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Analysis of Waterborne Disease Causing Bacteria from Traditional Drinking Water Sources in Pithoragarh City, Uttarakhand, India

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Abstract: Water is necessary for life to offer major health advantages. Access to clean and safe water is essential for the well-being and survival of living organisms. The objectives of this study were to study the water quality of traditional sources from Pithoragarh city to water-borne disease. Collected water samples were analyzed annually for the study of physico-chemical and seasonal bacterial analysis every month. Nutrient agar culture media and then selective media were used for isolating the different bacterial colonies, along with IMViC tests, were used for bacterial identification, whereas CFU was for quantifying viable cells. Physico-chemical parameters fluctuations across noticed were -- pH (7.24-7.54), water temperature (WT) (16.33-17.58 °C), Atmospheric temperature (AT) (17.58-19.25 °C), Total dissolved solids (TDS) (190-519.08 mg.ml^{-1}), DO (8.45-9.10 mg.ml^{-1}), CO_2 (4.27-5.88 mg.ml^{-1}), alkalinity (102.25-148.83 mg.ml^{-1}), Biological oxygen demand (BOD) (1.69-2.3 mg.ml^{-1}), and Electrical conductivity (EC) (567-601.41 µs/cm). Five bacterial species were confirmed by observing the colonies' patterns, colours, shapes, and sizes. IMViC tests confirm the coliform bacteria was lowest in January and highest in four months, i.e., April, July, August, and December. *E. coli* has the highest CFU mean values in post-monsoon seasons, followed by *Salmonella*, *Shigella*, and *Campylobacter*, with descending CFU values, whereas *Clostridium* exhibited the lowest CFU value. This study's significance is for public health and water source management in terms of waterborne disease-causing bacteria. Finally, this study confirms the presence of coliform bacteria in drinking water sources as a potential cause of human waterborne diseases.

Keywords: Natural water, Physico-chemical parameters, Bacterial diseases, Bacterial isolation, Traditional drinking water, Bacterial identification

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Introduction

Water is necessary for life and should always be available, safe, and in sufficient amounts to offer major health advantages (WHO, 2008). Since water makes up the majority of our body, it is

essential for all biological processes like digestion, assimilation, elimination, breathing, and maintaining temperature (Bassin *et al*., 2021). It is a fundamental human right to have access to clean drinking water, but if it is tainted with opportunistic pathogenic environmental bacteria, consumers may have health problems (WHO, 2004). Due to agricultural runoff, sewage effluents, and animal excrement interacting with these unprotected water sources, they may get contaminated with bacteria (Obi *et al.*, 2002; Sharma *et al.*, 2005). Water resources are being grossly misused and degraded. Because of the rising human population, urbanization, and industry, our pure water bodies are polluted with many types of microorganisms, and the ecological properties of these waterways are changing in unfavorable conditions (Singh *et al.*, 2010). The physicochemical parameters of water are essential for understanding its quality and suitability for various purposes, influenced by natural factors and human activities and suitability for various purposes (Gorde and Jadhav, 2013).

Many serious human illnesses, including cholera, typhoid, and other diarrheal illnesses, are water-borne pathogens that enter water sources through faecal and sewage pollution. The primary human pathogens, including *Salmonella*, *Clostridium*, *Shigella*, *Campylobacter*, *Escherichia coli*, and other dangerous bacteria, are frequently found in contaminated water (Panneerselvam and Arrumugam, 2012). Dangerous bacteria have been found in drinking water that cause illnesses including dysentery, diarrhoea, typhoid, etc. (Mulamattathil *et al.*, 2014; Amenu, 2014). The existence of harmful microorganisms in different water resources has also been discussed by few investigators (Pachepsky *et al.*, 2011; Bradford *et al.*, 2014).

This study aimed to address the gap in knowledge regarding waterborne diseases and traditional water sources in Pithoragarh City, Uttarakhand, India. Assessing the physico-chemical parameters of water evaluated its quality and ensure the health of human well-being. It is expected to provide valuable insights into the prevalence, identification, and potential risks associated with waterborne bacteria, enabling the development of targeted interventions for disease prevention and water resource management. There are four main objectives of this study i.e., physico-chemical analysis of water, isolate, identify, and quantify the waterborne diseases causing bacteria from drinking water sources in district Pithoragarh, India. This study's significance will be in terms of public health, water source management, antibiotic resistance, and regional impact, as it focuses on identifying waterborne disease-causing bacteria.

Materials and Methods

Study area and sampling:

The water samples were collected from traditional drinking water sources at ten different study sites near Pithoragarh City, located at 29.5829º N, 80.2182º E, in the northern part of Uttarakhand, India. These study sites are: Shive dhara, Hanuman mandir dhara, Rai dhara, Linthura dhara, Bin naula, Chungi dhara, Kumor naula, Ancholi dhara, Pavdeev dhara, and Mahadev dhara. The samples were placed into several sterile conical flasks coated in aluminium foil. The cap cover was then carefully put back on, and the neck was then plugged about 30 cm below the water's surface. Each bottle was then marked with the name of the sample site, the date, and the time. The samples (kept in icebox) were then immediately transported to the lab for bacteriological analysis. All water sampling was done according to APHA (2012).

Physico-chemical analysis:

The water samples were collected aseptically in 1000 ml sterile bottles. Water quality was assessed including pH, WT, AT, TDS, DO, CO_2, Alkalinity, BOD, and EC (APHA, 2012).

Isolation of bacteria by culture media:

General culture media was prepared by the APHA (2012) method. Different selective media were prepared for different bacteria, such as Eosin

Table 1: Bacterial morphological characters, adopted and modified (Park *et al*., 2012; Singh and Sao, 2015; Batra, 2018; Aktar *et al*., 2018)

S. No.	Sample location	Characteristics of Morphology			
		Colour	Shape	Size	Surface
1.	*E. coli*	White/metallic sheen	Round/Circular	1-3 mm	Smooth, Rough
2.	*Salmonella*	Pink colonies with black centers	Rod	2 mm	Smooth
3.	*Shigella*	Yellow colour	Irregular	1-2 mm	Smooth
4.	*Campylobacter*	Grey/white or creamy grey	Circular	1-2 mm	Smooth
5.	*Clostridium*	White or light yellow	Translucent	3-5 mm	Smooth
6.	*Yersinia*	Grey-white	Circular	1-3 mm	Smooth

methylene blue agar media for *Escherichia coli* (Leininger *et al.*, 2001), xylose lysin deoxycholate agar media for *Shigella spp*. (Gaurav *et al.*, 2013), brilliant green agar media for *Salmonella spp*. (Murchie *et al.*, 2007), skirrow's Campylobacter media for *Campylobacter spp*. (Bi *et al.*, 2013), lactose gelatin media for *Clostridium spp*. (Lin and Labbe, 2003), and cefsulodin irgasam novobiocin media for *Yersinia spp.* (Schiemann, 1979). Different colonies were confirmed as mentioned in Table 1.

Identification of bacteria by biochemical test (IMViC):

IMViC tests are commonly used to identify and differentiate between different bacterial species based on their metabolic properties. IMViC is an acronym for four different tests: indole, methyl red, Voges-Proskauer, and citrate, as illustrated in Table 2. The IMViC test was performed and determined by method of APHA (2012), along with Edberg *et al.* (2000) and Zahera *et al.* (2011). The test results were determined as positive and negative as per Table 3.

Quantification of bacteria by CFU determination:

The colony-forming unit was measured by quantifying the number of viable cells or bacteria present in a sample on a solid agar medium under specific conditions, which is important for assessing the level of contamination or infection. Serial dilution was the first step in this method. Then the selected culture media were prepared, poured into petri plates, and incubated at 37 °C for 24-48 h. The bacterial colonies were then counted, and the CFU was calculated using a formula given by APHA (2012).

Statistical analysis:

Summary statistical analysis of annual data was analyzed by mean, mode, median, standard deviation, PAST program 4.08 version (Hammer *et al.* 2001), MS Excel data analysis software, and ANOVA (Heiberger and Neuwirth, 2009).

Results

The analysis of the water quality of different drinking water sources was measured monthly from each sources. The results of different parameters during one year showed that the mean value of pH varies from 7.24 to 7.54, WT from 16.33 to 17.58 °C, AT from 17.58 to 19.25 °C,, TDS was 190 to 519.08 $mg.ml^{-1}$, DO was 8.45 to 9.10 $mg.ml^{-1}$, CO_2 from 4.27 to 5.88 $mg.ml^{-1}$, alkalinity from 102.25 to 148.83 $mg.ml^{-1}$, BOD from 1.69 to 2.3 $mg.ml^{-1}$, and EC from 567 to 601.41 µs/cms (Table 4). There is a significant value for WT and CO_2 ($p < 0.05$) and no significant difference in the

Table 2: The standard method determines IMViC test adapted and modified by Dikhit and Sohani (2022)

Biochemical Testing	Chemical	Reagent	Results	
Indole Test	Tryptone broth	Kovacs reagent	Red coloured ring appearance	+
			Yellow coloured ring appearance	-
Methyl Red Test	MRVP broth	Methyl red	Appearance of reddish brownish colour	+
			Appearance of yellow colour	-
Voges-Proskauer Test	MRVP broth	Alpha naphthol and 40% KOH	The appearance of pink color	+
			Appearances of yellow colour	_
Citrate Utilization Test	Citrate	NA	Appearance of blue colour	+
			Appearance of green colour	-

Table 3: The standard method determines the IMViC test (Aryal, 2022)

S. No.	Bacteria	Indole	Methyl Red	Vogus-Prosker	Citrate
1.	*E. coli*	+	+	-	-
2.	*Salmonella*	Variable	+	-	-
3.	*Shigella*	-	+	-	-
4.	*Campylobacter*	-	-	-	+
5.	*Clostridium*	-	+	-	-
6.	*Yersinia*	+	-	+	_

true average values of parameters between WT and pH, WT and AT, WT and TDS, WT and DO, WT and alkalinity, WT and BOD, and WT and EC (Table 5).

Six different plates of selective agar media were prepared for *E. coli*, *Salmonella*, *Shigella*, *Clostridium*, *Campylobacter*, and *Yersinia* (Fig. 1). The presence of only five colonies of bacteria in the different media and the absence of *Yersinia* colonies in the selective media showed that *Yersinia* was absent in the water sample. Selective agar media plates showed good growth indicating the effectiveness of the media and the presence of the respective bacteria. The presence of only one type of colony of the respective bacteria showed that there was no contamination in the selective media. The presence of different bacteria was further confirmed by the colony's colour, shape, size and surface (Table 1).

On the basis of IMViC test positive and negative results as shown in Table 6 and Figure 2, it can be concluded that the spot SD showed maximum bacteria in the month of May, HM in April, RD in July and December, LD in August, CD in February, BN in April and August, KN in October, AD in July, PD in December, and MD in June. The spot SD showed minimum bacteria in the month of November, HM in March, May, and

Table 4: Annual data of physico-chemical parameter of different water sources

S. No.	Sites	Perameters								
		pH	**WT (ºC)**	**AT (ºC)**	**TDS (mg.l^{-1})**	**DO (mg.ml^{-1})**	**CO_2 (mg.ml^{-1})**	**Alkalinity (mg.ml^{-1})**	**BOD (mg.ml^{-1})**	**EC (µs/cm)**
1.	**Site 1 (SD)**	7.2-7.7 7.39±0.12	10-22 17.16± 4.28	11-23 18.66±4.24	494-540 519.08± 15.24	7.4-12 9.02±1.13	3.3-8.6 5.88±1.71	114-296 148.83±52.61	1.4-3 22.3±0.49	366-761 599.08± 153.96
2.	**Site 2 (HM)**	7.2-7.8 7.5±0.2	8-22 16.33± 4.73	8-26 18±6.79	260-292 276.16±10.13	7.4-12 8.88±1.19	2.8-7.7 5.3±1.31	108-206 130.58±33.13	1.3-2.5 1.69±0.43	359-763 601.41±154.69
3.	**Site 3 (CD)**	6.9-7.6 7.24±0.21	9-22 17±4.32	6-27 17.58±7.77	106-212 190±27.80	7.8-11.6 9.10±1.08	2.5-11 5.86±2.42	66-133 108.66±20.57	1.7-2.1 1.85±0.13	350-752 595.66±151.26
4.	**Site 4(BN)**	6.8-7.7 7.39±0.22	8-22 16.75±4.76	7-27 17.91±7.22	361-389 374.5±7.97	8.1-10.8 8.70±0.71	3-6.8 4.62±1.18	89-129 102.25±30.91	2.2-4.3 3.55±0.55	360-766 600.08± 153.54
5.	**Site 5 (RD)**	7.2-7.8 7.54±0.20	11-22 17.16±4.06	8-25 18.33±6.06	233-276 248.83±11.49	7.8-12.4 9.15±1.15	2.3-6.2 4.27±1.27	89-156 118.41±19.30	1.3-9.1 2.69±2.09	344-787 595.33±159.47
6.	**Site 6 (LD)**	6.8-7.8 7.3±0.26	11-22 17.58±3.80	8-26 18.75±6.36	407-478 433.25±23.63	7.5-9.6 8.57±0.48	2.2-8.6 5.70±1.94	86-160 116.91±17.40	1.6-3.6 2.49±0.58	220-766 567.75±194.87
7.	**Site 7 (KN)**	6.8-7.6 7.28±0.21	11-22 17.58±4.01	7.3-25 18.60±5.55	423-504 466.58±29.02	6.7-10.1 8.45±0.83	2.8-10.7 5.66±2.48	112-153 124.83±13.24	2.8-4.6 3.60±0.56	327-754 584.08±158.12
8.	**Site 8 (AD)**	7.1-7.7 7.46±0.15	8-22 16.83±4.89	8-26 18.83±5.45	114-423 381.08±84.96	8.1-10.8 8.74±0.88	3.1-8.8 5.30±1.69	85-228 131.41±42.05	1.6-3.6 2.6±0.47	338-759 587.41±153.94
9.	**Site 9 (MD)**	7.2-7.9 7.47±0.22	10-21 16.91±4.14	8-26 18.75±5.84	245-285 261.41±13.68	7.2-13.2 8.94±1.48	2-7.3 4.83±1.90	110-236 132.75±39.08	1.4-2.5 2.05±0.30	332-745 585.5±145.94
10.	**Site 10 (PD)**	6.9-7.8 7.33±0.27	10-22 17.5±4.23	8-26 19.25±6.06	311-434 335.66±33.84	7.8-11.6 9.02±1.15	2.8-9.4 5.45±1.95	104-196 124.5±28.02	1.5-2.6 1.93±0.36	356-776 597.08±151.54

Table 5: Analysis of variance for physico-chemical parameters of different water sources

Parameters	Sum of square	df	Mean of square	f	p	Significant
WT/pH	97.3067	11	8.84607	0.9549	0.5298	NS
WT/AT	563.843	11	51.2584	20.79	8.78E-06	NS
WT/TDS	697.485	11	63.4077	0.8557	0.5997	NS
WT/DO	697.485	11	63.4077	0.8557	0.5997	NS
WT/CO2	165.895	11	15.0813	2.877	0.04683	S
WT/Alkalinity	2507.93	11	227.994	0.6677	0.743	NS
WT/BOD	99.6186	11	9.05624	0.7009	0.7173	NS
WT/EC	130073	11	11824.8	0.9555	0.5294	NS

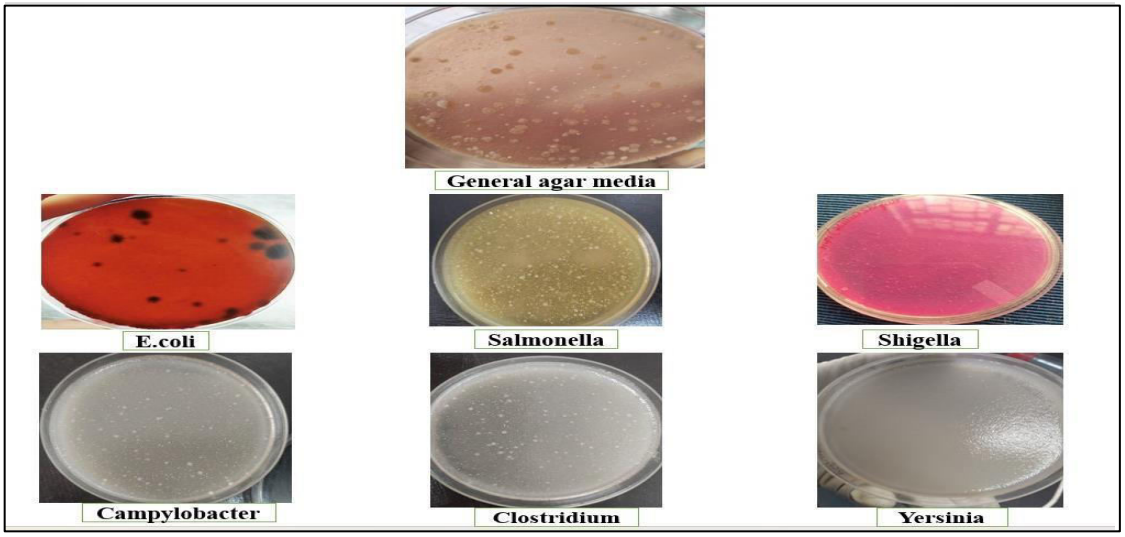

Fig. 1: Bacterial colonies on general agar medium and different selective media.

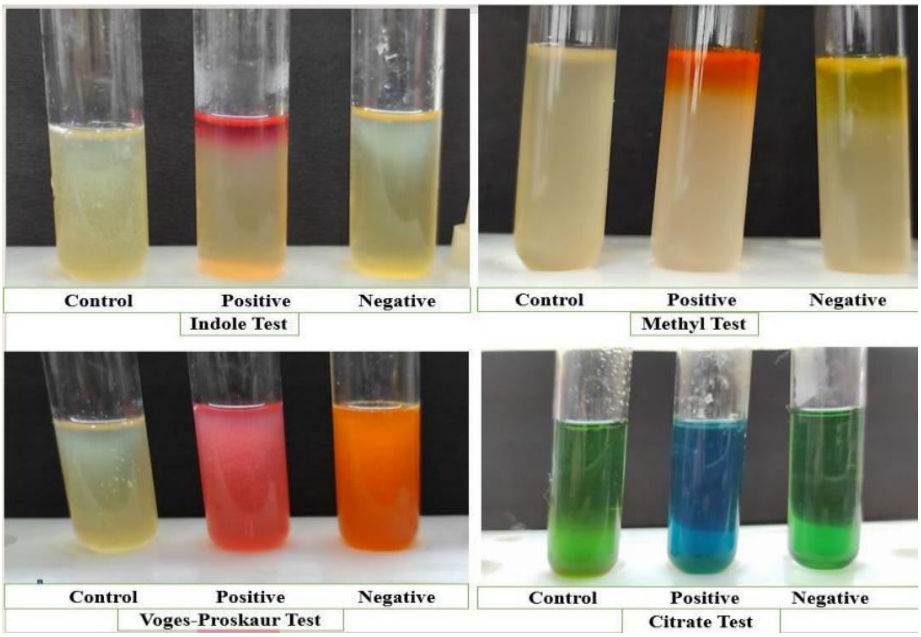

Fig.2: Biochemical (IMViC) test result.

Table 6: Showing the result of IMViC Test

Site	Name of the bacteria	Biochemical test (IMViC Test)											
		Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
SD	*E.coli*	-	+	+	-	+	-	+	+	+	+	+	+
	Salmonella	+	-	+	+	+	+	-	+	+	+	-	+
	Shigella	+	+	-	+	+	+	+	-	+	-	-	+
	Campylobacter	-	+	-	-	+	-	+	-	+	-	-	-
	Clostridium	-	+	-	-	+	-	+	-	+	-	-	-
HM	*E.coli*	+	-	-	+	-	+	-	-	-	-	-	+
	Salmonella	-	-	-	+	-	-	-	-	+	-	-	-
	Shigella	-	-	-	-	-	-	-	-	-	+	-	-
	Campylobacter	-	-	-	+	-	-	-	+	-	-	+	-
	Clostridium	-	+	-	-	-	-	-	-	-	-	-	-
RD	*E.coli*	-	+	+	-	+	-	+	+	+	-	+	+
	Salmonella	-	+	-	-	+	+	+	-	-	+	+	-
	Shigella	-	+	+	-	-	+	+	+	-	-	-	+
	Campylobacter	-	-	-	-	-	-	-	-	-	-	-	+
	Clostridium	+	-	-	+	+	-	+	-	-	+	+	+
LD	*E.coli*	-	+	-	-	+	-	+	+	+	+	+	+
	Salmonella	-	-	+	-	-	-	-	+	-	-	-	-
	Shigella	+	-	-	+	+	-	-	+	+	-	-	+
	Campylobacter	-	-	-	-	-	-	-	+	-	-	-	-
	Clostridium	-	+	-	-	-	-	-	-	-	-	-	-
CD	*E.coli*	-	+	-	-	-	+	-	-	-	+	-	-
	Salmonella	+	-	-	+	-	-	-	-	-	-	-	-
	Shigella	-	+	-	-	-	-	-	-	-	+	+	-
	Campylobacter	+	-	-	-	-	-	-	-	-	-	-	-
	Clostridium	-	+	-	-	-	-	-	-	-	-	-	-
BN	*E.coli*	+	-	+	+	-	+	-	+	+	+	+	+
	Samonella	+	-	-	+	+	-	+	+	+	-	+	-
	Shigella	+	-	+	+	-	+	-	+	+	-	-	+
	Campylobacter	-	+	+	+	-	+	-	+	-	+	-	-
	Clostridium	-	+	-	+	-	+	-	+	-	+	-	+
KN	*E.coli*	-	-	-	-	-	-	-	-	+	+	-	-
	Samonella	-	-	+	-	-	-	-	+	-	-	-	-
	Shigella	-	-	-	-	+	-	-	-	-	+	+	-
	Campylobacter	-	-	+	-	-	-	-	+	-	+	-	-
	Clostridium	-	-	-	-	-	-	-	-	-	-	-	+
AD	*E.coli*	+	+	+	-	+	-	+	+	+	+	-	-
	Samonella	-	-	+	-	+	-	-	+	+	-	-	+
	Shigella	-	+	-	-	-	+	+	-	-	+	+	-
	Campylobacter	+	-	-	+	-	+	+	+	-	-	+	-
	Clostridium	+	-	+	-	-	-	+	-	+	-	-	-
PD	*E.coli*	-	-	-	-	-	-	-	-	-	+	+	+
	Samonella	-	-	+	-	-	-	-	-	-	+	-	+
	Shigella	-	+	+	-	-	+	-	-	+	-	-	+
	Campylobacter	-	+	+	-	+	-	-	-	+	+	-	+
	Clostridium	-	-	-	+	+	+	-	-	-	+	+	+
MD	*E.coli*	-	-	+	+	+	+	+	+	+	+	+	+
	Samonella	+	-	-	+	+	+	-	-	+	-	+	-
	Shigella	+	+	+	-	+	+	+	+	-	-	-	+
	Campylobacter	-	-	-	+	-	+	-	+	-	-	+	-
	Clostridium	-	-	+	-	-	-	-	-	+	-	-	-

Table 7: Seasonal variation of CFU value of reported five bacteria

S. No.	Season	*E. coli*	*Shigella*	*Salmonella*	*Campylobacter*	*Clostridium*
1	Pre- monsoon	708.3±235.34	523.29±111.17	504.17±101.63	381.64±176.62	376.65±247.47
2		618.06±167.74	449.63±97.62	514.74±130.14	252.33±224.19	340.28±166.42
3		610.73±196.95	496.18±126.86	522.42±144.43	405.74±173.79	318.18±194.73
4		601.76±185.81	472.84±154.06	512.74±167.14	437.74±113.32	316.09±198.96
5	Monsoon	588.87±196.87	470.51±131.43	517.16±159.22	427.29±119.08	324.41±193.18
6		602.72±207.45	497.19±128.60	530.64±154.65	394.62±96.02	355.53±64.51
7		591.63±166.30	477.74±124.97	492.75±178.94	419.88±88.80	345.75±165.78
8		622.28±197.59	487.64±105.07	503.86±128.37	424.40±98.76	410.3±107.66
9	Post-monsoon	645.28±221.58	494.98±107.73	533.43±175.13	438.42±99.33	389.43±97.52
10		682.74±203.97	518.92±106.42	553.51±170.93	436.75±83.42	395.64±68.79
11		657.97±238.69	491.32±115.52	544.29±178.31	459.50±93.97	402.74±94.22
12		636.07±210.58	496.75±114.51	531.82±169.37	386.21±168.69	334.31±131.31

July, RD in January, April, and September, LD in June, CD in March, BN in May, KN in January, February, April, June, and July, AD in April, PD in January, and MD in February and October. Finally, we can conclude that the presence of all bacteria in the month of January was at its lowest level, and the highest levels were in the April, July, August, and December months.

Quantify the bacteria by using the colony-forming unit (CFU) method. Table 7 shows the CFU of several bacteria groups at ten sites in three seasons (pre-monsoon, monsoon, and post-monsoon) during the course of one year. The maximum mean CFU value for *E. coli* was 708.3, observed during the pre-monsoon period. For *Shigella*, the highest CFU value was recorded at 523.29 during the pre-monsoon. Similarly, the peak CFU value for *Salmonella* was 553.51, also noted in the post-monsoon season. *Campylobacter* reached its highest CFU value of 459.50 during the post-monsoon season. In contrast, *Clostridium* exhibited its maximum CFU value during the post-monsoon season (Fig. 3).

Discussion

In the present study, we analyze the physico-chemical parameters of different sources of drinking water, which are similar to the findings of Patil (2010), Jasmin and Mallikarjuna (2014), and Qureshi *et al.* (2021), who worked on the physic-chemical characteristics of groundwater quality parameters.In the present study the temperature of the water varies in different seasons, which is similar to the findings of Menberg *et al.* (2014) and Gunawardhana and Kazama (2011), who studied the analysis of climate change impacts on

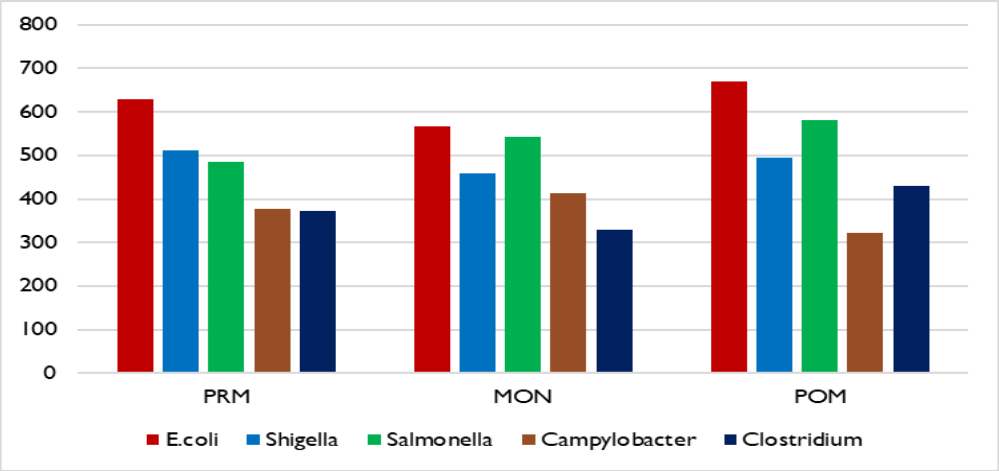

Fig. 3: CFU mean value in different seasons of different bacteria (PRM-pre-monsoon, MON monsoon and POM-post-monsoon).

groundwater temperature. The presence of bacteria was confirmed by observing the various colonies' patterns in different selective media, which is similar to the finding of Dinakaran (2022). Antony *et al*. (2012) and Akbar (2013) studied the analysis of microbiological drinking water quality, which is comparable to our analysis of the presence of several colonies of bacteria in different selective media. In this study, we identified different coliform bacteria, which is similar to the finding of Eckner (1998), who worked on the detection of waterborne coliform bacteria. In the present study, we found positive and negative IMViC test results for five different bacteria. Similarly, Aishvarya *et al.* (2018) also investigated the bacteriological evaluation of the river Jataganga. We concluded that the presence of all bacteria in the month of January was at its lowest level, and the highest levels were in April, July, August, and December, similar to Ibrahim *et al.* (2019) who also reported difference in bacterial presence in water samples on the basis of positive and negative biochemical results. Lawan *et al*. (2020) studied the presence of coliform bacteria, which is also similar to findings of this study. CFU value for several bacteria groups at ten sites in three different seasons (pre-monsoon, monsoon, and post-monsoon) revealed that there was a maximum for *E. coli* in the month of January, while a minimum value was observed for *Campylobacter* in the same month. Islam *et al*. (2011) also found a bacteriological evaluation of drinking water sources. Singh *et al.* (2009) performed groundwater bacteriological evaluation for quantifying the bacteria. Enitan *et al*. (2020) examined the hydrochemical, bacteriological, and categorization of groundwater quality, and Samuel *et al*. (2018) determined the CFU value of bacteria from the public hand-pump borehole water, which is also similar to our study.

Conclusion

It is concluded that the traditional water sources of Pithoragarh city are contaminated by pathogenic microorganisms, particularly coliform bacteria. Comprehensive analysis indicates satisfactory water quality, ensuring safety for consumption and environmental health. If this water is used for drinking purposes without being properly treated, it may potentially cause serious illnesses known as waterborne diseases and also confirm the seasonal variations of different bacterial species. Establishing an appropriate treatment facility is advised to get rid of tainted bacteria before they are used for drinking. As demonstrated in the current study, the traditional water sources supported the growth of bacteria, which has the potential to cause human diseases like dysentery, diarrhea, typhoid, etc.

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