Evaluating Seasonal Dynamics of Traditional Drinking Water Sources in Pithoragarh: A Physicochemical and Microbiological Perspective

Shailu Garkoti[1], Jaya Kandpal[1],Rakesh Verma[2*]

[1]Department of Zoology, Govt. PG College Bajpur, Kumaun University Nainital, Uttarakhand, India
[2*]Department of Zoology, S.S.J. University, L.S.M. Campus Pithoragarh, Uttarakhand, India

***Corresponding Authors**: Rakesh Verma
*E mail: rv.pith@gmail.com

Abstract
Water is the most widely distributed and abundant substance in nature, but only 3% is fit for human consumption. This study deals with the water quality analysis of the traditional water sources in the Pithoragarh city of Uttarakhand. These water sources are considered sacred traditional sources of drinking water in the Kumaun region. In the present study, samples were taken from 10 different sites and the physicochemical parameters analyzed, isolation, identification, and characteristics of bacteria with the antibiotic's sensitivity test were tested. The water pH ranges from 6.2 to 7.9, with temperatures of 8-18°C (water) and 10-21°C (air). Total dissolved solids were 114-498 mg/L, dissolved oxygen 5.8-7.4 mg/L, carbon dioxide 0.4-2.8 mg/L, alkalinity 56-202 mg/L, biochemical oxygen demand 1-2.9 mg/L, and electrical conductivity 309-798 µS/cm. ANOVA showed low significance ($P<0.05$) between water and air temperatures, but highly significant differences ($P<0.001$) for water temperature with TDS, CO_2, DO, BOD, alkalinity, and EC. Bacteria such as *E. coli*, *Salmonella*, *Shigella*, *Campylobacter*, and *Clostridium* were isolated on selective agar, but *Yersinia* showed no growth across all months and sites. Biochemical tests revealed site-specific bacterial presence, with all five bacteria positive in May and September at site 1 (SD) and varying positive results in summer months across other sites. Negative results were frequent in winter and early spring. The study revealed distinct antibiotic resistance patterns across bacterial species. *E. coli*, *Salmonella*, *Shigella*, and *Campylobacter* showed 50-90% resistance to tested antibiotics, with limited susceptibility. *Clostridium* exhibited the highest resistance, with 90% resistance to Metronidazole and 75% to Vancomycin. This study highlights the urgent need to protect traditional drinking water sources in Pithoragarh by addressing bacterial contamination and antibiotic resistance, guiding essential public health interventions.

Keywords: Physicochemical, Traditional water, Antibiotics, Resistance, Contamination

Introduction:

Water is the most widely distributed and abundant substance in nature, but only 3% is fit for human consumption. The sea contains 97% of the water unfit for human consumption; of this 3%, 2% is found in glaciers and the polar ice cap and 1% is usable for drinking (Mishra, 2023). Water is among the most investigated ingredients in the world, due to its obvious significance and it is amazing to study that it is so little understood, not just through the general public but also by scientists, who work on it every day (Chaplin, 2001). Water plays an important role in social life. According to UNICEF and WHO, 2.2 billion people worldwide cannot access clean drinking water. Particularly in at-risk populations, this pervasive problem exposes millions to waterborne illnesses and other health hazards (Baye, 2021). 90% of Uttarakhand's drinkable water comes from natural springs and rivers, illustrating the area's dependence on abundant natural water supplies. For their everyday water needs, 60% of Kumaon's rural residents rely directly on these natural springs (Chhimwal et al., 2022).

The physicochemical parameters of water, play a vital role in evaluating its overall quality and determining its appropriateness for specific uses. Water parameters provide insight into the water's physical and chemical characteristics, influencing its ecological health, portability, and industrial utility. Natural factors such as climate conditions, and biological activity, contribute to variations in these parameters. human activities, such as agricultural practices, industrial discharges, urbanization, and waste management, significantly impact water quality, often introducing pollutants or altering its natural balance. Understanding these parameters is essential for water resource management, environmental conservation, andpublic health protection (Gorde & Jadhav 2013). Bacteriological analysis tests are procedures used to examine samples for the presence of bacteria and other microorganisms. These tests provide insights into the sample's microbial content, aiding in assessing its safety, contamination level, and potential health risks. This analysis is often used to evaluate the safety and cleanliness of water substances (Chigbu & Sobolev, 2007). To isolate bacteriafrom water samples, selective media are used to encourage the development of some bacterial species while suppressing the growth of others. This procedure aids in identifying and differentiating the bacteria that are present in the sample. To further categorize and validate the bacterial species, biochemical tests are conducted upon isolation, such as the IMViC series (Saimin et al., (2020).

Antibiotic sensitivity testing is a technique used to assess the effectiveness of different antibiotics against a particular bacterial illness. This test determines which medications are most efficient in treating the infection (Leekha et al, 2011). The Zone of Inhibition (ZOI), a distinct area surrounding an antibiotic disc where microbial growth is inhibited, is a crucial sign of how well an antimicrobial drug is working to stop bacterial growth. Bacterial susceptibility is indicated by a Zone of Inhibition that meets or surpasses theestablished breakpoints, indicating potential effectiveness in treating illnesses. A smaller zone denotes resistance, making the antibiotic less likely to be effective against the bacteria, and an intermediate zone, which represents intermediate susceptibility, suggests a partial inhibitory impact (Alanis, 2005; Rahman et al., 2004).

The aim of the present study was conducted in Pithoragarh city, Uttarakhand, located at 29.35°N and 80.13°E, with an elevation of 1,627 meters in the Himalayan Kumaon region. Traditionally, Naulas (natural aquifers) and Dharas have been the primary drinking water sources in this area, revered as holy by the locals and worshiped since ancient times. However, with modernization, these traditional water sources have lost their prominence. The primary objective of this study was to assess the water quality of these sources to determine their suitability for consumption by analyzing the physicochemical parameters, isolation, identification, and characteristics of bacteria with the antibiotic sensitivity test.

Materials And Methods:

Study area: The water samples were collected from traditional drinking water sources at ten different study sites near Pithoragarh City, located at 29.5829ºN, 80.2182ºE, in the northern partof Uttarakhand, India for physiochemical analysis. These study sites are Shive dhara (SD), Hanuman mandir dhara (HD), Rai dhara (RD), Linthura dhara (LD), Bin naula (BN), Chungi dhara (CD), Kumor naula (KN), Ancholi dhara (AD), Pavdeev dhara (PD), and Mahadev dhara (MD). Detailed GPS locations of sampling sites are given in Table 1.

Sample collection & analysis: The water samples were collected aseptically in 1000 ml of sterile bottles for one year and tested in the laboratory of L. S. M. Campus of Pithoragarh and analysed. This study was focused on the 10 different water sources. Monthly samples were collected and the evaluation of water quality was based on multiple critical parameters, suchas pH (Hydrogen ion concentration), WT (Water temperature), AT (Atmospheric temperature), TDS (Total dissolved solids), DO (Dissolve oxygen), CO_2 (Carbon dioxide), Total Alkalinity, BOD (Biological oxygen demand), and EC (Electric conductivity) (APHA, 2012).

Isolation of bacteria by using selective media: Selective media contain specific components that support the growth of target organisms while inhibiting unwanted microbes. Selective media will be prepared separately for the different bacteria, such as Eosin methylene blue agar media for *Escherichia coli* (Leininger et al., 2001), xylose lysin deoxycholate agar media for *Shigella* (Gaurav et al., 2013), brilliant green agar media for *Salmonella* (Murchie et al., 2007), skirrow's campylobacter media for *Campylobacter* (Bi et al., 2013), lactose gelatin media for *Clostridium* (Lin & Labbe, 2003), and cefsulodin irgasan novobiocin media for *Yersinia* (Schiemann, 1979).

Characteristics of Bacteria: The bacterial culture characteristics such as color, shape, and surface of *E. coli*, *Shigella*, *Salmonella*, *Campylobacter*, and *Clostridium* displayed distinct morphological patterns based on observation, that varied across different months and sampling locations (Singh & Sao, 2015; Jan et al., 2016 & Batra et al., 2018).

Identification by Biochemical test (IMViC): The identification of isolated bacteria was confirmed by using biochemical tests, these all tests were based on bacterial metabolic activities. There was a total of four reactions: the Indole test, Methyl Red test, Voges Proskauertest, and Citrate utilization test, was done by the method given by (APHA, 2012) and followed by Saimin et al., (2020) and Dikhit & Sohani, (2022).

Antibiotic sensitivity test: After selective media, the next step was to prepare the Mueller Hilton agar media for antibiotic sensitivity testing to assess the susceptibility of the isolated bacteria to various antibiotics. This process involves spreading the bacterial culture evenly across the agar surface, followed by the placement of different antibiotic discs. Different antibiotics with different concentrations for the different bacteria were used. Different antibiotic concentrations for different studies of bacteria were set according to standard procedure (Bauer et al., 1966 & Hudzicki, 2009).

Zone of inhibition determination: For ZOI determination Kirby-bauer disc method was used. The zone of inhibition test is a simple method to assess the effectiveness of an antibiotic against bacteria. It was measured as the diameter of the clear area in millimeters. After incubating at 37ºC for 24-48 hours, measure and record the diameter of clear zones (inhibition zones) around each disc with the help of a measuring scale (Hi-Media). The diameter of the inhibition zone surrounding each antibiotic disc, as given by the manufacturer, was used to categorize organisms as either sensitive, intermediate, or resistant to an antibiotic (Bauer et al., 1966; Jayana et al., 2009).

Statistical analysis: The mean, standard deviation, PAST program 4.08 version (Hammer & Harper, 2001), MS Excel data analysis software, and ANOVA, were used to analyze the summary statistical analysis of the annual data (Heiberger et al., 2009).

Table 1. Name of different spots of the study sites

Site	Name of sites	Type of sources	Latitude	Longitude	Altitude in meter
Spot site 1 (SD)	Shiv dhara	Dhara	29.584412 N	80.211285 E	1607.23±7
Spot site 2 (HD)	Hanumanmandir dhara	Dhara	29.579093 N	80.211033 E	1550.94±2
Spot site 3 (RD)	Rai dhara	Dhara	29.589285 N	80.220025 E	1491.04±42
Spot site 4 (LD)	Linthura dhara	Dhara	29.587299 N	80.214856 E	1605.25±17
Spot site 5 (BN)	Bin naula	Naula	29.580651 N	80.228408 E	1475.4 ±69
Spot site 6 (CD)	Chungi dhara	Dhara	29.577872 N	80.236985 E	1524.1±6
Spot site 7 (KN)	Kumor naula	Naula	29.577421 N	80.215245 E	1522.0± 4.0
Spot site 8 (AD)	Aincholi dhara	Dhara	29.570591 N	80.209735 E	1575.9 ±27
Spot site 9 (MD)	Pavdeev dhara	Dhara	29.586200 N	80.194609 E	1494.0± 3.9
Spot site 10 (PD)	Mahadev dhara	Dhara	29.576370 N	80.199353 E	1494.0 ±5.2

Table 2: Annual variations of all physiochemical parameters in all spot sites

Name of sites	pH	WT (ºC)	AT (ºC)	TDS (mg/L)	DO (mg/L)	CO_2 (mg/L)	Alkalinity (mg/L)	BOD (mg/L)	EC (µs/cm)
SD	6.2-7.5 6.9±0.40	10-18 14.66±2.67	10-19 16.5±3.03	234-434 395.33±53.29	5.8-7 6.39±0.36	0.6-2 1.44±0.50	56-201 137.75±43.67	1.9-2.9 2.16±0.31	338-776 598.08±148.21
HM	6.4-7.5 7.01±0.39	8-17 14.33±2.87	11-20 16.75±3.01	114-423 381.08±84.96	5.9-7.1 6.54±0.37	0.6-2.1 1.43±0.55	68-198 231±37.53	1.7-2.9 2.14±0.32	338-759 587.4±153.94
RD	6.4-7.6 7.04±0.43	10-18 14.58±2.60	10-21 16.75±3.30	298-481 406.33±43.87	5.8-7.2 6.45±0.41	0.5-2.3 1.44±0.59	76-201 139.41±40.53	1.5-2.9 2.12±0.36	338-765 594.08±148.86
LD	6.4-7.5 7.07±0.32	8-17 14.66±2.90	11-18 15.83±2.75	256-488 399.75±52.55	5.9-7.3 6.49±0.44	0.9-2.1 1.49±0.47	98-199 155.16±36.17	1.6-2.7 2.11±0.35	334-765 593.16±156.32
BN	6.3-7.9 6.94±0.49	8-17 14.33±2.87	11-20 16.75±3.01	345-480 406.5±31.16	6-7.4 6.5±0.40	0.6-2.6 1.46±0.63	101-184 137.166±29.50	1-2.9 2.15±0.54	328-759 594.83±147.25
CD	6.1-7.3 6.91±0.43	9-18 14.33±3.02	12-20 16.83±2.85	314-490 403.91±46.52	5.9-7.2 6.47±0.42	0.8-2.2 1.61±0.49	59-198 127±43.38	1.5-2.6 2.08±0.32	338-759 605.41±139.48
KN	6.4-7.4 7.01±0.34	9-18 14.5±2.77	12-21 16.91±2.96	304-498 410.83±59.18	5.9-6.9 6.42±0.31	1-2.1 1.53±0.43	72-202 143.58±43.80	1.3-2.9 2.13±0.43	338-797 601.58±148.74
AD	6.7-7.6 7.10±0.35	7-17 14.25±3.07	12-21 16.91±2.96	254-494 399.41±53.82	5.9-7.2 6.46±0.39	0.5-2.8 1.76±0.64	89-185 131.41±32.18	1.9-2.4 2.10±0.19	338-790 603.25±138.62
MD	6.5-7.5 7.03±0.32	9-17 14±2.41	10-21 16.75±3.30	204-418 371.75±66.07	6.2-7.1 6.55±0.32	0.4-2.2 1.52±0.59	93-176 126.66±24.93	1.7-2.8 2.19±0.39	309-789 587.75±154.59
PD	6.7-7.5 7.15±0.28	8-18 14.91±3.02	11-21 17.66±3.42	274-490 413.16±58.59	5.9-7.2 6.51±0.37	1.4-2.2 1.77±0.21	87-181 138.75±29.30	1.3-2.8 2.08±0.37	472-798 624.25±129.80

Table 3: Analysis of variance (ANOVA) test between different paraments

S. No.	Variation between parameters	BG WG T	Sum of Squares	df	Mean Square	F	P value	Sig
1	WT/ AT	BG	39.75800417	1	39.75800417	4.986975658	0.036031173	*
		WG	175.3920917	22	7.972367803			
		T	215.1500958	23				
2	WT/ pH	BG	308.1666667	1	308.1666667	79.23103937	9.60074E-09	***
		WG	85.56831667	22	3.889468939			
		T	393.7349833	23				
3	WT/ TDS	BG	888037.6345	1	888037.6345	753.1082135	1.64129E-18	***
		WG	25941.59459	22	1179.163391			
		T	913979.2291	23				
4	WT/ CO_2	BG	957.4803375	1	957.4803375	242.5999023	2.2971E-13	***
		WG	86.828425	22	3.946746591			
		T	1044.308763	23				
5	WT/DO	BG	358.05375	1	358.05375	92.05988721	2.54849E-09	***
		WG	85.56585	22	3.889356818			
		T	443.6196	23				
6	WT/ BOD	BG	873.0234375	1	873.0234375	225.1172674	4.88659E-13	***
		WG	85.317825	22	3.878082955			
		T	958.3412625	23				
7	WT/Alkalinity	BG	90183.334	1	90183.334	267.8319487	8.40433E-14	***
		WG	7407.754592	22	336.7161178			
		T	97591.0886	23				
8	WT/EC	BG	2051881.913	1	2051881.913	197.1775813	1.83933E-12	***
		WG	228937.8021	22	10406.26373			
		T	2280819.715	23				

Between Group (BG), Within Group (WG), Total (T). Non-significant value (P>0.05), *=low significant (P≤0.05), **=intermediate significant, ***=highly significant (P≤0.001)

Table 4: Cultural characteristics of different bacteria on selective media agar

S.no	Sites	Bacterial characterization	
		Morphology	
1.	***E. coli***	**Color**	White/Metallic
		Shape	Round/Circular
		Surface	Smooth/Rough
2.	***Salmonella***	**Color**	Pink
		Shape	Round
		Surface	Smooth
3.	***Shigella***	**Color**	Yellow
		Shape	Irregular/Round
		Surface	Smooth
4.	***Campylobacter***	**Color**	White/Cream/Grey
		Shape	Round/Circular
		Surface	Smooth
5.	***Clostridium***	**Color**	Yellow/White
		Shape	Round/
		Surface	Smooth

Table 5: Showing the annual result of the IMViC test of all sites

Site	Name ofBacteria	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*	+	-	+	+	+	+	+	+	+	-	+	+
	Salmonella	+	-	+	+	+	+	+	+	+	+	+	+
	Shigella	+	-	+	+	-	+	-	+	+	-	-	+
	Campylobacter	-	+	+	+	-	+	+	+	-	+	-	-
	Clostridium	-	-	-	+	-	+	-	+	-	+	-	+
KN	*E. coli*	-	-	-	-	+	+	-	+	-	+	+	-
	Salmonella	+	+	+	+	+	+	+	+	+	+	-	+
	Shigella	-	-	-	-	+	+	-	+	+	+	+	-
	Campylobacter	-	-	+	+	-	+	+	+	-	+	+	-
	Clostridium	-	-	-	-	-	-	+	-	+	-	-	+
AD	*E. coli*	-	+	+	+	+	+	+	+	+	+	-	+
	Salmonella	-	+	+	-	-	-	-	+	+	-	-	+
	Shigella	-	-	-	+	+	+	+	-	-	+	-	-
	Campylobacter	+	-	-	+	-	+	+	+	-	-	-	-
	Clostridium	+	-	-	-	-	-	+	-	-	-	-	-
MD	*E. coli*	+	-	+	+	+	+	-	+	-	+	+	+
	Salmonella	+	+	-	+	-	-	+	+	-	+	-	-
	Shigella	-	-	+	+	-	+	-	+	+	-	+	-
	Campylobacter	-	+	-	-	+	-	+	+	-	+	-	-
	Clostridium	+	-	-	+	+	+	-	-	-	-	-	-
PD	*E. coli*	+	+	+	+	+	+	+	+	+	+	+	+
	Salmonella	-	+	+	+	-	+	+	+	+	-	+	-
	Shigella	+	-	+	-	-	+	+	+	-	-	-	+
	Campylobacter	-	-	-	+	+	+	-	+	-	-	-	-
	Clostridium	-	+	-	-	+	-	-	-	+	-	-	-

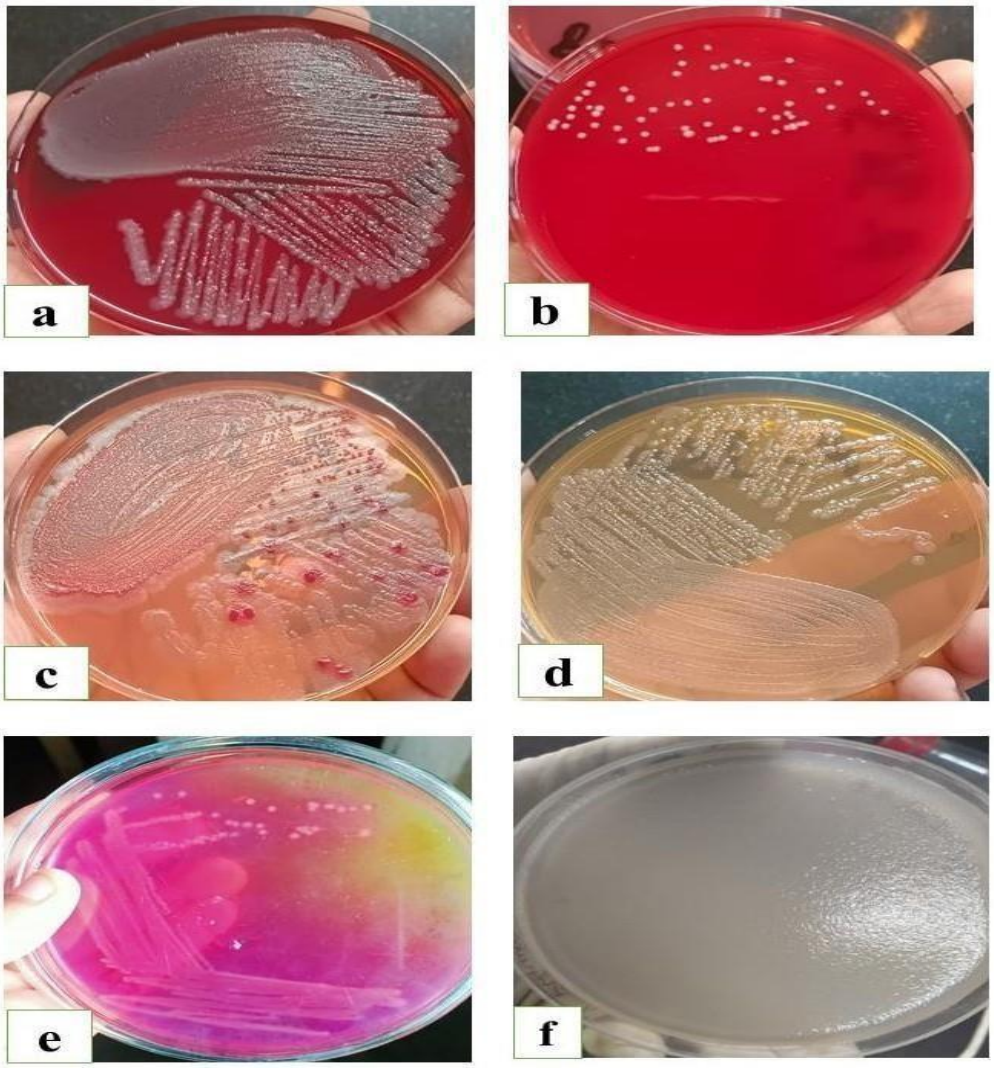

Figure 1: Showing the different colonies on different selective media (a) *E. coli* (b) *Shigella* (c) *Salmonella* (d) *Campylobacter* (e) *Clostridium* and (f) *Yersinia* (No growth)

Table 6. Annual antibiotic sensitivity profile of different antibiotics against different bacteria

S.no.	Name of Bacteria	Name of antibiotics	Disc concentration	Different spot-wise antibiotic sensitivityprofile		
				Resistant	Intermediate	Susceptible
1.	*E. coli*	Amxicillin	10 µg	55%	35%	10%
		Choromophenicol	30 µg	50%	45%	5%
2.	*Salmonella*	Ciprafloxin	5 µg	60%	40%	0.0%
		Gentamycin	10 µg	50%	45%	5%
3.	*Shigella*	Ciprafloxin	5 µg	60%	40%	0.0%
		Gentamycin	10 µg	50%	40%	10%
4.	*Campylobacter*	Erythromycin	15 µg	50%	50%	0.0%
		Tetracyclin	30 µg	80%	15%	25%
5.	*Clostridium*	Vancomycin	30 µg	75%	5%	20%
		Metronidazole	5 µg	90%	10%	0.0%

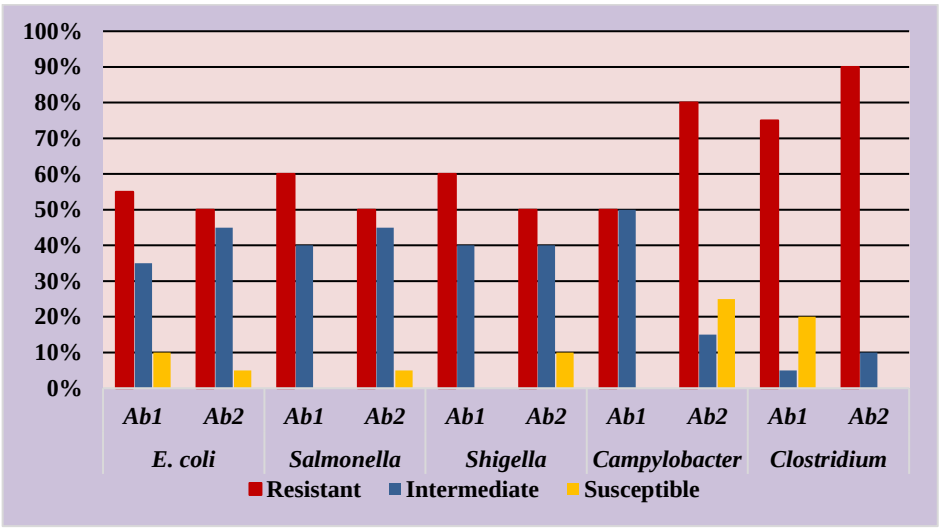

Figure 2: Annual antibiotic sensitivity test of different antibiotics against different bacteria

Result

Annual data on the physicochemical parameters of different water sources are shownin Table 2. The pH of the water varies between 6.2 and 7.9, while the water temperature (WT) and air temperature (AT) range from 8 to 18°C and 10 to 21°C, respectively, The values of total dissolved solids (TDS) range from 114 to 498 mg/L, while dissolved oxygen (DO) levels vary from 5.8-7.4 mg/L, carbon dioxide (CO_2) levels between the 0.4-2.8 mg/L. Water alkalinity ranges from 56 to 202 mg/L, and biochemical oxygen demand (BOD) readings range from 1 to 2.9 mg/L. The range of electrical conductivity (EC) was 309-798 µS/cm. In Table 3 ANOVA analysis revealed significant variations across multiple water quality parameters. The relationship between water temperature and air temperature showed low significance ($P<0.05$). In contrast, highly significant differences ($P<0.001$) were observed for water temperatureagainst other parameters, including total dissolved solids, carbon dioxide, dissolved oxygen, biochemical oxygen demand, alkalinity, and electrical conductivity. These results suggested strong interdependencies among these parameters, which varied significantly with environmental conditions.

The isolation of different bacteria on a selective agar medium, for nurturing and cultivating bacteria, was carried out. We observed the growth of different bacteria such as *E. coli*, *Salmonella*, *Shigella*, *Campylobacter*, and *Clostridium* based on colonies present on this agar medium, but *Yersinia* colony growth was absent across all months and at all sites. The bacterial culture characteristics of *E. coli*, *Shigella*, *Salmonella*, *Campylobacter*, and*Clostridium* displayed distinct morphological patterns that varied across different months and sampling locations, as exhibited in Table 4 and Figure 1. The results of the biochemical test were analyzed, at site 1 (SD), all five bacteria tested positive in May and September, with minimal presence in November. At site 2 (HM), bacteria were mostly present in July but absent in January, March, September, November, and December. At site 3 (RD), June and August showed positive results, while January recorded the most negative results. At site 4 (LD), bacteria were present in June but absent in November and December. At site 5 (CD), August and September showed positive results, while March recorded negative results. Similarly, positive results were observed in April and August at site 6 (BN), June and August at site 7 (KN), July at site 8 (AD), April and August at site 9 (MD), and June and August at site 10 (PD), with varying negative results across months (Table 5).

Our study also exhibited distinct antibiotic resistance patterns across different bacterial species, reflecting varied responses to antibiotics at different concentrations. For each bacterial species, two antibiotics (Ab1, Ab2) were tested, as shown in Table 6. In the case of *E. coli*, Amoxicillin (10 µg) exhibited 55% resistance, 35% intermediate response, and 10% susceptibility, while Chloramphenicol (30 µg) showed 50% resistance, 45% intermediate, and only 5% susceptibility. For *Salmonella*, Ciprofloxacin (5 µg) revealed 60% resistance, 40% intermediate, and no susceptibility, while Gentamycin (10 µg) showed 50% resistance, 45% intermediate, and 5% susceptibility. Similarly, *Shigella* showed 60% resistance to Ciprofloxacin (5 µg) and no susceptibility, while Gentamycin (10 µg) demonstrated 50% resistance, 40% intermediate, and 10% susceptibility. For *Campylobacter*, Erythromycin (15 µg) exhibited 50% resistance, 50% intermediate, and 0% susceptibility, while Tetracycline (30 µg) showed a high resistance rate of 80%, with 15% intermediate and 25% susceptibility. Lastly, *Clostridium* demonstrated significant resistance, with Vancomycin (30 µg) showing 75% resistance, 5% intermediate, and 20% susceptibility, while Metronidazole (5 µg) displayed 90% resistance, 10% intermediate, and no susceptibility, as presented in Table 6 & Figure 2.

Discussion

In the present study, we analyze the physiochemical parameters of different sources of drinking water, which were similar to the findings of Patil, (2010); Jasmin & Mallikarjuna (2014), and Qureshi et al., (2021), who worked on the physicochemical characteristics of groundwater quality parameters. The water quality parameters vary in different seasons, similar to the findings of Gunawardhana et al., (2011), who studied the analysis of climate change impacts on

groundwater temperature, which was comparable to our analysis. The presence of bacteria was confirmed by observing the selected colony patterns in different selective media, comparable to the results of Dinakaran et al., (2023), Antony et al., (2012), and Akbar et al., (2013) studied the analysis of microbiological drinking water quality, which was comparable to our analysis of the presence of selected colonies of bacteria in different selective media.

In our study, we observed the different morphology characteristics of other bacteria such as color, shape, and surface, similar to the observation of Singh & Sao (2015), and Batra, (2018). In our study, we observed changes in media color, with both positive and negative outcomes observed. These color changes are a direct indication of the biochemical reactions, findings align with the observations of Aishvarya et al., (2018), Ibrahim et al., (2019), and Jupriet al., (2023), who also reported media color changes in their analyses of drinking water contamination. We also conducted antibiotic sensitivity tests on various bacterial isolates from drinking water sources, similar to the work of Rahman et al., (2010); Hernandez-Camarena et al., (2015), and Bamigboye et al., (2020). who examined the zones of inhibition of coliform bacteria in drinking water by using the Kirby-Bauer disc diffusion method.

Conclusion

Assessment of water quality, bacterial contamination, and antibiotic resistance in traditional water sources of Pithoragarh, Uttarakhand, provides crucial insights into the potential health risks associated with these sources. The assessment of water quality reveals its adherence to safety standards, ensuring it is suitable for consumption and public health. The analysis showed significant variations in water quality parameters, with strong interdependencies influenced by environmental conditions. Water temperature had a highly significant impact on parameters. These findings highlight the need for regular monitoring to maintain water quality. The assessment of traditional water sources in Pithoragarh, Uttarakhand, revealed significant variations in water quality, bacterial contamination, and antibiotic resistance. Physicochemical parameters often exceeded permissible limits, and microbial analysis indicated the presence of potentially harmful bacteria, including antibiotic- resistant strains. These findings highlight the urgent need for improved water management practices and monitoring systems to ensure public health and environmental sustainability.

Acknowledgment

The authors are thankful to the Department of Zoology, L.S.M Campus Pithoragarh, Uttarakhand for providing the facilities to conduct the research successfully.

References:

1. Aishvarya, N., Malviya, M. K., Tambe, A., Sati, P., Dhakar, K., & Pandey, A. (2018). Bacteriological assessment of river Jataganga, located in Indian Himalaya, with reference to physicochemical and seasonal variations under anthropogenic pressure: A case study. *J. Environ. Microbiol*, 1(1), 10-16.
2. Akbar, A., Sitara, U., Khan, S. A., Muhammad, N., Khan, M. I., Khan, Y. H., & Kakar, S. U. R. (2013). Drinking water quality and risk of waterborne diseases in the rural mountainous area of Azad Kashmir Pakistan. *International Journal of Biosciences*, 3(12), 245-251.
3. Alanis, A. J., 2005. Resistance to antibiotics: are we in the post-antibiotic era. Archives of medical research, 36(6), 697-705.
4. Antony, R., Krishnan, K. P., Laluraj, C. M., Thamban, M., Dhakephalkar, P. K., Engineer, A. S., & Shivaji, S. (2012). Diversity and physiology of culturable bacteria associated with a coastal Antarctic ice core. *Microbiological research*, 167(6), 372-380.
5. APHA, AWWA, and WEF. (2012). Standard methods for the examination of water and wastewater. (22nd Ed.), American Public Health Association -American Water Works Association - Water Environment Federation.
6. Bamigboye, C. O., Amao, J. A., Ayodele, T. A., Adebayo, A. S., Ogunleke, J. D.,Abass, T. B., & Oyedemi, A. A. (2020). An appraisal of the drinking water quality of groundwater sources in Ogbomoso, Oyo state, Nigeria. Groundwater for Sustainable Development, 11, 100453.
7. Batra P, Barkodia M, Ahlawat U, Sansanwal R, Sharma T, Wati L. Endophytes (2018). An environmental friendly Bacteria for plant growth promotion. Int. J Curr. *Microbiol. Appl. Sci.*, 7(2), 1899-1911.
8. Bauer, A. W., Kirby, W. M. M., Sherris, J. C., & Turck, M. (1966). Antibiotic susceptibility testing by a standardized single disk method. American journal of clinical pathology, 45(4), 493-496.
9. Baye, D. (2021). Sustainable Development Goals (SDG) target 6.2 in Ethiopia: challenges and opportunities. *Open Access Library Journal*, 8(5), 1-28.
10. Bi, S. L., Shi, L., Yan, H., & Meng, H. C., 2013. Comparison of various culture methods (Skirrow medium, a blood-free medium, and a filtration system enrichedin Bolton and Preston broths) for isolation of Campylobacter spp. from raw meat samples. Annals of Microbiology, 63, 179-185.
11. Chaplin, M. F. (2001). Water: its importance to life. *Biochemistry and molecular biology education*, 29(2), 54-59.
12. Chhimwal, M., Kaur, S., Srivastava, R. K., Hagare, D., & Shiva Prasad, H. J. (2022). Water quality of springs and lakes in the Kumaon Lesser Himalayan Region of Uttarakhand, India. *Journal of Water and Health*, 20(4), 737-754.
13. Chigbu, P., & Sobolev, D. (2007). Bacteriological analysis of water.In *Handbook of water analysis* (pp. 111-148). CRC Press.

14. Dikhit, M. S., & Sohani, S. (2022). Microbial investigation through Isolation and identification of water bodies from selected hotels of Indore city, *Mukt Shabd Journal*, 11(12), 1-8.
15. Dinakaran, V. T., Chandrasekaran, M., Sivanandham, V., Murugaiah, S. G.,Santhaseelan, H., Krishnan, M., & Rathinam, A. J. (2023). Seasonal effluxes of microbial pollution and physiochemical assemblages along south east coast of India during southwest and northeast monsoon. *Regional Studies in Marine Science*, 59, 102804.
16. Gaurav, A., Singh, S. P., Gill, J. P. S., Kumar, R., & Kumar, D., 2013. Isolation and identification of Shigella spp. from human fecal samples collected from Pantnagar, India. Vet World, 6(7), 376.
17. Gorde, SP and Jadhav, MV (2013). Assessment of water quality parameters: A review. Int. J. Eng. Res. Appl., 3 (6): 2029 – 2035.
18. Gunawardhana, L. N., Kazama, S., & Kawagoe, S. (2011). Impact ofurbanization and climate change on aquifer thermal regimes. *Water resources management*, *25*, 3247-3276.
19. Hammer, Ø., & Harper, D. A., 2001. Past: paleontological statistics software package for education and data analysis. Palaeontologia electronica, 4(1), 1.
20. Heiberger, R. M., & Neuwirth, E., 2009. R through Excel: A spreadsheet interface for statistics, data analysis, and graphics (pp. 323-330). New York: Springer.
21. Hernandez-Camarena, J. C., Graue-Hernandez, E. O., Ortiz-Casas, M., Ramirez Miranda, A., Navas, A., Pedro-Aguilar, L., & Bautista-de Lucio, V. M. (2015). Trends in microbiological and antibiotic sensitivity patterns in infectious keratitis:10-year experience in Mexico City. *Cornea*, 34(7), 778-785.
22. Hudzicki, J. (2009). Kirby-Bauer disk diffusion susceptibility test protocol. *American society for microbiology*, *15*(1), 1-23.
23. Ibrahim, S. A., Muhammad, S. Y., & Ahmed, I. (2019). Bacteriological Assessment of Some Sachet Water Samples Sold in Dala Compared with That of Fagge, Kano Metropolis. *East African Scholars Journal*, 2(5), 143-148.
24. Jan, S., Khan, I., Dar, G. H., Kamili, A. N., & Tak, I. U. R. (2016). Ecologicaland microbiological characteristics of the Jhelum River in Kashmir Himalaya. *J Bacteriol Parasitol*, 7(3), 1000277.
25. Jasmin, I., & Mallikarjuna, P. (2014). Physicochemical quality evaluation of groundwater and development of drinking water quality index for Araniar River Basin, Tamil Nadu, India. *Environmental monitoring and assessment*, *186*, 935- 948.
26. Jayana, B. L., Prasai, T., Singh, A., & Yami, K. D. (2009). Assessment ofdrinking water quality of madhyapur-thimi and study of antibiotic sensitivity against bacterial isolates. *Nepal Journal of Science and Technology*, 10, 167-172.
27. Jupri, A., Vofi, Y., Faturrahman, R. I., Ernawati, B., Kiswowo, D., & Jannah, W. (2023). Microbiological analysis of drinking water sourcing from the spring of joben pesanggrahan, montong gading, east lombok. *Anim. Vet. Sci*, 11(5), 756- 763.
28. Leekha, S., Terrell, C. L., & Edson, R. S., 2011. General principles of antimicrobial therapy. In Mayo Clinic Proceedings, 86(2), 156-167.
29. Leininger, D. J., Roberson, J. R., & Elvinger, F., 2001. Use of eosin methylene blue agar to differentiate Escherichia coli from other gram-negative mastitispathogens. Journal of Veterinary Diagnostic Investigation, 13 (3), 273-275.
30. Lin, Y. T., & Labbe, R., 2003. Enterotoxigenicity and genetic relatedness of Clostridium perfringens isolates from retail foods in the United States. Applied and Environmental Microbiology, 69(3), 1642-1646.
31. Mishra, R. K. (2023). Freshwater availability and its global challenge. *British Journal of Multidisciplinary and Advanced Studies*, *4*(3), 1-78.
32. Murchie, L., Whyte, P., Xia, B., Horrigan, S., Kelly, L., & Madden, R. H., 2007. Prevalence of Salmonella in grade A whole shell eggs on the island of Ireland. Journal of Food Protection, 70(5), 1238-1240.
33. Patil, V. T., & Patil, P. R. (2010). Physicochemical analysis of selected groundwater samples of Amalner Town inJalgaon District, Maharashtra,India. *Journal of Chemistry*, *7*(1), 111-116.
34. Qureshi, S. S., Channa, A., Memon, S. A., Khan, Q., Jamali, G. A., Panhwar, A., & Saleh, T. A. (2021). Assessment of physicochemical characteristics in groundwater quality parameters. *Environmental Technology & Innovation*, *24*, 101877.
35. Rahman, M. M., Akhter, S., Jamal, M. A., Pandeya, D. R., Haque, M. A., & Rahman, A. (2010). Control of coliform bacteria detected from diarrhea associated patients by extracts of Moringa oleifera. *Nepal Med Coll J*, 12(1), 12- 19.
36. Rahman, M., Kuhn, I., Rahman, M., Olsson-Liljequist, B., & Mollby, R., 2004. Evaluation of a scanner-assisted colorimetric MIC method for susceptibility testing of gram-negative fermentative bacteria. Applied and environmental microbiology, 70(4), 2398-2403.
37. Saimin, J., Hartati, H., Purnamasari, Y., Mulyawati, S. A., Tien, T., & Ayitrina, P.(2020). Microbiological and biochemical contamination analysis of refilled drinking-water in Abeli, Kendari, Southeast Sulawesi. *The Indonesian Biomedical Journal*, 12(2), 124-129.
38. Schiemann, D.A., (1979). Synthesis of a selective agar medium for Yersinia enterocolitica. Can. J. Microbiol. 25, 1298–1304.
39. Singh, M. R., & Sao, S. (2015). Isolation and Identification of Bacteria from Hasdeo River Water in Champa Region and Level of Microbial Pollution. *International Journal of Research*, 97-101.