Original Article

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Presumptive Coliform Count in Water Sample Collected from Different Sites of a University, Moradabad, Uttar Pradesh, India

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Abstract

Introduction: Safe water is the most important than any other things for human health on this earth. The World Health Organization (WHO) report approximately 65% of rural and 36% of urban India's were without access to safe drinking water. The aim of our study is to determine presumptive coliform count in water sample collected from different sites of a University, Moradabad, Uttar Pradesh, India.

Materials and Methods: The microbial quality of drinking water sample from different sites was detected using multiple tube fermentation technique test (most probable number/100 ml) for the presence of total coliform count. Bacterial isolate was identified by culture, morphological, and biochemical characterization.

Results: A total 50 water sample were collected from different water cooler located at the different sites of the university. Out of 50 water sample, 22 (44%) positive and 28 (56%) were negative. Out of various positive water samples from various sites, girls and boys hostel had a highest degree of bacterial contamination. It was found were that 10 (45.46%) of water sample are contaminated with a multiple coliform bacteria and 12 (54.54%) of water sample found to be contaminated with a single isolate of coliform bacteria. Out of mix coliform bacteria *Escherichia coli* and *Klebsiella,* we found that out of 22 positive sample, 11 (50%) water sample were satisfactory, 5 (22.72%) were suspicious sample, and 6 (27.28%) were unsatisfactory sample.

Conclusion: Most of the sites met the WHO recommended standard microbiological parameters. However, some sites do not meet the WHO recommended standard. So, we would like to recommend the proper sanitation, regular treatment, supervision of water sources, and regular bacteriological assessment of all water sources for drinking should be planned and conducted.

Key words: *Escherichia coli*, MacConkey broth, Most probable number, Presumptive coliform

INTRODUCTION

One of the most important elements for all forms of life is water. On earth, in the maintenance of life, it is indispensable. For the composition and renewal of cells, it is also essential. Beside this, human being is continuing to contaminate water source and provoking water-related illness.[1,2]

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In general, water is mainly used for drinking especially as domestic purposes. A wide variety of inorganic compound requires to all living organism for growth, repair, maintenance, and reproduction.[3]

The most important, as well as one of the most abundant of those compounds is water, and it is particularly vital to living organism.[4]

In human life, water plays an essential role. The World Health Organization (WHO) reports approximately 65% of rural and 36% of urban India's were without access to safe drinking water.[5]

Pathogenic microorganisms include bacteria, viruses, and protozoa that are transmitted by water to humans and grow

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in the human intestinal tract most of these microorganisms and are transmitted via feces.[6]

Enteric pathogens such as coliform group of bacteria *Salmonellae, Vibrio* and dysentery-causing agents contaminate water. The human fecal material carried in sewage is often dumped in lakes and river. This increases contamination of water. Therefore, for microbial contamination water supply has to be regularly checked. The most reliable indicators of fecal contamination are coliform bacteria. However, strong evidence of fecal pollution is presence of *Streptococci.*[7]

Because of implied public health impacts the microbiological quality of drinking water has attracted great attention worldwide.[8]

In addition, various kinds of disease could be infected in humans by water and has been traced to be one of the ways. Typhoid fever, cholera, and bacillary dysentery are some water born disease. In untreated or poorly treated sewage, usually pathogens are spread by water contamination in water born infection.[9]

Escherichia coli is the most dangerous form enters the water supply and occurs in fecal contaminants in the case of water pollution. Many diseases caused by ingestion of contaminants into the water supply. Examples *Shigella* species, *Salmonella* species, *Vibrio cholera,* and *E. coli.*[4]

The coliform is Gram-negative, non-sporing, motile or non-motile aerobic and facultative anaerobic, rod-shaped bacteria, within 48 h at 35°C that ferment lactose with gas formation. All over the world, indicators organism are coliforms that are used is establish the degree of fecal pollution in water.

Members of coliforms bacteria are *Escherichia, Enterobacter, Proteus, Klebsiella, Yersinia, Hafnia, Serratia. E. coli,* and *Enterobacter aerogenes* are most important found as commensals which are abundantly found in the intestinal tract of all humans and are regularly discharged in the feces. *E. coli* and *E. aerogenes* are definitely found in any material which is focally polluted. In the world, we can say that any material with these coliforms indicator fecally polluted.[10]

Relationship exists directly among sanitation, water, nutrition, and human well-being health. In India, the major causes of many diseases are by consumption of contaminated drinking water, lack of personal and food hygiene, improper disposal of human excreta, and improper disposal of solid and liquid waste.[11]

The quality of drinking water may be maintained by protection of water sources, control of treatment processes, and management of the distribution and handling of water.[12]

By standard water treatment practices, the majority of bacterial pathogen are removed or inactivated. Water treatment in standard drinking water includes sedimentation, coagulation/flocculation, sedimentation, filtration, and disinfection.[13]

MATERIALS AND METHODS

The study was conducted in the Department of Microbiology Teerthanker Mahaveer Medical College and Research Centre.

Study Design and Period

The study was conducted on drinking water source to assess the extent of bacterial contamination from March 2015 to January 2016 in the Teerthanker Mahaveer University Moradabad, Uttar Pradesh.

A total 50 sample were collected from different drinking water cooler located at the different sites of the University, Moradabad, Uttar Pradesh.

Sample Collection Technique

Cotton wool soaked in 70% ethanol was used to sterilize the tip of tap from which sample was collected the tap was allowed to run for 2 min before sterile 250 ml screw capped glass bottle were carefully uncapped and filled with water and recapped.

Collection time, its source, and the name of site were noted on the sample of the bottle.

The water was collected using sterile bottles and transported for testing immediately to the department of medical laboratory science and pathology laboratory by ice cold containers within 50 min of collection.

Determination of Total Coliform

Testing water samples for the presence of coliforms. There will be three principal tests - The presumptive, confirmed, and completed the test.

Presumptive coliform test

Multiple tube fermentation method: Presumptive coliform count-multiple tube test.

The test is called presumptive because the reaction observed may occasionally be due to the presence of some other organisms and the presumption that reaction is due to coliform organisms has to be confirmed.

An estimate of the number of coliform organisms is usually made by adding varying quantities of water (0.1-50 ml) to double strength MacConkey's broth and single strength MacConkey's broth containing bromocresol blue sterilized in bottle/tubes containing Durham's tube (for indication of gas production).

Confirmed test

Confirmed test done by transferring a loopful of culture from a positive tube from the presumptive test into a tube of brilliant green lactose bile broth (oxoid) with Durham tubes. The tubes were incubated at 37°C for 24-48 h for total coliforms and 44.5°C for 24-48 h for fecal coliform and observed for gas production.

Completed test

Completed test was carried out in accordance with (WHO, 2012) by streaking a loopful of broth from a positive tube into eosin methylene blue (EMB) agar plate for pure colonies. The plates were incubated at 37°C for 24-48 h. Colonies developed on EMB agar, or MacConkey's agar was further identified as coliforms fecal coliforms *(E. coli)* using culture characteristic, morphology, and biochemical test. For fecal coliforms, colonies with green metallic sheen were Gram-stained, and the IMVIC test was carried out to identify the colony as *E. coli*. The most probable number (MPN) per 100 ml water was determined using the completed test.

Determination of coliforms count

Number of positive test tube with acid (yellow coloration) and gas production were matched with the McCrady's Statistical table, and MPN of coliform present in 100 ml of sample was thus determined.

For the confirmation test, a loopful of cultures from presumptive test inoculated on MacConkey agar, EMB agar, blood agar, nutrient agar, xylose lysine deoxycholate agar, and lysine iron agar. The culture plate will be incubated at 37°C for 24 h.

RESULTS

A total 50 water sample were collected from different water cooler located at the different sites of the university.

Out of 50 water sample, 22 (44%) were positive and 28 (56%) were negative from various sites of the university, which is shown in Table 1 and Figure 1.

Out of various positive water samples from various sites hospital, Medical college, Paramedical college, Girls and boys hostel, Nursing college, Physiotherapy college, College of education and stadium, dental college and dental outpatient department (OPD), college of computer science and information technology (CCSIT). Girls and boys hostel had the highest degree of bacterial contamination followed by Hospital, Medical college, Nursing college, College of education, CCSIT, which is shown in Table 2 and Figure 2.

In our study, it was found that 10 (45.46%) of water sample are contaminated with a multiple coliform bacteria and 12 (54.54%) of water sample found to be contaminated with a single isolate of coliform bacteria. Out of 12 single coliform bacteria, *E. coli* 8(36.37%) the most common isolate which is followed by *Klebsiella* 2 (9.09%), *Enterobacter,* and *Citrobacter* 1 (4.54%) which is shown in Table 3 and Figure 3.

Table 1: Percentage of total positive and negative sample

Total sample	50	Percentage
Positive sample	22	44
Negative sample	28	56

Table 2: Site wise distribution of positive and negative sample

Site of sample	Number of positive samples	Number of negative samples	Total number of samples
Hospital	3	2	5
Medical college	2	3	5
Girls and boys hostel	7	12	19
Paramedical college	1	1	2
Nursing college	2	1	3
Physiotherapy college	1	0	1
Engineering college	1	4	5
College of education and stadium	2	2	4
Dental college and dental OPD	1	2	3
CCSIT	2	1	3
Total	22	28	50

OPD: Outpatient department, CCSIT: Computer science and information technology

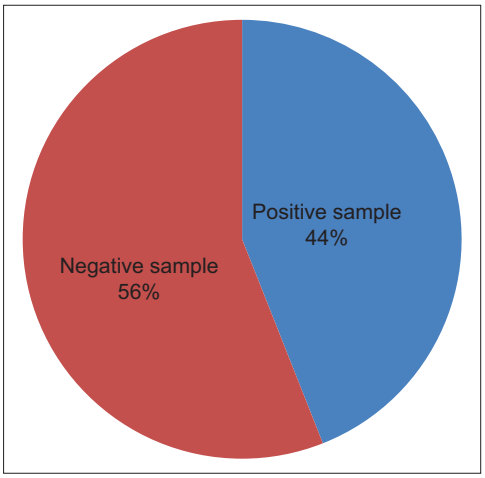

Figure 1: Percentage of total positive and negative sample

Table 3: Frequency of distribution of isolated organism in different sites

Sites of sample	Organism isolated				
	E. coli	*K. pneumoniae*	*E. aerogenes*	*C. koseri*	Mix organism
Hospital	-	-	-	-	3
Medical college	1	-	-	-	1
Girls and boys hostel	2	1	-	1	3
Paramedical college	1	-	-	-	-
Nursing college	1	-	-	-	1
Physiotherapy college	1	-	-	-	-
Engineering college	-	-	-	-	1
College of education and stadium	1	-	1	-	-
Dental college and Dental OPD	1	-	-	-	-
CCSIT	-	1	-	-	1
Total positive sample (%)	8 (36.37)	2 (9.09)	1 (4.54)	1 (4.54)	10 (45.46)

OPD: Outpatient department, CCSIT: Computer science and information technology, *E. coli: Escherichia coli, K. pneumonia: Klebsiella pneumoniae, E. aerogenes: Enterobacter aerogenes, C. koseri: Citrobacter koseri*

Table 4: Distribution of mix isolated organism

Organism	Number of samples
E. coli and *Klebsiella*	4
E. coli and *Klebsiella* and *Enterobacter*	2
Klebsiella and *Citrobacter*	1
Citrobacter and *Enterobacter*	1
E. coli and *Citrobacter*	2
Total	10

E. coli: Escherichia coli

In our study, out of mix coliform bacteria, *E. coli* and *Klebsiella* was found to be more followed by *E. coli* and *Klebsiella* and *Enterobacter, E. coli* and *Citrobacter*, *Klebsiella* and *Citrobacter*, *Enterobacter* and *Citrobacter,* which are shown in Table 4 and Figure 4.

In our study, we found that out of 22 positive sample, 11 (50%) water sample were satisfactory, 5 (22.72%) were suspicious sample, and 6 (27.28%) were unsatisfactory sample, which is shown in Table 5 and Figure 5.

DISCUSSION

In developing countries, the most common cause of gastroenteritis which affects humanity is due to lack of safe and clean drinking water supply. We expect water to be free from bacterial contaminants and other impurities because it is vital to our life. Even if water appears clear, it may not necessarily be safe and acceptable. The water that is suitable for human consumption must be free of chemical and pathogenic agents, pleasant to taste and usable for domestic purposes. So, potable water is an important source of infectious disease. So, water purification is most important for ensuring public health.

In this study, on the basis of the result obtained the coliform bacteriological quality of different sites of University. Drinking water cooler quality of the paramedical college, physiotherapy college, dental college, and OPD were satisfactory as compared to girls and boys hostels.

E. coli were more frequently detected in water cooler sample of girls and boys hostels as compared to hospital and engineering college.

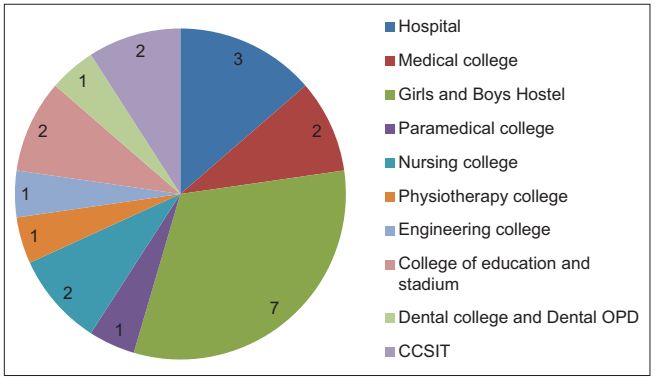

Figure 2: Distribution of site wise positive sample

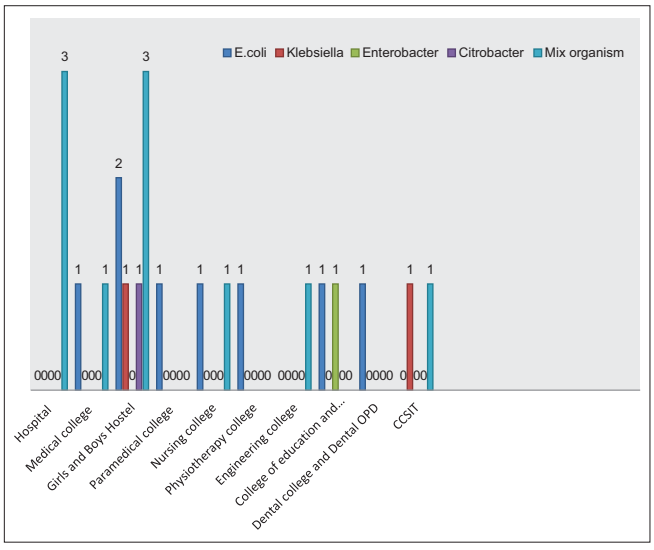

Figure 3: Distribution of organism isolated from different site

Table 5: Profile of total positive sample (*n*=22)

Sites of water sample	Number of positive samples	Number of satisfactory samples	Number of suspicious samples	Number of unsatisfactory samples
Hospital	3	2	1	0
Medical college	2	1	0	1
Girls and boys hostel	7	3	1	3
Paramedical college	1	1	0	0
Nursing college	2	0	1	1
Physiotherapy college	1	1	0	0
Engineering college	1	0	1	0
College of education and stadium	2	1	1	0
Dental and college dental OPD	1	1	0	0
CCSIT	2	1	0	1
Total	22	11 (50%)	5 (22.72%)	6 (27.28%)

OPD: Outpatient department, CCSIT: Computer science and information technology

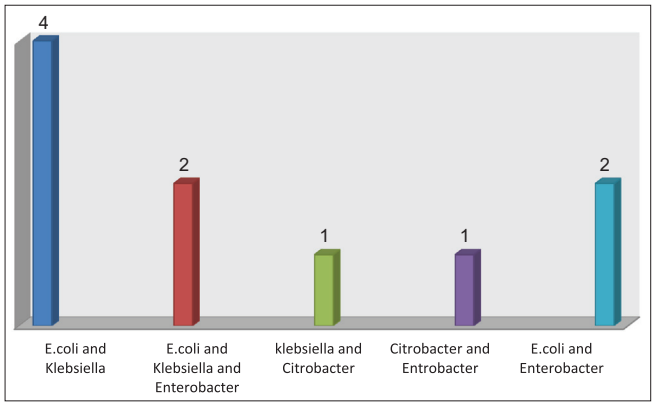

Figure 4: Distribution of mixed isolated organism

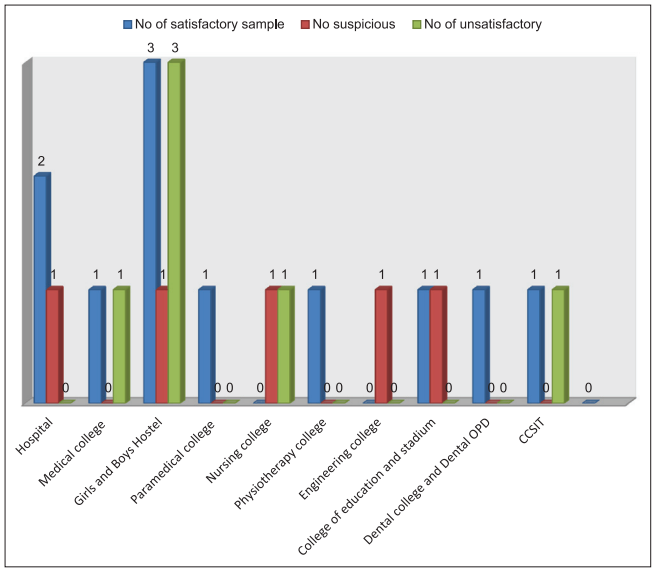

Figure 5: Profile of total positive sample

Mix organisms were more detected in water cooler sample in different sites of TMU as compared to the individual organism.

Hospital, paramedical college, engineering college, college of education and stadium, dental college, physiotherapy college drinking water cooler is safe as compared to girls and boys hostel, CCSIT, nursing college medical college water cooler. The reasons for the contamination are improper chlorination of water and irregular checking of water.

The water cooler is revealed the high number of *E. coli;* hence, it required the proper maintenance by change the filter and washing the filter time to time as per guidelines.

Our study is comparable with the study of Thakur *et al.* In that study, 17 water sample were collected from different source. Out of those, unsatisfactory 52.94%, satisfactory 11.76%, excellent 29.4%, and suspicious 5.88%. The most common isolates were *E. coli* and *E. aerogenes.* Both the enteric bacteria are considered as water pollution indicator organism[14].

While in the study of Ngwa and Chrysanthus most predominant bacteria was *Klebsiella* species 73.3%, followed by *Salmonella typhi,* 66.7%, *E. coli* 53.3%, *Enterobacter species* 26.7%, and *Proteus mirabilis* 6.7%.[15]

E. coli has been used as an indicator of fecal pollution in water for many decades. In the intestinal tract of human and animals bacterium is present in large numbers and is more numerous than disease-causing bacteria and viruses. The advantage of *E. coli* is that, it is not capable of growing and multiplying in water (except warm and food laden waters). Thus, the presence of this bacterium in water is indicators of fecal pollution.[16]

CONCLUSION

The result obtained from this study revealed that the microbiological parameters of water sampled from different sites of University were obtained and received. Most of the sites met the WHO recommended standard microbiological parameters. However, some sites do not meet the WHO recommended standard.

Several water born disease and chronic health problem may be caused by consuming unsafe drinking water. Hence, safe drinking water to each and every individual the earth is necessary. That is why proper treatment of water should be employed to avoid health problems.

The bacteriological analysis of drinking water revealed that the some samples of drinking water from different sites were contaminated with coliform and other pathogenic bacteria. The pathogen like *E. coli* and *Klebsiella* were isolated by selective media.

Hence, we would like to recommend the proper sanitation, regular treatment, supervision of water sources, and regular bacteriological assessment of all water sources for drinking should be planned and conducted.

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