CHAPTER-I

1. INTRODUCTION

Water is the vital natural resources which is essential for all sorts of living as well as non living elements and is an indispensable factor. Water has a major impact on the quality of life. The ensuring of good quality drinking water is a basic factor in ensuring public health, the protection of the environment and sustainable development. Water intended for human consumption should be safe and wholesome. Safe drinking water is the water that is acceptable for humans to drink and to use for other domestic purposes such as food preparation, washing, bathing, irrigation etc. There can be no state of positive health and well being without safe water (Park, 2002).

Water is so important for all living beings that it should always remain pure but unfortunately it gets polluted through various sources. All over the world water pollution is posing threat to human life both in rural and urban areas. Water pollution is the cause of many diseases and consequently of atmospheric pollution. Most inhabitants are served by surface waters i.e. rivers, streams and lakes. The raw water from these sources is frequently polluted with human waste or sewage and industrial waste. When water gets contaminated with various pathogenic as well as opportunistic microflora and toxic chemical compounds, it serves as the vehicle of transmission of a number of infectious diseases. Unfortunately, over a billion people in the developing world do not have access to safe water supply. The WHO has estimated that up to 80% of all sickness and disease in the world is caused by inadequate sanitation, polluted or unavailability of water. The pollution of drinking water is responsible for a large number of mortalities and morbidities due to water-borne diseases like typhoid, cholera, dysentery, hepatitis as well as many protozoan and helminthic infestations (WHO, 1996).

The growing imbalance between supply and demand has resulted in pollution and environmental degradation. The quality of water for drinking has deteriorated because of the inadequacy of treatment plants, direct discharge of untreated sewage into rivers, and inefficient management of piped water distribution system (UNEP, 2001).

Despite major effort to deliver safe piped, community water to the world's population, the reality is that water supplies delivering safe water will not be available to all people in near term (Agrawal, 1981). As a consequence of such water quality, water borne disease such as diarrhea, dysentery and gastroenteritis often occurs. These diseases are prevalent in both urban and rural areas throughout the nation. Diseases caused by contaminated water are among the ten most prevalent water borne disease in Nepal (DoHS, 1998). Thousands of people die or suffer from water and sanitation related diseases. Drinking water has direct impact on human health and can be extremely dangerous when it becomes the vehicle of transmission of disease (Sharma, 2000).

Important source of water pollution is domestic sewage system, which pollutes well and rivers which are important source of drinking water. Sewage is not only the cause of water pollution; industrial waste is also a significant polluter giving rise to contamination with heavy metals. Most of the industries discharge untreated water into the river. The effluent discharged by the factories contains detergents, non biodegradable materials and toxic chemicals hazardous to health and hygiene. Other sources of water pollution are distillery potassic fertilizers, electroplating plant, which contain harmful heavy metals and cyanides which causes excessive acidity in water. Intensive use of pesticides and fertilizers has increased the level of nitrates in water (Pandey, 2006).

There are frequent report of spread of typhoid, diarrhea and cholera throughout water supply in Nepal. In each and every summer, water borne epidemics hit different parts of the country including Kathmandu valley. Drinking water is only the vehicle for the transmission of water borne diseases (WHO, 2002). Annual report from DoHS (2004/2005) showed that there were 2332 cases of typhoid, 18611 cases of diarrheal disease, 9322 cases of intestinal worms, 543 cases of jaundice and infectious hepatitis in Kathmandu valley. The emergence of antibiotic resistant (AR) bacteria has become a growing public health concern in recent years. The presence and persistence of AR bacteria, particularly multiple-antibiotic resistant (MAR) bacteria is a serious threat to mankind. Antibiotic susceptibility testing is an essential component of the practice. It allows physicians to make accurate choices with all forms of antimicrobial therapy, prophylaxis, and pathogen directed therapy. Without surveillance for the development

of antibiotic resistance, serious infections may lead to death due to inadequate antibiotic therapy.

Though Nepal is known as the world's second richest nations in terms of water resources, more than one third of people in the country have problems in obtaining drinking water (Subba, 2001). Therefore the residents depend on alternative source of drinking water like dug wells, tube wells, stone spouts etc. Dug wells, tube wells and stone spouts extract water from shallow unconfined aquifers and surface waters which is at risk from contamination by pathogenic bacteria, pesticides, nitrate, industrial effluents, and domestic sewage through seepage. Drinking water quality assessment has always been crucial with reference to public health importance. This study is thus conducted to reveal the drug resistant coliform bacteria from water sample also helps to find out possible epidemics of water borne diseases caused by the consumption of contaminated drinking water and provide implication in infection control.

CHAPTER – II

2. OBJECTIVES

2.1 General Objective

To assess the quality of drinking water and detect drug resistant coliform from water sample.

2.2 Specific Objective

1. To enumerate coliform and thermotolerant coliform from different water samples.
2. To detect the pathogenic bacteria from water samples.
3. To describe the antibiotic susceptibility pattern of isolates.
4. To estimate the frequency of multiple drug resistant coliforms.

CHAPTER – III

3. LIERATURE REVIEW

3.1 Water pollution

Water pollution is defined as any physical, chemical or biological alternation in composition or condition of water directly or indirectly as a result of human activities, so that it become less suitable for any or all of the functions and purposes for which it would be suitable in its natural state (WHO, 2006) and also has defined as any physical, chemical or biological factor causing aesthetic or detrimental effects of aquatic life and on those who consume water (Trivedi and Goel, 1986).

Drinking water is derived from two basic sources: surface waters such as rivers and reservoirs and groundwater. All water contains natural contaminants particularly inorganic contaminants that arise from the geological strata through which water flows and, to a varying extent, anthropogenic pollution by both microorganisms and chemicals. (Fawell and Nieuwenhuijsen , 2003). Source water is vulnerable to contamination from many origins. Humans, livestock and wild animals are all sources of faecal contamination. It has been shown that many rivers in Europe are significantly contaminated with microbes arising from wastewater and/or livestock. Source water and particularly surface water, is often used for purposes such as irrigation, recreation and transport, which may also affect water quality. Groundwater contamination may be induced by different practices in the management of domestic wastewater and livestock manure (Dechesne and Soyeus, 2007).

Pathogens present in the surface waters originate from both point and diffuse sources and concentrations may vary considerably over time. Increased microbial impacts have been observed during rain periods with substantial runoff, typically resulting in elevated microbial concentrations in surface water. Point sources for pathogens may include municipal wastewater discharges and heavily polluted tributaries within a river system. Diffuse sources, on the other hand, include urban, agricultural and forestry runoffs with microbial impact from livestocks and wild animals in the catchment area (Astrom et al., 2007). Pathogens may be dispersed in the environment through the use of sewage sludge as fertilizers. Agricultural practices are important

sources of contamination especially from *Cryptosporidium* oocysts, *Giardia* cysts and *Campylobacter* spp. direct runoff into surface water, animal waste is often collected in impoundments from which effluent may infiltrate groundwater. Other sources of faecal contamination that may be a threat to water sources are storm water discharges, combined sewer overflows, accumulation of pathogens in sediments and wild animals (WHO, 2006). Furthermore, the microbial load to the raw water within the catchment is influenced by natural factors, such as (rain, sunlight and temperature), hydrology and topography (Astrom et al., 2007).

Water pollution is the most serious environment quality issue in Nepal. The quality of both surface water and ground water sources in different parts of Nepal is degraded. In general, surface water possesses a high possibility of organic, bacterial and viral contamination (Joshi et al., 2000). It is caused by the disposal of solid and liquid wastes on land and surface water. The most significant waste is sewage, industrial effluent, agricultural residues and chemicals (Poudyal, 2000). Moreover, continued discharge of domestic and industrial waste water directly into the river is one of the main causes of water pollution in the stream. All domestic sewers are discharged directly into the rivers without treatment. Industrial waste is also major cause of surface water pollution. As per the Nepal state of the Environment, 40% of the country's 4,271 industrial units are the main source of water pollution in Nepal (UNEP, 2001).

3.2 Physicochemical parameters of water

The ordinary consumer judges the water quality by its physical characteristics. The provision of drinking water that is not only safe but also pleasing in appearance, taste and odor is a matter of high priority (Park, 2002). Chemical tests identify impurities and other dissolved substances that affect water used for domestic purposes.

3.2.1 Temperature

Temperature is one of the most important parameter of water and is basically important for its effects on the chemistry and biological reactions in the organisms in water. A rise in temperature of the water leads to reduced solubility of gases and amplify tastes and odors (Trivedy and Goel, 1986). High water temperature enhances the growth of microorganisms and may increase taste, odor, color, and corrosion

problem. Water in the temperature range of 7^0C to 11^0C has pleasant taste and is refreshing. Thus, cool water is generally more palatable than warm water (WHO, 2006).

3.2.2 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). It is the major of the intensity of acidity or alkalinity and measures the concentration of hydrogen ions in water. pH less than 7 may cause corrosion and encrustation in the distribution system where as the disinfection with chlorine is less effective if pH of water exceeds 8.0 (WHO,1993). The pH value of drinking water from any sources should be within range, 6.5-8.5 (Trivedy and Goel, 1986). The pH of the water entering the distribution system must be controlled to minimize the corrosion of water mains and pipes in house hold water systems. Alkalinity and calcium management also contribute to the stability of water and control its aggressiveness to pipe and appliance (WHO, 2004).

3.3 Microbial indicator of water quality

Drinking water contaminated with sewage or other excreted matter from man and animal may contain various pathogenic bacteria, viruses, protozoa and parasites which make it dangerous for human consumption. Water borne diseases are spread through the drinking water sources. So from public health point of view, microbiological impurities in water are the most important and the supplies should be tested regularly to confirm their freedom from contamination (Colle et al., 2006).

The routine examination of environmental samples for the presence of all pathogens is often tedious, difficult and time consuming task. This problem is overcome by looking for certain indicator microorganisms whose presence indicates the pathogenic microorganisms may also be present. Frequent examination for fecal indicator organisms remains the most sensitive and specific way of assessing the hygienic quality of water. Faecal indicator should fulfill certain criteria to give meaningful results. An indicator should always be present when pathogens are present. Indicators and pathogens should have similar persistence and growth characteristics. Indicators and pathogens should occur in a constant ratio so that counts of the indicators give

good estimate of the numbers of pathogens present. Tests for the indicator should be easy to carry out and applicable to all types of water. The test should detect only the indicator organisms thus not giving false-positive reactions (Maier et al., 1996).

The indicator organisms generally used are three main types of bacteria which are coliform, faecal streptococci and *Clostridium perfringens*. Our immediate concern is the coliform bacteria since they are most common bacteria globally used as indicators. Coliforms are of two types: faecal and total, faecal is exclusively of faecal origin and total is found in faeces as well as in unpolluted soil and vegetation. Since total coliform can also exist in vegetation and soil, their presence is granted only on presumptive indication of contamination. But faecal coliform imply definite excrement contamination. Faecal coliform comprise two main types of bacteria namely *Escherichia coli* and *Klebsiella* spp Most of the faecal coliforms are *E. coli* and only less than 1 % of them are pathogenic (ENPHO/DISVI, 1992).

3.3.1 Total Coliform

Coliform organisms have long been recognized as a suitable microbial indicator of drinking-water quality, largely because they are easy to detect and enumerate in water. Coliform organisms, better referred to as total coliforms to avoid confusion with others in the group, are not an index of faecal pollution or of health risk, but can provide basic information on source water quality (Dufour et al., 2003).

Coliform are Gram-negative, non-spore-forming rod-shaped bacteria capable of growth in the presence of bile salts or other surface-active agents with similar growth-inhibiting properties, oxidase-negative, fermenting lactose at 35-37ºC with the production of acid, gas, and aldehyde within 24- 48 hours. These definitions presume the use of cultural methods for identification and enumeration. There has recently been a move towards a genotypic definition based on the recognition that in order to ferment lactose, organisms must possess β-galactosidase activity. Using this approach total coliforms are defined as members of a genus or species within the family Enterobacteriaceae capable of growth at 37ºC and possessing β-galactosidase. (Dofour et al., 2003). Traditionally, coliform bacteria were regarded as belonging to the genera *Escherichia, Citrobacter, Klebsiella* and *Enterobacter*, but the group is more heterogeneous and includes a wider range of genera, such as *Serratia* and

Hafnia. The total coliform group includes both faecal and environmental species (WHO, 2008).

Total coliforms include organisms that can survive and grow in water. Hence, they are not useful as an index of faecal pathogens, but they can be used as an indicator of treatment effectiveness and to assess the cleanliness and integrity of distribution systems and the potential presence of biofilms (WHO, 2008). Contamination indicated by the presence of total coliform is indicative of inadequate disinfection of drinking water (Hach, 2000).

Total coliforms should be absent immediately after disinfection, and the presence of these organisms indicates inadequate treatment. The presence of total coliforms in distribution systems and stored water supplies can reveal regrowth and possible biofilm formation or contamination through ingress of foreign material, including soil or plants (WHO, 2008).

Coliform bacteria have been recognized as a suitable microbial indicator of drinking water quality. They make up around 10 percent of the intestinal microflora of the human and animal intestine. The term coliform organism refers to gram negative, oxidase negative, non sporing rods capable of growing aerobically on agar medium containing bile salts and able to ferment lactose within 48 hours at 35-37^{0}C with the production of both acid and gas (Cheesebrough, 1984; Anderson and Davidson, 2002). The coliform group includes genus, *Escherichia, Klebsella, Enterobacter* and *Citrobacter*. The levels of coliform organisms present in the drinking water should not exceed the maximum permissible value of less than one cell per 100 ml of water (WHO, 1993).

The coliform groups of organisms are suitable as indicators because coliform organisms are constantly present in the intestinal tract of both humans and warm-blooded animals. They are present in intestinal tract in large numbers, eg: *E. coli* is present in numbers 10^9/gm of faeces. These organisms generally live longer in water than pathogens. They are relatively easy to detect and assay in water. The presence of coliforms in water is regarded as a warning signal; the water is subject to potentially dangerous pollution (Maier et al., 1996).

3.3.2 Thermotolerant coliform

Total coliform bacteria that are able to ferment lactose at 44–45°C are known as thermotolerant coliforms. In most waters, the predominant genus is *Escherichia*, but some types of *Citrobacter*, *Klebsiella* and *Enterobacter* are also thermotolerant. *Escherichia coli* can be differentiated from the other thermotolerant coliforms by the ability to produce indole from tryptophan or by the production of the enzyme β-glucuronidase. *Escherichia coli* is present in very high numbers in human and animal faeces and is rarely found in the absence of faecal pollution, although there is some evidence for growth in tropical soils. Thermotolerant coliform species other than *E. coli* can include environmental organisms (WHO, 2008).

Thermotolerant coliforms other than *E. coli* may originate from organically enriched water such as industrial effluents or from decaying plant materials and soils. In tropical and subtropical waters, thermotolerant coliform bacteria may occur without any obvious relation to human pollution and have been found on vegetation in the tropical rainforest. This means that the occurrence of the thermotolerant coliform group in subtropical or tropical waters or those enriched with organic wastes does not necessarily suggest faecal contamination by humans (Dufour et al., 2003).

3.3.3 *Escherichia coli*

Escherichia coli is considered the most suitable index of faecal contamination. In most circumstances, populations of thermotolerant coliforms are composed predominantly of *E. coli*; as a result, this group is regarded as a less reliable but acceptable index of faecal pollution. *Escherichia coli* (or, alternatively, thermotolerant coliforms) is the first organism of choice in monitoring programmes for verification, including surveillance of drinking-water quality. (Ashbolt et al., 2001).

Escherichia coli occurs in high numbers in human and animal faeces, sewage and water subject to recent faecal pollution.Water temperatures and nutrient conditions present in drinking-water distribution systems are highly unlikely to support the growth of these organisms. The presence of *E. coli* (or, alternatively, thermotolerant coliforms) provides evidence of recent faecal contamination, and detection should lead to consideration of further action, which could include further sampling and

investigation of potential sources such as inadequate treatment or breaches in distribution system integrity (WHO, 2008).

3.3.4 Intestinal Enterococci

Intestinal enterococci are a subgroup of the larger group of organisms defined as faecal streptococci, comprising species of the genus *Streptococcus*. These bacteria are Gram-positive and relatively tolerant of sodium chloride and alkaline pH levels. They are facultatively anaerobic and occur singly, in pairs or as short chains. Faecal streptococci including intestinal enterococci all give a positive reaction with Lancefield's Group D antisera and have been isolated from the faeces of warm-blooded animals. The subgroup intestinal enterococci consists of the species *Enterococcus faecalis*, *E. faecium*, *E. durans* and *E. hirae.* This group was separated from the rest of the faecal streptococci because they are relatively specific for faecal pollution (WHO, 2008).

The intestinal enterococci group can be used as an index of faecal pollution. Most species do not multiply in water environments. The numbers of intestinal enterococci in human faeces are generally about an order of magnitude lower than those of *E. coli.* Important advantages of this group are that they tend to survive longer in water environments than *E. coli* (or thermotolerant coliforms), are more resistant to drying and are more resistant to chlorination (WHO, 2008).

Intestinal enterococci are typically excreted in the faeces of humans and other warm blooded animals. Some members of the group have also been detected in soil in the absence of faecal contamination. Intestinal enterococci are present in large numbers in sewage and water environments polluted by sewage or wastes from humans and animals. The presence of intestinal enterococci provides evidence of recent faecal contamination, and detection should lead to consideration of further action,which could include further sampling and investigation of potential sources such as inadequate treatment or breaches in distribution system integrity (WHO, 2008).

3.3.5 *Clostridium perfringens*

Clostridium spp are Gram-positive, anaerobic, sulfite-reducing bacilli. They produce spores that are exceptionally resistant to unfavourable conditions in water

environments, including UV irradiation, temperature and pH extremes, and disinfection processes, such as chlorination. The characteristic species of the genus, *C. perfringens*, is a member of the normal intestinal flora of 13–35% of humans and other warm-blooded animals. Other species are not exclusively of faecal origin. Like *E. coli*, *C. perfringens* does not multiply in most water environments and is a highly specific indicator of faecal pollution (WHO, 2008)

In view of the exceptional resistance of *C. perfringens* spores to disinfection processes and other unfavourable environmental conditions, *C. perfringens* has been proposed as an index of enteric viruses and protozoa in treated drinking-water supplies. In addition, *C. perfringens* can serve as an index of faecal pollution that took place previously and hence indicate sources liable to intermittent contamination (WHO, 2008).

Clostridium perfringens and its spores are virtually always present in sewage. The organism does not multiply in water environments. *Clostridium perfringens* is present more often and in higher numbers in the faeces of some animals, such as dogs, than in the faeces of humans and less often in the faeces of many other warm-blooded animals. The numbers excreted in faeces are normally substantially lower than those of *E. coli*. The presence of *C. perfringens* in drinking-water can be an index of intermittent faecal contamination. Potential sources of contamination should be investigated. Filtration processes designed to remove enteric viruses or protozoa should also remove *C. perfringens*. Detection in water immediately after treatment should lead to investigation of filtration plant performance (WHO, 2008).

3.4 Other common intestinal pathogens contaminating drinking water

3.4.1 *Salmonella* spp

Salmonella are ubiquitous in the environment and can be detected at low concentration in most surface water. It belongs to the family Enterobacteriaceae. It is Gram-negative, straight rod shaped, non capsulated, non-sporing with peritrichous flagella, aerobic and facultative anaerobic bacilli. They grow between 15-45^{0}C with an optimum temperature of 37^{0}C. XLD is best selective media and selenite F broth is probably the best enrichment media for it. Most strain produces H_2S in TSI with

production of acid and gas (Colle et al., 1996). These organisms are usually present in small numbers compared to coliform (APHA, 1998). *S.* Typhi is mainly water borne (Cheesebrough, 2000). It causes enteric fever (typhoid and paratyphoid) and other *Salmonella* spp causes diarrhoeal disease. At present, 107 types can be distinguished by phage typing which is the value in epidemiologic studies. For paratyphoid fever, three bioserotypes of *S.* enteridis are recognized, paratyphoid A, B and C (Bebenson, 1995).

3.4.2 *Shigella* spp

It belongs to the family Enterobacteriaceace. The isolation of *Shigella* from drinking water indicates recent faecal contamination (WHO, 1996). Transmission is mainly by the faecal oral route with poor sanitation, unhygienic conditions and overcrowding, facilitating the rapid spread of infection. Only small numbers of organisms are required to cause disease (Cheesbrough, 2000). It is Gram-negative non-spore forming, non-motile rods, capable of growth under both aerobic and anaerobic conditions. They are non lactose fermenting and non citrate utilizing. All *Shigella* form acid from glucose, but except for a few serotypes, not gas. Colonies are pale and yellowish in MacConkey agar. XLD is probably the best selective medium and producing red colonies (Colle et al., 1996).

3.4.3 *Vibrio cholerae*

Vibrio species are primarily aquatic occurring in fresh water, estuarine and marine habitats in association with aquatic organisms include example copepods (Singleton et al., 2001). *V. cholerae* is transmitted by the faecal oral route with most epidemics occurring when water supplies become faecally contaminated (Cheesbrough, 2000). It belongs to the family Vibrionacea. *Vibro* spp are Gram negative, motile by one or more polar flagella, non-sporing, slightly curved rods with a single polar flagellum (Holt et al., 1994). TCBS agar is an excellent selective medium for the primary isolation of vibrionaceae, in which sucrose-fermenting yellow colonies of diameter 2-3 mm after overnight incubation at 35-37^{0}C (Cheesbrough, 2000). Some of the major emerging and re-emerging water-borne agents are: *V. cholerae* biotype ElTor serotype 0139. This microorganism is responsible for cholera, which is a painless form of diarrhea, characterized by rice-watery stool (Anderson and Davidson, 2002).

3.4.4 *Proteus vulgaris*

It belongs to the family Enterobacteriaceae. They are Gram- negative, actively motile, non capsulated, pleomorphic rod. Motility is not as easily observed at 35-37^{0}C (Cheesebrough, 2000). *Proteus* are Methyl red positive, they hydrolyse urea rapidly, H_2S positive (Colle et al., 1996). *P. vulgaris* are found widely distributed in soil, polluted water, intestine of healthy man and animals. *P. vulgaris* may cause urinary infection.

3.5 Sources and access of drinking water in Kathmandu

Nearly all of the surface sources and ground water sources have been exploited. The growing imbalance between supply and demand has led to chronic shortages and competition that have resulted in pollution and environmental degradation. Apart from quantitative shortages, the quality of drinking water in the Kathmandu Valley is becoming a serious public health issue for the past few years. The quality of water for drinking has deteriorated because of untreated sewage into rivers and inefficient management of the piped water distribution system (UNEP, 2001).

3.6 Drinking water as a vehicle of diseases

For pathogens transmitted by the faecal–oral route, drinking-water is only one vehicle of transmission and the greatest risk from microbes in water is associated with consumption of drinking water that is contaminated with human and animal excreta, although other sources and routes of exposure may also be significant (WHO, 2010).

Enteropathogenic microbes are usually adapted to multiplying in the intestines of humans and animals and surface water is only a niche in their circulation through the environment and human or animal populations (Medema et al., 2003).

Most bacterial pathogens potentially transmitted by water infect the gastrointestinal tract and are excreted in the faeces of infected humans and other animals. However, there are also some waterborne bacterial pathogens, such as *Legionella, Burkholderia pseudomallei* and atypical mycobacteria that can grow in water and soil. The routes of transmission of these bacteria include inhalation and contact (bathing), with infections occurring in the respiratory tract, in skin lesions or in the brain (WHO, 2010).

The most important bacterial diseases transmitted through water are listed in Table -2.

Table-1: Bacterial disease transmitted through water

Disease	Causal bacterial agent
Cholera	*Vibrio cholerae*, serovarieties O1 and O139
Gastroenteritis caused by vibrios	Mainly *Vibrio Parahaemolyticus*
Typhoid fever and other serious salmonellosis	*Salmonella enterica* subsp. *enterica* serovar ParaTyphi *Salmonella enterica* subsp. *enterica* serovar Typhi *Salmonella enterica* subsp. *enterica* serovar Typhimurium
Bacillary dysentery or shigellosis	*Shigella dysenteriae* *Shigella flexneri* *Shigella boydii* *Shigella sonnei*
Acute diarrheas and gastroenteritis	*Escherichia coli*, particularly serotypes such as O148, O157 and O124

Source: Cabral, 2010

3.7 Outbreaks of waterborne disease in Nepal

The WHO has estimated that up to 80% of all sickness and disease in the developing countries is caused by inadequate sanitation, polluted water, or unavailability of water (Cheesebrough, 1993). The number of outbreaks that has been throughout the world demonstrates that transmission of pathogens by drinking water remains a significant cause of illness. However, estimates of illness based solely on detected outbreaks are likely to be underestimating the problem. Out breaks of waterborne epidemic is rampant in Nepal as in most of the third world countries. Mortality and morbidity due to such disease still top the list. Every year the onset of the epidemics comes also with the monsoon. Many outbreaks of waterborne diseases probably are not recognized; therefore, their incidences are not reported. But there are real incidents of waterborne disease, in which improvements in drinking water quality could have saved many lives. As mentioned in the UNICEF situation analysis (UNICEF, 1987), in Nepal water and hygienic related diseases, are responsible for 15% of all cases and 8% of all deaths in the general population. In 1985, over 50% of hospital patients in Nepal were found to be suffering from gastro-intestinal disorder normally caused by waterborne pathogens. In 1990, cholera outbreak during summer hit different parts of the country

including the capital city and caused an enormous loss of lives (ENPHO/DISVI, 1990a). In 1990, Public Health Division recorded 23,888 gastro-enteritis cases in 39 districts with maximum 8,437 in the Kathmandu valley. In 1991 as well, the disease started to spout up at the beginning of the summer, striking badly the western, mid-western and central region of the country, which the eastern region was less affected. In between April to August, Public Health Division reported 43,520 gastro-enteritis cases with 1,252 deaths (ENPHO/DISVI, 1991).

Ise et al., (1996) reported a cholera outbreak in 1994 (July- September) in Kathmandu Valley. The authors indicated that the waterborne infection was related to the consumption of contaminated river water. *Vibrio* cholera O1 and enteropathogenic *E. coli* were detected in the stool of the patients. Pokhrel & Kubo (1996) reported that the cholera outbreak in Kathmandu in 1995 was due to the seasonal variation, maximum number of incidences occurring in the rainy seasons.

Diarrhoeal disease outbreak in 2009, mostly affected Mid-Western development region, Jajarkot being most affected. Others most affected districts were Rukum, Dailekh, Surkhet, Achham and Bajhang. There were 58874 people affected and 314 deaths were reported. The highest number of death was reported in Jajarkot with 48 deaths. The causative agent of the outbreak was found to be *Enterotoxigenic E. coli* (LT and ST) and *Aeromonas* species. *Vibrio cholera* O1 Ogawa was also reported from the 30% of the sample. (http:/ /www.un.org.np/resources/diarrhea-outbreak/)

I. Principle of Laboratory diagnosis of coliform

Tests for detection and enumeration of indicator organisms, rather than of pathogens, are used. The coliform group of bacteria, as herein defined, is the principal indicator of suitability of water for domestic, industrial, or other uses. The cultural reactions and characteristics of this group of bacteria have been studied extensively. Experience has established the significance of coliform group density as a criterion of the degree of pollution and thus of sanitary quality. The significance of the tests and the interpretation of results are well authenticated and have been used as a basis for standards of bacteriological quality of water supplies. The membrane filter technique, which involves direct plating for detection and estimation of coliform densities, is as

effective as the multiple-tube fermentation test for detecting bacteria of the coliform group (APHA, 1999).

The membrane filter method gives a direct count of total coliforms and faecal coliforms present in a given sample of water. A measured volume of water is filtered, under vacuum, through a cellulose acetate membrane of uniform pore diameter, usually 0.45 µm. Bacteria are retained on the surface of the membrane which is placed on a suitable selective medium in a sterile container and incubated at an appropriate temperature. If coliforms and/or faecal coliforms are present in the water sample, characteristic colonies form that can be counted directly.The technique is unsuitable for natural waters containing very high levels of suspended material, sludges and sediments, all of which could block the filter before an adequate volume of water has passed through. When small quantities of sample (for example, of sewage effluent or of grossly polluted surface water) are to be tested, it is necessary to dilute a portion of the sample in sterile diluent to ensure that there is sufficient volume to filter across the entire surface of the membrane. If the quality of water is totally unknown, or there is doubt concerning the probable bacterial density, it is advisable to test two or more volumes in order to ensure that the number of colonies on the membrane will be in the optimum range for counting (i.e. 20-80 colonies per membrane). If a suitable volume of sample cannot be filtered through a single membrane, the sample may be filtered through two or more and the numbers of colonies on the membranes added to give the total count for the sample. Membrane filtration and colony count techniques assume that each bacterium, clump of bacteria, or particle with bacteria attached, will give rise to a single visible colony. Each of these clumps or particles is, therefore, a colony forming unit (cfu) and the results are expressed as colony forming units per unit volume. In the case of thermotolerant coliform bacteria the result should be reported as thermotolerant coliforms [No.] cfu per 100 ml. (UNEP/WHO, 1996).

3.8 Study on the quality of drinking water in Kathmandu valley

ENPHO/DISVI (1990b) conducted a study on water quality of 21 stone spouts of Kathmandu city. The study showed that samples from all the spouts were faecally contaminated. Out of total, 81% of the stone spouts showed very high contamination,

above 100 coliform per 100ml of sample and 19% showed less than 100 coliform per 100 ml.

A study conducted by Bottino et al., (1991) in Kathmandu revealed that most water samples were within the permissible value regarding the chemical parameters except ammonia, nitrate and iron. Pradhananga et al., (1993) recorded the lower pH value of most water samples from the Pashupati area.

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

A study by ENPHO (1993) on heavy metal pollution on water and waste water in Kathmandu valley showed that iron concentration in dug wells of Thimi is higher than WHO guideline value though other metals are in traces. Iron in deep wells could be due to the natural property of the underlying sediments.

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

Maharjan (1998) reported that the temperature of water samples ranged from19.01^0C to 22.0^0C, 19.2^0C to 20.5^0C, 19.0^0C to 25.0^0C, 18.1^0C to 22^0C and 17.5^0C to 21.4^0C for shallow pumps, shallow wells, stone spouts, protected pumps and unprotected pumps respectively. Most of the sources (88.6 %) were recorded within permissible limit of pH value while some (11.4%) were found with pH values slightly less than the WHO guideline value.

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

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

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) also assessed water samples of Kathmandu city from different sources and reported 8.3 % of water samples to have crossed the guideline value for pH.

NGOFUWS (2006) carried out a study on traditional stone spouts in five municipal areas of Kathmandu valley. The study covered 84 samples of different places of Kathmandu valley. During the chemical test, in pH (18% samples) was found to be above WHO permissible guideline value. During the coliform test for 72 hours observation, only one sample showed negative test result while all the 83 samples have positive test result coliform is present.

Adhikari et al., (1986) carried out coliform tests of 100 samples of drinking water (taps water, natural spouts and ponds) from different areas in the Kathmandu valley and found unsatisfactory results with more than 1800 coliforms per 100 ml of water. CEDA in 1989 tested water samples from different localities in Kathmandu and reported that all samples were contaminated with faecal matter indicating that tap and ground water sources were unsafe for drinking.

Vaidya (2006) conducted study on traditional dug wells in Lalitpur Sub-Metropolitan City. The study had identified 318 numbers of dug wells in the city. Out of 318, 59 were selected to assess the drinking water quality. The pH of 51% of water sample was found to exceed the WHO guideline value. Similarly, 98%, 28.8% and 17% of water samples were found to exceed WHO guideline value for ammonia, iron content

and hardness respectively. In microbial study, the wells under chemical treatment showed absence of Coliform, whereas others showed presence of Coliform.

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

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 unto 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.

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

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

Karmacharya and Pariyar (1999) tested 250 samples to assess the quality of ground water. In their study, 43 dug wells, 43 stone spouts, 14 shallow tube wells and 28 deep tube wells were selected. The study revealed that 6% of the sample for pH, 41% of the sample for turbidity, 28.4% of the sample for conductivity, 2.4% of the sample for total hardness, 34% of the sample for ammonia, 51% of the sample for nitrate and 32% of sample for iron exceeded the WHO standard.

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

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%) was reported.

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.

Shakya (2005) conducted a study on the water quality of 20 stone spouts of Lalitpur and found that the level of nitrate, phosphate and ammonia in the water of spouts were higher than who guideline value. 30% of the spouts had pH level higher than WHO guideline. Chloride level did not meet the WHO guideline in 95% of the spouts, while iron concentration was within the limit. The water needs proper treatment before drinking.

Regmi (2001) studied on the water quality of stone spouts of Kathmandu City. 10 sample stone spouts has been studied and found that water is microbiological contaminated while some spouts such as Kapurdhara, Chhauni, Koteshor-2, New Baneshwor, Bhatbhateni are good for drinking, bathing and washing.

Prasai et al., (2007) conducted a study to evaluate the quality of drinking water of the Valley. A total of 132 drinking water samples were randomly collected from 49 tube wells, 57 wells, 17 taps and 9 stone spouts in different places of Kathmandu Valley. Total plate and coliform count revealed that 82.6% and 92.4% of drinking water samples found to cross the WHO guideline value for drinking water. During the study, 238 isolates of enteric bacteria were identified, of which 26.4% were *Escherichia coli* , 25.6% were *Enterobacter* spp, 23% were *Cirtobacter* spp, 6.3%were *Pseudomonasaeruginosa*, 5.4% were *Klebsiella* spp, 4 % *Shigella* spp, 3 % were *Salmonella*Typhi, 3% were *Proteus vulgaris*, 3% were *Serratia* spp and 1 % were *Vibrio cholerae*.

Jayana et al., (2009) studied the drinking water quality of Madhyapur Thimi Municipality. A total of 105 samples comprising 50 wells, 45 tap waters and 10 stone spout were studied. Total coliform count showed 64.67% of the sample crossed the WHO guideline value. Eleven different kind of enteric bacteria were isolated from the sources, *Enterobacter* species being the predominant.

Warner et al., (2008) in a similar study found that most problematic were total coliform and *Escherichia coli* bacteria, which were present in 94% and 72% of all the water samples.

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 106 CFU/ml in river water and lowest bacterial count was found to be 3.0 X 103 CFU/ml in Kuleshwor tap water. Similarly, the highest coliform count was 1100 CFU/100 ml and lowest coliform count was found to

be 500 CFU/100 ml in sample taken from Sundharighat tank. Both the study showed that the most water get contaminated during storage or in the distribution system.

Shrestha (2008) carried out a study on the water quality of 20 public wells for 8 months (February to September 2006) of Madhyapur Thimi Municipality. The physico-chemical parameters such as pH, electrical conductivity, total hardness, dissolved oxygen, free carbon dioxide, total alkalinity, calcium magnesium, chloride and phosphate vary significantly from sites 1 to 20 with the variation in the month. The Nitrate-nitrogen content of all well water samples were found within WHO limit during investigation period. The microbiological analysis was done using MF technique and it showed that all the water samples were faecally contaminated making the water unfit for consumption.

Gopali (2008) carried out a study to assess the microbiological and physico chemical quality of chlorinated drinking water of Kathmandu city. Water sample were collected from the 7 distribution points of Sundari ghat reservoir and 13 distribution points of Balaju reservoir. 15.78% of samples were found to be exceeding the WHO guideline value for turbidity and all the other physico chemical parameters (pH, turbidity, residual chlorine) were within the WHO guideline value. 65.78% of sample from sundarighat reservoir and 91.42% of sample from Balaju reservoir were found to be contaminated with the total coliform.

Maharjan (2009) carried out the study to enumerate the traditional stone spouts in Kathmandu metropolitan city and water quality assessment of the stone spouts. It was found that 165 stone spouts are existing till date. A total of 50 samples from different stone spouts were collected for physico chemical and microbiological analysis. pH of 26% of sample were found to be below WHO guideline and also national standard value. 28% of the sample exceeded the WHO guideline value for iron. Almost all the samples were found to be microbiologically contaminated with coliforms.

Shrestha (2009) studied on the drinking water quality of Kathmandu metropolitan city. A total of 86 samples were collected randomly from different locality of Kathmandu. It was found that pH of 87.21% sample was within WHO permissible limits and all of the sample showed the absence of residual chlorine. 100% of the

sample showed the presence of coliform. Coliform count ranged from 44->300 cfu/100ml of water sample.

3.9 Antibiotic Resistance

Madigan et al., (1997) defines antibiotic as ‘‘a chemical agent produced by one organism that is harmful to other organisms’’. Antibiotics are also defined as compound or substance that either kills or inhibits the growth of a microorganism, such as bacteria, fungi and protozoa and of three major sources of origin: (i) naturally isolated; (ii) purely chemically synthesized; or (iii) semi-synthetically derived. Antibiotics are also defined according to their mechanism for targeting and identifying microorganisms –broad-spectrum antibiotics are active against a wide range of microorganisms; narrow-spectrum antibiotics target a specific group of microorganisms by interfering with the metabolic process specific to those particular organisms (Mossialos et al., 2010).

It has been observed that antibiotic susceptibility of bacterial isolates is not constant but dynamic and varies with time and environment (Hassan, 1995) and changes in the incidence and levels of antibiotic resistance in natural population are not confined to particular segments of the bacterial population and reflect responses to the increased exposure of bacteria to antimicrobial compounds over the past several decades (Hound and Ochman, 2000).

Multiple drug resistance is defined as resistance to ≥2 antibacterial drug classes (Ortega et al., 2004). Xenobiotics, vietenery antibiotics, clinical antibiotics serves as the major sources for creating selective pressure for spread of MDR strain of *E. coli*(Hawkey and Jones, 2009).

3.10 Studies on bacterial resistance to antibiotics

Maharjan (1998) studied the antibiotic resistance pattern from ground water samples randomly collected from Patan area and reported that 82.5% isolates were resistant to at least one antibiotic and 2.5% of the total isolates were resistant to five or more antibiotics. Resistance was more commonly directed towards Nitrofurantoin (68.3%) followed by Ampicillin (44.2%) and Tetracycline (28.3%). All the isolates were sensitive to Amikacin and Gentamicin. 71.4% of the *E. coli* isolates were resistant to at least one antibiotic and 23.8% were resistant to at least two antibiotics.

Lazar et al., (2002) performed antimicrobial susceptibility testing of 12 *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.

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

Prasanna (2006) performed antibiotic sensitivity testing to the isolates from 100 water samples collected randomly from different sources of Kathmandu valley. The result showed Tetracycline 90% sensitive, Ampicillin 100% resistant, Chloramphenicol 100% sensitive, Ofloxacin 80% sensitive and Cephalexin 90% resistant. Frequency of MAR against antibiotics within species are as follows E. coli 20%, Enterobacter spp 12%, Citrobacter spp 5%, Klebsiella spp 20% and Salmonella spp 25%

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 which included *E. coli* (n=8). Ten antibiotics were used: Ampicillin, Amoxicillin-clavulanic acid, Tetracycline, Chloramphenicol, Nalidixic acid, Rifampicin, Amikacin, Gentamicin, Kanamycin and Streptomycin. 93% showed resistance to at least one antimicrobial. *E. coli* was the most sensitive genus to antimicrobials because only 12.5% of the isolates demonstrated multiple resistance. During rainy season 100% *E. coli* isolates were resistant to Ampicillin, Amoxicillin-clavulanic acid, Tetracycline, Chloramphenicol, Nalidixic acid and Streptomycin and during dry season 71%, 14%, 14%, 57% and 100% were resistant to Ampicillin, Kanamycin and Rifampicin.

Lourenco et al., (2007) isolated enterobacterial strains from estuarine water of Sao Vicente,Brazil. Out of 142 isolates, *E. coli* (40.1%) was the predominant one. The isolates were subjected to antimicrobial susceptibility testing using seven different antibiotics. Analysis of the antimicrobial activity of the tested drugs against the isolates showed Gentamycin, Netilmicin and Ciprofloxacin with the highest activity (100%), followed by Cefepime (97.3%), Cefoxitin (84.2%), Amoxicillin/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%). All the isolates were resistant to at least one antibiotic. *P aeruginosa* was found resistant to almost all antibiotics used. Of the total isolates, resistance 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%) was shown.

Shrestha (2008) assayed eight antibiotics against 33 isolates out of 296 from the drinking water of Kathmandu. Most of the isolates (93.9%) were resistant to Ampicillin while only two strains showed the partial sensitivity towards Ampicillin.

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 drug resistance pattern.

Tagoe (2011) determined the antibiotic susceptibility pattern of bacterial isolates in Sachet water. Isolates showed 100% resistance to Ampicillin, Flucloxacillin and Penicillin, while none of them was resistant to Gentamycin. The resistance to other antibiotics ranged from 93.3% for Erythromycin and Cefuroxime, 60% for Cotrimoxazole and 20% for Tetracycline.

Jackson (2011) isolated E.coli, Salmonella serovar typhimurium and Vibrio cholera 01 from water of Western Kenya. All the bacterial isolates were sensitive to Ciprofloxacin, E. coli isolates were resistant to Ampicillin, Tetracycline, Cotrimoxazole, Chloramphenicol and Gentamycin while S. typhimurium isolates exhibited resistance to Ampicillin, Tetracycline and Cotrimoxazole. The V. cholera 01 isolates were resistant to Tetracycline and Ampicillin.

CHAPTER – IV

4. MATERIALS AND METHODS

4.1 Materials

The list of materials: - equipments, chemicals, media and reagent are listed in appendix I.

4.2 Methods

For this study water from wells, tap water and stone spouts were collected randomly from different localities of Kathmandu, Lalitpur and Bhaktapur. The study period was from January to August 2011.

4.2.1 Sampling Site

The study was conducted in Kathmandu, Lalitpur, Bhaktapur and Kritipur Municipality and sampling site are listed in appendix- II.

4.2.2 Sample Collection for water analysis

Sample collection was done according to the standard method described by APHA, 1998. Chlorinated tap water sample for microbial testing were collected in the sterile bottle containing 0.2ml of 3% sodium thiosulphate solution prior to sterilization. In each sampling site, water sample from dug well was collected with the help of clean sampling plastic bottle tied with the rope. Sample was collected from surface of the well. Water sample from Stone spout was collected in the sampling bottle by holding the base with one hand while the other hand was used to remove and replace the screw cap. For the sample collection from tap water, at first the tip of the tap was sterilized. Then the tap was opened and left for some time and water sample was collected in sterilized sampling bottle. Care was taken to prevent accidental contamination of the water during its collection. A code number, sampling date and time was written to each bottle using a marker.

4.2.3 Transportation and preservation of sample

The collected water samples were kept within an ice-box at 4ºC during transportation and analyzed in Central Department of Microbiology, Kritipur on the same day

immediately after its delivery and always within 6 hours of collection. Physico-chemical analysis was done in the sampling site on the same day. Two types of parameter, physico-chemical and microbial were analyzed for water quality assessment.

4.3 Study of Physico-Chemical Parameter of water samples

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

Temperature was determined with the help of a standard mercury thermometer graduated up to 50°C. Hydrogen ion concentration in the sample was measured with the help of the p^{H} meter by inserting the electrode into the water samples.

4.4 Microbial Examination of Water

Microbial Examinations were carried out according to standard methods as described by APHA (1998).

4.4.1 Total coliform count

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

Standard total coliform membrane filter procedure

First of all, sterile filter holder with stopper was assembled on the filter flask. 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. The sterile funnel was securely placed on the filter base.

The water sample was well mixed by inverting the bottle several times, and then 100 ml of the water sample was poured into the funnel. The sample was slowly filtered under partial vacuum by using electric vacuum pump. The funnel was removed and the membrane was directly transferred, keeping its upper side upwards, onto a plate of EMB agar with the help of sterile forceps. Care was taken not to entrap air bubbles between the membrane and the medium. Then it was incubated for 24 hrs at 37^{0}C in

inverted position. After proper incubation total colony forming unit (CFU) were counted. For this, all typical and atypical colonies were counted.

4.4.2 Thermotolerant coliform count

Thermotolerant coliform counts were also enumerated by MF technique as described by APHA, 1998.

Standard thermotolerant coliform membrane filter procedure

First of all, sterile filter holder with stopper was assembled on the filter flask.

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. The sterile funnel was securely placed on the filter base.

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. The sample was slowly filtered under partial vacuum by using electric vacuum pump. The funnel was removed and the membrane was directly transferred, keeping its upper side upwards, onto a plate of EMB agar with the help of sterile forceps. Care was taken not to entrap air bubbles between the membrane and the medium.

Then it was incubated for 48 hrs at 44.5^0C in inverted position. After proper incubation total colony forming unit (CFU) were counted. For this, all typical and atypical colonies were counted.

4.4.3 Detection of *E. coli*

The colonies producing the greenish metallic sheen on EMB agar plate (from 37^0C) were sub cultured on Nutrient Agar plate and incubated at 37^0C for 24 hours. Then the morphological characteristics of isolated colonies were noted and further subjected to gram staining and biochemical tests for identification based on Bergey's Manual of Determinative Bacteriology, 1994.

4.4.4 Detection of coliforms other than *E. coli*

The pink colonies observed on EMB agar plate (from both 37^0C and 44.5^0C) were sub cultured on Nutrient Agar plate and incubated at 37^0C for 24 hours. Then the morphological characteristics of isolated colonies were noted and further subjected to

gram staining and biochemical tests for identification based on Bergey's Manual of Determinative Bacteriology, 1994.

4.4.5 Isolation of *Salmonella* spp and *Shigella* spp

The water samples were enriched in selenite F broth as an enrichment medium for the isolation of *Salmonella* spp and *Shigella* spp. In this enrichment medium selenite inhibits coliforms while permitting *Salmonella* spp and *Shigella* spp to grow.

Enrichment

Water sample of 5 ml was inoculated into 45ml of selenite F broth and mixed thoroughly.

It was then incubated at 37°C for overnight. A loopful of the upper part of broth was cultured on a selective media i.e. Salmonella- Shigella (SS) agar. The plate was incubated at 37ºC for 24 hours.

Isolation and identification

The pale colony with black center and all other typical colonies were further streaked onto NA for purification. Isolated colonies were subjected to gram staining and biochemical tests for identification based on Bergey's Manual of Determinative Bacteriology, 1994.

4.5 Antibiotic susceptibility test

Antibiotic susceptibility test of the isolates towards various antimicrobial discs was done by modified Kirby-Bauer disk diffusion method as recommended by National Committee for Clinical Laboratory Standards (NCCLS) using MHA (Vandepitte et al., 2003).

Organisms were classified as sensitive or resistant to an antibiotic according to the diameter of the inhibition zone surrounding each antibiotic disc. Based on the sensitivity pattern of the isolates, isolates resistant to three or more than three classes of antibiotics were considered as multiple antibiotic resistant bacteria.

I. Procedure for Antibiotic Susceptibility Test

Cells were grown at 37^0C in 5ml of nutrient broth for about 4 hours using pure cultures as inoculums. The turbidity developed was compared with standard Mc

farland solution. A sterile cotton swab was dipped into the properly prepared inoculums and firmly rotated against the upper inside wall of the tube to expel excess fluid, and then swabbed onto Mueller-Hinton agar. During swabbing the plate was streaked with the swab three times turning the plate 60^0C between each streaking to achieve a lawn of confluent bacterial growth. The plate was kept at room temperature for 5 to 10 minutes, but no longer than 15 minutes to dry the inoculums. 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 pre diffusion and then incubated at 37^0C for 24hrs. The diameter of the zone of inhibition was measured at the end of the incubation period.

4.6 Quality control

Strict quality control was maintained to obtain reliable microbiological results. The quality of each agar plate prepared was maintained by incubating one plate of each batch in the incubator. A control strain of ATCC was given in Appendix I used for the identification test, standardization of Kirby-Bauer test, correct interpretation of inhibition zones of diameter. Quality of sensitivity test was maintained by maintaining the thickness of MHA at 4 mm and the pH of 7.2-7.4. Similarly antibiotics discs having correct amount as indicated was used. Strict aseptic condition was maintained while carrying out all the procedures.

Quality of antibiotic discs was assessed using reference cultures of *E. coli* ATCC 25922 and *Staphylococcus aureus* ATCC 25923.

4.7 Data Analysis and Statistical Tools

All laboratory data were analysed using MS Excel 2007. Associations among variables were assessed using Chi square test and expressed in terms P value (95% CI) using SPSS V. 16 software, whenever required.

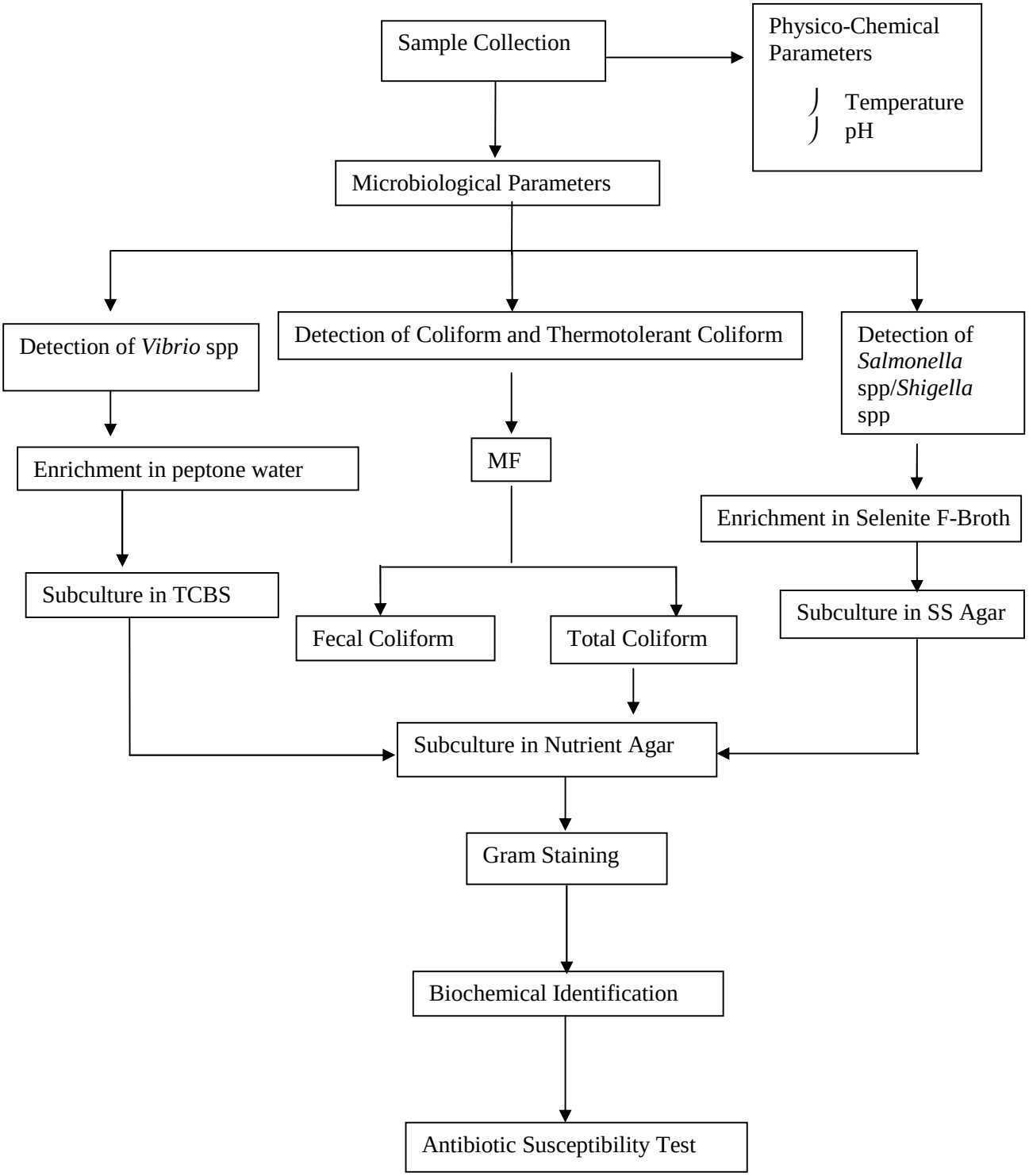

Figure-1: Flow Chart Diagrammatic Representation of Overall Process

CHAPTER – V

5. RESULTS

This study was carried out from January 2011 to August 2011. During the study period physical parameters and bacteriological assessment along with isolation and identification of the isolates, in addition, antibiotic susceptibility and resistance pattern of the isolates was determined. A total of 66 drinking water samples, 28 from tap water; 24 from well water and 14 from spout water were collected from different place of Kathmandu valley and were assessed for Physico-Chemical (Temperature, pH) and microbiological parameters.

5.1 Physico-Chemical quality of drinking water

The temperature and pH of all the samples noted were enlisted in appendix-III. Temperature of Tap water was between 12.2°c to 16°c and pH was found in between 6.5 to 7.8, temperature of Well water was found between 10°c to 14.1°c and pH was found in between 6.3 to 7.8. Temperature of Spout water was between 12.1°c to 16°c and pH was found in between 6.3 to 8.5.(Table-2)

Table-2: Physical quality of drinking water

S.n	Source	Parameters	Min	Max
1	Tap	Temperature	12.1°c	16°c
		pH	6.5	7.8
2	Well	Temperature	10°c	14.1°c
		pH	6.5	8.5
3	Spout	Temperature	12.1°c	16°c
		pH	6.5	8.1

5.2 Microbiological quality of drinking water

5.2.1. Total coliform count in drinking water

Out of 66 water samples analyzed, 72.7% (n=48) samples showed the presence of coliform (Figure-2). Highest number of total coliform (85.7%) was detected in spout

water followed by well water (79.2%) (Table-3). The coliform count in 48 samples ranged from 6- >300 CFU/100ml of water samples.

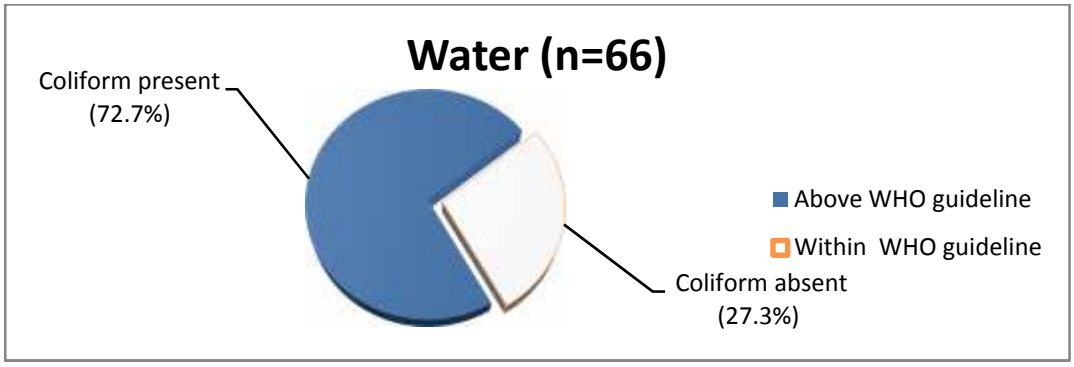

Figure-2: Total coliform present in water sample

Table-3: Total coliform count in drinking water

S.n	Samples source	Above WHO guideline	Within WHO guideline	Totals (%)
1	Tap	60.7% (n=17)	39.3% (n=11)	100% (n=28)
2	Wells	79.2% (n=19)	20.8% (n=5)	100% (n=24)
3	spouts	85.7% (n=12)	14.3% (n=2)	100% (n=14)

5.2.2 Presence of thermotolerent coliform in drinking water

Out of 66 samples analyzed, 75.7% (n=50) samples showed the presence of thermo tolerant coliform crossing the WHO guideline (Figure-3). Highest number of total coliform (87.5%) was detected in well water followed by spout (71.4%) (Table-4). Thermo tolerant coliform count ranged from 5>300 c.f.u/100ml sample.

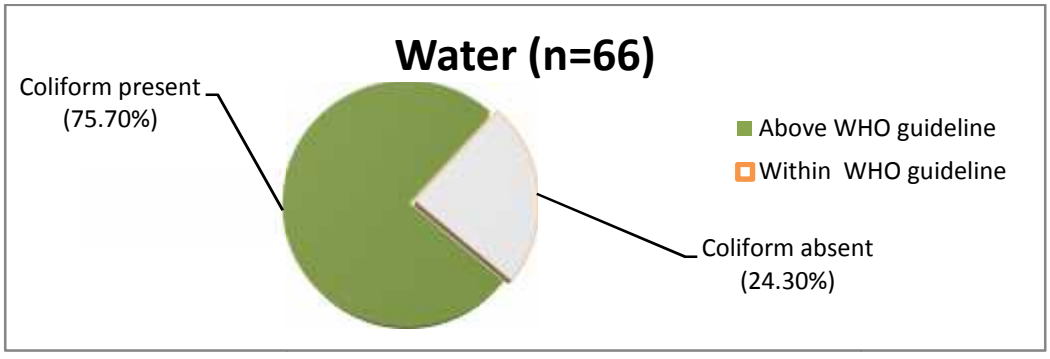

Figure-3: Thermotolerant coliform present in tested water sample

Table-4: Thermotolerent coliform count of drinking water

S.n	Samples source	Above WHO guideline	Within WHO guideline	Totals(%)
1	Tap	67.9% (n=19)	32.1% (n=9)	100% (n=28)
2	Wells	87.5% (n=21)	12.5% (n=3)	100% (n=24)
3	spouts	71.4% (n=10)	28.6% (n=4)	100% (n=14)

5.2.3 Relation of coliform with source

There was no significant relation between water sources with presence of coliform ($P > 0.05$) and thermotolerant coliform ($P > 0.05$).

5.2.4 Occurrence and distribution of bacteria in drinking water

All the bacterial isolates from different water sources were enlisted in Appendix–IV. In this study 97 different isolates of organisms were isolated and identified as *Escherichia coli* 32 % (n=31), *Citrobacter* spp 24.7% *(*n=24), *Enterobacter* spp 21.6% (n=21) and *Klebsiella* spp 18.6% (n=18) .The highest number of organism isolated was *E. coli* which is 32% (n=31) and the lowest number of organism isolated was *Salmonella* spp which is 3.1% (n=3). (Table-5)

Table-5: Occurrence and distribution of bacteria in drinking water

Organism isolated	No of Isolates	Organism isolated (%)
E. coli	31	32
Citrobacter spp	24	24.7
Enterobacter spp	21	21.6
Klebsiella spp	18	18.6
Salmonella Typhi	3	3.1
Total	97	100

5.2.5 Source wise distribution of bacteria

Out of 97 organisms isolated 17 organisms were isolated from tap water, 54 organisms from well and 28 organisms from spout. The recovered percentage of the isolates from tap water were *E. coli* 35.3% (n=6), *Klebsiella* spp 17.7% (n=3),

Enterobacter spp 23.5% (n=4) and *Citrobacter* spp 23.5% (n=2). The recovered percentage of the isolates from well water were *E. coli* 30.8% (n=16), *Klebsiella* spp 19.2% (n=10), *Enterobacter* spp 21.1% (n=11), *Citrobacter* spp 25% (n=13) and *Salmonella* spp 3.9% (n=2). The recovered percentage of the isolates from spout were *E. coli* 32.2% (n=9), *Klebsiella* spp 17.8% (n=5), *Enterobacter* spp 21.4% (n=6) , *Citrobacter* spp 25% (n=7) and *Salmonella* spp 3.6% (n=1). (Table-6).

Table-6: Source wise distribution of bacteria

Variable	**Organism**	**Recovered**	**Percentage**
Tap water	*1. E. coli*	6	35.3%
	2.Klebsiella spp	3	17.7%
	3. Enterobacter spp	4	23.5%
	4. Citrobacter spp	4	23.5%
	Total	17	100%
Well water	*1. E. coli*	16	30.8
	2. Klebsiella spp	10	19.2
	3. Enterobacter spp	11	21.1
	4. Citrobacter spp	13	25
	5. *Salmonella* Typhi	2	3.9
	Total	52	100%
Spout water	*1. E. coli*	9	32.2
	2. Klebsiella spp	5	17.8
	3. Enterobacter spp	6	21.4
	4. Citrobacter spp	7	25
	5.Salmonella Typhi	1	3.6
	Total	28	100

5.2.6 Distribution of bacterial isolates from different source

Out of 97 organism isolated from different source, 17 organisms were isolated from tap water, 52 organisms were isolated from well water and 28 organisms were isolated from stone spouts which is 17.5%, 53.6% and 28.9% respectively. (Table-7)

Table-7: Distribution of bacterial isolates from different source

S.n	Samples source	Isolates	Isolates %
1.	Tap (n=28)	17	17.5%
2.	Wells (n=24)	52	53.6%
3.	Spouts (n=14)	28	28.9%
	Total	97	100%

5.3 Antibiotic Susceptibility Pattern of bacterial isolates

All the coliform isolates were subjected to the antibiotic susceptibility test to eight different antibiotics. The antibiotics used were Ofloxacin (Of), Tetracycline (T), Co-trimoxazole (Co), Amikacin (AK), Cefexime (Cfx), Chloramphenicol (C), Amoxicillin (Am), Nalidixic Acid (NA). Antibiotic pattern of all the bacterial isolates were enlisted in appendix- VIII.

5.3.1 Antibiotic Susceptibility Pattern of *Escherichia coli*

Out of 31 isolates, most of the isolates, 93.5% and 80.6% were resistance to tetracycline and Amoxicillin respectively whereas all the isolates were sensitive towards Chloramphenicol ,Ofloxacin and Co-trimoxazole (Table-8, Table-9).

Table-8: Antibiogram of *E. coli*

S.no	Source	Sample code	Colony code	Of	C	Am	Ak	Cfx	Na	Co	T
1	Tap	KT1	EC1	S	S	R	I	R	S	S	R
2	Tap	KT3	EC2	S	S	R	R	R	S	S	R
3	Tap	KupT3	EC3	S	S	R	S	I	I	S	R
4	Tap	BalT1	EC4	S	S	I	I	I	S	S	R
5	Tap	BalT2	EC5	S	S	R	I	R	R	S	R
6	Tap	BhtT3	EC6	S	S	R	S	I	I	S	R
7	Well	KW1	EC7	S	S	R	S	R	I	S	R
8	Well	KW2	EC8	S	S	R	I	R	R	S	R
9	Well	KW3	EC9	S	S	I	S	I	S	S	I
10	Well	KW4	EC10	S	S	R	R	R	I	S	R
11	Well	KupW1	EC11	S	S	R	I	R	S	S	R
12	Well	KupW2	EC12	S	S	R	R	R	R	S	R

13	Well	JhmW1	EC13	S	S	R	S	I	R	S	R
14	Well	JhmW3	EC14	S	S	I	I	I	S	S	R
15	Well	SW2	EC15	S	S	R	S	R	I	S	R
16	Well	SW3	EC16	S	S	I	R	I	I	S	R
17	Well	BalW1	EC17	S	S	R	I	R	R	S	I
18	Well	BalW2	EC18	S	S	R	S	I	I	S	R
19	Well	BalW3	EC19	S	S	R	R	R	S	S	R
20	Well	BhtW1	EC20	S	S	R	S	I	R	S	R
21	Well	BhtW2	EC21	S	S	R	R	R	I	S	R
22	Well	BhtW3	EC22	S	S	R	I	S	R	S	R
23	Stone Spout	KD2	EC23	S	S	I	S	I	S	S	R
24	Stone Spout	KupD1	EC24	S	S	R	R	R	S	S	R
25	Stone Spout	KupD2	EC25	S	S	R	R	R	R	S	R
26	Stone Spout	SD2	EC26	S	S	R	S	I	I	S	R
27	Stone Spout	BalD1	EC27	S	S	R	S	I	R	S	R
28	Stone Spout	BalD2	EC28	S	S	R	I	R	I	S	R
29	Stone Spout	BhtD1	EC29	S	S	R	I	I	R	S	R
30	Stone Spout	BhtD2	EC30	S	S	R	R	R	I	S	R
31	Stone Spout	BhtD3	EC31	S	S	R	I	R	R	S	R

Table-9: Antibiotic Susceptibility Pattern of *E. coli*

Antibiotic	**Sensitive**		**Intermediate**		**Resistant**		
Used	**No.**	**%**	**No.**	**%**	**No.**	**%**	**Total**
Amoxicillin	0	0	6	19.4	25	80.6	31
Amikacin	12	38.7	11	35.5	8	25.8	31
Cefixime	1	3.2	15	48.4	15	48.4	31
Tetracycline	0	0	2	6.5	29	93.5	31
Chloramphenicol	31	100	0	0	0	0	31
Ofloxacin	31	100	0	0	0	0	31
Nalidixic acid	10	32.3	13	41.9	8	25.8	31
Co-trimoxazole	31	100	0	0	0	0	31

5.3.2 Antibiotic Susceptibility Pattern of *Citrobacter* spp

Out of 24 *Citrobacter* isolates exhibits 100% sensitive towards Co-trimoxazole, Ofloxacin, and 95.8% towards Chloramphenicol while it shows 100% resistance towards Amoxicillin followed by Tetracycline (62.5%) and Cefexime (45.9%). (Table-10, Table-11)

Table-10: Antibiogram of *Citrobacter* spp

S.no	Source	Sample code	Colony code	Of	C	Am	Ak	Cfx	Na	Co	T
1	Tap	KupT1	C1	S	S	R	S	I	S	S	I
2	Tap	BalT1	C2	S	S	R	S	R	S	S	R
3	Tap	BhtT1	C3	S	S	R	S	R	I	S	I
4	Tap	BhtT2	C4	S	S	R	S	I	S	S	R
5	Well	KW1	C5	S	I	I	R	I	I	S	S
6	Well	KW3	C6	S	S	I	R	S	S	S	S
7	Well	KupW1	C7	S	S	I	S	S	R	S	S
8	Well	JW1	C8	S	S	R	S	R	I	S	R
9	Well	JW5	C9	S	S	R	S	R	I	S	R
10	Well	SW2	C10	S	S	I	R	I	R	S	I
11	Well	SW3	C11	S	S	R	S	R	I	S	R
12	Well	SW5	C12	S	S	R	S	R	I	S	R
13	Well	BW2	C13	S	S	R	R	I	R	S	R
14	Well	BW3	C14	S	S	I	R	S	I	S	I
15	Well	BW4	C15	S	S	R	S	R	I	S	R
16	Well	BhtW1	C16	S	S	R	S	S	R	S	I
17	Well	BhtW3	C17	S	S	R	S	R	I	S	R
18	Stone Spout	KD2	C18	S	S	R	S	S	I	S	R
19	Stone Spout	KupD3	C19	S	S	R	S	R	S	S	R
20	Stone Spout	SD1	C20	S	S	R	S	R	S	S	R
21	Stone Spout	BD1	C21	S	S	R	I	R	I	S	R
22	Stone Spout	BD2	C22	S	S	I	R	S	S	S	I
23	Stone Spout	BhtD1	C23	S	S	R	I	S	S	S	R
24	Stone Spout	BhtD3	C24	S	S	R	I	S	I	S	R

Table-11: Antibiotic Susceptibility Pattern of *Citrobacter* spp

Antibiotic Used	Sensitive		Intermediate		Resistant		Total
	No.	%	No.	%	No.	%	
Amoxicillin	0	0	6	25	18	75	24
Amikacin	15	62.5	3	12.5	6	25	24
Cefixime	8	33.3	5	20.8	11	45.9	24
Tetracycline	3	12.5	6	25	15	62.5	24
Chloramphenicol	23	95.8	1	4.2	0	0	24
Ofloxacin	24	100	0	0	0	0	24
Nalidixic acid	8	33.3	12	50	4	16.7	24
Co-trimoxazole	24	100	0	0	0	0	24

5.3.3 Antibiotic Susceptibility Pattern of *Enterobacter* spp

Out of 21 isolates, *Enterobacter* spp shows susceptibility pattern of 100% sensitive towards Co-trimoxazole, Ofloxacin and Chloramphenicol and resistance Amoxicillin (52.4%) and tetracycline (47.7%). (Table-12, Table-13)

Table-12: Antibiogram of *Enterobacter* spp

S.no	Source	Sample code	Colony code	Of	C	Am	Ak	Cfx	Na	Co	T
1	Tap	KT5	ET1	S	S	R	S	S	S	S	S
2	Tap	JT4	ET2	S	S	R	S	S	S	S	R
3	Tap	ST3	ET3	S	S	S	S	S	S	S	S
4	Tap	BhtT4	ET4	S	S	R	I	S	S	S	R
5	Well	KW2	ET5	S	S	S	S	S	I	S	I
6	Well	KW4	ET6	S	S	R	I	I	S	S	R
7	Well	KupW2	ET7	S	S	R	S	S	I	S	R
8	Well	JW1	ET8	S	S	R	S	S	S	S	R
9	Well	JW3	ET9	S	S	R	S	S	I	S	R
10	Well	JW5	ET10	S	S	I	S	I	S	S	I

11	Well	SW5	ET11	S	S	R	I	S	S	S	R
12	Well	BW1	ET12	S	S	I	S	S	S	S	I
13	Well	BW2	ET13	S	S	I	S	S	S	S	I
14	Well	BW4	ET14	S	S	I	I	S	S	S	I
15	Well	BhtW2	ET15	S	S	R	I	S	S	S	R
16	Stone spout	KupD1	ET16	S	S	R	S	I	S	S	R
17	Stone spout	KupD2	ET17	S	S	I	S	S	S	S	I
18	Stone spout	SD2	ET18	S	S	R	S	S	S	S	R
19	Stone spout	Bhtd1	ET19	S	S	I	S	S	S	S	I
20	Stone spout	Bhtd2	ET20	S	S	I	S	S	I	S	S
21	Stone spout	Bhtd4	ET21	S	S	I	S		I	S	S

Table-13: Antibiotic Susceptibility Pattern of *Enterobacter* spp

Antibiotic Used	**Sensitive**		**Intermediate**		**Resistant**		**Total**
	No.	**%**	**No.**	**%**	**No.**	**%**	
Amoxicillin	2	9.6	8	38	11	52.4	21
Amikacin	16	76.1	5	23.9	0	0	21
Cefixime	18	85.7	3	14.3	0	0	21
Tetracycline	4	19	7	33.3	10	47.7	21
Chloramphenicol	21	100	0	0	0	0	21
Ofloxacin	21	100	0	0	0	0	21
Nalidixic acid	16	76.1	5	23.9	0	0	21
Co-trimoxazole	21	100	0	0	0	0	21

5.3.4 Antibiotic Susceptibility Pattern of *Klebsiella* spp

Out of 18 isolates, *Klebsiella* were 100% sensitive towards Co-trimoxazole, Ofloxacin and Chloramphenicol, and 100% resistance towards amoxicillin, followed by Tetracycline (62.7%) and Cefexime (44.4%). (Table-14, Table-15)

Table-14: Antibiogram of *Klebsiella* spp

S.no	Source	Sample code	Colony code	Of	C	Am	Ak	Cfx	Na	Co	T
1	Tap	KT4	K1	S	S	R	S	S	S	S	S
2	Tap	JT5	K2	S	S	R	S	R	S	S	R
3	Tap	BalT3	K3	S	S	R	S	S	S	S	S
4	Well	KW2	K4	S	S	R	S	R	S	S	R
5	Well	KW3	K5	S	S	R	S	S	S	S	S
6	Well	KW4	K6	S	S	R	R	R	R	S	R
7	Well	KupW1	K7	S	S	R	R	R	I	S	R
8	Well	KupW2	K8	S	S	R	R	R	R	S	R
9	Well	JW3	K9	S	S	R	I	R	R	S	R
10	Well	SW2	K10	S	S	R	S	S	S	S	I
11	Well	BW1	K11	S	S	R	I	R	S	S	R
12	Well	BW4	K12	S	S	R	S	S	S	S	I
13	Well	BhtW2	K13	S	S	R	I	S	R	S	R
14	Stone spout	KW2	K14	S	S	R	R	R	S	S	R
15	Stone spout	KupD3	K15	S	S	R	R	R	S	S	S
16	Stone spout	BD2	K16	S	S	R	R	S	I	S	R
17	Stone spout	Bhtd2	K17	S	S	R	S	S	R	S	R
18	Stone spout	Bhtd4	K18	S	S	R	S	S	I	S	R

Table-15: Antibiotic Susceptibility Pattern of *Klebsiella* spp

Antibiotic	**Sensitive**		**Intermediate**		**Resistant**		
Used	**No.**	**%**	**No.**	**%**	**No.**	**%**	**Total**
Amoxicillin	0	0	0	0	18	100	18
Amikacin	9	50	3	16.7	6	33.3	18
Cefixime	10	55.6	0	0	8	44.4	18
Tetracycline	4	22.2	2	11.1	12	66.7	18
Chloramphenicol	18	100	0	0	0	0	18
Ofloxacin	18	100	0	0	0	0	18
Nalidixic acid	10	55.5	3	16.7	5	27.8	18
Co-trimoxazole	18	100	0	0	0	0	18

5.3.5 Antibiotic Susceptibility pattern of *Salmonella* Typhi

Out of the 3 isolates all the *Salmonella* Typhi isolates were sensitive towards Cotrimoxazole and Chloramphenicol. Whereas 100 % of the isolates were resistant to Amoxicillin and tetracycline followed by Nalidixic acid and Cefexime which is 66.7%. (Table-16, Table-17)

Table-16: Antibiogram of *Salmonella* Typhi

S.no	Source	Sample code	Colony code	Of	C	Am	Ak	Cfx	Na	Co	T
1	Well	BW3	S1	S	S	R	S	R	I	S	R
2	Well	BhtW1	S2	I	S	R	I	R	R	S	R
3	well	BhtW3	S3	I	S	R	S	I	R	S	R

Table-17: Antibiotic Susceptibility pattern of *Salmonella* spp

Antibiotic	**Sensitive**		**Intermediate**		**Resistant**		
Used	**No.**	**%**	**No.**	**%**	**No.**	**%**	**Total**
Amoxicillin	0	0	0	0	3	100	3
Amikacin	2	66.7	1	33.3	0	0	3
Cefixime	0	0	1	33.3	2	66.7	3
Tetracycline	0	0	0	0	3	100	3
Chloramphenicol	3	100	0	0	0	0	3
Ofloxacin	1	33.3	2	66.7	0	0	3
Nalidixic acid	1	33.3	0	0	2	66.7	3
Co-trimoxazole	3	100	0	0	0	0	3

5.4 Distribution of MDR Isolates

Out of 97 isolates taken for antibiotic susceptibility test 60.8% (n=69) were found to be MDR isolates. MDR pattern of *E. coli*, *Citrobacter* spp, *Enterobacter* spp, *Klebsiella* spp and *Salmonella* Typhi were 92.3% (n=28), 62.5% (n=15), 47.6% (n=10), 72.2% (n=13), and 100% (n=3) respectively. (Table-18)

Table-18: Distribution of MDR Isolates

Bacteria	**No. of isolates**	**No. of MDR Isolates**	**MDR Isolates (%)**
E. coli	31	28	92.30%
Citrobacter spp	24	15	62.50%
Enterobacter spp	21	10	47.60%
Klebsiella spp	18	13	72.20%
Salmonella Typhi	3	3	100%
Total	97	69	71.10%

CHAPTER - VI

DISCUSSION AND CONCLUSION

6.1. Discussion

Human health depends on safe water more than any other thing. Basically the life of human is related to safe water. Most of the problems in developing countries are mainly due to the lack of safe drinking water. And it is apparent that health of individuals depends on safe drinking water. Unfortunately, in many countries around the world, including Nepal water has become a scarce commodity as only a small proportion of the populace has access to treated water. Alternative sources of water such as rainwater and ground water have become major sources of drinking water for people living in new settlements and some residents who do not have access to treated water in Nepal. The need to assess the quality of water from some of these alternative sources has become imperative because they have a direct effect on the health of individuals (WHO, 1996).

According to Rao (1991) about 75-80% people of developing countries are exposed to unsafe drinking water. Water contaminated with antibiotic-resistant bacteria is a major threat to public health, as the antibiotic resistance determinants can be transferred to bacteria of human clinical significance (Blake et al., 2003). The prevalence of antimicrobial resistance has increased during the recent decades (Threlfall et al., 2000) and may be due to selection pressure caused by the indiscriminate use and misuse of antimicrobials (Bywater et al., 2004). As a result, enteric diseases often reaching the epidemic proportion devastate the several parts of country. Hence this study was under taken to reveal the antimicrobial resistance pattern of colforms from different water sources (Tap, Stone spouts and traditional dug wells) of Kathmandu valley.

Temperature is one of the parameter that directly affects, or be affected by other parameter. In this research, minimum temperature 10°C and maximum temperature 16°C was observed in well water. Although the variations among water samples were not wide, significant differences were recorded between water samples of all water sources. It is established that climatologic factors affect temperature. In Nepalese

context, Shrestha, (2008) and Bhattarai et al., (2008) found that the temperature of the water samples was lower in winter season as the present study was done. In this study, all drinking water samples were found to lie within pH range of WHO guideline value. Similar measurements have been reported by Panta, (2011), Maharjan, (2009) and Diwakar et al., (2008) pH of a water body is very important in determination of water quality since it affects other chemical reactions such as solubility and metal toxicity. All water samples (100%) showed their pH values within the permissible level as recommended by the WHO (6.5-8.5). This could therefore be regarded as neutral and unpolluted (Fakayode, 2005). pH is an operational water quality parameters and a large variety of pollutants such as discharges from industries containing detergents, heavy metals, bleaching materials, acids, alkalis etc affect the pH of receiving water. pH less than 7.0 may cause corrosion of metal pipes thereby releasing toxic metals like Zn, Pb, Cd and Cu, etc. and higher than 8.0 adversely affect the disinfection process. Chlorination may markedly less effective in increasing pH values.

Similar result was obtained in the studies conducted by Gyewali (2007), Gopali (2008) and Shrestha (2008), Diwakar (2008) who reported that the pH of the water samples lie within the permissible limit. However, other studies conducted previously have shown a deviation in the pH values of water from the normal value. Prasai (2002) reported 8.3 % of water samples to have crossed the guideline value for pH. Bajracharya (2007) reported that 85.09% of the samples were within standard, 14.91% below the standard and 0 % above the standard with regard to pH. Prasanna (2006) reported that 10 (10%) of water sample were found to lie above WHO guideline value. Jayana (2008) reported that 84.76 % samples were within the permissible limit while 13.33 % samples showed below the limit and 1.9% samples crossed the upper limit of WHO guideline value.

The routine examination of environmental samples for the presence of intestinal pathogens is often a tedious, difficult and time consuming task. Thus, this problem is overcome by looking for certain indicator microorganisms whose presence indicates the presence of other pathogenic microorganisms also (Maier et al, 1996). Frequent examination for faecal indicator organisms remains the most sensitive and specific way of assessing the hygienic quality of water. The bacteria selected as indicator of

faecal pollution should be universally present in the faeces of humans and warm blooded animals in large quantity than any pathogen yet are unable to proliferate in water to any extent. Moreover, they should be more resistant than pathogens to the stresses of aquatic environment and disinfection processes (Colle et al., 2006). There are different types of bacteria used as indicators but our immediate concern is the coliform bacteria since they are the most common bacteria globally used as indicators. Thus, this study indicates that most of the water sources are highly faecally contaminated. Water quality thus indicates that pollution of water is increasing alarmingly and it has created serious threat to human health and environment.

In present study, total coliform growth was found higher in spout water (85.7%), followed by well water (79.2%) then tap water (60.7%) and Similarly, the thermotolerant coliform growth was found higher in well water (87.5%) followed by spout water (71.4%) the tap water (67.9%). Thermotolerant coliforms were seen in some driking water samples but no coliforms growth at 37^{o}C, it might be due to coliforms present in drinking water samples were in decline phase or it might be due to the mucoid colony which overlapped the coliforms.

While comparing the coliform count found in the tested water sample with the WHO guideline and National standard value, it was found that 48 (72.7%) samples crossed the guideline value indicating that only 18 (27.3 %) samples are safe from total coliform point of view. While 39.2% of tap water samples were lie within WHO guide line. It indicates that the tap water pipeline might be cross contaminated with sewerage system or might be due to the lickage of pipe line. The coliform detection also indicated inadequate mass chlorination.

The coliform contamination in the ground water may be due to sewage infiltration, seepage and disposal of domestic wastes nearby the sources. The construction of septic tank nearby the ground water sources also facilitates the contamination of water with coliform bacteria. While 20.8% of well water and 14.3% of spout water sources were within WHO guide line. This may because the treatment system in city supply water might kill the coliform bacteria. Coliform bacteria should not be detectable in treated water supplies and, if found, suggest inadequate treatment, post-treatment

contamination, or excessive nutrients. In this sense, the coliform test can be used to assess treatment efficiency and the integrity of the distribution system.

As source of water in stone spouts is surface water, it is easily being polluted by anthropogenic activities. These may have been contaminated due to infiltration of various kinds of pollutants, toxic wastes from chemical plants, biological wastes from hospitals resulting in plumes of pollutants travelling in the ground water and contaminating the underground environment. Domestic wastewater, industrial waste, increase in the use of agrochemicals and hazardous waste disposal sites add ground water contamination. Moreover stone spouts are neither protected properly nor is the source treated.

This indicates that the water from all sources were highly contaminated particularly in well and spout sources and the contamination is recent.
High contamination on stone spout and ground water may be due to direct discharge of untreated sewage or municipal wastes into surface waters or in open places near to sources, which was observed in most of the places. Contamination with in such environment due to such unusual practices, contaminants can easily leach down to groundwater table leading high microbial contamination to shallow water.

Maximum thermotolerant *E. coli* along with thermotolerant coliform detected in shallow water is probably due to poor drainage facility and improper construction pattern of septic reservoirs, infiltration of domestic or wild animal fecal matter Construction of septic tank close to the groundwater sources may be a reason of high microbial contamination to groundwater, which was common almost in all the places as observed. As a consequence, the effluent from septic tank can easily percolate down to groundwater and leads high microbial contamination to ground water table.

The coliform group consists of several genera of bacteria belonging to the family *Enterobacter*iaceae. Traditionally these genera included *Escherichia*, *Citrobacter*, *Enterobacter* and *Klebsiella*. With the advent of new technologies for bacterial analyses, the working definition of a coliform has again changed. Lactose fermentation is one of the key criteria in the coliform definition and fermentation of lactose is determined, in part, by the presence of a specific enzyme, ß-galactosidase.

The presence of ß-galactosidase in a member of the *Enterobacter*iaceae is considered specific to coliforms (Stevens et al., 2003).

These indicators, used to assess the potential public health risk of drinking water, are key elements of most drinking water quality guidelines. The presence of coliform organisms has health significance for consumer. The presence of these bacteria in drinking water may indicate contamination resulting from the failure in disinfection process (Tortorello, 2003) and the presence of pathogenic organisms that are sources of water borne diseases. However, absence of these bacteria in water does not necessarily guarantee the absence of pathogens (Krewski et al., 2004). Water is considered safe when it is free of *E. coli* (Wanke, 1990).

Higher percentage (53.6%) of organisms were isolated from well water, followed by spout (28.9%) and only 17.5% of organism were isolated from tap water. Among the isolates, recovery of *Escherichia coli* was highest among isolates which constitutes of 32%,followed by *Citrobacter* spp (24.7%), *Enterobacter* spp (21.6%) and *Klebsiella* spp (18.6%) . The lowest number of organism isolated was *Salmonella* Typhi which is 3.1%.

While within sources, the recovered percentage of the isolates from tap water were *E. coli* 6 (40%),*Klebsiella* spp 3 (20%), *Enterobacter* spp 4 (26.7%) and *Citrobacter* spp 2 (13.3%); from well water were *E. coli* 16 (29.6%),*Klebsiella* spp 10 (18.5%), *Enterobacter* spp 11(20.4%) , *Citrobacter* spp 15 (27.8%) and *Salmonella* spp 2 (3.7%) and the spout were *E. coli* 9 (32.2%),*Klebsiella* spp 5 (17.8%), *Enterobacter* spp 6 (21.4%) , *Citrobacter* spp 7 (26%) and *Salmonella* spp 1 (3.6%).

Apart from total coliform and fecal coliform other microorganisms that are pathogenic to human have also been detected in this study and the result has been supported by results obtained by other reaserches in the past. In this study *Salmonella* Typhi was detected in well 2 (3.7%) and in spout 1 (3.6%) and not detected in tap water.

The results obtained were comparable with previous studies. Jayana (2009) isolated 142 enteric bacteria of 11 different types of which 24.6% was *E. coli*, 20.4% was *Citrobacter* spp, 27% was *Proteus vulgaris*, 5.6% was *Klebsiella* spp, 3.5% was

Proteus mirabilis, 2.8% was *Shigella dysentery*, 2.1% was *Salmonella* Typhi, 2.1% was *Pseudomonas aeroginosa*, 1.4% was *Salmonella* Paratyphi and 0.7% was *Vibrio cholera*.

Similarly, Prasai (2002) isolated 238 enteric bacteria and identified as *E. coli* (26.4%), *Enterobacte* spp (25.6%), *Citrobacter* spp (22.6%), *Pseudomonas* aeroginosa (6.3%), *Klebsiella* spp (8.4%), *Shigella* spp (3.78%), *Salmonella* Typhi (3.3%), *Proteus vulgaris* (2.9%), *Serratia* spp (2.52%) and *Vibrio cholerae* (0.84%). Likewise, Kafle (2007), also found 72 % of the sample showed total coliform and 62 % showed fecal coliform. When analysed sourcewise 100 % of the sample from stone spouts showed coliform. In this study, *Salmonella* spp and *Shigella* sppwas also found in 5 % and 3 % of the sample respectively. The detection of pathogenic enteric bacteria in drinking water in Kathmandu valley reveals the alarming situation for water borne epidemics in the valley.

Antimicrobial resistance is a serious threat to the treatment of infectious disease and a leading public health concern of 21st century. Antimicrobial resistant *E. coli* has been detected in many places including drinking water. The ingestion of water contaminated with antimicrobial resistant bacteria may contribute to the prevalence of antimicrobial resistance in humans (Coleman, 2008). Infection with antibiotic resistant bacteria makes the therapeutic options for infection treatment extremely difficult or virtually impossible in some instances. Therefore, the determination of antimicrobial susceptibility of isolate is often crucial for optimal antimicrobial therapy of infected patients (Atif et al., 2010). Antimicrobial resistant bacteria can be found in all environments. In the recent days, the incidence of multiple antibiotics resistant bacteria in aquatic environment has increased resulting in a serious environmental and health problems, since they harbor various resistance genes (Costa et. al., 2006). Bacteria possessing these genes has emerged from the abusive use of antimicrobial drugs, mainly in hospitals, agricultural and animal farming (Davies, 1997).

In this study, a total of 97 isolates were taken for antibiotic susceptibility test against different antibiotics. Among the tested antibiotics, isolates tested showed more resistant towards Ampicillin and were more sensitive towards Amikacin. The misuse of particular antibiotics may be the important factor in developing the antibiotic

resistant bacteria. However other factors such as environmental conditions may also interfere in developing the resistant bacteria.

The antibiotic susceptibility pattern among 31 *Escherichia coli* isolates showed that 100 % of the isolates were sensitive towards Chloramphenicol Ofloxacin and Co-trimoxazole. The higher resistance was seen to Tetracycline (93.5 %) followed by Amoxycillin (80.6 %), Cefexime (48.4%), Nalidixic acid (25.8), Amikacin (25.8). In a study of rural ground water by Bisonette et al, 1995, 87 % of the coliform was resistant to at least one antibiotic. High resistance to Tetracycline was found in a study by Pandey and Musarrat 1993, in the isolates obtained from urban drinking water. Similarly, in a study by Patoli et. Al, (2010) multi drug resistance was seen in 62.96 % of *E. coli* isolates from drinking water isolates. Maximum resistance was observed against Nalidixic Acid (92.6%), followed by Ampicillin (88.89%), whereas none of the *E. coli* isolates showed resistance against Amikacin.

Klebsiella spp were 100% sensitive towards Co-trimoxazole, Ofloxacin and Chloramphenicol, and 100% resistance towards amoxicillin, followed by Tetracycline (62.7%) and Cefexime (44.4%). *Enterobacter* spp shows susceptibility pattern of 100% sensitive towards Co-trimoxazole, Ofloxacin and Chloramphenicol and resistance Amoxicillin (52.4%) and Tetracycline (47.7%). *Citrobacter* isolates exhibits 100% sensitive towards Co-trimoxazole, Ofloxacin, and 95.8% towards Chloramphenicol while it shows 100% resistance towards Amoxixciilin followed by Tetracycline (62.5%) and Cefexime spp (45.9%). MDR isolates constitutes *E. coli* (90.3%), *Citrobacter* spp (62.5%), *Klebsella* spp (72.2%), *Enterobacter* spp (47.6 %).

In previous study, Prasanna (2006) performed antibiotic sensitivity testing to the isolates from 100 water samples collected randomly from different sources of Kathmandu valley. The result showed Tetracycline 90% sensitive, Ampicillin 100% resistant, Chloramphenicol 100% sensitive, Ofloxacin 80% sensitive and Cephalexin 90% resistant. Frequency of MAR against antibiotics within species are as follows *E. coli* 20%, *Enterobacter* spp 12%, *Citrobacter* spp 5%, *Klebsiella* spp 20% and *Salmonella* spp 25%. 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%). Of the total isolates, resistance 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%) was shown.

The higher number of MDR isolated were due to high resistance pattern of organism towards Amoxicillin and Tetracycline. It may be due to the over use of these antibiotics in husbandry and also due to unsafe disposal of animal excreta and dumping sewages. It also indicates that prolonged exposure to these antibiotics may result in the resistance towards other antibiotics which concern of great public health issue.

The antibiotic susceptibility pattern of 3 *Salmonella* Typhi taken in this study showed that 100% of the isolates were Multiple Drug Resistant (MDR). All the isolates were sensitive towards Cotimixazole and Chloramphenicol. Whereas 100 % of the isolates were resistant to Amoxicillin and Tetracycline followed by Nalidixic acid and Cefexime which is 66.7%. In a study by Shrestha et al, (2009) *Salmonella* isolates from drinking water showed resistance against Amoxycillin (70 %), Cephalexin (20 %) and Ceftizoxime (14.28 %). Banani et al, (2006) subjected the *Salmonella* isolates to antibiotic sensitivity test. All of the tested isolates were susceptible to Ciprofloxacin, Ceftriaxone, Ceftiofur, Ceftizoxime and Florfenicol. The percentage of *Salmonella* isolates susceptible to Chloramphenicol, Sulphamethoxazole-trimethoprime, Cephalexin, Nalidixic acid, Tetracycline, Nitrofurantoin, Amoxicillin and Ampicillin were 92.3, 83.3, 44.9, 25.4, 19.8, 12.4 and 11.1 respectively. White et al, 2003 found majority of the *Salmonella* isolates were susceptible to antimicrobials tested however resistance was observed to Tetracycline (26%), Streptomycin (23%), Sulfamethoxazole (19%), Chloramphenicol (8%) and Ampicillin (8%). Twenty-eight (36%) *Salmonella* isolates were resistant to at least one antimicrobial and 10 (13%) isolates displayed resistance to four or more antimicrobials. Slight differences in the sensitivity pattern to antibiotics was seen. The antibiotic susceptibility pattern between serovars potentially differ markedly from one locality to the next (Hong-Yu Qu et al, 2007).

6.2 Conclusion

Prevalence of MDR coliforms is high in drinking water in Kathmandu valley especially in stone spouts and well water and isolation of *Salmonella* from drinking water indicates significant health risk to exposed population. The water quality of stone spouts and wells are very poor and need to be treated before consumption. The prevalence of Multiple Drug Resistant organisms poses a great challenge to clinicians and the consumption of water containing these antibiotic resistant organisms may prolong the treatment of water-borne diseases.

CHAPTER - VII

SUMMARY AND RECOMMENDATION

7.1 Summary

Water is essential for human life. For the reduction of water born disease, the routine examination of water quality is of great concern as all the water borne diseases are caused by fecal contamination of water resources and unhygienic practices. The bacteriological quality of different drinking water sources of Kathmandu valley such as tap water, well water, stone spout, was studied. Moreover, antibiotic susceptibility of different isolates was also performed.

1. This study was conducted at Laboratory of Central Department of Microbiology from January 2011-August 2011. A total of 66 water samples were collected randomly from different drinking water sources, 28 from tap water, 24 from well water and 14 from spout water.
2. Temperature of the samples showed no much variation. Minimum temperature 10°C was observed in well water and maximum temperature 16°C was observed in well and spout water. The pH, all water sample were within the WHO guideline value.
3. Total coliform growth was found higher in spout water (85.7%), followed by well water (79.2%) then tap water (60.7%). Similarly, the thermotolerant coliform growth was found higher in well water (87.5%, followed by spout water (71.4%) the tap water (67.9%).
4. In this study 97 different types of organisms were isolated and identified as *Escherichia coli* (32%), *Citrobacter* spp *(*24.7%), *Enterobacter* spp (21.6%) and *Klebsiella* spp (18.6%).The highest number of organism isolated was *E. coli* which is 31(32%) and the lowest number of organism isolated was *Salmonella* Typhi which is 3(3.1%).
5. Out of 97 organisms isolated 15 organisms were isolated from tap water, 54 organisms from well and 28 organisms from spout. The recovered percentage of the isolates from tap water were *E. coli* 40% (n=6), *Klebsiella* spp 20% (n=3), *Enterobacter* spp 26.7% (n=4) and Citrobacter spp 13.3% (n=2). The

recovered percentage of the isolates from well water were *E. coli* 29.6% (n=16), *Klebsiella* spp 18.5% (n=10), *Enterobacter* spp 20.4% (n=11), Citrobacter spp 27.8% (n=15) and *Salmonella* Typhi 3.7% (n=2). The recovered percentage of the isolates from spout were *E. coli* 32.2% (n=9), *Klebsiella* spp 17.8% (n=5), *Enterobacter* spp 21.4% (n=6). *Citrobacter* spp 26% (7) and *Salmonella* Typhi 1 (3.6%).

6. The antibiotic susceptibility pattern among *Escherichia coli* isolates showed that 100 % of the isolates were sensitive towards Chloramphenicol ofloxacin and Co-trimoxazole. The higher resistance was seen to Tetracycline (93.5 %) followed by Amoxycillin (80.6 %), Cefexime (48.4%), Nalidixic acid (25.8) , Amikacin (25.8).

7. *Klebsiella* spp were 100% sensitive towards Co-trimoxazole, Ofloxacin and Chloramphenicol, and 100% resistance towards amoxicillin, followed by Tetracycline (62.7%) and Cefexime (44.4%). *Enterobacter* spp shows susceptibility pattern of 100% sensitive towards Co-trimoxazole, Ofloxacin and Chloramphenicol and resistance Amoxicillin (52.4%) and tetracycline (47.7%). *Citrobacter* isolates exhibits 100% sensitive towards Co-trimoxazole, Ofloxacin, and 95.8% towards Chloramphenicol while it shows 100% resistance towards Amoxixciilin followed by Tetracycline (62.5%) and Cefexime (45.9%).

8. All the *Salmonella* Typhi isolates were sensitive towards Co-trimoxazole and Chloramphenicol. Whereas 100 % of the isolates were resistant to Amoxycillin and tetracycline followed by Nalidixic acid and cefexime which is 66.7%.

9. All the organisms isolated were tested for antimicrobial sensitivity. MDR isolates were *E. coli* (90.3%), *Citrobacter* spp (62.5 %), *Klebsella* spp (72.2%), *Enterobacter* spp (47.6 %) and *Salmonella* Typhi *(*100 %) were multiple antibiotic resistant.

7.2 Recommendations

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

1. A regular monitoring of water quality for improvement is necessary for both well and spout water sources.
2. The proper well and stone spout location and construction, control of human activities to prevent sewage from entering water body is the keys to the avoiding bacterial contamination of drinking water.
3. The water from the stone spouts and well should be consumed only after the treatment like chlorination, boiling and filtration since pathogenic organism was detected.

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