatment steps and management of distribution systems (WHO, 2004). Several treatment processes are adopted by urban drinking water supply system such as pretreatment, aeration, coagulation, flocculation, sedimentation, filtration and disinfection as steps of multiple barrier approach to obtain water of increasingly improved quality and to make sure that the failure of any one process will not result in deterioration of drinking water quality. The drinking water source or water exiting a treatment plant may provide a safe and potentially disinfected supply, but water may become re-contaminated through distribution and storage, if it is touched by unclean hands, dirty cups or dippers or if it is

held in contaminated or uncovered storage vessels. In such circumstances, safe household water management bears utmost importance in maintaining or improving the microbiological quality of the water through collecting, distributing, transporting and storing in the home (WHO, 2004). Chlorination is the most affordable and widely used method of disinfection at both urban treatment and household level in many developing countries like Nepal that cannot adopt expensive sophisticated techniques. Other common methods of safe household water management include boiling, filtration, solar disinfection of water (SODIS), use of metal vessels for their oligodynamic action or combination of these techniques (Murcott, 2006).

3.5 Water quality indicators

Water quality can be ensured only through regular monitoring and surveillance from the standpoint of physical, chemical and microbiological standards. A large number of parameters signifying the quality of water in various uses have been proposed. A regular monitoring of some of them not only prevents diseases and hazards but also checks the water resources from going further polluted (Trivedy and Goel, 1986). The quality of water does, however, have a great influence on public health; in particular the microbiological quality of water is important in preventing ill health. Poor microbiological quality is likely to lead to outbreaks of infectious water-related diseases and may causes serious epidemics to occur.

3.6 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.

3.6.1 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.

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, 1993). Cool water is generally more palatable than warm water, and temperature will impact on the acceptability of a number of other inorganic constituents and chemical contaminants that may affect taste. High water temperature enhances the growth of microorganisms and may increase taste, color and corrosion problems (WHO, 2004).

3.6.2 pH

pH is the negative log10 of the hydrogen ion concentration in a solution. Measurement of pH is the most important and frequently used tests in water chemistry (APHA, 1998). 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 (Nepal Standard, 2062). 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 corrosiveness to pipe and appliance (WHO, 2004).

3.7 Microbiological indicators of drinking water quality

The detection of water-borne pathogens becomes complex, qualitatively unreliable and does not ensure complete safety of water for consumer. Therefore, microorganisms of faecal origin, whose presence presumes that contamination has occurred and suggests the nature and extent of contaminants, have been chosen as indicator organisms (Metcalf and Eddy, 2000). Faecal indicator bacteria including total and faecal coliforms have been used in many countries as a monitoring tool for microbiological impairment of water and for prediction of presence of bacterial, viral and protozoan pathogens. These microorganisms are of faecal origin from higher mammals and birds, and their

presence in water may indicate faecal pollution and possible association with enteric pathogens. The microbiological examination offers the most sensitive test for the detection of recent and potentially dangerous faecal pollution and provides hygienic assessment of water quality with high sensitivity and specificity.

WHO has recognized the following three groups in order to elucidate the term microbial indicator:

• General (process) microbial indicators,
• Faecal indicators (such as *E. coli*)
• Index organisms and model organisms.

Process indicator: A group of organisms that demonstrates the efficacy of a process, such as total heterotrophic bacteria or total coliforms for chlorine disinfection.

Faecal indicator: A group of organisms that indicates the presence of faecal contamination, such as the bacterial groups thermotolerant coliforms or *E. coli*. Hence, they only infer that pathogens may be present.

Index and model organisms: A group or species indicative of pathogen presence and behaviour respectively, such as *E. coli* as an index for *Salmonella* and F-RNA coliphages as models of human enteric viruses.

The basic criterion for making final choice of an appropriate indicator is as follows (WHO, 1996; NHMRC, 2001; and Payment, 1998).

- An indicator should always be present in faecally contaminated water.
- It should be consistently present in fresh faecal waste and should be non-pathogenic.
- It should not grow in natural waters.
- It must occur in greater numbers than the associated pathogens.

- Simple, reliable, and inexpensive methods should exist for the detection, enumeration and identification of indicator organisms.
- It should be more resistant to environmental stress or treatment and persist for greater length of time than pathogen.

The major indicator organisms of faecal pollution are *E. coli*, the thermotolerant and other coliforms, the faecal streptococci and the spores of sulfite reducing clostridia (WHO, 1993). The coliform and thermotolerant bacteria belong to the Enterobacteriaceae family.

3.7.1 The current applicability of faecal indicators

Many members of the total coliform group and some so-called faecal coliforms (e.g. species of *Klebsiella* and *Enterobacter*) are not specific to faeces, and even *E. coli* has been shown to grow in some natural aquatic environments (Solo Gabriele *et al.,* 2000). Hence, the primary targets representing faecal contamination in temperate waters are now considered to be *E. coli* and enterococci. For tropical waters, where *E. coli* and enterococci may grow, alternative indicators such as *Clostridium perfringens* may be preferable. Due to the lower persistence of vegetative cells of the faecal bacteria compared to viruses and parasitic protozoa, poor correlations have been reported between waterborne human viruses or protozoa and thermotolerant coliforms. It is evident from drinking water outbreaks where coliform standards were met (Kramer *et al.,* 1996).

3.7.2 Enterobacteriaceae

Members of the Enterobacteriaceae are gram negative, non-sporing rods; often motile, usually by means of peritrichate flagella. Capsulate or non-capsulate. Easily cultivable on ordinary laboratory media including Mac Conkey's lactose bile-salt agar. Aerobic and facultatively anaerobic. All species ferment glucose with the formation of acid or of acid and gas. Reduce nitrate to nitrite with the exception of some strains of *Erwinia* and

Yersinia. Oxidase negative and catalase positive except *Shigella dysenteriae* type 1. Typically intestinal parasites of man and animals, though other species may occur in other parts of the body, on plants and in the soil. Many species are pathogenic. The G+C content of the DNA in most of the Enterobacteriaceae is in the range of 49-59 mol% but in *Proteus* and *Providencia* it is 37-42 mol% and *Yersinia* it is 46-47 mol% (Topley and Wilson, 1990).

3.7.3 Coliform

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 organisms" refers to gram negative, oxidase negative, non-sporing rods capable of growing aerobically on agar medium containing bile salt, able to ferment lactose within 48 hours at 35-37^{o}C with the production of acid with or without gas (Cheesbrough, 1993). The most common indicators to date have been "total coliform" bacteria, containing a subset of the faecal coliform. Using total coliform includes counting faecal and non-faecal coliform, which may result in data that are misleading and do not relate to the risk of water-borne illness. Coliform bacteria belong to the family Enterobacteriaceae and include *E. coli, Enterobacter, Klebsiella* and *Citrobacte*r (WHO, 1993).

3.7.4 Thermotolerant coliform

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

3.7.5 *E. coli*

German bacteriologist Theodor Escherich first isolated *E. coli* from the faeces of newborns in 1885 and described as *Bacterium coli commune*. It was later renamed *Escherichia coli* by Castellani and Chalmers in 1919 , and for many years the bacterium

was simply considered to be a commensal organism of the large intestine. It was not until 1935 that a strain of *E. coli* was shown to be the cause of an outbreak of diarrhoea and gastroenteritis among infants.

i. General characteristics

Usually motile and usually produce gas from fermentable carbohydrates. Mannitol is fermented but not mannose and inositol and most strains acidify lactose promptly. Indole is usually produced; give a positive methyl-red reaction and a negative Voges-Proskauer reaction. Do not grow on Simmon's citrate medium. Most strains fail to hydrolyze urea or to produce H_2S detectable in triple sugar iron agar. Phenylalanine is not deaminated and gluconate is not oxidized. There is no growth in Moller's KCN medium and gelatin is not liquefied. Most strains decarboxylate lysine. Inhabit the intestine of man and animals, cause both suppurative and diarrhoeal diseases. G+C content of DNA 50-51 mol% (Topley and Wilson, 1990).

Strains of *E. coli* are usually motile and some strains, especially those from extraintestinal infections, possess a polysaccharide capsule. They grow well on ordinary media forming large (2-3mm diameter) circular, low convex, smooth and colourless colonies in 18 hours on nutrient agar, and large red colonies on Mac Conkey's lactose agar; blood agar is discoloured around the growth and there may be β-haemolysis. They grow over a wide range of temperature (15-45^{o}C), some strains are more heat resistant than other members of the Enterobacteriaceae and will survive 60^{o}C for 15 minutes or 55^{o}C for 60 minutes. They are more sensitive to heat than members of the genus *Salmonella* to certain dyes, particularly brilliant green (Collee *et al.,* 1996).

E. coli is characterized by the possession of the enzymes β-galactosidase and β-glucuronidase (WHO, 1993) and substrates of β-glucuronidase have been incorporated into selective media for the simultaneous detection and identification of *E. coli*.

The GI tract of most warm-blooded animals is colonized by *E. coli* within hours or a few days after birth. The bacterium is ingested in foods or water or obtained directly from other individuals handling the infant. The human bowel is usually colonized within 40 hours of birth. *E. coli* can adhere to the mucous overlying the large intestine. *E. coli* originated from faeces capable of growing at $44.5^{O}C$ in lactose or mannitol medium with production of acid or gas. Being facultative organism *E. coli* is adapted to its intestinal (anaerobic) and its extraintestinal (aerobic or anaerobic) habitats.

ii. Indicator value of *E. coli*

E. coli is the best coliform indicator of faecal contamination from human and animal wastes. *E. coli*'s presence is more representative of faecal pollution because it is present in higher numbers in faecal material and generally not elsewhere in the environment. In human and animal faeces, 90- 100% of coliform organisms isolated are *E. coli* (Hurst *et al*., 2002). As a component of the assessment of public health risk through monitoring of water and treated wastewater, it seems to be the most sensitive indicator. The large numbers of *E. coli* present in human gut and the fact that they are not generally present in other environments support their continued use as the most sensitive indicator of faecal pollution. *E. coli* is retained as primary compliance parameter for faecal contamination by major International bodies such as United States Environmental Protection Agency (USEPA), European Union (EU) and Australian drinking water guidelines (Edberg *et al.*, 2000).

iii. Antigenic structure

Kauffman (1947) proposed a serological classification scheme for *E. coli* based on the presence of lipopolysaccharide 'O', flagellar 'H' and polysaccharide 'K' antigens detected by agglutination reactions with specific antisera.

a. Somatic antigen (O antigen)

These antigens represent the side chains of repeating sugar units projecting from the outer lipopolysaccharide layer of the bacterial cell wall. The O-antigens are heat- stable

and alcohol-stable. Over 170 different O antigens are described which are designated as 1, 2, 3, and so on. The normal colon strains of *E. coli* belong to early O groups (1, 2, 3, 4, 5, etc.) and the enteropathogenic strains belong to the later O groups (26, 55, 86, 111, 112, etc). Numerous cross reactions occur between *E. coli* O antigens, and between these and O antigens of other genera of Enterobacteriaceae.

b. Flagellar antigen (H antigen)

These antigens represent the determinant groups in the flagellar protein. They are heat-labile as well as alcohol-labile. The detached flagella remain immunogenic, but not the bacterium. More than 50 H antigens are described and all of them are monophasic. The organism has to be grown in semisolid agar for H antigen determination.

c. Surface antigen (K antigen)

It refers to the acidic polysaccharide surface (capsular) antigens even if no detectable capsule is present. It is divided into two groups- group I and II. The former is of high molecular weight and heat-stable while the latter is of low molecular weight and heat-labile. Intestine harbours several different serotypes of *E. coli* and most of them do not possess K antigens. Over 80 K antigens are known so far.

d. Fimbrial antigen (F antigen)

Many Enterobacteriaceae contain fimbriae. The common pili which are chromosomally determined, present in large number cause mannose sensitive haemagglutination which are probably not related to pathogenesis. Other than common pili, some strains of Enterobacteriaceae possess sex pili which are determined by conjugative plasmids and appear to be organs of conjugation. Type I fimbriae mediates adhesion of bacterium to human and animal cells that contain mannose residue.

iv. *E. coli* in human infections

Although *E. coli* is a normal bowel inhabitant, its pathogenic classification is somewhere between that of the overt pathogens and opportunistic organisms. Over 700

serotypes of *E. coli* are recognized based on O, H, and K antigens. From their normal site in the human body they are able to cause frequent opportunistic infections. Pathogenic strains of *E. coli* are responsible for three types of infections in humans: urinary tract infections (UTI), neonatal meningitis, and intestinal diseases (gastroenteritis). The diseases caused by a particular strain of *E. coli* depend on distribution and expression of an array of virulence determinants, including adhesins, invasins, toxins, and abilities to withstand host defenses. Furthermore, as the leading cause of nosocomial infections among Enterobacteriaceae, *E. coli* is likely to have greater virulence capabilities than the other species categorized as "opportunistic" Enterobacteriaceae (Bailey and Scott, 2007).

v. *E. coli* strains as primary intestinal pathogens

As a pathogen, *E. coli* is best known for its ability to cause intestinal diseases. Five serogroups of *E. coli* that cause diarrhoeal diseases are now recognized: enterotoxigenic *E. coli* (ETEC), enteroinvasive *E. coli* (EIEC), enterohemorrhagic *E. coli* (EHEC), enteropathogenic *E. coli* (EPEC), and enteroaggregative *E. coli* (EAEC).

a. Enterotoxigenic *E. coli* (ETEC)

ETEC is an important cause of diarrhoea in infants and travelers in underdeveloped countries or regions of poor sanitation. The diseases vary from minor discomfort to a severe cholera-like syndrome. ETEC are acquired by ingestion of contaminated food and water, and adults in endemic areas evidently develop immunity. ETEC strains produce heat-labile enterotoxin (LT) or heat stable enterotoxin (ST) or both. The disease requires colonization and elaboration of one or more enterotoxins. The infective dose of ETEC for adults has been estimated to be at least 10^8 cells; but the young, the elderly and the infirm may be susceptible to lower numbers. ETEC adhesins are fimbriae which are species-specific. These fimbrial adhesins adhere to specific receptors on enterocytes of the proximal small intestine. Symptoms of ETEC infections include diarrhoea without fever.

b. Enteroinvasive *E. coli* (EIEC)

EIEC closely resemble *Shigella* in their pathogenic mechanisms and the kind of clinical illness they produce. EIEC penetrate and multiply within epithelial cells of the colon causing widespread cell destruction resulting dysentery-like diarrheoa with fever. EIEC apparently lack fimbrial adhesins but do possess a specific adhesin that, as in *Shigella*, is thought to be an outer membrane protein. Also, like *Shigella*, EIEC are invasive organisms. Unlike typical *E. coli*, EIEC are non-motile, do not decarboxylate lysine and do not ferment lactose. Pathogenicity of EIEC is primarily due to its ability to invade and destroy colonic tissue.

c. Enteropathogenic *E. coli* (EPEC)

EPEC induces a profused watery, sometimes bloody, diarrhoea. They are a leading cause of infantile diarrhoea in developing countries. Outbreaks have been linked to the consumption of contaminated drinking water as well as some meat products. Pathogenesis of EPEC involves a plasmid-encoded protein referred to as EPEC adherence factor (EAF) that enables localized adherence of bacteria to intestinal cells and a non fimbrial adhesin designated intimin, which is an outer membrane protein that mediates the final stages of adherence. The diarrhoea and other symptoms of EPEC infections probably are caused by bacterial invasion of host cells and interference with normal cellular signal transduction, rather than by production of toxins. Some types of EPEC are referred to as diffusely adherent *E. coli* (DAEC), based on specific patterns of adherence.

d. Enteroaggregative *E. coli* (EAEC)

The distinguishing feature of EAEC strains is their ability to attach to tissue culture cells in an aggregative manner. These strains are associated with persistent diarrhoea in young children. They resemble ETEC strains in that the bacteria adhere to the intestinal mucosa and cause non-bloody diarrhoea without invading or causing inflammation. They also produce a haemolysin related to the haemolysin produced by *E. coli* strains

involved in urinary tract infections. The role of the toxin and the haemolysin in virulence has not been proven.

e. Enterohaemorrhagic *E. coli* (EHEC)

EHEC are recognized as the primary cause of haemorrhagic colitis (HC) or bloody diarrhoea, which can progress to the potentially fatal haemolytic uraemic syndrome (HUS). EHEC are characterized by the production of verotoxin or Shiga toxins. The toxin plays a role in the intense inflammatory response produced by EHEC strains and may explain the ability of EHEC strains to cause HUS. The toxin is phage encoded and its production is enhanced by iron deficiency. There are many serotypes of Shiga toxin-producing *E. coli*, but only those that have been clinically associated with HC are designated as EHEC. Of these, O157:H7 is the prototypic EHEC and most often implicated in illness worldwide. The infectious dose for O157:H7 is estimated to be 10 - 100 cells. EHEC infections are mostly food or water borne and have implicated undercooked ground beef, raw milk, cold sandwiches, water, unpasteurized apple juice and vegetables.

vi. Source and occurrence

Enteropathogenic *E. coli* are enteric organisms, and humans are the major reservoir, particularly of EPEC, ETEC and EIEC strains. Livestock, such as cattle and sheep and, to a lesser extent, goats, pigs and chickens, are a major source of EHEC strains. The latter have also been associated with raw vegetables, such as bean sprouts. The pathogens have been detected in a variety of water environments.

vii. Routes of exposure

Infection is associated with person-to-person transmission, contact with animals, food and consumption of contaminated water. Person-to-person transmissions are particularly prevalent in communities where there is close contact between individuals, such as nursing homes and day care centres.

viii. Significance in drinking-water

Waterborne transmission of pathogenic *E. coli* has been well documented for recreational waters and contaminated drinking-water. Control measures that can be applied to manage potential risk from enteropathogenic *E. coli* include protection of raw water supplies from animal and human waste, adequate treatment and protection of water during distribution. There is no indication that the response of enteropathogenic strains of *E. coli* to water treatment and disinfection procedures differs from that of other *E. coli*. Hence, conventional testing for *E. coli* provides an appropriate index for the enteropathogenic serotypes in drinking-water. This applies even though standard tests will generally not detect EHEC strains.

3.7.6 Other members of coliform group

Klebsiella spp. produces the mucoid colonies on MacConkey agar (Cheesbrough, 2000). The outermost layer of *Klebsiella* spp. consists of a large polysaccharide capsule that distinguishes the organism from other members of the family. *Klebsiella* spp. is excreted in the faeces of many healthy humans and animals, which are readily detected in sewage polluted water (WHO, 2004). *Klesiella* spp. can cause nosocomial infections and contaminated water and aerosol may be a potential source of the organisms in hospital environments and other health care facilities (Collee *et al.*, 1996).

Enterobacter spp. can be found in the intestinal tract of humans and animals and in soil, sewage, water and dairy products. They are one of the commonest opportunistic pathogens isolated from urine, respiratory tract and blood (Cheesbrough, 1993).

Citrobacter spp. is isolated from human or animal faeces, food, water, sewage, soil, etc. They are opportunistic pathogens and occasionally isolated from urine, blood, pus and other specimens (Cheesbrough, 1993). *Citrobacter freundii* and *Citrobacter diversus* have been associated with cases of diarrhoea (WHO, 2004).

3.7.7 Other common water-borne pathogens

Salmonella

Salmonella spp. is one of the common pathogens found in water, is gram negative, motile, non-capsulated, nonsporing bacilli that do not ferment lactose, produce hydrogen sulphide or gas from carbohydrate fermentation (*Salmonella* Typhi). Salmonellae are detected at low number in most surface water and are found in small number compared to coliform (APHA, 1998). Salmonellae are excreted in the faeces of infected humans or animals. In regard to enteric illness, *Salmonella* spp. can be divided into two fairly distinct groups: the typhoidal species/serovars (*Salmonella* Typhi and *Salmonella* Paratyphi) and the remaining non-typhoidal species/serovars (WHO, 2004).

***Shigella* spp.**

Shigella spp. cause serious intestinal diseases, including bacillary dysentery. All species can produce severe disease, but illness due to *S. sonnei* is usually relatively mild and self limiting. In the case of *Shigella dysenteriae*, clinical manifestations may proceed to an ulceration process, with bloody diarrhoea and high concentrations of neutrophils in the stool (WHO, 2004). These are non-lactose fermenting and non citrate utilizing. All Shigellae produce acid from glucose but not gas (with few exceptions). The isolation of *Shigella* spp. from drinking water indicates recent faecal pollution and infect human only (WHO, 1996).

***Vibrio* spp.**

Vibrio spp. is small, curved bacteria that are both catalase and oxidase positive. Among the vibrios, special attention has focused on the identity of those causing cholera. *Vibrio chlolerae* is the only pathogenic species of significance from freshwater environments. The isolation of *V. cholerae* O1 from water used for drinking is of major public health importance and is evidence of faecal contamination (WHO, 1996). A number of serotypes can cause diarrhoea, only O1 and 0 139 currently cause the classical cholera symptoms in which cases suffer fulminating and severe watery diarrhoea bacteraemia (WHO, 2004).

***Proteus* spp.**

Proteus spp. hydrolyses urea rapidly after four hours and produce H_2S in TSI (WHO, 2004; Cheesborough, 2000). *Proteus* spp. is found widely distributed in soil, polluted water, intestine of healthy man and animals. *Proteus mirabilis* is the commonest species of *Proteus* in human infection which cause urinary tract infection. It is often found in domiciliary patients with diabetes or structural abnormalities of the urinary tract and hospital patients after instrumentation. *Proteus vulgaris* may cause urinary tract infection (Collee *et al.,* 1996).

***Pseudomonas* spp.**

Pseudomonas spp. are oxidase positive, non spore forming widespread bacteria with some being opportunistic pathogens. *P. aeruginosa* is commonly found in faeces, soil, water and sewage but cannot be used as an index of fecal contamination, since it is not invariably present in faeces and sewage and may also multiply in the enriched aquatic environment and on the surface of organic materials in contact with water. However, its presence may be one of the factors taken into account in assessing the general cleanliness of water distribution systems (Payment *et al.,* 2003; WHO, 2004). It is a classical opportunistic pathogen with innate resistance to many antibiotics and disinfectants and causes skin infection especially at burn sites, wounds, respiratory infection, otitis externa, eye infection usually hospital acquired and septicaemia (Cheesbrough, 2000).

3.8 Membrane filtration method

Until the 1950s practical water bacteriology relied almost exclusively, for indicator purposes, on the enumeration of coliforms and *E. coli* based on the production of gas from lactose in liquid media and estimation of most probable numbers using the statistical approach initially suggested by McCrady in 1915. In Russia and Germany, however, workers attempted to culture bacteria on membrane filters, and by 1943 Mueller in Germany was using membrane filters in conjunction with Endo-broth for the analysis of potable waters for coliforms. By the 1950s membrane filtration was a

practical alternative to the MPN approach, although the inability to demonstrate gas production with membranes was considered a major drawback (Waite, 1985).

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. Production of metallic sheen confirms the presence of coliforms.

3.9 Studies on physico-chemical quality of drinking water in Nepal

Several studies on the physico-chemical parameters were made in water of Kathmandu valley and several parts of Nepal.

Shrestha (2002) analyzed water samples from various sources and reported the physicochemical parameters of most of the samples lying within the WHO guideline value except for conductivity, turbidity and iron. Similarly, Prasai (2002) analyzed water samples of Kathmandu city from different sources and reported 8.3 % of water samples to have crossed the WHO guideline value for pH. JICA/ENPHO (2005) assessed the physicochemical parameters in pre-monsoon and reported one- third of the water samples (34.3%) to be below WHO guideline value.

Bajracharya (2007) showed that the temperature showed marked variation ranging from 21.1^{o}C to 30.1^{o}C for tap water. Similarly, a comparison of pH value with WHO guideline (6.5-8.5) revealed that 85.09% of the samples were within guideline, 14.91% below the guideline and 0 % above the guideline. Jayana (2007) reported a distinct variation in the temperature of tested water samples ranging from 12.2^{o}C to 29.8^{o}C in tap water. The pH value of water range from 6.6 to 8.5 in tap water. Gyewali (2007) assessed the physicochemical parameters of water samples taken from seven different sources. Temperature of the water samples were found approximately near value depending upon the season of sample collection ranging from 14^{o}C to 17^{o}C. The pH value of all the water samples was found within the WHO guideline value (6.5-8.5)

ranging from 7.08 to 7.80. Gopali (2008) showed that the temperature of water did not show variability with values lying between 23.2^{o}C and 30.2^{o}C. Also the pH value was found to be ranging from 7.05 to 8.08. Similarly, Shrestha (2008) reported variation in the temperature as the lowest and the highest temperature recorded being 23.2^{o}C and 29.6^{o}C respectively. The pH value of the water samples ranged from 6.70 to 8.50.

3.10 Studies on microbial quality of drinking water in Nepal

In poor and developing countries like Nepal poor water quality, water pollution and their consequences have always been the topic of sensational story. The vital connection between the water and health though been established already, is given little emphasis in government policy. It has been reported that 66% of the rural people and more than 80% of the urban people has access to the piped water with the per capita water consumption in the rural area being far less than that in urban area of 45 liter and 60 liter respectively (Pradhan, 2004).

Karki (2001) studied the solar disinfection of 35 drinking water samples which were artificially contaminated with *E. coli* ATCC 25922. Out of these, 28 water samples showed more than 99.99% disinfection, 2 water samples showed 60-70% and 5 water samples showed only upto 25% disinfection. Similarly for *S.* Typhi, among 20 water samples tested, 15 water samples showed more than 99.99% disinfection, 2 water samples showed 70-80 % disinfection and 3 water samples showed only up to 30 % disinfection.

Rai *et al*., (2001) analyzed a total of 57 drinking water samples, 41 from natural water sources and 16 from piped tap water collected from different areas in Kathmandu Valley. 43(75%) samples had one or more species of organisms, out of which 22(51%) were *E. coli*. Among the 17 *E. coli* strains isolated, over two-thirds (15) were EPEC.

Prasai (2002) examined a total of 132 samples of drinking water of Kathmandu valley from different sources in which 92.4% of samples showed the presence of coliform

bacteria with the highest recovery of *E. coli* (26.4%) followed by *Citrobacter* spp.(22.6%), *P. aeruginosa* (6.3%), *Klebsiella* spp.(5.4%), *Shigella* spp.(3.78%) , *Salmonella* Typhi (3.3%), *Proteus vulgaris* (2.9%) , *Serratia* spp. (2.52%), *Vibrio cholerae* (0.0845%).

Shrestha (2002) analyzed a total of 95 water samples for bacteriological parameters from various sources. The raw water samples and settled water samples showed 100% presence of coliform, whereas reservoir water and Kuleshwor water showed 53.33 % and 76% respectively. The overall study found 85.26% of the samples to have exceeded WHO guideline value for total coliform.

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 well and open well were contaminated.

Bajracharya (2007) assessed the quality of drinking water of Kathmandu and reported 90.35% samples showing the presence of the coliform with ten different kinds of enteric bacteria with the highest recovery of *Citrobacter* spp.(26.22 %), followed by *E. coli* (25%), *Enterobacter* spp. (20.73%), *Shigella* spp.(8.54%), *Proteus vulgaris* (7.93%), *P. aeruginosa* (3.66%), *Salmonella* Paratyphi (3.05%), *Klebsiella* spp. (2.44%), *Proteus mirabilis* (1.83%) and *Salmonella* Typhi (0.61%).

Bhatta *et al*., (2007) studied the occurrence and diversity of *Salmonella* serovars in urban water supply systems of Nepal and detected the occurrence of *Salmonella* in 42 out of 300 water samples. A total of 54 isolates were identified to genus level by standard tests, subsequently confirmed by serotyping, phage typing and PCR detection of virulence genes. The predominant was *Salmonella* Typhimurium followed by *Salmonella* Typhi, *Salmonella* Paratyphi A and *Salmonella* Enteritidis.

Jayana (2007) assessed a total of 105 drinking water samples from the different sources of Madhyapur Thimi and reported 64.76 % of the samples crossed the WHO guideline value for total coliform count. *Enterobacter* spp. was the most predominant organism (29.5%) followed by *E. coli* (24.6%), *Citrobacter* spp. (20.4%), *Proteus vulgaris* (7%), *Klebsiella* spp. (5.6%), *Proteus mirabilis* (3.5*%), Shigella dysentery* (2.8%), *Salmonella* Typhi (2.1%), *Pseudomonas* spp. (2.1%), *Salmonella* Paratyphi (1.4%) and *Vibrio cholerae* (0.7%).

Gyewali (2007) assessed the water quality of Kathmandu valley, taken from seven different sources. All the water samples showed the presence of coliform bacteria. The highest bacterial count was 6.4 X 10^6 CFU/ml in river water and lowest bacterial count was found to be 3.0 X 10^3 CFU/ml in Kuleshwor tap water. Similarly, the highest coliform count was 1100 CFU/100 ml and the lowest coliform count was found to be 500 CFU/100 ml in samples taken from Sundharighat tank. Both the study showed that the most water get contaminated during storage or in the distribution system.

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

Gopali (2008) assessed the microbial quality of chlorinated drinking water of Kathmandu valley. 111 water samples were collected from the 7 distribution points of Sundarightat reservoir (38) and 13 distribution points of Balaju reservoir (73). The microbiological analysis of water samples from Sundarighat reservoir revealed the presence of total colifrom within WHO guideline value in 34.21% of total samples and 68.78% were found to contain total coliforms above the WHO guideline value in Sundarighat reservoir. In Balaju only 9.58% were found to meet the WHO guideline value whereas the rest 90.41% exceeded the guideline.

Shrestha (2008) assessed the municipal drinking water quality of the Kathmandu. A total of one hundred and seventy water samples were collected. 85.9% of the samples revealed the presence of heterotrophic bacteria while 88.2% of the total sample showed the presence of total coliform. Fifteen different genera of enteric bacteria with the total of two hundred and ninety six isolates were identified, *E. coli* (22.6%) being the predominant followed by *Klebsiella oxytoca* (16.55%), *Pseudomonas aeruginosa* (11.14%), *Citrobacter freundii* (10.13%), *Enterobacter aerogenes* (9.8%), *Shigella* spp. (7.09%), *Salmonella* Paratyphi (6.75%), *Proteus mirabilis* (6.41%), *Proteus vulgaris* (3.04%), *Acenetobacter* spp. (2.70%), *Citrobacter diversus* (2.02%), *Salmonella* Typhi and *Vibrio cholerae* (0.67%), *Serratia* spp. and *Vibrio parahaemolyticus* (0.33%).

3.11 Antibiotic susceptibility testing

Antibiotic susceptibility tests measure the ability of an antibiotic to inhibit bacterial growth in vitro (WHO, 2000). In the treatment and control of infectious diseases, especially when caused by pathogens that are often drug resistant, susceptibility testing is used to select effective antimicrobial drugs. Susceptibility testing is not usually indicated when the sensitivity reaction of a pathogen can be predicted (Cheesbrough, 2000). The most widely used testing method is the standardized agar disk diffusion method also known as the NCCLS (National Committee for Clinical Laboratory Standards) or modified Kirby-Bauer method (Lalitha, 2004). In this test, small sterile filter paper disks of uniform size (6 mm) impregnated with a defined concentration of an antibiotic are placed on the surface of an agar media previously inoculated with the test organism compared with turbidity standard so as to obtain uniform confluent growth after incubation. The disks are placed in even array on the plate, at well-spaced intervals from each other. When the disks are in firm contact with the agar, the antibiotic diffuses into the surrounding medium and comes in contact with the multiplying organisms. The plates are incubated at 35^{O}C for 18 to 24 hours. After incubation, the plates are examined for the presence of zones of inhibition of bacterial growth (clear rings) around the antibiotic disks. The organism is scored as susceptible (S), intermediate (I) or resistant (R) by measuring the diameter of the zone (in

millimeters) and comparing with the values listed in a standard zone size interpretative chart (NCCLS, 2002). The size of the zone of inhibition depends on a number of factors, including the rate of diffusion of a given antibiotic in the medium, the degree of susceptibility of the organism to the antibiotic, the number of organisms inoculated on the plate, and their rate of growth. It is essential, therefore, that the test be performed in a fully standardized manner so that the values read from the chart provide an accurate interpretation of susceptibility or resistance (Morello *et al.,* 2002).

3.12 Studies on bacterial resistance to antibiotics

Most of the works about surveillance on antibiotic resistance have been carried out in bacteria isolated from clinical samples; however, studies should also be expanded to those bacteria recovered from environmental samples in order to evaluate their role as possible reservoir of resistance genes and their capacity to transfer them to human pathogenic organism (Harakeh *et al.,* 2006). Studies on antibiotic resistance among water isolates have been made in Nepal and other countries which revealed variation in the pattern of the antibiotic resistance.

Papandreou *et al*., (2000) monitored the sensitivity of 239 Gram-negative bacteria (of faecal and non-faecal origin), isolated from the old drinking water distribution network of Patras in Southwestern Greece, to 20 antibiotics. The levels of antibiotic resistance obtained were: Cephalothin (86.7%), Ampicillin (77.5%), Carbenicillin (71%), Cefoxitin (55.4%), Cefuroxime (51.2%), Ticarcillin (31.3%), Ceftizoxime (31.2%), Chloramphenicol (30.3%), Cefotetan (25.2%), Cefotaxime (17.9%), Sulfisoxazole (15.2%), Ceftriaxone (12.5%), Tetracycline (11.9%), Trimethoprim/sulfamethoxazole (7.4%) and Piperacillin (2.4%).

Lazar *et al.,* (2002) performed antibiotic susceptibility testing of *E. coli* strains isolated from chronically polluted waters using Ampicillin, Tetracycline, Gentamicin, Kanamycin, Chloramphenicol, Ceftazidime and Cefotaxime. All strains were multiple

antibiotic resistant, 16% of them being resistant to 3, 4 and 6 antibiotics, 32% to 5 and 8% to all 7 antibiotics, respectively.

Shrestha (2002) studied the antibiotic resistance pattern of *E. coli* isolated from five different sources. 10 different antibiotics were used against the *E. coli* isolates and found that all the *E. coli* isolates were resistant to Ampicillin followed by 51.8%, 11.11%, 14.8% and 7.40 % of the total isolates being resistant to Nitrofurantoin, Nalidixic acid, Co-trimoxazole and Kanamycin respectively. The study also reported 74.07% of the total isolates being resistant to at least one antibiotic and 3.07% of the total isolates were multiple antibiotic resistant.

Lin *et al*., (2004) studied antibiotic resistance profiles of environmental isolates from Mhlathuze River, KwaZulu-Natal. The antibiotic resistance patterns of 113 enteric bacteria isolated from the Mhlathuze River showed that 94.7% were resistant to at least one class of antibiotic while 75.2% were multi-resistant. All isolates were sensitive to Gentamicin. The levels of resistance exhibited by isolates to Penicillin, Rifampicin, Novobiocin, Ampicillin and Cephalothin were 72.6%, 69.2%, 52.1%, 43.6% and 28.2% respectively.

Walia *et al.,* (2004) found that 70%, 55% and 15% of *E. coli* tested from drinking water in Michigan were resistant to Carbenicillin, Tetracycline and Streptomycin, respectively.

Tambekar *et al.,* (2006) isolated 85 strains of thermotolerant *E. coli* from 1000 water samples from different sources. These 85 isolates showed resistance to Ofloxacin (92%), Novobiocin (86%), Cefdinir (82%), Ciprofloxacin (79%), Cefazolin (64%), Ceftriaxone (58%) and Nitrofurantoin (51%). It was also observed that Azithromycin, Gentamicin, Amikacin, Chloramphenicol, Co-trimoxazole and Tetracycline were the most effective against the *E. coli* strains.

Roy *et al.,* (2006) cultured 32 *E. coli* isolates from different sources in Tamilnadu state of India. All the *E. coli* isolates showed high resistance to multiple antibiotics with 100% resistance observed against Ampicillin/cloxacillin, Chloramphenicol, Tetracycline, and Co-trimoxazole followed by Ciprofloxacin (67.7%), Gentamicin (61.3%), and Nitrofurantoin (51.3%).

Alhaj *et al*., (2007) studied the prevalence of antibiotic resistance in *E. coli* isolates from different sources. Seventy *E. coli* isolates from humans and environments were tested for susceptibility to 10 antibiotics by diffusion method. Resistance was found in 61.2% of the isolates. The observed resistances were to Kanamycin and Tetracycline (81.4%), Chloramphenicol (75.7%), Gentamicin (74.3%), Cefetoxin (44.3%), Norfloxacin (27.1%) and Ciprofloxacin (24.3%).

Watkinson *et al.,* (2007) determined the antibiotic resistance patterns of 462 *E. coli* isolates from different sources. The antibiotics chosen were Ampicillin, Cephalothin, Nalidixic Acid, Sulfafurazole, Gentamicin and Tetracycline. The highest incidence of bacterial resistance recorded was that for Tetracycline (51%), followed by those for Cephalothin (41%) and Sulfafurazole (32%).

Lima-Bittencourt *et al.,* (2007) performed antibiotic susceptibility test upon 102 enterobacterial isolates predominantly *E. coli* which was the most sensitive genus to antibiotics because only 12.5% of the isolates demonstrated multiple resistance. During rainy season 100% *E. coli* isolates were resistant to Ampicillin, Amoxycillin-clavulanic acid, Tetracycline, Chloramphenicol, Nalidixic Acid and Streptomycin and during dry season 71%, 14%, 14%, 57% and 100% were resistant to Ampicillin, Tetracycline, Nalidixic Acid, Kanamycin and Rifampicin respectively.

Lourenco *et al*., (2007) isolated 142 enterobacterial strains from estuarine water of Sao Vicente, Brazil with *E. coli* (40.1%) as the predominant one. The isolates were subjected to antibiotic susceptibility testing using seven different antibiotics. Analysis

of the antibiotic activity of the tested drugs against the isolates showed Gentamicin, Netilmicin and Ciprofloxacin with the highest activity (100%), followed by Cefepime (97.3%), Cefoxitin (84.2%), Amoxycillin/clavulanic acid (57.8%), and Ampicillin (47.3%).

Jayana (2007) assayed ten antibiotics against the total of 142 isolates of Kathmandu and reported the maximum resistance commonly directed toward Erythromycin (79.5%) and Penicillin G (62.67%). The other resistances were towards Ampicillin (34.5%), Ofloxacin (5.6%), Chloramphenicol (5.6%), Amoxycillin (61.9%), Cephotaxime (41.7%), Amikacin (14.7%), Ceftriaxone (15.4%) and Tetracycline (21.1%).

Shrestha (2008) assayed eight antibiotics against 33 isolates out of 296 from the drinking water of Kathmandu. The level of resistance exhibited by isolates to specific antibiotics was found as follows: Ampicillin (93.93 %), Tetracycline (27.27%), Nitrofurantoin (24.24%), Co-trimoxazole (18.18 %), Nalidixic Acid (15.15 %). All *E. coli* isolates showed resistance towards Ampicillin while all *E. coli* showed sensitivity towards Ciprofloxacin and Norfloxacin. 28.5% of *E. coli* showed the multi antibiotic resistance pattern.

Liliana *et al.,* (2008) performed antimicrobial susceptibility testing against *E. coli* isolated from surface water, ground water and drinking water sources. The resistance of *E. coli* to Ampicillin, Cephalothin, Ampicillin-sulbactam, Sulfamethoxazole-trimethoprim, Chloramphenicol and Nalidixic Acid was 44.1%, 26.5%, 5.9%, 5.9%, 4.4% and 4.4% respectively.

In another study, Tambekar *et al.,* (2008) carried out antibiotic susceptibility tests on 75 *E. coli* strains isolated from various water sources. Most of the isolated strains of *E. coli* were resistant to Linomycin (85%) and Vancomycin (66%) and sensitive to Levofloxacin (91%), Tobramycin (91%), and Moxifloxacin (90%).

Soge *et al.,* (2009) performed antimicrobial susceptibility on gram negative bacterial isolates obtained from rural south-western Ugandan groundwater. The antibiotics used were Ampicillin (10 µg), Cefotaxime (30 µg), Ceftazidime (30 µg), Chloramphenicol (30 µg), Kanamycin (30 µg), Tetracycline (30 µg), Trimethoprim/sulfamethoxazole (25 µg). Of the 52 Gram-negative isolates examined, 26 were susceptible to all antibiotics and 26 isolates were resistant to more than one antibiotics examined, with 14 isolates exhibiting multi-drug resistance.

3.13 Oligodynamic action

Oligodynamic action is the ability of small amount of heavy metal to exert a lethal effect on bacterial cell. Von Naegeli who pointed out that "definite metals and metal compounds confer in minute quantity of water solutions the ability to change and finally kill cells in a characteristic way" and noted the oligodynamic action of metals and metal compounds in 1893. Many metallic elements have been observed to inhibit the growth of bacteria and to inactivate enzymes. Practical application of such activity of metals has been made in the purification of water and in the preservation of tomato juice, of cider, and of hides. This antimicrobial effect is shown by metals such as mercury, silver, copper, lead, zinc, gold, aluminum and other metals and the concentration of the metal needed for this antimicrobial effect is extremely small. The zone of inhibition surrounding the metal on inoculated plate demonstrates the phenomenon of oligodynamic action.

Toxicity occurs through the displacement of essential metals from their native binding sites or through ligand interactions. In general, nonessential metals bind with greater affinity to thiol-containing groups and oxygen sites than do essential metals. Alterations in the conformational structure of nucleic acids and proteins and interference with oxidative phosphorylation and osmotic balance are other toxic properties. The redox properties that make some metals, such as copper, essential elements of biological systems, may also contribute to their inherent toxicity. For example, redox cycling between cupric and cuprous ions can catalyze the production of highly reactive hydroxyl

radicals, which can subsequently damage lipids, proteins, DNA and other biomolecules. Both gram positive and gram negative bacteria are affected by the oligodynamic action of heavy metals but the resistance towards such metal may develop. There are several bacterial strains that contain genetic determinant of resistance to heavy metal (Bopp *et al.,* 1983). These determinants for the resistance are often found on plasmids and transposons (Summers, 1985).

3.14 Metal tolerance mechanisms

Because heavy metals are increasingly found in microbial habitats due to natural and industrial processes, microbes have evolved several mechanisms to tolerate the presence of heavy metals or to use them as terminal electron acceptors in anaerobic respiration. To have a toxic effect heavy metal ion must first enter the cell. Because some heavy metals are necessary for enzymatic functions and bacterial growth, uptake mechanisms exist that allow for the entrance of metal ions into the cell. To survive under metal-stressed conditions, bacteria have evolved several types of mechanisms to tolerate the uptake of heavy metal ions. These mechanisms include the efflux of metal ions outside the cell, accumulation and complexation of the metal ions inside the cell, and reduction of the heavy metal ions to a less toxic state. Most mechanisms studied involve the efflux of metal ions outside the cell, and genes for this general type of mechanism have been found on both chromosomes and plasmids (Spain, 2003).

3.15 Studies on Oligodynamic action

Jayana (2007) tested six heavy metals for their oligodynamic action against the water isolates of Kathmandu. The study showed that the silver, copper, and zinc were very effective compared to brass against the water isolates while aluminum and steel were found to be very ineffective.

Huang (2007) tested the in vitro efficacy of copper and silver ions in eradicating *P. aeruginosa*. The results showed that copper ion concentrations tested (0.1-0.8 mg/L) achieved more than 99.99% reduction of *P. aeruginosa* whereas silver ions

concentration of 0.08 mg/L achieved more than 99.99% reduction of *P. aeruginosa* in 6 hours. Combination of copper and silver ions exhibited a synergistic effect against *P. aeruginosa.*

Shrestha (2008) studied oligodynamic effect of metals silver, copper and brass on different bacteria isolated from water samples. The load of *E. coli* was reduced totally after 4 hours of incubation with copper and brass pot but 100% reduction in its load was achieved only after 24 hours with silver pot. Whereas in case of MDR *E. coli* isolated from water, the microbial load was reduced totally after 24 hours of incubation with silver and brass pots while this was achieved only after 48 hours of incubation with copper.

Fisher (2008) studied the speeding effect of SODIS by combined effect of hydrogen peroxide, temperature, pH, and copper plus ascorbate on the photoinactivation of *E. coli.* The study showed that these additives make SODIS more rapid and effective in both sunny and cloudy weather, developments that could help make the technology more effective and acceptable to users.

Midwest Research Institute (USA) conducted a study in which bacteria were introduced into 50 foot coils of different plumbing tube materials. Water with a suspension of *E. coli* was then pumped through the coils and changes in bacteria viability were periodically determined. While in different types of plumbing material the level of bacteria remained the same, or in some cases even increased, in the copper loop only 1% of the *E. coli* bacteria remained viable after five hours. Based on a similar study, The Midwest Research Institute subsequently also reported that water distribution systems made of copper have a greater potential for suppressing growth and for decreasing persistance of *L. pneumophila* cells in potable water than do distribution systems constructed of plastic materials or galvanized steel (Wells, 2001).

A study conducted at the centre for Applied Microbial Research, Public Health Laboratories Service (PHLS) in England, compared the growth of *Legionella pneumophila* on copper and other plumbing materials by using a continuous culture model system. They found that the bacteria levels were reduced on copper surfaces compared with a glass control and other plumbing materials at all the temperatures tested (20^{o}C, 40^{o}C, 50^{o}C and 60^{o}C) and in the three different waters used (Copper Plumbing Company ,2004).

CHAPTER – IV

4. MATERIALS AND METHODS

4.1 MATERIALS

All the materials, chemicals, equipments, media and reagents used to accomplish this study are given in the Appendix.

4.2 METHODS

A total of 102 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

The study was conducted in eleven different areas of Kathmandu district viz. Kuleshwor, Balkhu, Bagbazaar, Maitidevi, Handigaon, Kirtipur, Gaushala, Teku, Kamalpokhari, Kalikastan and Kalopul.

4.2.2 Collection of water samples

Water samples for bacteriological analysis were collected in pre-sterilized bottles (15 lbs at 121^{o} C for 15 minutes) of 500 ml capacity containing 0.3 ml of 3% (w/v) sodium thiosulphate solution added prior to the sterilization. Bottles were tightly capped and care was taken to prevent contamination. For physico-chemical examination water samples were collected in clean plastic bottles, washed and rinsed 3-4 times with water to be tested.

The water was sampled for bacteriological analysis as given by Cheesbrough (2000) as follows:

1. Any external fittings from tap were removed, such as an antisplash nozzle or rubber tube.
2. Outside nozzle of the tap was cleaned carefully.

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 ethanol 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 labeled with the sample code number.

4.2.3 Transportation and preservation of sample

Immediately after collection, samples were transported to the laboratory of Central Department of Microbiology, Kirtipur holding in an ice-box at 4^{o}C. Water samples were examined as soon as possible on arrival or within 6 hours of collection. In some cases when immediate analysis was not possible, the samples were preserved at 4^{o}C.

4.2.4 Water quality analysis

Physicochemical (pH and temperature) and microbiological parameters were analyzed for assessment of drinking water quality.

4.2.4.1 Analysis of physico- chemical parameters of water samples

Standard Methods for the Examination of Water and Wastewater (APHA, 1998) was followed to analyze physico-chemical parameters of water. The temperature and pH of water samples were recorded at the site during sampling period.

a. Temperature

Temperature was determined with the help of a standard mercury thermometer graduated up to 50^{o}C. Thermometer bulb was immersed into the water soon after collection and the reading was noted.

b. pH

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

4.2.4.2 Total coliform and thermotolerant coliform count

In this study, total coliform and thermotolerant coliform were enumerated by the membrane filtration (MF) technique as described by APHA, 1998.

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, on to a plate of M- Endo agar with the help of sterile forcep. Care was taken not to entrap air bubbles between the membrane and the medium.
7. Then it was incubated at 37^{o}C for 24 hours for total coliform and 44.5^{o}C for thermotolerant coliform in an inverted position.
8. After proper incubation total colony forming unit (CFU) were counted.

4.2.4.3 Isolation and identification of *E. coli*

Greenish metallic sheen producing colonies from thermotolerant coliform positive M-Endo agar plates were sub-cultured onto MacConkey agar and incubated at 44.5^{o}C for 24 hours. All lactose fermenting colonies were subcultured on Nutrient agar for pure culture. Identification of *E. coli* was done based on colony characteristics,

morphological characteristics and biochemical properties on respective media according to Bergey's Manual of Determinative Bacteriology, 1986.

A) Cell morphology

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

B) Study of biochemical tests

Biochemical tests are based on the ability of microorganisms to produce enzymes thus utilizing different substrates as described by Cheesbrough, 1993. The isolated pure colonies were inoculated into different biochemical media for different tests which are as follows:

Table 4.1 Biochemical tests performed for identification of *E. coli* isolates

S.N.	Tests	Biochemical Media
1.	Catalase	3% H_2O_2
2.	Oxidase	10% Tetramethyl - p- phenylene diamine dihydrochloride.
3.	Indole Production	Sulfide- Indole- Motility medium (SIM)
4.	Methyl Red test	Glucose phosphate peptone water or MR-VP medium.
5.	Voges- Proskauer test	Glucose phosphate peptone water or MR-VP medium.
6.	Citrate utilization test	Simmon's citrate agar.
7.	Fermentation of glucose, lactose and sucrose, H_2S and gas production	Triple sugar Iron Agar (TSIA)
8.	Aerobic or anaerobic utilization of carbohydrate	Hugh and Leifson Medium.
9.	Urease Production	Urea base agar.

4.2.5 Study of antibiotic susceptibility of *E. coli* isolates

Antibiotic susceptibility of isolated *E. coli* strains was assayed using a modified Kirby-Bauer disk diffusion method (Bauer *et al.*, 1966). Cells were grown at 37^{o}C in 5ml of nutrient broth for about 4 hours 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 Muller-Hinton agar. During swabbing the plate was streaked with the swab three times turning the plate 60^{o}C 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^{o}C 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 as listed by manufacturer. Organisms considered to be intermediate resistance were scored as sensitive.

4.2.5.1 Preparation of swab

Cotton wool swabs on wooden applicator sticks should be prepared. They were sterilized in tubes either in the autoclave or by dry heat.

4.2.5.2 Preparation of barium sulphate standard:

- Preparation of 1% v/v H_2SO_4

1 ml of conc. H_2SO_4 was added to 99 ml of distilled water and mixed well.

- Preparation of 1% w/v $BaCl_2 .2H_2O$

0.5 gram of $BaCl_2 .2H_2O$ was dissolved in 50 ml of distilled water.

- 0.6 ml of $BaCl_2 .2H_2O$ was mixed with 99.4 ml of H_2SO_4 and dispensed in capped tube

- It was stored in well sealed container in dark at room temperature**.**

4.2.6 Study of Oligodynamic action against *E. coli* isolates

The study of effect of metals against test organisms was done as described by Benson, 1979. Cells were grown at 37^{o}C in 5ml of nutrient broth for few hours using pure cultures as inoculum for turbidity development. A sterile cotton swab was dipped into the properly prepared inoculum and firmly rotated against the upper inside wall of the tube to express excess fluid, and then swabbed onto nutrient agar. The plate was kept at room temperature for 5 to 10 minutes. Five metallic disks of silver, copper, brass, aluminium and steel were carefully placed on the solidified agar surface in the plate at equal distance and sufficiently separated from each other with the help of a flamed forceps. The metallic disks before placing on the agar were cleaned, one at a time, as follows:

- Washed first with soap and water, then rinsed with water.
- With flamed forceps dipped in alcohol and then flamed.

Then the plate was incubated for 24 hrs. at 37^{o}C. The zone of inhibition for each disk was measured at the end of the incubation period.

CHAPTER-V

5. RESULTS

This study was carried out from August 2008 to March 2009. A total of 102 tap water samples were randomly collected from different areas of Kathmandu city. The study aimed at determination of some physico-chemical parameters and bacteriological quality of water samples along with the isolation of *E. coli* and study of antibiotic susceptibility pattern and oligodynamic action against the isolates.

5.1. Physico-chemical parameters of water

5.1.1 Temperature

The variation in temperature was observed with the highest and the lowest temperature being 25.7^{o}C and 11.8^{o}C respectively and the average temperature being 18.7^{o}C. The highest temperature was recorded in the month of August from Kuleshwor while the lowest temperature was recorded in the month of December from Teku.

5.1.2 pH

No variation was seen in the pH values of the water samples with all lying close to the neutral pH and within the WHO guideline (6.5-8.5). The pH value of the water ranged from 6.9 in Gaushala to 7.9 in Balkhu with an average value of 7.4.

Table 1: Location wise result for physico-chemical parameters

S.N.	Location	Mean temperature (^{O}C)	Mean pH
1.	Kuleshwor	25.5	7.4
2.	Balkhu	24.3	7.7
3.	Bagbazaar	23.8	7.6
4.	Maitidevi	20.5	7.4
5.	Handigaon	16.4	7.2
6.	Kirtipur	13.6	7.6
7.	Teku	12.1	7.5
8.	Gaushala	13.6	6.9
9.	Kalopul	14.9	7.2
10.	Kamalpokhari	18.7	7.1
11.	Kalikastan	22.2	7.5

5.2 Bacteriological quality of water

A total of 102 tap water samples were analyzed for the presence of total coliform and thermotolerant coliform by millipore membrane filtration method.

5.2.1 Total Coliform count and Thermotolerant Coliform count:

Out of 102 water samples, 88 samples (86.2%) were found to contain the total coliform beyond the WHO guideline value (0 CFU/100 ml), while the rest of the water samples i.e., 14 (13.7%) meet the WHO guideline. The coliform count ranged from 0 to >300 CFU/100 ml.

Similarly, 20 samples (19.6%) were found to contain thermotolerant coliform beyond the WHO guideline value (0 CFU/100 ml) while rest of the samples i.e., 82 (80.3%) were found to meet the WHO guideline. The thermotolerant count ranged from 0 to 52 CFU/100 ml.

Table 2: Total coliform count exceeding WHO guideline value

Total no. of samples	No. of samples exceeding WHO guideline value	Percentage
102	88	86.2

Table 3: Location wise total coliform count exceeding WHO guideline value

S.N.	Location	No. of sample	No. of sample exceeding WHO guideline	Percentage
1.	Kuleshwor	10	10	100
2.	Balkhu	10	10	100
3.	Bagbazaar	8	8	100
4.	Maitidevi	10	10	100
5.	Handigaon	8	4	50
6.	Kirtipur	10	4	40
7.	Teku	10	10	100
8.	Gaushala	10	10	100
9.	Kalopul	8	4	50
10.	Kamalpokhari	10	10	100
11.	Kalikastan	8	8	100

Table 4: Thermotolerant coliform count exceeding WHO guideline value

Total no. of sample	No of sample exceeding WHO guideline value	Percentage
102	20	19.6

Table 5: Location wise thermotolerant coliform count exceeding WHO guideline value

S.N.	Location	No. of sample	No. of sample exceeding WHO guideline	Percentage
1.	Kuleshwor	10	2	20
2.	Balkhu	10	3	30
3.	Bagbazaar	8	5	62.5
4.	Maitidevi	10	3	30
5.	Handigaon	8	0	0
6.	Kirtipur	10	0	0
7.	Teku	10	0	0
8.	Gaushala	10	0	0
9.	Kalopul	8	0	0
10.	Kamalpokhari	10	3	30
11.	Kalikastan	8	4	50

5.2.2 Isolation and identification of *E. coli*

E. coli was isolated and identified using the conventional biochemical method from the water samples that were tested positive for thermotolerant coliform. Thus, a total of 20 *E. coli* isolates were obtained.

Table 6: Location wise isolation of *E. coli*

S.N.	Location	Total no. of samples	No. of isolates	Percentage recovery
1.	Kuleshwor	10	2	20
2.	Balkhu	10	3	30
3.	Bagbazaar	8	5	62.5
4.	Maitidevi	10	3	30
5.	Handigaon	8	0	0
6.	Kirtipur	10	0	0
7.	Teku	10	0	0
8.	Gaushala	10	0	0
9.	Kalopul	8	0	0
10.	Kamalpokhari	10	3	30
11.	Kalikastan	8	4	50

5.3 Antibiotic susceptibility pattern of *E. coli* isolates

E. coli isolates were subjected to the antibiotic susceptibility test to ten different antibiotics using Kirby-Bauer disk diffusion method. The antibiotics used were Tetracycline(T), Co-trimoxazole(Co), Amikacin(AK), Cephalexin(Cp), Chloramphenicol(C), Amoxycillin(Am), Ceftriaxone(Ci), Ciprofloxacin(CF), Nalidixic Acid(NA) and Gentamicin(G). Resistance of the isolates was predominantly directed towards Cephalexin(65%) followed by Amoxycillin(45%) and Tetracycline(15%). All the isolates were 100% sensitive to Co-trimoxazole, Amikacin, Chloramphenicol, Ceftriaxone, Ciprofloxacin, Nalidixic Acid and Gentamicin. Isolates that were resistant to at least one antibiotic were the most common (n=14 i.e, 70%) followed by those resistant to at least two or more (n=10 i.e, 50%) while only one (n=1 i.e, 5%) was resistant to three antibiotics.

Table 7: Antibiotic susceptibility pattern of *E. coli*

Isolate no.	Antibiotics									
	T	Co	Ak	Cp	C	Am	Ci	CF	NA	G
1.	S	S	S	R	S	I	S	S	S	S
2.	S	S	S	R	S	R	S	S	S	S
3.	S	S	S	R	S	R	S	S	S	S
4.	S	S	S	S	S	I	S	S	S	S
5.	S	S	S	R	S	R	S	S	S	S
6.	S	S	S	R	S	R	S	S	S	S
7.	S	S	S	I	S	I	S	S	S	S
8.	R	S	S	R	S	I	S	S	S	S
9.	S	S	S	R	S	R	S	S	S	S
10.	S	S	S	I	S	I	S	S	S	S
11.	S	S	S	I	S	I	S	S	S	S
12.	R	S	S	R	S	R	S	S	S	S
13.	I	S	S	R	S	R	S	S	S	S
14.	S	S	S	I	S	I	S	S	S	S
15.	R	S	S	R	S	I	S	S	S	S
16.	S	S	S	R	S	R	S	S	S	S
17.	I	S	S	I	S	R	S	S	S	S
18.	S	S	S	I	S	I	S	S	S	S
19.	S	S	S	R	S	S	S	S	S	S
20.	S	S	S	R	S	S	S	S	S	S

N.B: T-Tetracycline (30 µg), Co-Co-trimoxazole (25 µg), Ak-Amikacin (30 µg), Cp-Cephalexin (30 µg), C-Chloramphenicol(30 µg), Am-Amoxycillin(10 µg), Ci-Ceftriaxone (30 µg), CF- Ciprofloxacin(5 µg), NA- Nalidixic Acid(30 µg), G-Gentamicin(30 µg)

Table 8: Percentage of antibiotic susceptibility of *E. coli* isolates

Antibiotic used	Sensitive(S)		Intermediate(I)		Resistant(R)	
	No. of isolates	Percent	No. of isolates	Percent	No. of isolates	Percent
Tetracycline (30 µg)	15	75	2	10	3	15
Co-trimoxazole (25 µg)	20	100	0	0	0	0
Amikacin (30 µg)	20	100	0	0	0	0
Cephalexin (30 µg)	1	5	6	30	13	65
Chloramphenicol(30µg)	20	100	0	0	0	0
Amoxycillin(10 µg)	2	10	9	45	9	45
Ceftriaxone(30 µg)	20	100	0	0	0	0
Ciprofloxacin(5 µg)	20	100	0	0	0	0
Nalidixic Acid(30 µg)	20	100	0	0	0	0
Gentamicin(30 µg)	20	100	0	0	0	0

Table9: Frequency of antibiotic and multiple-antibiotic resistance among *E. coli*

Total no. of isolates	Antibiotic resistant		Multiple antibiotic resistant	
20	No. of isolates	Percentage	No. of isolates	Percentage
	14	70	10	50

5.4 Oligodynamic action against *E. coli* isolates

Five different types of metals (silver, copper, brass, aluminum and steel) were tested for their inhibitory effect against the isolates. Silver was found to be highly effective whereas copper effective in inhibiting the *E. coli* isolates as shown by their highest zone of inhibition but brass, aluminum and steel were found totally ineffective. Silver was relatively more effective than copper as it produced greater average zone of inhibition

(6.4 mm) and inhibited all of the isolates while the average zone of inhibition produced by copper was 4.1 mm and it could inhibit 70% (n=14) of the isolates.

Table 10: Oligodynamic action of metals upon *E. coli* isolates

Isolate no.	Zone of inhibition (ZOI) (mm)				
	silver	copper	brass	aluminium	steel
1	6	5	0	0	0
2	7	6	0	0	0
3	6	5	0	0	0
4	5	0	0	0	0
5	7	6	0	0	0
6	8	6	0	0	0
7	7	6	0	0	0
8	5	0	0	0	0
9	7	7	0	0	0
10	6	5	0	0	0
11	7	6	0	0	0
12	5	5	0	0	0
13	6	0	0	0	0
14	7	6	0	0	0
15	6	0	0	0	0
16	6	0	0	0	0
17	6	0	0	0	0
18	7	6	0	0	0
19	7	7	0	0	0
20	7	6	0	0	0
Average ZOI	6.4	4.1	0	0	0

CHAPTER- VI

6. DISCUSSION AND CONCLUSION

6.1 DISCUSSION

Water and sanitation are the primary drivers of public health which means that once we can secure access to clean water and to adequate sanitation facilities for all people, irrespective of the difference in their living conditions, a huge battle against all kinds of diseases will be won. The majority of diseases in developing countries are infectious in nature caused by bacteria, viruses and other microbes, which are shed in human faeces and pollute water supplies, which people use for drinking and other purposes. In Nepal, health problems of general people due to poor quality water are a major issue. The water borne diseases are among the top ten diseases in the country that can be minimized by adopting effective preventive measures such as health and sanitation awareness, protection of water sources, avoiding any kind of contamination and so on. If effective efforts are given to these preventive measures, water borne diseases can be minimized (Pradhan, 2004).

This study was undertaken to assess the microbial quality of drinking water of Kathmandu city along with the measurement of some physicochemical parameters (pH and temperature). Antibiotic susceptibility pattern and oligodynamic action against *E. coli* isolates were also studied.

In this study, distinct variation in temperature of tested water samples was observed. The temperature ranged from 11.8^{o}C to 25.7^{o}C. Previous studies have also shown a variation in temperature of drinking water of Kathmandu. Studies conducted by Joshi *et al.*, (2004), Bajracharya (2007), Jayana (2007), Gopali (2008), Shrestha (2008) are in agreement with this study in terms of temperature variation.

Water bodies undergo temperature variation along with normal climatic fluctuations and seasonal variation. Temperature of water is influenced by latitude, altitude, season, time of day, air circulation, cloud cover and the flow and depth of water body (Chapman, 1993). A rise in temperature of the water leads to the speeding up of the chemical reactions in water, reduces the solubility of gases, enhances growth of microorganisms and amplifies the tastes, odor and corrosion problem however there is no standard guideline value regarding the temperature. Low temperature suppresses and higher temperature increases the chlorination process. Water in the temperature range of 7^{o}C to 11^{o}C has a pleasant taste and is refreshing (WHO, 2004).

Uniformity was seen among the pH values of the water samples. The pH value of the water ranged from 6.9 to 7.9 with an average value of 7.5. Thus, all the samples (100%) were within the WHO guideline (6.5-8.5). Similar result was obtained in the studies conducted by Sharma (1986), Bottino *et al.,* (1991), Thapa (1997), Maharjan (1998), Prasai (2002), Gyewali (2007), Gopali (2008) and Shrestha (2008) who reported that the pH of the water samples lie within the permissible limit.

In drinking water, acidic pH may cause corrosion of metal pipes in the distribution system and alkaline pH adversely affects the disinfection process as more alkaline water requires a longer time or a higher FRC level at the end of the contact time for adequate disinfection. Chlorination may be markedly less effective in increasing pH values. High pH induces the formation of trihalomethanes which are toxic. pH is an important factor in fixing alum dose in drinking water treatment. At high pH, dissolved minerals such as iron and calcium precipitate, adding turbidity and increasing soil removal demand upon filter system. High pH in the presence of high total alkalinity contributes to scaling of filters, heaters and piping. As pH drops below seven, the condition of the water becomes progressively acidic, producing eye irritation, corrosion and other undesirable effects (Gopali, 2008).

The bacteriological quality of the water samples revealed the presence of total coliform in 86.2% of the samples to be beyond the WHO guideline (0 CFU/100 ml), while the rest of the water samples i.e, 13.7% meet the guideline. The coliform count ranged from 0 to >300 CFU/100 ml. Similarly, 19.6% of the samples were found to contain thermotolerant coliform beyond the WHO guideline value (0 CFU/100 ml) while rest of the samples i.e., 80.3% were found to meet the WHO guideline. The thermotolerant count ranged from 0 to 52 CFU/100 ml.

This study along with the previous studies carried till date have shown that the drinking water quality of Kathmandu is deteriorating with time in all types of sources. The result of this study in terms of microbial quality of drinking water is in agreement with the studies carried out by Sharma (1978), Sharma (1986), Adhikari *et al.,* (1986), CEDA (1989), ENPHO/DISVI (1995), Maharjan (1998), Prasai (2002), Shrestha (2002), Warner *et al.,* (2007), Jayana (2007), Bajracharya (2007), Gyewali (2007), Gopali (2008) and Shrestha (2008) all of which indicate that the public water supply is far from satisfactory in almost all localities. This can be attributed to insufficient treatment of surface waters for the drinking water supply, malfunctioning of sewage collection systems, and defective water distribution pipelines leading to contamination of potable water by faecal coliform and other pathogenic bacteria (Bhatta *et al.,* 2007).

Altogether 27 *E. coli* isolates were obtained from 102 water samples. The data generated from the studies carried out on water quality in Nepal indicate *E. coli* as the predominant enteric bacteria isolated from faecally contaminated water. Sharma (1986) and Thapa (1997) both isolated *E. coli* as predominant bacteria from water samples. Shrestha (2002) obtained a total of 27 *E. coli* isolates from 95 drinking water samples. Warner *et al.,* (2007) found that 72% of all the water samples were found to contain *E. coli.* Similarly, studies done by Rai *et al*., (2001), Prasai (2002), Bajracharya (2007), Jayana (2007) and Shrestha (2008) reported predominance of *E. coli* as major bacterial isolate with the percentage recovery of 51%, 26.4%, 25%, 24.6% and 22.6% respectively.

The presence of coliform in a high proportion of water samples when coupled with the detection of *E. coli* in water sample is cause for concern. Although *E. coli* is normal inhabitant of the gastrointestinal tract of warm-blooded animals, certain serotypes have been associated with waterborne disease outbreaks and mortality in humans (Bruneau *et al.,* 2004). Several classes of enteropathogenic *E. coli* have been identified on the basis of different virulence factors, including enterohaemorrhagic *E. coli* (EHEC), enterotoxigenic *E. coli* (ETEC), enteropathogenic *E. coli* (EPEC), enteroinvasive *E. coli* (EIEC), enteroaggregative *E. coli* (EAEC) and diffusely adherent *E. coli* (DAEC) (WHO, 2006).

The result highlights the deteriorating quality of water which may result in outbreak of water-borne diseases. The unsatisfactory drinking water quality may be attributed to the failure of the disinfection of the raw water at treatment plant or to the infiltration of contaminated sewage through cross connection, leakage points and back siphonage. The chlorine dosing in the reservoirs may be inadequate to maintain FRC concentration up to the recommended limit. If the cross contamination in the pipelines is high in areas at the vicinity of the treatment plants, most of the FRC may be consumed by the pollutants in these areas and very little will be left for distant areas (DISVI, 1990). Furthermore, several investigators have shown that coliform could survive standard chlorine residual into the distribution system (Momba *et al.,* 1999).

Coliform have been extensively used as an indicator of the microbial quality of drinking water. Strains of total coliform bacteria may colonize surfaces within systems and become part of a biofilm (Lechevallier *et al.,* 1990). Their ability to thrive in an environment in a drinking water distribution makes total coliform an unreliable index of faecal contamination. *E. coli* is the indicator of choice used in WHO guidelines for drinking water quality (WHO, 2006). The data suggest that *E. coli* is extensively derived from the faeces of warm blooded animals, fails to multiply in the natural environment and has been shown to be highly resistant to treatment process. Its

presence in drinking water is interpreted as an indication of recent or substantial post treatment faecal contamination or inadequate treatment (Payment *et al.,* 2003).

Microbial quality of water consumed is critical in controlling infectious diseases and other health problems. Therefore regular monitoring of drinking water not only prevents diseases and hazards but also checks the water resources from going further polluted. There can be no state of positive health and well being without safe water. The conservation of water sources is very important to provide safe water. The ultimate objective for monitoring the microbiological quality of water is to identify and then minimize the public health risk from consuming water intended for drinking. So, the conservation, management and establishment of sanitary practices for the disposal of sewage and the increasing use of disinfection methods resulted in a dramatic decrease in waterborne diseases by protecting public water supplies from contamination and providing safe drinking water.

20 *E. coli* isolates were assayed against ten antibiotics. These antibiotics were chosen because of their wide-spread use and importance in the treatment of gram-negative bacterial infections. Resistance was found most commonly directed toward Cephalexin (65%) and Amoxycillin (45%) while only 15% exhibited resistance towards Tetracycline. All the isolates were 100% sensitive to Co-trimoxazole, Amikacin, Chloramphenicol, Ceftriaxone, Ciprofloxacin, Nalidixic Acid and Gentamicin. Isolates that were resistant to at least one antibiotic were the most common (70%) followed by those resistant to at least two or more (50%).

Varying patterns of resistance of *E. coli* and other bacteria to different antibiotics has been reported within and outside the country. Maharjan (1998) reported resistance mainly toward Nitrofurantoin, Ampicillin and Tetracycline. 71.4% of *E. coli* was resistant to at least one antibiotic while 23.8% was multiple antibiotic resistant. Shrestha (2002) reported that the *E. coli* isolates were resistant to Ampicillin, Nitrofurantoin, Nalidixic Acid, Co-trimoxazole and Kanamycin. 74.07% of total *E. coli* isolates were

resistant to at least one antibiotic and MAR was found in 3.07%. Jayana (2007) reported resistance directed toward Erythromycin, Penicillin G, Ampicillin, Ofloxacin, Chloramphenicol, Amoxycillin, Cephotaxime, Amikacin, Ceftriaxone and Tetracycline. Shrestha (2008) reported that all the *E. coli* isolates showed resistance towards Ampicillin. 28.5% of *E. coli* showed the multi drug resistant pattern.

Many works have been done in other countries on the antibiotic susceptibility testing upon *E. coli* and other bacterial isolates from water. Study conducted by El-Zanfaly *et al.,* (1987) in drinking water of Cairo, Egypt showed that most of the *E. coli* isolates were resistant to Ampicillin. Antai (1987) in Port Harcourt, Nigeria disclosed that the *E. coli* strains were most often resistant to Ampicillin, Cephalothin and Tetracycline. Ramtete *et al*., (1991) found that most of the coliform isolates were resistant to Ampicillin. Similarly Pandey and Musarrat (1993) reported the Ampicillin and Tetracycline resistant *E. coli* in the water of India. Pathak *et al*., (1994) showed that resistance to Ampicillin was found maximum while almost 50% of the total isolates exhibited the multiple antibiotics resistance. Bissonette *et al*., (1995) in West Virginia, USA revealed resistance of isolates towards Ampicillin, Nitrofurantoin, Tetracycline, Chloramphenicol and Nalidixic Acid. Parveen *et al*., (1997) reported that *E. coli* isolates from point sources were significantly resistant to antibiotics. Boon *et al*., (1999) in Southeastern Australia found *E. coli* to be resistant to Ampicillin, Chloramphenicol, Kanamycin, Nalidixic Acid, Neomycin and Streptomycin. Papandreou *et al*., (2000) in southwestern Greece observed highest levels of antibiotic resistance for Cephalothin, Ampicillin, Carbenicillin, Cefoxitin and Cefuroxime. Lazar *et al.,* (2002) reported all *E. coli* strains to be multi antibiotic resistant using Ampicillin, Tetracycline, Gentamicin, Kanamycin, Chloramphenicol, Ceftazidime and Cefotaxime. Walia *et al.,* (2004) found *E. coli* from drinking water in Michigan resistant to Carbenicillin, Tetracycline and Streptomycin. Lin *et al*., (2004) reported resistance of environmental isolates to Penicillin, Rifampicin, Novobiocin, Ampicillin and Cephalotin. Roy *et al.,* (2006) in Tamilnadu state of India found that all the *E. coli* isolates showed high resistance to multiple drugs which included Ampicillin/cloxacillin, Chloramphenicol, Tetracycline,

Co-trimoxazole, Ciprofloxacin, Gentamicin and Nitrofurantoin. Alhaj *et al*., (2007) who studied the prevalence of antimicrobial resistance in *E. coli* isolates found that the most prevalent resistances were to Kanamycin, Tetracycline, Chloramphenicol and Gentamicin. Watkinson *et al.,* (2007) recorded the highest incidence of *E. coli* resistance towards Tetracycline, Cephalothin and Sulfafurazole. Liliana *et al.,* (2008) found *E. coli* isolated from different water sources to be resistant to Ampicillin, Cephalothin, Ampicillin-sulbactam, Sulfamethoxazole-trimethoprim, Chloramphenicol and Nalidixic Acid.

Among drinking-water isolates, the emergence of resistance and decreasing levels of susceptibility of *E. coli* to a wide spectrum of antibiotics is a matter of concern because it may limit the availability of antibiotics for clinical management of waterborne outbreaks in the future (Siya Ram *et al.,* 2008). *E. coli* recovered from contaminated water distribution system expressed a high level of resistance to antimibiotics that are commonly used in clinical medicine (Tetracycline, Amoxycillin, Ampicillin, etc.). Antibiotic resistance in *E. coli* is of particular concern because it is the most common Gram-negative pathogen in humans. In addition, antibiotic resistant *E. coli* strains have the ability to transfer antibiotic resistance determinants not only to other strains of *E. coli*, but also to other bacteria within the gastrointestinal tract (Salvadori *et al.,* 2004). This could contribute to the spread and persistence of antibiotic-resistant bacteria and resistance determinants in humans and the environmen[t]. Conjugative experiments have shown that *E. coli* harbors self transmissible plasmids carrying resistant genes for Tetracycline, Amoxycillin and other antibiotics. It seemed that all these resistant genes may be carried on one or more self transmissible plasmids (Jabri *et al.*, 2008).

Indiscriminate use of antibiotics by healthcare providers or by way of self-prescribing and overcounter availability are major risk factors for the development of high levels of antibiotic resistance, which is common in developing countries. Other factors are overcrowding, poor hygienic practices prevalent in rural people of low socio-economic

status, and an increasingly mobile population contributed to facilitate the dissemination of antibiotic resistance determinants among the pathogens (Spratt, 1994). Some other factors contributing towards resistance include incorrect diagnosis, unnecessary prescriptions, improper use of antibiotics by patients, and the use of antibiotics as livestock food additives for growth promotion (Bouza E. & Cercenado E., 2002). Microbes undergo mutation of genes, which can spread from cell to cell by mobile genetic elements such as plasmids, transposons and bacteriophages. The antibiotic resistance character is most often encoded on plasmids, which can easily be transferred among isolates.

Presence of antibiotics at sub-inhibitory concentrations in the environment allows the selection of resistant strains (Hirsch *et al.*, 1999). Studies conducted in various countries have detected a number of antibiotics in the low microgram per liter or the nanogram per liter range in different environmental compartments (Kummerer, 2004).Added to this fact bacteria with acquired resistance have also been found in surface water, ground water and drinking water in different countries (Schwartz *et al.*, 2003). Furthermore, the 5-10 % of the bacteria present in water is viable but non-culturable and that is why the study of antibiotic resistance allows evaluating a small fraction of the impact of antibiotics in aquatic ecosystems (Seveno *et al.*, 2002).

An increasing number of studies have documented an additional mechanism for maintaining antibiotic-resistant bacteria in the environment through co- or cross-resistance to metals or co-regulation of resistance pathways (Stepanauskas *et al.*, 2005). Many have speculated and have even shown that a correlation exists between metal tolerance and antibiotic resistance in bacteria because of the likelihood that resistance genes to both (antibiotics and heavy metals) may be located closely together on the same plasmid in bacteria and are thus more likely to be transferred together in the environment (Spain, 2003).

The findings of this study indicate that the *E. coli* recovered in this study expressed a high level of resistance to antibiotics that are commonly used in clinical medicine (Tetracycline, Amoxycillin, Cephalexin). Tetracycline resistance in bacteria is mediated by four mechanisms: efflux, ribosomal protection, enzymatic inactivation, and target modification. The leading tetracycline resistance mechanism in *E. coli* is the extrusion of drug from the cytoplasm by efflux in an energy-dependent fashion via proton exchange, thereby reducing the intracellular concentration of the drug (Tuckman *et al.*, 2007). Widespread resistance to the broad-spectrum Tetracyclines has been caused by heavy clinical use and misuse in the human population, use in agriculture as a growth promoter and as an infection control agent in domestic animals, aquaculture, and horticulture (Chopra, 2002).

Results revealed dominance of *E. coli* isolates resistant to β-lactam antibiotics, which is worrying as such microorganisms can contribute, at the environmental level, to the spread of genetic markers to other bacteria as well as to the hindering of treatments with β-lactams, the antibiotics group most used in the control of infectious diseases (Lourenco *et al*., 2007). One of the defence mechanisms against β-lactams in gram negative bacteria including *E. coli* is the production of β-lactamases that hydrolyse the amide bond of the four membered β-lactam ring. The spread of β-lactamase genes has been greatly exacerbated by their integration within mobile genetic elements, such as plasmids or transposons, which facilitate the rapid transfer of genetic material between microbes. The other mechanism includes alteration of the antibiotic target site by the β lactam-resistant cell-wall transpeptidases. The final mechanism is prevention of access of the antibiotic to the target by broad-specificity drug-efflux pumps which is now recognized as a major contribution to antibiotic resistance in gram-negative species like *E. coli* (Wilke *et al.*, 2005).Beside these, the active nucleus of the β-lactams is so similar among many congeners that extensive cross-resistance can be expected.

The evaluation of oligodynamic action of metals upon *E. coli* isolates was another objective of the study. Five types of metal disks made of silver, copper, brass,

aluminum and steel were used for their oligodynamic action where the silver proved to be highly effective, copper effective while brass, aluminum and steel totally ineffective towards the bacterial isolates. Studies reported by Clark (1956), ENPHO/DISVI (1991), Shahi *et al.*, (1996), Maharjan (1998), Jayana (2007) and Shrestha (2008) have indicated copper and silver most effective, brass effective and aluminium and steel ineffective in their oligodynamic action.

Copper and silver ions are known to react with protein molecules containing amino, hydroxyl, imidazole, carboxylate, thiol, and/or phosphate particles. The accumulation of copper ions in the cell wall protein results in the failure of key enzymes involved in the selective transmittance of specific nutrients into the bacterial cell wall and membrane. Silver ions cause enzyme proteins to denature to the point that structural changes to the enzyme results in the failure of any further functioning. It has been shown in that silver ions, once they have penetrated the cell walls and membrane of both gram-positive and gram-negative bacteria, reach the genomic DNA, which results in the failure of the bacterial cells to reproduce (Kim *et al.*, 2002).

Decreased effectiveness of silver and copper towards the isolates has been observed as compared to aforementioned studies. Although silver is seen more effective than copper in inhibiting all the isolates subjected to oligodynamic action, the zone of inhibition is relatively smaller as compared to above mentioned studies. On the other hand, the zone of inhibition exerted by copper is smaller than silver and it could not inhibit some isolates at all. The decreased effectiveness of these metals can be attributed to potential increased tolerance of *E. coli* towards them or question can be raised about the purity of metals used to make these disks. The presence of MAR *E. coli* in this study might be the potential reason for the organism being resistant towards the metals such as silver and copper.

Metals are so widely used in mining, industry, and agriculture that high levels of metals may exist in some environments. As such, bacteria have evolved several types of

mechanisms to resist toxicity due to high metal concentrations. Data accumulated have shown that *E. coli* encodes efflux proteins that mediate resistance to copper and silver by cation efflux. These proteins form a tetrapartite resistance system that directly transports these ions from the periplasm across the outer membrane (Franke *et al.*, 2003).

6.2 CONCLUSION

The presence of *E. coli* and faecal coliforms in drinking water supplied by a defective water distribution system impacted by leaking sewage lines and open drains may pose health risks to people consuming such water for drinking. In spite of the small sample size, the results of this study emphasize the human health risk associated with exposure to contaminated drinking water due to the presence of potential enteropathogenic antibiotic resistant *E. coli* and other enteric pathogens. Therefore, the presence of *E. coli* and other coliforms in drinking water distribution systems of developing nations like Nepal requires increased surveillance for risk assessment and prevention strategies to protect public health. Furthermore, heavy metal contamination of aqueous environment may lead to increased tolerance of bacterial isolates towards oligodynamic metals and their subsequent decreased effectiveness in reducing microbial load by using filters and vessels made out of them which otherwise are effective and cheap methods of water disinfection in developing countries.

CHAPTER-VII

7. SUMMARY AND RECOMMENDATIONS

7.1 SUMMARY

The bacteriological quality of drinking water is of great concern as it is directly connected to the health and well being of the population. All the water borne diseases are caused by faecal contamination of drinking water sources and unhygienic practices. However, the incidence of water borne diseases can be reduced by safe disposal of waste, protection of drinking water sources, good hygienic and sanitary practices. This study is dedicated to microbial and some physicochemical quality assessment of urban drinking water supply of Kathmadu city along with the isolation, identification, study of antibiotic susceptibility and oligodynamic action on *E. coli* isolates.

1. A total of 102 tap water samples were randomly collected from eleven different areas of Kathmandu city. Physico-chemical and bacteriological parameters were analyzed to assess the drinking water quality.

2. Distinct variation in temperature was observed with the highest and the lowest temperature being 25.7^{o}C and 11.8^{o}C respectively. No variation was seen in the pH values of the water samples with all lying close to the neutral and within the WHO guideline (6.5-8.5).

3. Out of 102 water samples, 88 samples (86.2%) were found to contain the total coliform while 20 samples (19.6%) were found to contain thermotolerant coliform beyond the WHO guideline value (0 CFU/100 ml).

4. A total of 20 *E. coli* isolates were obtained from 20 water samples tested positive for thermotolerant coliform.

5. Antibiotic resistance of *E. coli* isolates was predominantly directed towards Cephalexin(65%) followed by Amoxycillin(45%) and Tetracycline(15%). All the isolates were 100% sensitive to Co-trimoxazole, Amikacin, Chloramphenicol, Ceftriaxone, Ciprofloxacin, Nalidixic Acid and Gentamicin.

6. Study on oligodynamic action revealed silver to be the most effective, copper effective while brass, aluminium and steel totally ineffective towards *E. coli* isolates.

7.2 RECOMMENDATIONS

Following recommendations can be suggested based on the outcome of this study:

- Regular bacteriological quality assessment and disinfection of all drinking water sources should be planned and conducted.

- Regular monitoring of chlorination in treatment plants and establishment of re-boosting stations may help maintain standard free residual chlorine concentration (0.2 mg/L) and keep drinking water safe to some extent.

- Common and cheap methods of safe household water management like boiling, filtration, solar disinfection of water (SODIS), chlorination and using metal vessels for their oligodynamic action or combination of these techniques should be employed.

- Copper and if affordable silver should be used in the filters and making water vessels or their combinations can be used to exert synergistic action upon the water-borne pathogens.

- Drinking water sources should be protected from potential heavy metal contamination.

- Serotyping of *E. coli* should be performed to eliminate the possibility of the presence of enteropathogenic strains.

- Indiscriminate use of antibiotics should be discouraged.

- MAR indexing of *E. coli* should be carried out to trace the source of faecal contamination in water.

- Molecular level study should be carried out in order to establish the epidemiological relationship among the water isolates and the possible sources.