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Study of Bacteriological Treatment of Water for Rural Communities

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Abstract

This study assessed the drinking water quality of rural communities of Baluwa and Gokarna Village Development Committee. Sixteen water samples were taken for bacteriological investigation from different water sources and analyzed for their total coliform count of *Escherichia coli, Salmonella sps.* and *Klebsiella sps.* Water samples were collected from various sources such as streams, water reservoir, tap water, wells, springs and spouts. These water samples were investigated for bacteriological contamination, based upon the minimum requirement developed by WHO (1993). Enteric pathogenic bacteria *Escherichia coli* was detected from almost all water samples while *Salmonella sps.* and *Klebsiella sps.* were reported from well, spring and spout water . Total coliform count ranged from 150 to 1100 cells/100ml. Chemically, water samples investigated were safe and within the tolerance limits set by WHO (1993). The study also assessed the efficiency of plant extracts against bacteria *Escherichia coli* and their application for water purification. Two plants *Azadirachta indica* and *Moringa oleifera* were used for water purification, using simple pot filtration method, especially applicable for rural communities. Plants gave satisfactory result, and *Azadirachta indica* gave 93% reduction in bacterial load, while *Moringa oleifera* gave 90% reduction.

Keywords: Azadirachta indica, Bacteriological study, *Escherichia coli, Moringa oleifera, Salmonella sps.*

Introduction

Water is the precious gift of nature and it is the vital resource for all living beings. It is a scarce precious commodity, getting scarcer everyday as communities, industries and agriculturists pour their filth, muck and untreated wastes into the water bodies. Water is drying at its source due to increasing environmental disturbances, and available surface waters are getting contaminated everyday. Only 30% of the world's people have a guaranteed supply of treated water, while rest 70% depend on wells, bore holes and uncertain sources of water supply (IWTC 1992). Water pollution is the most serious environmental quality issue all over the world, yet the people are less aware and give little emphasis on the vital connection between water and health. Nepal is facing enormous challenge to supply potable water to its population. The population of Nepal is predominantly rural, with only about 10% living in urban areas. At present, the population having access to piped water facilities is estimated to be 47%. Unfortunately, only 5% of the rural population have piped water supply (CBS 1995).

Many studies have implicated that the contaminated water is a principal factor for mortality and morbidity associated with enteric diseases. Thus water may be extremely dangerous when it becomes the vehicle for transmission of disease; it may cause explosive outbreaks. Water born illnesses are associated with ingestion of water containing pathogenic microorganisms such as bacteria, protozoa and viruses which is responsible for most mortality and morbidity in developing countries. About 80% of all diseases of the developing world are related to unsafe water supply and inadequate sanitation. Due to the absence of an adequate, reliable water supply and sanitation services, more than 15 million children aged 0 to 4 die each year (UNICEF 1997). In Nepal, water and hygiene related diseases are responsible for 8% of all death in general population. According to the country health profile (Ministry of Health, 1997), infective and parasitic diseases constitute the largest single (31.27%) cause of morbidity in the general population. Diarrhoeal diseases, arising mainly from unhygienic drinking water and unsanitary conditions of the water environment, account for nearly 1/3 of all child deaths. Amoebic dysentery is the most universally prevalent water borne disease throughout the country, and other very common diseases are gastroenteritis, typhoid fever, giardiasis and worm infestation. Prevalent rate of diarrhoea is about 15% for all age and 40% among children under five. Approximately 30,000 children under five die of diarrhoea each year. The incidence of diarrhoea per 1000 children under five years has increased from 143 to 154 in 1996 /1997 because of increase in diarrhoeal visits.

Today high technology facilitates the treatment of water to any desired level of purity, but such facilities are limited to developed countries. Most of the developing countries, constrained by socio-economic conditions and lack of industries, transportation, energy and technical know- how are not able to introduce modern facilities into their countries. The drinking water supply and treatment in such countries is in the preliminary stages of development (Ranjitkar 1985). Nepal is also one of the developing countries, which is facing enormous challenge to provide treated drinking water to all its population. Drinking water quality often remains unevaluated because of the high cost of specialized equipment and supplies needed for microbiological water quality testing. The country could apply and highly benefit from those water supply and treatment methodologies which require less capital expenditures in operation and maintenance. The maximum utilization of local resources in construction and operation, and minimum utilization of imported materials and equipment will be important to eliminate operational and maintenance problems. The fundamental purpose of water treatment is to protect the consumers from pathogens and impurities present in the water that may be injurious to human health. The purpose of water treatment is to provide water free from injurious or harmful agents.

The selection of appropriate system for community water treatment includes different factors including requirements and preferences of the user, laws and regulations, treated water availability requirements and finally minimum total cost comprising capital investment, operating costs, space requirements, operating material usage, water and energy consumption. Most of our Nepalese communities face financial problems, so people do not take interest to afford money for domestic water treatment, and thus they consume contaminated and polluted water unknowingly.

The use of indigenous plants in the treatment of water is commonly being practiced in different parts of world. The current trend in the world is to optimize the use of chemicals such as chlorine and coagulants in water treatment and to develop physical or biological method of treatment in order to reduce the doses of chemicals required, there by reducing the formation of disinfection by- products (WHO 1993). Thus use of indigenous plant and plant products can be performed safely and it has no side effects. It was after the discovery of microorganisms as causative agents for many infectious and septic diseases of human beings and animals that more interest was created in plant origin substances which were toxic to these microorganisms. It might be beneficial if these plants were investigated extensively for ascertaining their antimicrobial activity in vitro. The research presented in this study therefore considers many of the important practical aspects of the study of plants for their antimicrobial properties, which has been investigated earlier by other workers. This study was concentrated on antimicrobial activities of two plants against water pollution indicator bacteria *Escherichia coli*, and the use of these plants for water purification, using simple filtration method.

Nepal is rich in its majestic green vegetation including medicinal herbal plants. The natural flora with medicinal value in Nepal may be considered as one of the important natural resources for the development of economic conditions. Due to wide topographic variation including tropical plains, temperate hills and valleys and snow - capped high mountain peaks of alpine zone, Nepal possesses rare and valuable plants having antimicrobial properties. During Vedic ages the crude plants and their water extracts have been used as drugs for curing infectious diseases (Bhatta 1970).

Several researchers in different parts of the world, including Nepal are experimenting on antimicrobial activities of plant and plant products. More attention of researchers is drawn towards the substances derived from plants which are known to exert toxic effects upon infectious microorganisms but not on human beings.

Methodology

Sampling sites

The study was conducted in communities near to the Kathmandu city. Two Village Development

Committees mentioned below were selected for study - A). Gokarna VDC B) Baluwa VDC. Both VDC's lie in the north - east of the Kathmandu city. Gokarna is the suburban area of Kathmandu valley while Baluwa is a rural area close to Gokarna. Water samples were taken from different wards of VDC'S and investigated for bacteriological quality. People of Gokarna VDC have tap water facility joined to Sundarijal water treatment plant. People also consume water from wells, streams and spouts. In case of Baluwa VDC, mostly people depend upon natural sources of water such as streams, springs, spouts and tap water. In some localities, there is facility of tap water supply system but no disinfection system.

The study was conducted from January 1996 to July 1998. During this period, each water sample was taken 3 times for convenient study. A total of 16 water samples were taken randomly from the two mentioned VDCs.

Examination of water sample was based upon the minimum requirement of WHO (1993). All culture media used in this study were manufactured by Himedia, India Examination of water for microorganisms was done by: A; Most Probable Number (MPN) test. B; Eijkman's test ; A Most Probable Number (MPN) test

Most probable number (MPN) technique was performed inoculating water sample in lactose broth containing Durham tube and incubating for suitable period, the tubes were examined for gas production by the coliform organism. This test is known as presumptive test. 10ml of water sample were inoculated into 3 tubes of double strength 10 ml of lactose broth with Durham's tube, while 1ml and 0.1ml of water samples were inoculated into separate sets of 3 tubes of 5ml of single strength lactose broth, and incubated at 37° C for 48hrs.

Incubated tubes showing positive results from the presumptive test were subjected to a confirmative test. Confirmed test was done by transferring few loopfuls from every positive tube into a tube containing Brilliant green lactose bile broth with Durham's tube. The tubes were then examined for gas formation after incubation at 35° C for 48hrs. Inoculum taken from positive tube of the confirmatory test were streaked on plates of MacConkey agar and Violet red bile agar and incubated at 37° C for 24 hours. After incubation, colonial characteristics on different plates were observed.

Eijkman's Test

Eijkman's test is performed to confirm "Thermal Coliform" bacteria in positive tubes of presumptive test. It al so helps to determine whether these coliform bacilli are *E. coli.* The method involves inoculating loopfuls of each presumptive positive culture to thermostatically controlled tubes at 44° C containing 5ml lactose broth with Durham's tube. Immediately the tubes were reincubated in the incubator set at their correct temperature i.e. 44° C for 48hrs. After suitable incubation tubes were taken and examined for gas production.

Identification of Bacteria

Bacteria isolated on respective selective and differential medium were identified by standard microbiological method. The isolated bacteria were identified on the basis of colonial characteristics, morphological characteristics and biochemical properties.

Collection of Plants

Two medicinal plants i.e. *indica* and *oleifera* having antimicrobial properties were selected for the experiment. Indian Council of Medicine Research (Chopra, 1955) has suggested 36 medicinal plants said to be useful in Cholera and prolonged fevers such as the enteric group. The above mentioned two plants were included in the same group. They were collected from different locations of Nepal.

Plant Extract Preparation

Every plant material was successively extracted with phosphate buffer (pH4). Effective part of each plant material was finely grounded, and 1gm was dissolved in 100ml of phosphate buffer and stirred well. It was kept at 4°C for 24hrs. to get fine 1% stock solution of plant extract.

Determination of Minimum Inhibitory Concentration (MIC) of Plant Extract

Minimum Inhibitory Concentration (MIC) of a drug or plant extract is defined as the lowest concentration that will inhibit the growth of specific microorganism. Determination of MIC value of any plant extract is the simple way to measure the effectiveness of any plant extract. In another words, MIC is that concentration of inhibitory agent which just prevents the growth of a test organism in a suitable medium. MIC of plant extracts against test organism were done by two-fold dilution method, using sterile nutrient broth in the case of bacteria as a basal media, as described by Shadowy and Ingroff (1980).

Pot Filtration for Water Treatment

Pot filtration has been made very simple and easy to run which is in common practice in village households. Pot filter is composed of three pots of 7-8 litres (Figure 1). 2 holes of half a centimeter diameter were made at the bottom of upper two pots. The holes were covered with sterile stones of 2-4cms in size in the upper pot while clamps were fitted in the holes of middle pot during treatment. Flow rate of water could be made faster or slower with the help of clamps. Instead of clamps, filter rods which are commercially available can be fitted in the holes of middle pot.

Upper pot was covered with coarse sand with peat gravel of smaller size up to 2cm depth of pot. Middle pot was filled with test samples upto 2cm depth of pot. In case of plant sample, it was crushed and placed in the middle pot.

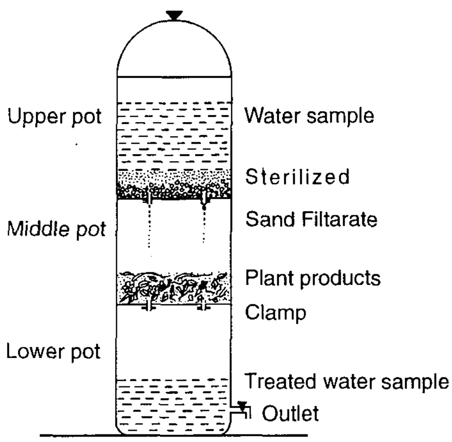

Figure 1. Diagramctric sketch showing internal systems of pot filteration method

Water Sample Water sample was made to contain 10^3cfu/ml, adding 24hrs fresh culture of *Escherichia coli.*

Method

Sterilized sand and peat gravel were put in upper pot upto 2cm depth. Crushed plant products were put in middle pot upto 2cm depth. Water sample was added in upper pot and subjected to treatment for 30 minutes. Treated sample was taken in a sterile conical flask and put to plate count method.

Pour plate Method

Total number of bacteria in water sample before treatment was confirmed by performing pour plate method. Different dilutions of water sample were made and pour plate method was performed to get average count of organisms. Similarly, total number of bacteria was enumerated in treated sample. Different dilutions of treated water sample were made and subjected to pour plate to get average count. These counts observed before and after treatment were compared, and efficiency of plant products were evaluated.

Results

Total Coliform Count

All water samples were analyzed for bacteriological study for coliforms. The coliform counts were higher from all water samples.

Water samples from Baluwa VDC

The total coliform count was found to be 460 cells/100 ml from sample S6 (Well water), stream water S8, and spout water S9 while lowest count was 150 cells/100ml from sample S3 (Tap water). Similarly, count was found to be 210 cells/100ml from S1 (Stream water), 210 cells from sample S2 (Reservoir water), 240 cells from sample S4 (Spring water), 240 cells from sample S5 (Spring water), and 240 cells from S7 (Spout water).

Water samples from Gokarna VDC

All seven water samples showed high value of total coliform count. Highest count was found from sample S5 (Well water), 1100 cells/ 100ml while lowest count was from sample S3 (Mulpani) 24 cells/ 100ml. Other counts were 43 cells from S1, 43 cells from S2, 75 cells from S4, 43 cells from S6 and 43 cells from S7 etc.

Isolation 0f Enteric Pathogens

Enteric pathogens like coliforms e.g. *E. coli*, *Klebsiella sps.* and *Salmonella sps.* were detected from most of water samples. *E. coli* was isolated from all sixteen samples, while *Klebsiella sps.* and *Salmonella sps.* were detected from spring, well and stream water samples. Lists of detected enteric pathogens are presented in Table 1 and Table 2.

MIC values of Plant Extracts

Two plant extracts were tested against *Escherichia coli* and the activities of plant extracts against tested bacteria have been found to exhibit good range of concentration. MIC value of seed extract of *Moringa oleifera* was found 625 ppm. MIC value of leaf extract of *Azadirachta indica* was found 1250 ppm.

Results of Water Treatment

Efficiency of plant materials for water treatment was determined by using pot filtration method. Plants taken were tested against *E. coli* and results of water treatment using pot filtration is shown in Table 4 and Table 5. The bacterial load in water samples was successively reduced after sand filtration in upper pot. The average percentage reduction in bacterial load after sand filtration was found to be 37% .

Similarly, in pot filtration method when water sample containing low number of bacteria was used for treatment, more successful results were observed. *A. indica* gave 93% reduction in bacterial load after 30 minutes treatment while *M. oleifera* gave 90% reduction.

Discussion

This study was undertaken with an aim to investigate drinking water quality of rural communities in Nepal. Drinking water samples were analyzed for bacteriological quality of different water sources such as streams, water reservoir, tap water, springs for coliform count, *E. coli, S. sps.* and *K. sps.* The purpose of study was also to find out efficiency of plants against *E. coli* and their application for water purification. None of the water samples from different sources was found bacteriologically safe in this study. All the water samples contained higher num-

Table 1. Microbial analysis of drinking water samples of Baluwa VDC

S. No.	Sample No. (Sources)	Total Coliform Count CFU/ml	Detected micro-organisms
1	S1 (Stream water)	210	*E. coli, Salmonella sps.*
2	S2 (Reservoir water)	210	*E. coli*
3	S3 (Tap water)	150	*E. coli*
4	S4 (Spring Water)	240	*E. coli, Salmonella sps., Klebsiella sps.*
5	S5 (Spring water)	240	*E. coli, Salmonella sps.*
6	S6 (Well water)	460	*E. coli, Salmonella sps.*
7	S7 (Spout water)	240	*E. coli, Klebsiella sps.*
8	S8 (Stream water)	460	*E. coli, Klebsiella sps, Salmonella sps.*
9	S9 (Spout water)	460	*E. coli, Klebsiella sps.*

Table 2. Microbial analysis of drinking water sample of Gokarna VDC

S. No.	Sample No. (Sources)	Total Coliform Count CFU/ml	Detected micro-organisms
1	S1 (Tap water)	43	*E. coli*
2	S2 (Tap water)	43	*E. coli*
3	S3 (Springs water)	24	*E. coli*
4	S4 (Tap water)	75	*E.coli, Klebsiella sps.*
5	S5 (Well water)	1100	*E.coli, Klebsiella sps., Salmonella sps.*
6	S6 (Tap water)	43	*E. coli*
7	S7 (Spout water)	43	*E.coli, Klebsiella sps.*

Table 3. Determination of MIC (Minimum Inhibitory Concentration) of some plant extracts

S. No.	Plant	Extracts	Test Organism: Escherichia coli
			MIC Value, ppm
1.	*Azadirachta indica*	Leaf extract	1250
2.	*Moringa oleifera*	Seed extract	625

Table 4. Results of water treatment using *Azadirachta indica* by pot filtration method

Plant Sample	No. of bacteria in water Sample				
	Before Treatment CFU/ml	After Sand Filtration CFU/ml	After Treatment for 30 mins CFU/ml	Decrease bacterial load (%)	Average Decrease (%)
Azadirachta indica	180	95	7	96%	
	400	195	24	94%	93%
	450	245	40	91%	
	390	210	31	92%	

Table 5. Results of water treatment using *Moringa oleifera* by pot filtration method

Plant Sample	No. of bacteria in water sample				
	Before treatment CFU/ml	After sand filtration CFU/ml	After treatment for 30 mins CFU/ml	Decrease bacterial load (%)	Average decrease (%)
Moringa oleifera	480	235	58	88%	
	750	360	67	91%	90%
	500	235	40	92%	
	1080	530	118	89%	

ber of coliform bacteria than WHO guidelines (1993). A number of pathogenic enteric bacteria were isolated and detected from different water sources. Coliform bacteria such as *E. coli, Klebsiella species.* and pathogenic bacteria *S. species* were detected. These bacteria were given importance in accordance with public health of the people. *E. coli* was detected from all water sources while *salmonella sps.* and *Klebsiella sps.* were detected mainly from well water, spring water and spout water but not from tap water and spring water .

The findings of this study are supported by results observed by several workers from rural villages of Nepal. Results of this study were also supported by findings of Joshi (1987) in bacteriological tests of drinking water sources of two villages of central Nepal which were near to sampling sites of this study, Chaubas (Shivapuri) and Syabru (Langtang). He reported the coliform count as ranging from 5-100 cells per 100 ml of water. Protected springs were less contaminated than unprotected ones.

DISVI (1990b) carried out microbiological tests of drinking water in seven rural areas of Illam and found that water samples from springs aquifers and river had unacceptable levels of faecal coliform bacteria ranging from 2 to 2,400 cells /100 ml. In other studies, ENPHO/DISVI (1990) investigated the bacteriological quality of spouts from 21 localities which were found to be feacally contaminated. In this study also, 90 percent of the samples contained an average coliform count of more than 100 cells /100ml. The probable sources of contamination around natural water sources are unhygienic environment, unmanaged sewage disposal, dust and dirt in the surroundings and recreational activities. At a number of areas, faecal matters from baby diapers, kitchen wastes and animal excreta accumulate at the public tab during washing and cleaning activities.

In most of the rural communities, people continue to use the most convenient sources of water, irrespective of quality. Collected water is contaminated both outside and within the domestic environment through poor hygiene and sanitary practices. In most of the rural villages, latrines are non-existent; thus people use to discharge excreta near water sources, making water contaminated. Village people are unaware about the quality of water, and are consuming it and regularly facing the problems created by water-borne infections. Many village communities meet their water demand by supplementing with ground water sources. Ground water is extracted through bores and wells. Use of tubewells and dugwells is widespread in areas where there is no easy access to surface water. Groundwater extracted from deep wells, and protected aquifers is usually free from pathogenic micro-organisms, and the distribution of such untreated ground water is in common practice in many countries. Unsanitary and recreational activities around ground water sources are the primary cause of pollution and microbial contamination of ground water sources.

During rainy season, volume of water bodies and flow of surface water sources increases. In this season, water bodies become contaminated due to seepage of sewage wastes and run off from adjacent land. Simultaneously total bacterial and total coliform count increases along with nature of pollution. During dry summer season, water volume decreases and contamination level increases that enhance the high bacterial count. This situation reflects inexplosive outbreaks of enteric diseases, and epidemics occur more during the dry summer season and rainy season than in winter season (IUCN 1992).

In this study, *A. indica* showed higher effectiveness while *M. oleifera* also gave satisfactory results. Several researchers from different parts of the world have studied the antimicrobial properties of plants and plant products. In Nepal, Shakya (1982), Shrestha and Sharma (1988), and Adhikari and Sharma (1988) have studied antimicrobial activities of different types of plant products against coliform bacteria including *E. coli.* Similarly, applications of plants for water treatment have been carried out in other parts of the world. Sutherland, Folkard and Grant (1990) carried out an economic assessment of replacing the use of Aluminium sulphate by *M. oleifera* coagulant in the Malawi. They found that crushed seeds of *M. oleifera* are a viable alternative to Aluminium sulphate (Alum) as a coagulant for water treatment applications in the developing world. After treatment, coliform reductions were in the order of 96%. In Sudan, the seeds from the *M. oleifera* tree is being practiced for water purification as a primary coagulant, which is favourable leading to turbidity reductions in raw water (Ranjitkar 1985). Similarly, there are many reports showing the effectiveness of *A. indica* (Neem plant) against infectious diseases.

The rate of kill of a bacterial population varies directly with the concentration of the disinfectant. Slightly raising or lowering the concentration of certain agents can increase or decrease their bactericidal effect, and effects of dilution on activity can be of crucial importance. The efficiency of disinfection greatly depends on the nature and numbers of the contaminating microorganisms. If the degree of microbial contamination is high, a long exposure time or a high concentration of disinfectant may well be required. Since active principle compound is in very small quantity in plant products and is in combined and crude form, a long exposure time is required to get satisfactory reduction in bacterial population. It is more suitable for water samples containing low number of organisms. For the treatment of water samples containing higher number of organisms using plant products, a long exposure time is needed (Hugo and Russell 1977).

A high incidence of enteric diseases associated with poor sanitation and polluted water is characteristics of the disease picture in many of the developing countries of the world. Thus rural people of developing world need local water treatment technologies which are reliable, rapid, simple and convenient .The development and introduction of appropriate technologies are of prime concern, if the developing countries are ever to share the benefits of improved water quality.

In Nepal, the diversity of the physiography due to the altitudinal and climatic variation has made it possible to grow all types of plants. The varied climatic conditions offer the added advantage of the natural occurrence of a wide range of economically important medicinal plants. Those growing wilds are too numerous, and to be acquainted with them all would entail a profound study of a major size. Therefore, efficacy of plant extracts for antimicrobial activities should be studied frequently, and they can be used, as appropriate, in rural areas for water treatment.

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