

## Original Research Article

## Presumptive Coliform Count in Water Sample collected from Different sites of a University, Moradabad, Uttar Pradesh, India

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**Abstract:** Water is one of the most significant or vital resource for humanity and basic human right contamination of water bodies is one of the areas of major concern in the public health. The aim of our study is to determine presumptive coliform count in water samples which are collected from different sites of TMU, Moradabad, U.P., India. Most probable number test was done to detect the coliform bacteria in drinking water sample. Samples were collected from different sites of TMU and bacterial isolate was identified by culture, morphology and biochemical characterization of bacteria. Out of 50 water sample, 16 (32%) were positive in which coliform bacteria were present whereas 34 (68%) were negative in which coliform bacteria were absent. Positive water samples were contaminated with a multiple coliform (43.75%) and single coliform (56.25%) bacteria isolate. Out of 9 single coliform bacteria *Escherichia coli* 4 (25%) *Pseudomonas* 2 (12.5%) *Citrobacter* 1(6.25%) and *Klebsiella* 2 (12.5%) we found. Out of 16 positive sample 9 (56.25%) water sample were satisfactory, 4(25%) were suspicious sample, and 3 (18.75%) were unsatisfactory sample. On the basis of the result obtained, some samples from different sites were polluted with coliform and other pathogenic bacteria. So we would like to suggest that all water sources of drinking water should be planned and conducted through the proper sanitation, regular treatment, and supervision of water sources and regular estimation of bacteria.

**Keywords:** Most probable number, MacConkey broth, presumptive coliform.

### INTRODUCTION

Water is a vital resource for all form of life on the earth. For the maintenance of life it is essential and also indispensable for the regeneration and composition of cells.

Regardless of this, human beings are pollute water sources results in water related diseases [1,2].

Water sources used by population (rural and urban) in developing countries are usually very prone to faecal pollution [3].

Water is mainly used for drinking and as household purposes. A wide variety of inorganic compound requires to all living organism for growth, maintenance, repair, and reproduction [4].

The World Health Organization reports just about sixty five percent of rustic and thirty six percent of urban India's were without access to safe drinking water [5].

Harmful bacteria, viruses and protozoa, are transmitted by water to humans and cultivate in the human intestinal tract that are invisible to the naked eye, so water, which taste and looks fine but may not necessarily be protected to drink. These microbes can survive in both such as surface and ground water supplies which may cause immediate sickness in humans if not properly treated. Poor water quality or bad sanitation was reported million deaths a year that caused by impure water [6].

Ethiopia's people (74%) had lack of protected drinking water in 2007. The majority of the population eighty percent (80%) and urban coverage is around

eighty nine percent (89%) live in rural areas, where most reports recommend that fewer than twelve percent have (12%) access to potable water. The rural populations (19%) have access to use to safe drinking water provisions [7].

Diseases related to pollution of drinking-water compose a major load on human health. Widespread health risk connected with drinking-water is microbial contamination. Sicknesses and diseases in the world are caused by insufficient sanitation, impure water or unavailability of water that ranges up to 80%. As to 2006 account of world health organization just about three out of five persons in developing countries don't have access to safe drinking water and only about one in four has any kind of hygienic facilities. Water can also play a role in the transmission of pathogens which are not fecal excreted. Contagion of drinking water with a type of *E. coli* known as O157:H7 can be fatal [8, 22].

Coliform group of bacteria (enteric pathogens) *Salmonellae*, *Vibrio* and dysentery-causing agents contaminate water. The fecal material of human carried in sewage is habitually dumped in river and lakes. This increases pollution of water. Therefore, for microbial contamination water supply has to be regularly checked [9].

The dangerous form is *Escherichia coli* which enter in the water supply and occurs in fecal pollutants in the case of water contamination. Many diseases caused by ingestion of contaminants into the water supply. Examples *Shigella species*, *Salmonella species*, *Vibrio cholera*, and *E. coli* [10].

The coliform is Gram-negative, rod-shaped, none spore forming, motile or non-motile aerobic and facultative anaerobic bacteria. The degree of fecal pollution in water is well indicated by coliform.

Members of coliforms bacteria are *Escherichia*, *Klebsiella*, *Enterobacter*, *Yersinia*, *Hafnia*, *Proteus*, *Serratia*. *E. coli* and *Enterobacter aerogenes* are most important organism found as commensals and abundantly establish 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 [11].

Management and control of water sources, treatment processes and handling of water can recover the distinction of drinking water [12]. The majority of bacterial pathogen detached or destroyed by standard water treatment practices includes sedimentation, coagulation/ flocculation, filtration and disinfection [13]

## MATERIAL AND METHODS

The study was conducted in Department of Microbiology Teerthanker Mahaveer Medical College and research center

### Study design and period

The study was conducted to assess the level of bacterial contamination in drinking water sources from January 2016 to February 2017 in TMU Moradabad (U.P)

### Sample collection technique

Heat sterilized bottles containing an adequate volume of sodium thiosulphate to neutralize the bactericidal effect of any chlorine or chloramines in water is used for the collection of water sample. 0.1 ml of a fresh 1.8% aqueous solution of sodium thiosulphate should to be present in each bottle of 100 ml volume [14].

Cotton wool soaked in 70% ethanol was used to sterilize the tip of the tap from which water sample collected the tap was allowed to run for two minutes before sterile 250 ml screw capped glass bottle were carefully uncapped and filled with the water and recapped. Time of collection, site name and its source was noted on sample bottle. The water collected using sterile bottles and transported for testing directly to the department of microbiology laboratory by ice cold container within fifty minutes [15, 16].

### Determination of total coliform

Three principal test such as the presumptive, confirmed and completed test can be used for testing of water sample presence of coliform.

### Presumptive coliform test

#### Multiple tube fermentation method

Presumptive coliform count –multiple tube test. The test is name presumptive as a result of the reaction observed may occasionally be due to the occurrence of another organism and also the presumption that reaction is due to coliform organism should to be confirmed.

The number of coliform organisms an estimate is usually made by adding variable quantities of water (0.1 to 50 ml) to double strength MacCaonkey broth and Single strength MacCaonkey broth containing bromocersol blue untainted in bottle/tuber containing Durham's tube (for indication of gas production). With the help of sterile pipettes all the three set of tubes filled with 10 ml, and 0.1 ml quantities of water sample.

Then, incubation was done at 37°C for 24 hours to 48 hours for estimation of total coliforms and for faecal coliforms at 44°C for 24-48 hours and observed for the production of acid and gas. Broth color change in yellow from reddish purple indicates the production of acid, while gas entrapment in the Durham tubes indicates the gas production. Then McCrady's statistical table was used for the estimation of MPN.

**Confirmed test**

This test was confirmed by transmitting a loopful of culture into a tube of Brilliant Green Lactose Bile broth (Oxoid) containing Durham tubes from a positive tube from the presumptive test. Then incubation was done at 37°C for 24 hours to 48 hours for total coliforms and faecal coliform at 44.5°C for 24-48 hours and observed for production of gas.

**Completed test**

Administration of complete test was done in accordance with World Health Organization, 2012 by streaking a loopful of broth into Eosin Methylene Blue agar plate from a positive tube for pure colonies. Then incubation of plates was done at 37°C for 24 hours to 48 hours. Growths of colonies on MacConkeys agar or EMB agar were observed. By using culture characteristic, morphology and biochemical test colonies were categorized as coliforms or faecal coliforms (*E. coli*). For fecal coliforms, colonies with green metallic sheen were Gram stained and the IMVIC test was carried out to recognize the colony as *E.coli*. by using the completed test the Most Probable Number per 100 ml water sample was determined [17, 18].

**Determination of coliform count**

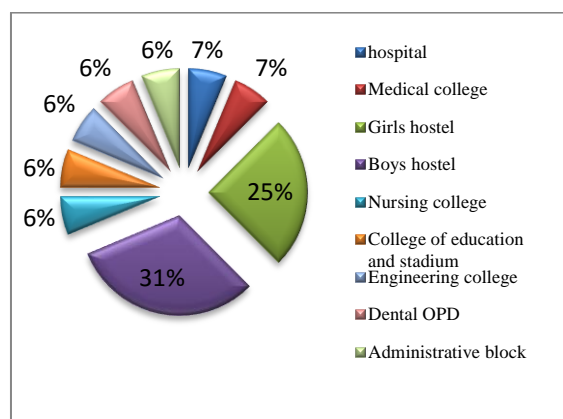
Number of positive test tube with acid (yellow coloration) and gas production were matched with the McCarty’s statistical table, and MPN of coliform present in 100 ml of sample was thus determined. For the confirmation test, a loopful of cultures from presumptive test inoculated on MacConkey Agar, EMB Agar, Nutrient Agar, blood agar, Xylose lysine Deoxycholate Agar and Lysine Iron Agar. The culture plate will be incubated at 37°C for 24 hours.

**RESULT**

Total 50 water samples were collected from different drinking water cooler located at the different sites of the University and tested by MPN method. Out of 50 water sample, 16 (32%) were positive in which coliform bacteria were present while 34 (68%) were negative in which coliform bacteria were absent. Out of various positive water samples from various sites, Hospital, Medical College, Nursing College, Dental OPD, College of education and stadium and girls and boys hostel had a highest degree of bacterial contamination. Followed by Hospital, medical college, Nursing College, Engineering College which is shown in table number 1.

**Table-1: showing site wise distribution of positive & negative sample**

Site of samples	Number of positive samples	Number of negative samples	Total no. of samples
Hospital	1	7	8
Medical college	1	3	4
Girls hostel	4	4	8
Boys hostel	5	3	8
CCSIT	0	5	5
Nursing college	1	4	5
College of education and stadium	1	3	4
Engineering college	1	2	3
OPD of physiotherapy	0	1	1
Dental OPD	1	2	3
Administrative block	1	0	1
Total	16	34	50



**Fig-1: Showing distribution of site wise positive sample**

**Table-2: Frequency of distribution of isolated organism in different sites**

Site of Samples	Organism				
	<i>E.coli</i>	<i>Klebsiella Pneumoniae</i>	<i>pseudomonas</i>	<i>Citrobacter Koseri</i>	<i>Mix organism</i>
Hospital	1	–	–	–	1
Medical college	–	–	–	–	2
Girls hostel	–	1	1	–	1
Boys hostel	1	–	1	1	1
CCSIT	–	–	–	–	–
Nursing college	–	1	–	–	–
College of education and stadium	–	–	–	–	1
Engineering college	1	–	–	–	–
Dental OPD	1	–	–	–	–
Administrative block	–	–	–	–	1
Total positive sample	4 (25%)	2 (12.5%)	2 (12.5%)	1 (6.25%)	7 (43.75%)

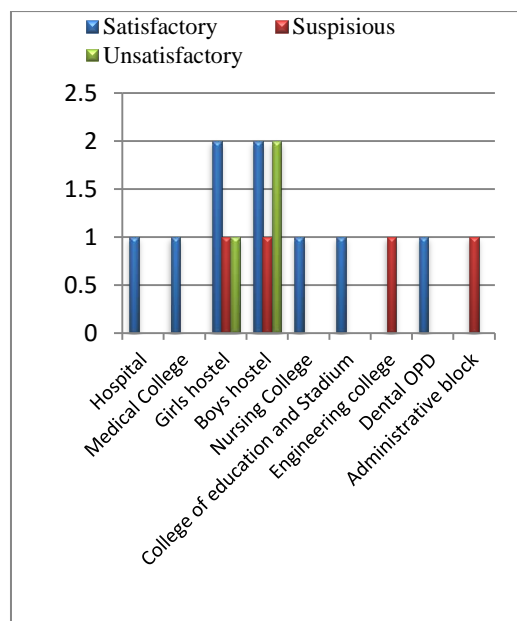
In our study, it was found that 7 (43.75%) of water sample contaminated with a multiple coliform bacteria and 9 (56.25%) of water samples found to be contaminated with a single isolate

We found that out of 16 positive sample 9 (56.25%) water sample were satisfactory, 4(25%) were

of coliform bacteria. Out of 9 single coliformbacteria *Escherichia coli* 4 (25%) *Pseudomonas* 2 (12.5%) *Citrobacter* 1(6.25%) and *Klebsiella* 2 (12.5%) we found that which is shown in table and figure suspicious sample, and 3 (18.75%) were unsatisfactory sample

**Table-3: profile of total positive sample (n=16)**

Site of water samples	Number of positive samples	Number of satisfactory samples	Number of suspicious samples	Number of unsatisfactory sample
Hospital	1	1	0	0
Medical college	1	1	0	0
Girls hostel	4	2	1	1
Boys hostel	5	2	1	2
Nursing college	1	1	0	0
College of education and stadium	1	1	0	0
Engineering college	1	0	1	0
Dental OPD	1	1	0	0
Administrative block	1	0	1	0
Total	16	9 (56.25)	4 (25%)	3 (18.75)



**Fig-5: Showing profile of total positive samples**

## DISCUSSION

A number of water born disease, and chronic health problem may be caused by consuming unsafe drinking water. In developing countries, the most common cause of gastroenteritis which affects humanity is due to lack of secure and uncontaminated drinking water supply. Hence, safe drinking water to each and every individual is necessary on earth. That is appropriate treatment of water should be used to keep away from health problems.

In this study, on the basis of the result obtained the coliform bacteriological quality different sites of university, drinking water cooler quality of Medical College, Hospital, College of education Nursing College, Dental OPD were satisfactory as compared to girls and boys hostels.

*Escherichia coli* were recurrently detected in water cooler samples of Hospital, Engineering College, girls and boys hostels as compared to Dental OPD and Nursing College. *Pseudomonas* was found in water cooler of girls and boys hostel.

Mix organisms were less detected in water cooler samples in different sites of university as compared to individual organisms.

*Escherichia coli* were observed mainly in the water cooler; hence the water cooler by washing and changing the filter regularly as per guidelines is necessary.

Our study is comparable with the study of Thakur M. *et al.* In which study, water samples were collected from different source. Among 17 water samples, 29.4% excellent, 11.76% Satisfactory, 5.88%

Suspicious and 52.94% unsatisfactory, *E. coli* and *Enterobacter aerogenes* were most common isolates. Both the organisms are considered as an indicator of water pollution [19].

Although in the study of Ngwa NR and Chrysanthus N, the majority widespread isolated bacteria 73.3% *Klebsiella species*, followed by 53.3% *E. coli*, 66.7% *Salmonella typhi* 26.7% *Enterobacter species*, and 6.7% *Proteus mirabilis* [20]

*E. coli* used as an indicator of faecal contamination in water for many decades. In human and animals intestinal tract, a vast number of bacterium is present and is more copious than disease causing bacteria and viruses. The reward of *E. coli* is that, it's not capable of growing and multiplying in water (except for food laden water and warm). Thus for faecal contagion the incidence of this bacterium in water is an indicators [21].

## CONCLUSION

The result obtained from our study revealed that, the microbiological parameters of water samples from different sites of a TMU were obtained and collected. Most of the sites met the WHO recommended standard microbiological parameters. But some sites doesn't met the WHO suggested standard.

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

The water cooler revealed the high number of *E. coli* and *Klebsiella* it means drinking water fecally

contaminated because *E.coli* as an indicator of faecal pollution. It means accurate maintenance is required by washing and changing the cartridge regularly as per guidelines of Supplier Company.

So we would like to suggest that all water sources of drinking water should be planned and conducted through the proper sanitation, regular treatment, and supervision of water sources and regular estimation of bacteria.

Water should pass three steps – storage, disinfection and filtration; storage eliminates 90 to 95 % of physical contaminations by mechanism of suspensions for certifying potable and protected water supply.

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