



INTERNATIONAL JOURNAL OF ENVIRONMENT

Volume-3, Issue-3, Jun-Aug 2014

ISSN 2091-2854

Received:30 June

Revised:10 July

Accepted:20 August

COLIFORM AND *VIBRIO CHOLERA*E ANALYSIS OF DRINKING WATER COLLECTED FROM CHOLERA OUTBREAK REGION OF BHAKTAPUR MUNICIPALITY

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Abstract

Water borne infections in Nepal, especially in Kathmandu valley is one the major public health problems, causing thousands of deaths every year. Among three cities in the valley, the water borne infection including cholera is most predominant in Bhaktapur district. So the study was carried out to know the microbial drinking water quality in the city and to determine the prevalence of water borne infections in the specified region of the district in 2012. Altogether eighty (two samples from a single site at different interval-2/3 days) water samples were collected from Bhaktapur Municipality, one of the most vulnerable regions for water borne diseases, following standard methods as described by APHA, 2010. All samples were transferred to Microbiology laboratory of Khwopa College, Dekocha, Bhaktapur and preceded immediately for Microbial analysis. The coliform density in the water samples were determined by Most Probable Number (MPN) method followed by microscopy, colonial morphology and biochemical characterization. Subsequently, the presence of *Vibrio cholerae*, a causative agent of Cholera was analyzed in the same samples by enrichment in alkaline peptone water followed by culture on Thiosulphate citrate bile-salt sucrose (TCBS) agar, a selective media for *Vibrio* spp. The biochemical tests were then performed to identify *V. cholerae*. Among eighty water samples, 87.5 percent water samples contained coliforms and half of which (45%) contained fecal coliforms, *Escherichia coli* and remaining 12.5 percent water samples contained no coliforms. *Vibrio cholerae* were isolated from four water samples (5%). The drinking water quality in the region was found to be very poor. Therefore, the people in the region were suggested to treat the drinking water by using any of physical or chemical disinfection methods prior to drinking.

Key words: MPN, Coliforms, TCBS agar, *Vibrio cholerae* and Oxidase test

Introduction

Water is essential for life: both in terms of quantity and quality. Every year, approximately 3.4 million people die due to water-related diseases with the majority being young children under the age of five. Diarrheal diseases alone account for 2.1 million deaths per year. Other types of diseases associated with poor water quality include cholera, typhoid, arsenic poisoning, schistosomiasis or “snail fever”, and trachoma, which cause blindness (Dies *et. al*, 2003). Lack of hygiene knowledge, unsafe drinking water and sanitation are major causes of Diarrhoea with highest number of morbidity and mortality in the developing countries including Nepal (WHO, 1993).

Water borne diseases outbreaks (WBDO's) occur when drinking water becomes contaminated by microbial pathogens or chemicals. Typically, WBDO's are caused by microbial pathogens such as bacteria, viruses or protozoan. These pathogenic organisms are transmitted via the fecal oral route. This means that the drinking water supply has somehow been contaminated by fecal materials from humans or other warm blooded mammals (Gleick, 2002). Among bacterial outbreaks, cholera is perhaps, currently the most characterized of the major waterborne infections, but less of a threat compared to Enterobacteriaceae. The primary causative agent of cholera, *Vibrio cholerae*, is part of the normal bacterial flora of estuaries and most strains are not harmful to humans. Two strains are associated with establishment of diarrhoeal disease in humans, serogroups *_O1* and *_O139*. These express the cholera enterotoxin (CT). Symptoms can vary from the most severe cholera gravis that is lethal, to inapparent infection (Woodall, 2010). Analysis of global trends in the incidence of cholera by five-year periods shows a steady increase since the beginning of the millennium. From 2004 to 2008, a cumulative total of 838,315 cases was notified to WHO, compared with 676,651 cases between 2000 and 2004, representing a 24% increase in the number of cases reported for this most recent five-year period as described in Cholera, 2010.

Many cities of Nepal including Bhaktapur municipality are continuously suffering a severe drinking water supply crisis, particularly in the dry seasons of every year. The drinking water supply in the city is intermittent. It has the poorest drinking water and sanitation coverage for its population among three districts in Kathmandu valley and a large percentage of its drinking water contains faecal coliforms (Diwakar *et. al*, 2008). Several bacterial, protozoal and viral water borne diseases have posed serious public health problem here. Diarrhoeal disease due to Enterobacteriaceae and Cholera are the two most prevalent water borne infections in the city. The explosive outbreaks of these diseases are the prime cause of death of thousands every year (Kansakar *et al.*, 2011; Tamang *et al.*, 2005)

Therefore, in this research, our prime focus is to study about the occurrence of coliforms (especially faecal coliforms) and *V. cholerae* in drinking waters from outbreak regions of the city so that we can report and recommend those people who are continuously drinking the contaminated water.

Methodology

Sampling method and processing

The study was carried out in Bhaktapur municipality, one of the most outbreak regions for water borne diseases. Water samples for bacteriological testing were collected from main supply, household storage, stone tap and outbreak reported houses in sterile bottles by standard

methods as mentioned in APHA, 2010 and transported immediately for study. All the samples collected from those sources were used by people for direct drinking without prior treatment. Small aliquots of sample were enriched in alkaline peptone water for isolation of *Vibrio* spp. (Chakraborty, 2001 and Monica, 1998). Microbiological analysis of water samples was conducted in Microbiological Laboratory of Khwopa College, Dekocha, Bhaktapur. To minimize the error in results, samples were collected two times from same sites in the random period of 2-3 days intervals.

Microbial examination of water sample

Microbial examination of water samples were done by Most Probable Number (MPN) method as described by APHA, 2010 for estimation of coliform and faecal coliform density, *Escherichia coli*. Detection of *V. cholerae* was done by the enrichment of water samples on alkaline peptone water, followed by isolation of the typical organism on selective medium, Thiosulphate Citrate Bile-salt Sucrose (TCBS) agar. Suspected *Vibrio* colonies were picked and subjected to biochemical tests including oxidase test to identify *V. cholerae* (Monica, 1998).

Results

Among eighty water samples, 37.5% contained less than 30 coliforms per 100 ml, most of which contained 23 coliforms. 12.5% water samples were found to be excellent containing no coliforms. While 17.5% water samples contained less than 10 coliforms most of which contain less than 5 and only four samples were populated with 9 coliforms. 10% water samples contained 43 coliforms and similar percent of water samples were highly saturated with maximum number of coliforms equivalent to 1100. In remaining water sample, coliforms density of 93, 120, 150, 210, and 460 were found to be distributed equally which was equivalent to 2.5% in each sample size (Figure 1).

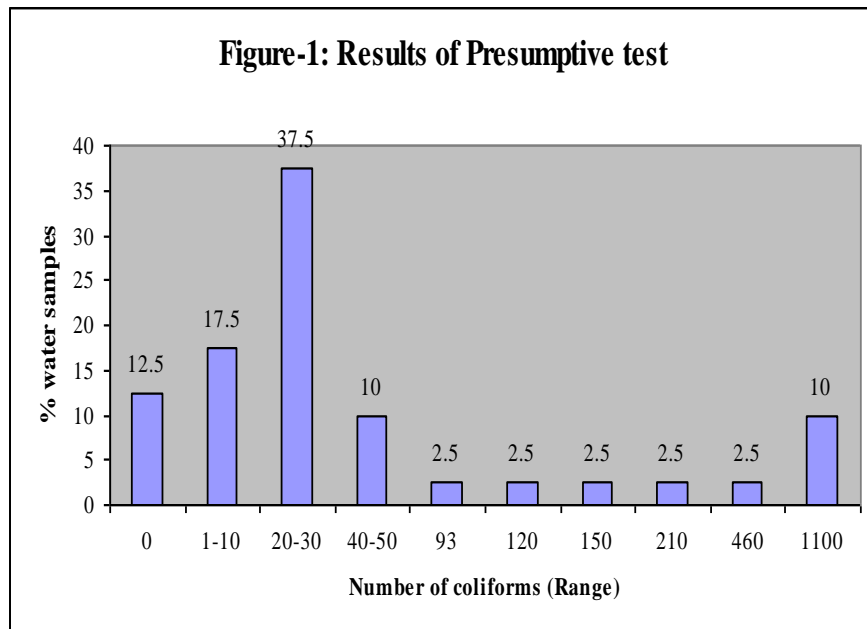


Figure 1. Results of Presumptive test

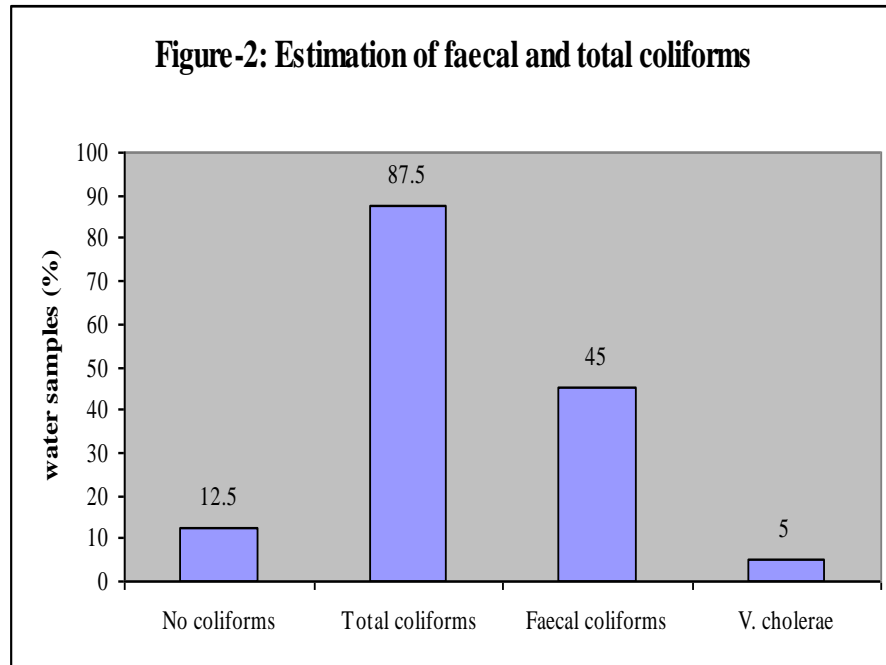


Figure 2. Estimation of faecal and total coliforms

Half or 50% water samples processed in this experiment showed typical colonies with green metallic sheen morphology on EMB agar indicating the presence of *E. coli*. Further, Eighteen samples (45%) containing *E. coli* were finally confirmed by positive indole and methyl red tests. However, remaining 55% water samples were free of faecal contamination i.e. absence of *E. coli*.

Vibrio cholerae were isolated from four water samples which were collected from outbreak reported houses (Figure-2).

Discussion

As samples were collected two times from same sites in the random period of 2-3 days intervals, the total samples were eighty. This was done to prevent the accidental results that might arise by any cause. The results obtained for every two samples from each site were tallied and found to be reproducible. Among the eighty water samples, 87.5% (70 water samples) contained coliforms and half of which contained faecal coliforms and remaining 12.5% (10 water samples) contained no coliforms. As presumptive test is only a preliminary test which is used for enrichment of coliforms in lactose media, the presence of gas production doesn't confirm the presence of coliform. Besides coliforms, some Gram positive bacteria like *Bacillus*, *Streptococcus*, *Clostridium* and *Lactobacillus* also give false positive result (Goel, 1997). The positive gas production in presumptive test were further confirmed by inoculating the samples in 2% Brilliant Green Lactose Bile Broth (BGLB) and on Eosin Methylene Blue (EMB) agar, both of which are selective for coliforms. The typical colonies on EMB media finally confirmed the presence of faecal coliforms. About 50% of water samples collected was found to be contaminated with faecal coliforms. The positive indole and methyl red tests add more to identify *E. coli*. According to WHO Guidelines for drinking-water quality, the drinking water should not contain any *E. coli* (WHO, 2003 and WHO, 2008). Therefore those water samples are

not portable without prior disinfection by any physical and chemical methods. As mentioned in Dewakar *et al.*, 2008 and Wagle, 2009, the drinking water quality of valley region is still very poor.

The yellow colonies from many water samples show the presence of *Vibrio* species as most of them give yellow color on TCBS media which is a selective for *V. cholerae* and other *Vibrio* spp. of faecal origin. Colonies suspicious for *V. cholerae* appeared on TCBS agar as yellow, shiny colonies, 2 to 4 mm in diameter. The yellow color is caused by the fermentation of sucrose in the medium. Sucrose-nonfermenting organisms, such as *V. parahaemolyticus*, produce green to blue-green colonies (Kay *et. al*, 1994). However all *V. cholerae* are not pathogenic. They are part of the normal bacterial flora of estuaries and most strains are not harmful to humans. Only two strains are associated with establishment of diarrhoeal disease in humans, serogroups _O1 and _O139. Therefore the yellowish colonies from TCBS media should be further analyzed by oxidase test in order to identify the virulent strains. The sucrose fermenting colonies were again carefully inoculated on alkaline peptone medium. The fresh growth of organism was used for oxidase test. Neither colonies from Nutrient agar nor that from TCBS was used. Due to lack of salts, nutrient agar does not allow optimal growth of *V. cholerae*. Likely TCBS agar may yield either false-negative or false-positive results (Kay *et. al*, 1994 and CDC, 1994). In a positive reaction, the bacterial growth becomes dark purple immediately. The positive oxidase strains are virulent ones. Therefore in this work the evidence of oxidase positive isolates shows that the water samples contain pathogenic strains. But the contamination is not at source. The possible source may be due to seepage, and cross contamination into distribution pipe lines.

The prevalence rate of cholera was 5% in oppose to the findings of Das *et al.*, (2008) and Tamang *et al.*, (2005) that documented 56.6% and 31% respectively but was comparable with the findings of Kansakar *et al.*, (2011) (11.1%). Epidemics or explosive outbreaks generally occur in underdeveloped areas with inadequate sanitation, poor hygiene and limited access to safe water supplies, whereas in some countries, a seasonal relation for cholera epidemics has been observed (Faruque *et al.*, 2005).

Conclusion

The drinking water quality in the region was found to be poor and non potable. Moreover the detection of *V. cholerae* in drinking water samples indicates the possible cholera outbreak in the region at any time in the future.

Acknowledgement

We express our sincere gratitude to Uttam for laboratory assistance and all the local people who helped us by providing information and water samples.

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