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Original Research Article

Bacteriological profile of water samples in and around Shimla hills: a study from the sub Himalayan region

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ABSTRACT

Background: The present study was done to assess the bacteriological quality of drinking water in and around Shimla in the event of an outbreak of hepatitis E in Shimla city, January 2016.
Methods: Total 1098 water samples from different sources were received from January to July 2016. The bacteriological analysis of water was done by the multiple tube technique. Results were interpreted after 48 hours of incubation of the water sample in MacConkey bile broth medium in accordance with Mc Crady probability table.
Results: A total of 1098 water samples were received in the Microbiology laboratory of which 129 (11.74%) were unsatisfactory, 25 (2.27%) satisfactory and rest 925 (84.24%) were excellent. Nearly 9.21% samples from water tanks, 2.3% from public taps were unsatisfactory but water samples from all the water ATM s were excellent. *Escherichia coli* were the commonest isolate 35.6%, *Klebsiella pneumoniae* 31.6%, *Klebsiella oxytoca* 19.3%, *Enterobacter sps* 8% and *Citrobacter sps* 2%. In the corresponding period 477 patients had come to the health facilities for clinical symptoms of jaundice. The serum samples from clinically suspected cases were subject to antibody testing for IgM HAV and IgM HEV and it revealed that 109 (23%) were positive for HAV while 253 (53%) were positive for HEV. During the above period 75 (15%) patients had co-infection with both HAV and HEV.
Conclusions: Bacteriological assessment of drinking water is essential and should be carried out on regular basis so as to prevent outbreaks of Hepatitis A and E and other water borne diseases.

Keywords: Water bacteriology, Coliforms, *Escherichia coli*, Multiple tube technique

INTRODUCTION

Water pollution is a global public health problem and poses a threat to human life. Worldwide nearly 4 billion clinical cases of diarrhoea and more than 3million deaths occur annually due to water borne infections.[1] According to WHO, globally approximately 1.1 billion drink unsafe water and nearly 88% of diarrhoeal diseases are attributed to unsafe water, sanitation and hygiene.[2] Recent WHO estimates a decline in the number of diarrhoeal deaths to 8,42,000 in low and middle income countries since the last decade due to provision of safe water, sanitation and hygiene.[2] In India, 37.7 million people suffer due to waterborne diseases annually and nearly 1.5 million children die due to diarrhoea alone.[3]

Water borne pathogens lead to the manifestation of clinical syndromes like diarrhoea, hepatitis and enteric fever etc. It is well established that infectious diseases are transmitted primarily through water supplies contaminated with human and animal excreta, particularly faeces. The human pathogens transmitted through the faeco-oral route, include bacteria like *Salmonella* spp., *Shigella* spp, pathogenic *Escherichia coli*, *Vibrio cholerae*, *Yersinia entercolitica*, *Campylobacter* spp, viruses such as hepatitis A and E, rotavirus, poliovirus and parasites such as *Entamoeba histolytica* and *Giardia* spp.[4] Waterborne pathogens like *Vibrio cholerae, Enteropathogenic E.coli, Salmonella* spp and Hepatitis E virus may cause outbreaks and have a high mortality rate. The outbreaks are more severe in the developing countries. For public and environmental health protection, it is mandatory to provide safe drinking water. The importance of safe water, sanitation and hygiene for health and overall development of the nation cannot be undermined. Access to safe drinking water is essential for health and a basic human right; apart from a component of effective policy for health protection. Microbial safety of drinking water is necessary and one of the major challenges of the 21st century. It is essential to monitor the drinking water quality to ensure that the water supply system is functioning correctly. A wide plethora of pathogenic agents can be found in water, and monitoring for their presence on a routine basis is impractical. Indicator microorganisms survive better and longer than pathogens with uniform and stable properties and may easily be detected by standard laboratory techniques. The coliforms have been recognized as a suitable microbial indicator of drinking water quality. Coliforms traditionally belonged to genus *Escherichia*, *Citrobacter*, *Enterobacter* and *Klebsiella*. According to modern taxonomical methods it is a heterogeneous group that includes *Enterobacter cloacae* and *Citrobacter freundii* that are found in faeces and environment. Detection of coliform bacteria in treated water supplies suggests inadequate treatment and post treatment contamination. Amongst the coliform *Escherichia coli* is a normal inhabitant of the intestinal tract of humans and other warm blooded animals is thus regarded as the most affordable, fast, sensitive, specific indicator of recent faecal contamination.[5]

Drinking water is defined as water intended for human consumption for drinking and cooking purposes from any source.[6] The current WHO bacteriological guidelines for drinking water recommend zero faecal coliforms for 100ml of water.[7] A significant number of diseases can be prevented in developing nation like ours with access to improved water sources, as piped water, public taps, stand pipes, tube wells and rain water collection. Improving access to safe drinking water can result in tangible health benefits so every effort should be made to achieve drinking water quality as safe as possible. The microbiological quality of drinking water is a concern to consumers, water suppliers, regulators and public health authorities.

Shimla is a hilly town situated in the lap of Western Himalayas. Latitude 30°6" N and longitude 77°11" E and is in the humid sub-temperate zone with a population of 1,69,518 people. Water is supplied through seven surface water sources which supply raw water to four water treatment plants (WTP) i.e. Dhalli WTP, Ashwani WTP, Churah WTP and Gumma WTP. Ashwani khud is the source of raw water for Ashwini WTP. Ashwani WTP was contaminated with sewage effluent from Malyana and Dhalli STP (sewerage treatment plants).This lead to an outbreak of jaundice which commenced in January 2016, confirmed to be due to Hep E virus by a team from NIV (National Institute of Virology), Pune. Thereafter the water supply from Ashwini WTP was completely stopped for Shimla town. The present study was undertaken to assess the bacteriological quality of water supplied in and around Shimla town in the wake of an outbreak of Hepatitis E virus in Shimla from January 2016 to April 2016.

METHODS

Water samples were received in the Department of Microbiology, Indira Gandhi Medical College, Shimla through a person appointed by the Municipal Corporation, Shimla, for the assessment of bacteriological quality of water from various public places of the town. From 18th January to 31st July 2016, a total of 1098 water samples from various water sources in and around Shimla were received and processed in the Department of Microbiology, Indira Gandhi Medical College, Shimla.

The samples were collected, transported and sent by the Municipal health authorities to the Department of Microbiology in accordance with WHO and the Indian Council Medical Research (ICMR) guidelines.[8,9] Two hundred thirty millilitres of water samples from each source were collected in sterile glass stopper bottles for microbiological examination. Water samples containing residual chlorine were neutralized by adding pre-sterilized 0.1 mL sodium thiosulphate (1.8% w/v) per 100 mL of water sample. The samples were stored at 2°C–8°C in the refrigerator to avoid changes in bacterial count until analysis or transported in a vaccine carrier from point of collection to the laboratory. The total coliform count test was based on the multiple tube fermentation method to estimate the most probable number (MPN) of coliform organism in 100 mL of water for diagnosis of bacteriological contamination. The test was carried out by inoculation (for 48 hours at 37°C) of measured quantities of sample water (5, 10, 50 mL) into tubes of double and single-strength Mac Conkey lactose bile salt broth with bromocresol purple as an indicator. The tubes showing gas formation were considered to be presumptive coliform positive. The results of MPN were interpreted based on McCrady probability tables from the number of tubes showing acid and gas (fermentation by coliform organisms) to define the sample as excellent, satisfactory or unsatisfactory.[10] Differential coliform count (Eijkman's test) was performed by incubating subcultures from the positive presumptive tests at 44°C and 37°C in lactose bile broth and the other subculture at 44°C in tryptophan broth. The presence of coliform bacilli was confirmed by the production of gas from lactose at 37°C, and that of *E. coli* was confirmed by the production of gas from lactose and indole from tryptophan at 44°C, followed by subculture on MacConkey agar.[11] All the media and reagents were procured from Hi-media Pvt Ltd. Mumbai, India. Further the coliforms and other organisms were analysed by subculture on MacConkey agar, biochemical reactions and other identification tests. Colonies from these plates were identified by conventional biochemical methods according to standard microbiological techniques.[12]

Further 466 serum samples of clinically suspected cases of jaundice were tested for IgM antibody to Hepatitis E and Hepatitis A virus by the ELISA kit (DS-EIA-Anti-HAV-M-RECOMB; MP diagnostic HEV IgM ELISA 3.0) according to the manufacturers' instructions. This would establish the clinical correlation of jaundice cases and the unsatisfactory water samples from different parts of the city.

RESULTS

A total of 1098 water samples from various sources in and around Shimla were received and processed in the department of Microbiology from January 2016 to July 2016. Of these 129 (11.74%) were unsatisfactory, 25 (2.27%) satisfactory and rest 925 (84.24%) were excellent as shown in (Figure 1, 2; and Table 1). The water samples were analyzed by the multiple tube method (presumptive coliform count done). Of these 125 samples were subjected to Eijkman test and 46 were differential coliform positive. There are 25 wards in and around Shimla and 41 tanks which store water for supply to the consumers in different parts of Shimla. Results indicated that all samples were found contaminated with total coliforms as well as faecal coliforms in the month of January, February, March, and April. After stringent action taken up by the public health authorities chlorination of water sources started and instructions were issued regarding testing of water samples from water sources and tanks on regular basis. There after water quality improved subsequently and no faecal coliforms detected. Although during rainy season other coliforms were detected.

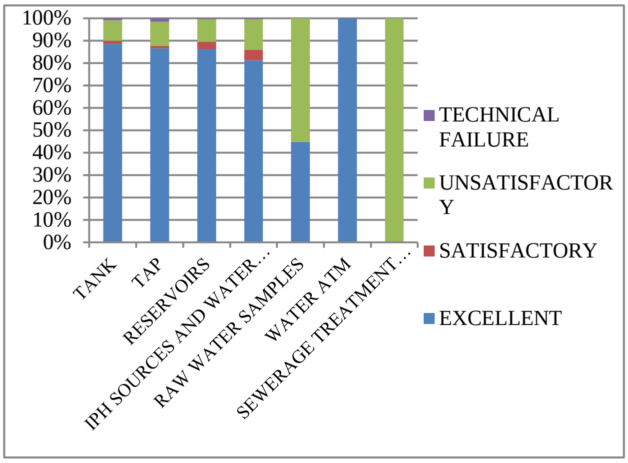

Figure 1: Bar chart depicting the water samples from various sources and their outcome.

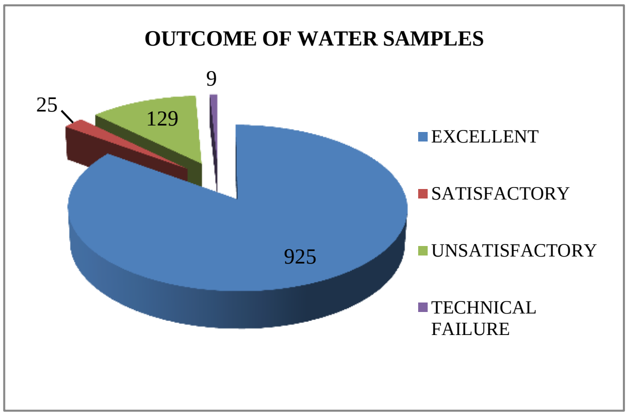

Figure 2: Pie chart showing the outcome of various water samples

Table 1: Table showing results of water sample testing.

Source	No of samples	Excellent	Satisfactory	Unsatisfactory
Water tank	369	327 (88.61%)	5 (1.35%)	34 (9.21%)
Public tap	245	212 (86.53%)	3 (0.27%)	26 (2.3%)
Reservoir	219	189 (86.3%)	7 (3.2%)	22 (10%)
IPH	214	174 (81.3%)	10 (4.67%)	29 (13.5%)
Raw water	29	13 (44.82%)	0 (0%)	16 (55.17%)
Water ATM	12	12 (100%)	0 (0%)	0 (0%)
Sewerage	2	0 (0%)	0 (0%)	2 (100%)

Amongst samples from different sources 9.21% of the samples from water tanks; 2.3% from public taps were unsatisfactory. It is commendable that all samples from different water ATMs were excellent as shown in table 1.The commonest isolate was *Escherichia coli* (35.6%); followed by *Klebsiella pneumonia* (31.6%); *Klebsiella oxytoca* (19.3%); *Enterobacter* (8%) and *Citrobacter* species (2%) as depicted in (Figure 3).

In the corresponding period 477 patients had come to the health facilities for clinical symptoms of jaundice. They were subject to antibody testing IgM HAV and IgM HEV and found that 109 were positive for HAV while 253 were positive for HEV. During the above period 75 patients had co-infection with both HAV and HEV. We did a seasonal time trend analysis for cases of HAV, HEV, with respect to unsatisfactory water samples in winter season (19 January to 31 March), summers from (1 April to 15 June) and monsoons (16 June to 31 July).

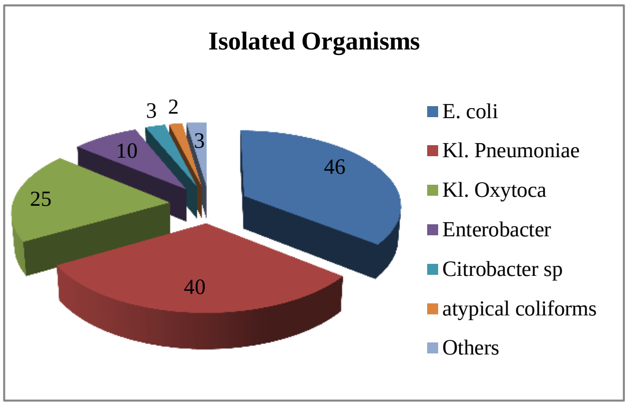

Figure 3: Pie chart depicting various organisms isolated from water samples.

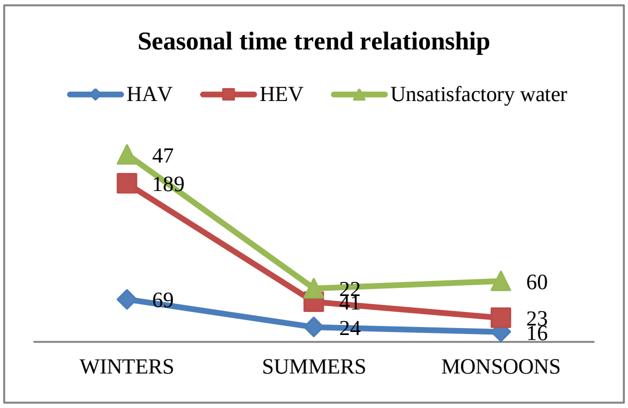

Figure 4: Seasonal time trend relationship of HAV and HEV with unsatisfactory water samples.

DISCUSSION

Water borne diseases cause nearly one third of intestinal infections worldwide.[13] Globally it has been estimated that lack of safe water, sanitation and proper hygiene measures has led to 40% of total deaths and 5.7% of total disease burden. A significant burden of disease could be prevented in developing countries through access to improved water sources. These have been defined by WHO as piped water, public taps, tube wells, bore holes, protected springs and rain water collection.[14] The microbiological quality of drinking water is of concern to consumers, water suppliers, regulators and public health authorities. Water is essential to sustain life, and every effort should be made to provide satisfactory supplies of drinking water to all. According to United States environmental protection agencies (USPEA), WHO guidelines all public water supplies must be tested regularly and be free from any coliforms.[15]

The present study of bacteriological assessment of drinking water sources in and around Shimla city revealed 11.74% of the water samples as unsatisfactory and not desirable for human consumption. Our findings are in contrast to other studies from North India where 48%, 47.5%and 38.6% of the water samples were unsatisfactory.[16-18] Our study highlighted that 9.21% of samples from water tanks were unsatisfactory in contrast to public taps (2.3%). This may be explained that water tanks are more liable for contamination from leaking sewage lines, sludge, animal droppings, birds and monkey faeces. Also the lids of water tanks are often opened by the monkeys declared as vermin in Shimla city.[19] It is pertinent to mention that water quality from all water ATMs in the city was excellent. Water ATMs have emerged as a boon to provide safe drinking water in accordance with the international drinking water quality standard, IS 10500. It works on the principle of reverse osmosis and UV based filtration technology where the water is filtered through 5 stages.

The outbreak of jaundice due to hepatitis E virus was an eye-opener to the municipal authorities and the IPH department regarding the mixing of sewage from Malyana STP with Ashwani khud WTP. Water supply from the Ashwani khud has been completely cut off till date. This water treatment plant was not working efficiently and was not able to meet the increased demands as the population has increased manifold since the British era.

Due to the large spurt in the number of jaundice cases in the city, serum samples were tested by ELISA technique for IgM antibody to HAV and HEV. Of these clinically suspected cases 53.03% cases were positive for IgM antibody to HEV, 22.85% IgM antibody to HAV and 15.2% cases co infection (both IgM antibody to HEV and HAV) was detected. In contrast another study reported seropositivity in 68.8% cases of hep E, 9.4% cases of hep A and dual infection in 1.8% cases.[20] Hepatitis E virus (HEV) and Hepatitis A Virus (HAV) are enterically transmitted viruses of public health importance due to their ability to cause sporadic, endemic and epidemic outbreaks mostly associated with sewage contamination of drinking water especially in developing countries. In India, HEV is reported to be endemic accounting for 50-70% of sporadic outbreaks of enterically transmitted hepatitis.[21] Several outbreaks due to Hepatitis A and E have been reported from different regions of the country.[22,23] In a previous study from Himachal Pradesh in 2007, 63.2% serum samples were positive for anti HAV IgM antibodies.[24]

A large plethora of pathogenic microorganisms that cause disease are present in water but monitoring for their presence on routine basis is not practical. According to WHO, *Escherichia coli* is the most discriminating marker for recent faecal contamination so an indicator of choice for drinking water portability in developed nations.[17] The detection of coliforms was done by presumptive coliform count and further confirmation of *Escherichia coli* by differential coliform or the Eijkman test.

The most common coliform detected was *Escherichia coli* 35.6% followed by *Klebsiella pneumoniae* 25.6%. Our study showed a lesser proportion of contamination with coliforms in contrast to another study where *Escherichia coli* were 68.5% and *Klebsiella* spp. 2.6%.[15] Although other coliforms are generally not harmful themselves, they indicate the possible presence of other pathogenic bacteria, viruses and protozoans. In the present study contamination of water treatment plants was 13.5% though in other regions up to 83% of samples from water treatment plants were contaminated.[11] A WHO report has stated that more people would die from consuming unsafe drinking water and unsanitary conditions by the year 2020 than would die from AIDS, if steps to improve water quality are not taken.[15] It is a need of the hour to ensure provision of safe drinking water to consumers. A safe and potable water supply is ensured through three stages of storage, filtration and disinfection. Storage is the first step where 90-95% impurities are removed by sedimentation. Penetration of light results in oxidation of organic matter by aerobic bacteria and decreases the free ammonia content of water and the bacteriological contamination by 90%. The 2nd stage is filtration where 98-99% drop in bacterial count occurs. Disinfection is the final stage that results in 98-99% drop in the bacteriological count. Active intervention from public health and the health department along with raising people's awareness regarding water hygiene are required for improving the quality of drinking water.

CONCLUSION

Safe drinking water for all is one of the major challenges of the 21st century. Routine basic microbiological analysis of drinking water should be carried out by assaying the presence of *Escherichia coli* by the multiple tube fermentation technique. Microbial contamination of drinking water has adverse health consequences so its control and prevention is of paramount importance and can never be compromised. Active intervention from public health officials and the health department along with raising people's awareness regarding water hygiene are required for improving the quality of drinking water. In the present study the outbreak of hepatitis E was managed effectively due to coordination between the epidemiologists and microbiologists. Further bacteriological surveillance of drinking water and supplies are required to ensure health safety of the residents.

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