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RESEARCH ARTICLE

Microbial Study of Water Samples Collected from Different Districts of Himachal Pradesh

Pathania S. Dhirendra[1*], Bhardwaj Payal[2], Pathania S. Mamta[3]

ABSTRACT

Water microbiology deals with the study of the living organisms of microscopic size present in water that causes infectious diseases of man. The primary test performed on water taken from 10 different sites act as an indicator of different types of water pollution, especially fecal pollution of water due to the presence of coliforms bacteria because they are invariably present in feces of human beings. The safety of drinking water is an ongoing concern within the global village. Traditionally, the safety of potable water supplies has been controlled by disinfection, usually by chlorination and coliform population estimates. However, it has been reported that coliform-free potable water may not necessarily be free of pathogens. Some diseases causing microorganisms enter the water from different sources and causes different types of diseases such as Polio, Typhoid, Hepatitis, Shigellosis, Salmonellosis can spread through this contaminated water which is a matter of concern.

Keywords: Fecal coliforms, Fecal pollution, Microbial, Water Microbiology.

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INTRODUCTION

India is rich in water resources, being endowed with a network of rivers and blessed with snow cover in the Himalayan range that can meet a variety of water requirements of the country.[1] The rivers of India play an important role in the lives of the Indian people. Water resources are of great significance for various activities such as drinking, irrigation, aquaculture, and power generation. The scientists at All India Institute of Medical Sciences (AIIMS), New Delhi, find an alarming prevalence of various diseases causing microbes in drinking water and recreational water. Different authors also reported that the Indian River system is polluted mainly because of the human impact.[2] The significance of water as a potent ecological factor can be appreciated only by studying its physicochemical and microbial characteristics. Major factors affecting the microbiological quality of surface waters are discharges from sewage works and runoff from informal settlements.[3] Indicator organisms are commonly used to assess surface waters' microbial quality, and fecal coliforms (FC) are the most commonly used bacterial indicator of fecal pollution. They are found in water that is contaminated with fecal wastes of human and animal origin.[4] Total coliforms (TC) comprise bacterial species of fecal origin as well as other bacterial groups (e.g., bacteria commonly occurring in soil). The coliforms are indicative of the general hygienic quality of the water and the potential risk of infectious diseases from water. High FC and TC counts in water are usually manifested in the form of diarrhea and sometimes by fever and other secondary complications. Bathing and swimming in streams and rivers are also common among children and adults in the local community.[5-11] The probability of ingesting an infective dose of disease-causing microorganisms is very high because water borne pathogens generally have low infective dose. These sources of bacterial contamination include runoff from feedlots, pastures, dog runs, and other

[1]Department of Higher Education, HP, Shimla, India

[2]M.Tech in Materials Science, University of Mysore, Karnataka, India

[3]Department of Botany, SILB, Solan Himachal Pradesh, India

Corresponding Author: Pathania S. Dhirendra, Department of Higher Education, HP, Shimla, India. E-Mail: dhirendra.pathania73@gmail.com

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land areas where animal wastes are deposited. Additional sources include seepage or discharge from septic tanks, sewage treatment facilities, and natural soil/plant bacteria. Insects, rodents, or animals entering the well are other sources of contamination. Old wells were dug by hand and lined (cased) with rocks or bricks. These wells usually have large openings and casings that often are not well-sealed. This makes it easy for insects, rodents, or animals to enter the well. Older water systems, especially dug wells, spring-fed systems, and cistern-type systems, are most vulnerable to bacterial contamination. Any system with casings or caps that are not water-tight are vulnerable. This is particularly true if the well is located so surface runoff might be able to enter the well. During the last 5 to 10 years, the construction of the well and water distribution system has improved to the point where bacterial contamination is rare in newer wells. The present study was undertaken to isolate the different microorganisms esp. coliform bacteria from water samples taken from different places of HP.[12-16]

MATERIALS AND METHODS

Collection of Samples (Table 1)

Different types of material like samples, Swab, Petri dish and other glasswares, burner, autoclave, hot air oven, incubator, laminar air chamber, sterile needle, sterile inoculation loop, cotton, glass slides, coverslips, sterile test tubes, durham tubes, micropipette, were used to perform various types of biochemical tests. Various media like nutrient agar medium, MacConkey Agar medium, etc., were used to grow the bacterial colonies. Gram stain was used to detect the various types of bacteria.[17-19]

For the biochemical studies, various tests like Indole, Methyl Red, Voges Proskauer, citrate utilization, catalase, and Carbohydrate fermentation were performed.

Table 1: Total 10 samples were collected from the different sources of H.P

S. No.	*Samples*	*Source*
1	Tap Water	Solan
2	Grassland Water	Solan
3	Handpump Water	Hamirpur
4	Well Water	Shimla
5	Well Water	Hamirpur
6	Rain Water	Solan
7	Beas River	Hamirpur
8	Sutlej River	Shimla
9	Ganga River	Haridwar
10	Sewage Water	Solan

Collection of Water Samples

Total 10 samples were collected from the different water sources of HP in sterilized bottles. For example, sample 1 is collected from tap water (Solan), sample 2 from grassland (Solan), sample 3 from handpump (Hamirpur), sample 4 from well (Shimla), sample 5 from well (Hamirpur), sample 6 of rainwater, sample 7 from Beas river, sample 8 from Sutlej river, sample 9 from Ganga river, the sample of sewage water (Figure 1).

Isolation of Bacteria

We isolate the microorganisms from the different water samples on the nutrient agar plates. These petri plates were incubated at 37°C for 48 hours in an incubator. After 24 hours' colonies were appeared on the nutrient agar plates (Figure 2).

Different colonies grow in a different manner on agar plates. Colony of well Hamirpur is a smooth, light cream color. Colony of Sutlej water is yellowish. Colony of rainwater is also creamish. The colony of handpump water grows vigorously. Colonies of rainwater and well Shimla is also creamish.

Identification of Bacteria

Gram's staining (Table 2)

Gram's staining is a common technique used to differentiate two large groups of bacteria based on their different cell wall constituents. The Gram's stain procedure distinguishes between gram-positive gram-negative groups by coloring these cells red, red or violet.

The result reveals that nine water samples may contain different bacteria: Bacillus subtilis, *Streptococcus* sp. staphylococcus epidermidis, staphylococcus aureus, Micrococcus lutes Enterobacter sp. Bacillus subtilis is found

Figure 1: Sterile bottles for water collection

in tap water and Ganges water sample (Table 3). It is a gram-positive bacterium and is non-pathogenic bacteria. Streptococcus sp. Is found in well (Shimla) and rain water sample. It is gram-positive bacteria and is also non-pathogenic. Staphylococcus epidermidis is gram-positive bacteria and found in Beas river sample. It is associated with intravascular diseases. Staphylococcus aureus is a gram-positive bacterium and found in hand pump and well (Hamirpur) water sample. It is an opportunistic pathogen. The bacteria are a leading cause of food poisoning, it is resulting from the consumption of food contaminated with enterotoxins. Food intoxication involved rapid onset of nausea, vomiting, abdominal pain, cramps, and diarrhea. Micrococcus luteus is gram-positive bacteria and found in Sutlej river water sample. Once it is not pathogenic but it is opportunistic pathogen in immunocompromisedpatients.

Enterobacter sp. is a gram-negative bacterium and found in a grassland water sample. It causes numerous infections, including cerebral abscess, pneumonia, meningitis and wound an abdominal cavity. *E. coli* is a gram-negative and is found in the sewage water sample

Most Probable Number (MPN)

The test is a method to estimate the concentration of viable microorganisms in a sample by means of replicate liquid broth growth in ten-fold dilutions and is particularly useful with samples that contain material that interferes with plate count enumeration methods (Figure 4).[19-21]

After 48 hours, there is acid and gas production in three test tubes of double strength and five test tubes of single strength. It gave an unsatisfactory grade to sewage water. The MPN count for sewage water is 1100. This shows that sewage water has a high quantity of *E.coli* and is highly contaminated with fecal matter (Figure 5).[22]

Cooke 1995 examined the drinking water for the presence or absence of *Salmonella*, *Citrobacter*, *E. coli,*

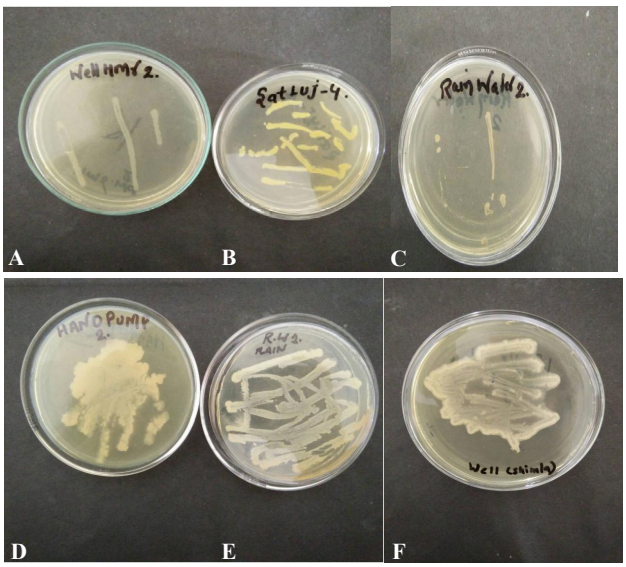

Figure 2: Nutrient agar plates (A-Well Hamirpur, B-Sutlej, C-Rain Water 2, D- Handpump Water, E-Rain Water 1, F-Well Shimla)

Table 2: Biochemical test for selected isolates

Samples / *Test*	*Tap water*	*Grassland water*	*Handpump water*	*Well Shimla*	*Well Hamirpur*
Gram's staining	+ve	-ve	+ve	+ve	+ve
Indole	-ve	-ve	-ve	-ve	-ve
Methyl red	+ve	-ve	+ve	+ve	+ve
Voges		+ve	+ve	-ve	+ve
Citrate	+ve	+ve	+ve	+ve	+ve
Nitrate	+ve	+ve	+ve	+ve	+ve
Catalase	+ve	+ve	+ve	-ve	+ve
Oxidase	+ve	-ve	-ve	+ve	-ve
			Fermantation test		
Glucose	+ve	+ve	+ve	+ve	+ve
Lactose	+ve	+ve	+ve	+ve	+ve
Mannitol	+ve	+ve	+ve	-ve	+ve
Sucrose	+ve	+ve	+ve	+ve	+ve
Identified bacteria	*Bacillus subtilis*	*Enterobacter sp.*	*Staphylococcus aureus*	*Streptococcus sp.*	*Staphylococcus aureus*

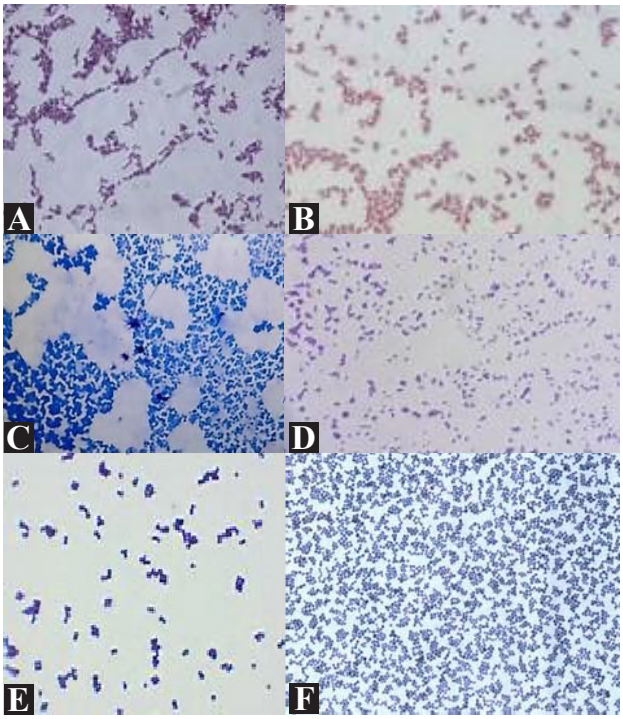

Figure 3: Gram's Staining (A-*Bacillus subtilis*, B-Enterobacter sp., C- *Staphylococcus aureus*, D-*Streptococcus* sp., E-Micrococcus luteus, F- *Staphylococcus* epidermidis)

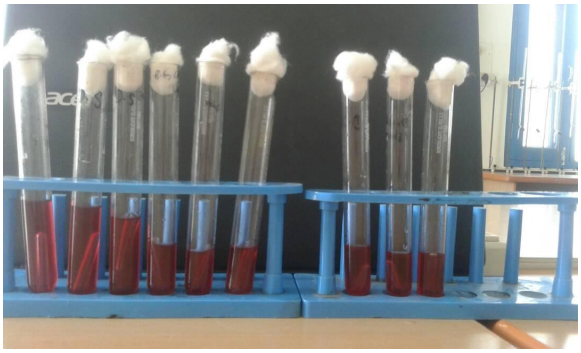

Figure 4: Control for MPN

Figure 5: Result for MPN after 48 hours

Table 3: Biochemical tests for selected isolates

Sample / *Test*	*Rain water*	*Beas water*	*Sutlej water*	*Ganga water*
Gram's staining	+ve	+ve	+ve	+ve
Indole	-ve	-ve	-ve	-ve
Methyl red	-ve	-ve	-ve	+ve
Voges	-ve	-ve	+ve	+ve
Citrate	+ve	+ve	-ve	+ve
Nitrate	+ve	+ve	+ve	+ve
Catalase	-ve	+ve	+ve	+ve
Oxidase	-ve	-ve	+ve	+ve
		Fermantation Test		
Glucose	+ve	+ve	+ve	+ve
Lactose	+ve	+ve	+ve	+ve
Mannitol	-ve	+ve	+ve	+ve
Sucrose	+ve	+ve	+ve	+ve
Identified bacteria	*Streptococcus sp.*	*Staphylococcus epidermidis*	*Micrococcus luteus*	*Bacillus subtilis*

and *Vibrio* species in the Indira Sagar/Omkeshwar project affected areas and rehabilitation/resettlement colonies of Sardar project in Madhya Pradesh.[3] Tambekar *et al.* (2008) revalidated the testing methods for assessing microbial safety of drinking water in the villages of Amrawati district of Maharashtra[4] for using bacteriological analysis with the

help of multiple tube fermentation techniques to determine the most probable number (MPN), membrane filter techniques, Eijekamn's test for thermotolerant coliform and Manja's Rapid hydrogen sulfide test for detection of fecal contaminations in drinking water.

CONCLUSIONS

A total of 10 samples were collected aseptically in a sterilized container from different water sources. Bacteriological examination based on the isolation of the microorganisms on the nutrient agar from the water sample.It was revealed that *Bacillus subtilis, Streptococcus sp.*, *Staphylococcus aureus Enterobacter sp. Staphylococcus epidermidis* and *Micrococcus luteus* were found in 9 samples. The sample which was collected from the grassland was contaminated with coliform. The sample, which was collected from the sewage, was highly contaminated with *E. coli.* Thus, the present study suggested that sources and water samples from Tap water, handpump, well were suitable for drinking. Another sample except grassland and sewage; were found suspicious hose standards could be improved and make them suitable for drinking. The samples which were collected from the Grassland and Sewage were highly contaminated with coliform.

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