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Research article

Qualitative and quantitative enumeration of *Coliform* bacteria in song river water in rural area of Dehradun

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ABSTRACT

As India's population is growing so is its water demand and corresponding water contamination. Water is a very essential element in formation of life and is an essential requirement for living organisms to thrive. Therefore, it is very important that the drinking water available must be free of any contamination and proper measures be taken in that direction. Faecal contamination poses crucial threat to people having an impaired immune system and can be life threatening. Microbiological contamination causes serious health issues in human being. In case microbiological contamination found in water, it means that water quality is very poor and this type of water causes Gastrointestinal, Urinary tract infection, dysentery, diarrhoea and typhoid disease.

Keywords: Faecal coliform, Pathogenic Bacteria, Song River, Dehradun, Water Quality

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INTRODUCTION

Water is necessary for healthy physiological function and is required for life. Human existence depends on the availability of high-quality drinking water. Saltwater covers more than 97 % of the water on the earth, with just 3% being freshwater, of which 69 percent is utilized for residential uses, 22% for industry, and 8% for irrigation. Lakes hold around 2% of the total volume of freshwater, whereas rivers hold just 0.0001% of the water on the planet's surface. Dams, wells, rivers, streams, and ponds that have been found to host waterborne pathogens are the major sources of drinking water in rural regions. The river is a natural watercourse with a continuous flow of 600water as its defining feature. In terms of microbial ecology, rivers and streams have received less attention. There are free-swimming microbes as well as microbes attached to the movable interfaces of aquatic aggregates in aquatic systems, as well as algae, fungus, and protozoa, which make up a large portion of mixed marine environments [1,2].

The ever-growing worldwide human population, as well as the related industrialization, agriculture, and urbanization, are increasing water consumption and reducing the supply of fresh drinkable water. As a result, rivers typically utilized for potable water or other community requirements are reported to be highly polluted in many emerging nations where agricultural and industrial production has increased significantly. The pollution is mostly caused by human activities, which are compounded by insufficient wastewater treatment facilities. As a result, the water's potential applications are reduced. According to the Millennium Ecosystem Assessment (2005), high nutrient loading limits aquatic systems' ability to supply ecosystem services like fresh water. Water quality analyses of major African rivers found significant temporal and geographical variation in water quality, owing mostly to human and seasonal causes. Contaminants in water have negative health consequences for people and other animals. The biology and physiology of marine animals are largely reliant on the habitat's water quality, according to DWAF (1996b). One of the most serious issues with water quality, particularly in underdeveloped nations, is microbial pollution [3,4]. The World Health Organization (WHO) estimates that polluted water kills millions of people annually

Soil contamination has resulted in excessive nutrient concentration, causing eutrophication and algal blooms, which have seriously harmed freshwater ecosystems and hampered their ability to provide essential environmental services to humankind. BOD (biochemical oxygen demand) emission levels show that industrial levels of pollution are higher in several Central and Northeast Asian nations, after the Southeast Asia countries. Metals, paper &pulp,

textiles, &food and beverage sectors are all major contributors to pollution. More than 60% of the inhabitants in Asia lack access to potable water pipes in their houses [5]. Bacterial contamination is the most significant determinant of water quality. The existence and concentration of harmful bacteria and viruses in water may have a significant influence on people's lives all over the globe. Freshwater, farm products, and even skin contact can all be harmed by pathogen-contaminated water supplies. Cutaneous disease, pulmonary disease, and bladder disease are all indications of water-borne diseases, as can severe dysentery, dizziness, puking, and even hepatitis. Keep in mind that not everyone will be impacted equally; small children, immune-compromised individuals, and the elderly are often more vulnerable [6]

Pathogenic bacteria from human and animal faeces pollute water, posing public and environmental health hazards. The proper indications must be identified, and it must be known which source of faeces is the root cause of the problem. *E. coli, Clostridium*, and *Enterococci* are used to detect microbial contamination in soil and water sample. They are also utilized as faecal contamination indicators to assess faecal contaminants and potential quality of water degradation in various origins of potable water. Faecal Coliforms are often used as a conventional indication of recent faecal contamination for years. In tropical countries, these microbiological markers have also been utilized. The maximum contaminant level is a regulatory limit on how much of a chemical is allowed in public water systems [7]. Even though the origin of quality of water in most tropic locales varies from that in temp areas in 3 ways: physicochemical, biological, and socioeconomic characteristics, tropical localities accept the MCLs set in temperate areas without inquiry [8,9]

MATERIAL AND METHOD

Collection of Samples

Sample of water was collected aseptic conditions in sterile bottles from several sampling sites by immersing the bottles straight into the water's surface. The water sample was labelled properly in the laboratory for analysing the bacteria. The sample collection site was song river in Dehradun, Uttarakhand, India. It flows from Dhanaulti to Narendra nagar, beginning as a spring-fed stream on the southern slopes of the Himalayan range's Radi Top of Missouri ridge. It is also a Ganga tributary river [10,11].

Physical and chemical testing of water

The testing of pH, TDS, turbidity, colour, temperature, odour, total hardness, magnesium, chloride, alkanity tests were done by American Public Health Association 23rd Edition 2017. Carbon Dioxide, arsenic, ammonia, copper, manganese, hydrogen sulphate tests were done by the High Media Mumbai test Kits [12,13].

Heterotrophic Plate Count

1 ml of water sample was aseptically distributed into sterile Petri plates using a sterile micropipette. Molted HPC (heterotrophic plate count) agar was poured & rotated clockwise and anticlockwise. At 37°C, plates were incubated for a day and then colony count was completed. The outcome was measured in cfu/ml of water [14,15]

Most Probable Number

MPN (Most Probable Number) technique was done by the method of APHA 23rd Edition, 2017. Take 15 tubes, 10 tubes of single strength, 5 tubes double strength of MacConkey broth. 0.1 ml and 1 ml in 5-5 set of single strength and 10 ml in double strength tubes. Incubate for 48 h at 35°C.All positive fermentation tubes' bacterial cultures were transferred to EC broth fermentation tubes and cultured for 48 hours at 44.5°C for faecal coliform confirmation. MPN values were determined using total and faecal coliform gas positive tubes and were represented as MPN per 100 ml. The in oculums were placed onto a Petri plate with EMB Agar and cultured for 24 hours at 35°C to confirm E. coli [16]

Membrane Filtration Test

Put 0.45 micron of filter paper between Millipore assemblies. Filter 100 ml of water sample through the parasitic pump. Remove filter paper with forceps and put this filter paper on the surface of Enrichment medium. [17]

Isolation of bacteria from Sample

Collect water sample and 1 ml was taken, serial diluted with DW. Serial dilution was done up to 10^8 from each dilution. 100µl sample was spread on selective media and nutrient agar plate. Incubate plates for 24h at 37°C. Subculture the colonies appearing in the plates and maintain at 4°C [18].

Characterization of Isolates from Sample

The colony morphology (colour, texture, and form) of the acquired bacterial colonies was evaluated macroscopically and microscopically using Gram staining (KOH string). A single isolated colony was chosen for smear preparation and staining in order to examine the isolates' morphological characteristics. IMVIC (Indole, Methyl Red, Voges Proskauer, Citrate Utilization) and Triple Sugar Iron Agar tests were used to further biochemically characterize bacterial isolates. To distinguish between positive and “false-positive” responses, appropriate positive and negative controls were utilized [19,20]

RESULT AND DISCUSSION

The major goal of this research was to identify harmful bacteria from Dehradun's song river. The idea behind this study was the occurrence of pathogenic bacteria in water which highly affect public health. Microbiological parameters such as MFT, MPN, Faecal coliforms, there are presence of coliforms and faecal coliforms bacteria in the song water. We isolate bacteria like *Salmonella spp., Pseudomonas spp., E. coli, Klebsiella spp.*

The result of physico-chemical parameters of this testing were within limit (Table In HPC, TNTC bacterial colony present in the nutrient agar plate (1ml, 0.1ml) was positive. In MPN,

Production of acid and gas in MPN (MacConkey broth) tubes and red turns yellowish colour in after 24 hours of incubation shows positive growth of bacteria (Table2). There was production of acid and gas in EC broth tubes and there were some tubes which turn green in colour after 24 hours of incubation shows positive growth of faecal coliform (Table3). In MFT, bacteria were trap in the 0.45um filter paper on different agar medium. TNTC colonies were found in the filter paper which indicates bacteria present in water sample shows positive test. On Endo-medium, E. coli form metallic sheen colony, salmonella differential, salmonella form purple/green colony, in cetrimide agar, pseudomonas form yellow/green colony, and in M-FC agar klebsiella form purple colony (Table 4). Bacteria colonies found in different serially diluted agar medium plate. We obtain 15 x 10^8 colonies on the salmonella plate, 8 x 10^8 colonies on endo agar plate, 8 x 10^8 colonies on cetrimide plate, 8 x 10^8 colonies on M-FC plate and 20 x 108 on nutrient agar plate. (Table 6)

Four bacteria were isolated from song water *Salmonella spp., Klebsiella spp., Escherichia coli, Pseudomonas* spp. all these bacteria are gram negative and rod-shaped bacteria pink in colour. When we hold bacteria culture with inoculating loop it forms stringy, viscous material pulled up from suspension. It means KOH test is positive butall bacteria i.e. salmonella spp., klebsiella spp., pseudomonas spp., E. coli bacteria are negative (Table 5). Formation of red colour in the top layer of the tubes. In klebsiella spp., and E. coli shows positive test while no red layer formation in the salmonella spp., and pseudomonas spp., shows negative test for indole. Formation of red colour in the tubes. In salmonella spp., klebsiella spp. indicates positive test while absence of red colouration in pseudomonas spp., E. coli shows negative test for MR-VP. Changing in colour from green to blue shows positive test while no colour change shows negative test. A positive test is observed for the *Salmonella spp., Pseudomonas spp., Klebsiella spp., E. coli* shows negative test which means no growth occur. Produces bubbles after adding hydrogen peroxide. A catalase test is positive for all the bacteria salmonella spp., pseudomonas spp., klebsiellaspp, E. coli. Formation of red colour in slant tube shows positive test while no colour change shows negative test. For *Klebsiella spp., Pseudomonas spp., Salmonella spp.,* shows positive test in E. coli shows negative test for TSI test.

Formation of pink colour from yellow shows positive test while no colour change shows negative test. In *Klebsiella spp*., shows positive test, in *Salmonella spp., Pseudomonas spp., E. coli* shows negative test (Table 8, Figure.1) Bacterial pathogens in song water have been a serious public health concern in recent years. E. coli is found in a vast variety of human and animal intestinal flora, where it typically causes little harm. Bacterial pathogens in song water have been a serious public health concern in recent years. *E. coli* is found in a vast variety of human and animal intestinal flora; there it typically induces little damage. *E. coli,* on the other hand, can cause serious illnesses such as urinary infections, bacteremia, and meningitis in different parts of the body. The most frequent clinical signs of *Salmonella* infections are gastroenteritis (moderate-to-severe dysentery, dizziness, and puking), bloodstream infection or septic condition (high rising flu with positive blood cultures), and typhoid fever, enteric fever (continuous flu either with dysentery). *Pseudomonas* may cause a variety of infections, as well as harm to the respiratory tract in patients who have underlying illnesses. *Klebsiella spp.* can lead to severe infections, such as pneumonia.

Table 1: Physical appearance and chemical analysis of water samples

Characteristic	River Water	Requirement (Acceptable Limit)	Permissible Limit in the Absence of Alternate Source
Temperature	28.6 ° c	…..	…..
Odour	Agreeable	Agreeable	Agreeable
pH	6.5	6.5-8.5	No relaxation
Turbidity, NTU	0.6	1	5
Colour Hazen	5	5	15
Total dissolved solids, mg/l	264	500	2000
Total hardness (CaCO3), MG/l	256.2	200	600
Magnesium (Mg), mg/l	25.34	30	100
Calcium (Ca), mg/l	160.12	500	2000
Free residual chlorine, (Cl2) mg/l	0.3	0.2	1
Total alkalinity mg/l	80.97	200	600
Chloride (Cl), mg/l	38.28	250	1000
Total arsenic (As) mg/l	Nil	0.01	0.05
Ammonia (N) mg/l	Nil	0.5	No relaxation
Copper mg/l	Nil	0.05	1.5
Manganese (Mn)	Nil	0.1	0.3
Hydrogen Sulphate mg/l	Nil	….	…..
Corbondioxide mg/l	4	…..	…..
MPN 100ML	1600	Not-detected	Not-detected
Facel Coliform	170	Not-detected	Not-detected
E. coli MFT	TNTC	Not-detected	Not-detected

Table 2: MPN for Coliforms in River Water

Location Dehradun	Source of sample	Combination of Positives Samples			MPN Index /100 ml
		10 ml	1 ml	0.1 ml	
Raiwala	River	5	5	4	1600

Table 3: MPN for faecal coliforms in River Water

Location Dehradun	Source of sample	Combination of Positives Samples			MPN Index /100 ml
		10 ml	1 ml	0.1 ml	
Raiwala	River	5	4	1	170

Table 4: Membrane filtration technique

Media	Membrane filtration Technique colony	Test Organisms identification in river water
Salmonella differential agar	TNTC	Salmonella Spies.
Endo agar	TNTC	E. Coli
Cetrimide agar	TNTC	Pseudomonas Spies.
M-FC agar	TNTC	Klebsiella Spies.
Nutrient agar	TNTC	Mix growth

Table 5: Morphology Characteristics of the isolates

Colony Characteristics	Cell Features		Test Organisms identification
Colour	KOH string	Gram's staining	
Purple and green	Stringy Viscous	-ve, rod	Salmonella Spies.
Metallic sheen	Stringy Viscous	-ve, rod	E. Coli
Yellow/green	Stringy Viscous	-ve, rod	Pseudomonas Spies.
Purple colony	Stringy Viscous	-ve, rod	Klebsiella Spies.

Table 6: Analysis of Water quality

Total Water samples	Samples Mean Bacterial Count (CFU/ml)	Media
1	15×10^8	Salmonella differential agar
	8×10^8	Endo agar
	8×10^8	Cetrimide agar
	8×10^8	M-FC agar
	20×10^8	Nutrient agar

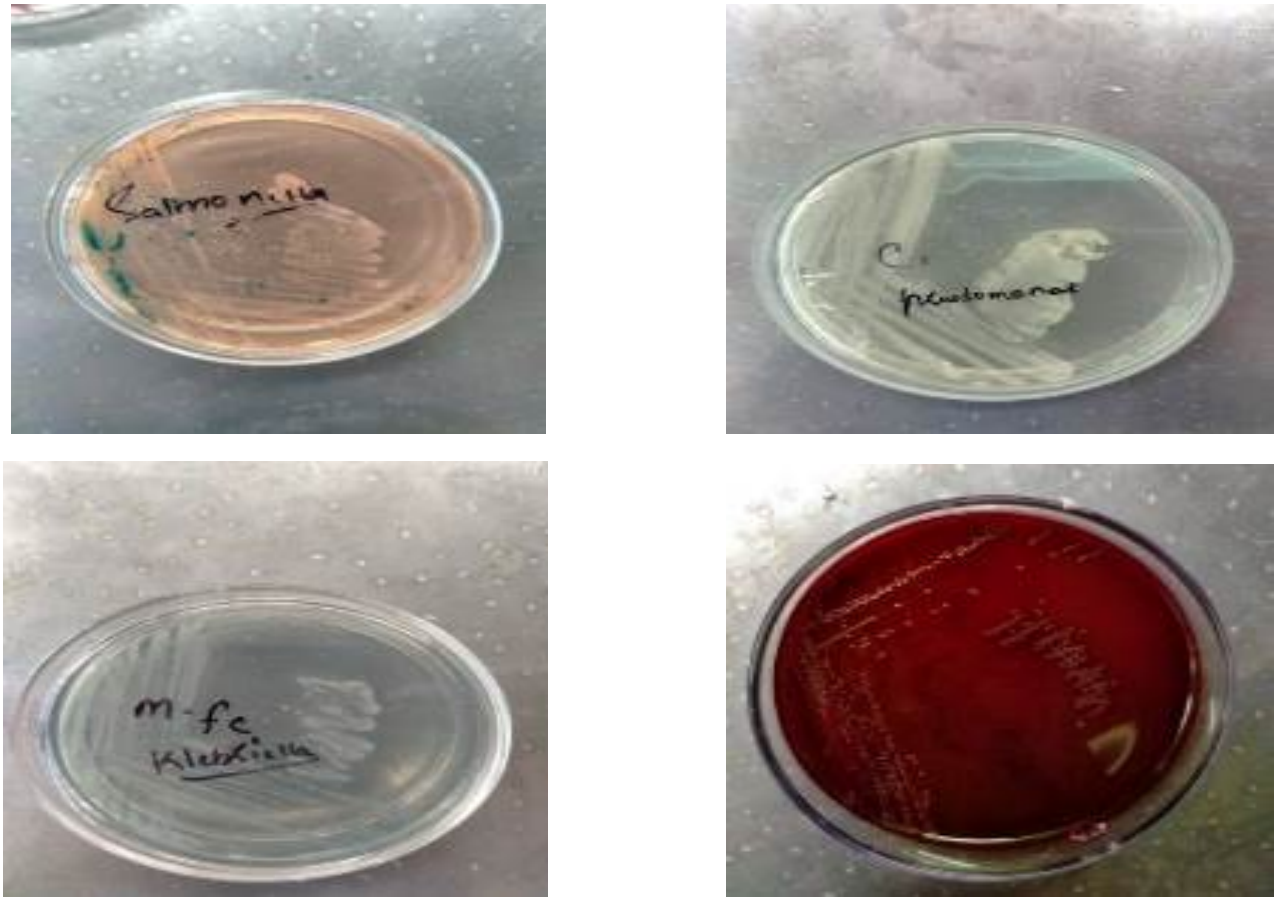
Table 7: Microbial examination of water sample

Bacterial isolates	Total Positive sample
Escherichia coli	1
salmonella sp.	1
Klebsiella sp.	1
Pseudomonas sp.	1

Table 8: Biochemical test for bacterial isolates

Tests isolates	Indole test	Methyl Red test	Voges Proskaur test	Citrate test	Catalase test	Urease test	TSI test
Escherichia coli	+ve	+ve	-ve	-ve	+ve	-ve	A/A with gas
Salmonella sp.	+ve	-ve	+ve	+ve	+ve	-ve	Al/A with H_2S
Klebsiella sp.	-ve	-ve	+ve	+ve	+ve	+ve	A/A with gas
Pseudomonas sp.	-ve	-ve	-ve	+ve	+ve	-ve	No gas & No H_2S

Figure 1: Bacterial culture growth



CONCLUSION

Drinking water that is safe to drink is the most essential necessity of any person or other creature. Because if the water is contaminated it can affect our life and health. In this research on song river, it was found that this water is contaminated. There was presence of disease-causing bacteria *Salmonella spp., klebsiella spp., Escherichia coli, Pseudomonas spp*., were found and, also faecal coliform was found. Therefore, this water is not suitable to drinking without any purification. Song River water is polluted and contaminated by coliform and faecal coliform bacteria treatment of polluted river water made the water potable by ensuring 100% disinfection of water. It is recommended that purification method like boiled water, UV treated water etc. be used to purify the contaminated water before it can be used for drinking. Monitoring of water is to be done within constant time period. Aware people not to throw garbage of any type in river because this river is also a tribute of river Ganga and most contamination happens through it.

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