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

Pathania Singh Dhirendra [1], Bhardwaj Payal [2], Pathania Singh Mamta [3]

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

[2]*Department of M. Tech in Material Sciences, University of Mysore, Karnataka, India.*

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

Corresponding Author:dhirendra_pathania73@gmail.com

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 faecal pollution of water due to the presence of coliforms bacteria because they are invariably present in faeces 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 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.

Key Words:— ***Living organisms, Faecal pollution, Drinking water, Chlorination.***

I. 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 (James *et al*., 2004). The rivers of India play an important role in the lives of the Indian people. Water resources are great significance for various activities such as drinking, irrigation, aquaculture and power generation. Report of the scientists at All India Institute of Medical Sciences (AIIMS), New Delhi, finds an alarming prevalence of various diseases causing microbes in drinking water and recreational water. Different authors also reported that Indian River system is polluted mainly because of the human impact (Bald win TJ,1998). Significance of water as a potent ecological factor can be appreciated only by studying its physio-chemical and microbial characteristics.

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Major factors affecting microbiological quality of surface waters are discharges from sewage works and runoff from informal settlements. Indicator organisms are commonly used to assess the microbiological quality of surface waters and faecal coliforms (FC) are the most commonly used bacterial indicator of faecal pollution. They are found in water that is contaminated with faecal wastes of human and animal origin. Total coliforms (TC) comprise bacterial species of faecal 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 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 river are also common among children and adults in the local community. The probability of ingesting infective dose of disease causing microorganism 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 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 five to 10 years, well and water distribution system construction has improved to the point where bacterial contamination is rare in newer wells. The present study was under taken to isolate the different microorganism esp. coliform bacteria from water samples taken from different places of H.P.

II. MATERIALS AND METHODS

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

Table.1. Collection of samples

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

Different types of material like samples, Swab, Petri dish and other glass wares, Burner, Autoclave, Hot air oven, Incubator, Laminar air chamber, Sterile needle, Sterile inoculation loop, Cotton, Glass slides, cover slips, 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.For the biochemical studies various test like Indole test, Methyl Red test, Voges Proskauer Test, citrate utilization test, catalase test, Carbohydrate fermentation were performed.

2.1 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 rain water, sample 7 from Beas river, sample 8 from Sutlej river, sample 9 from Ganga river, sample of sewage water (Fig. 1)

Fig.1. Sterile bottles for water collection**.**

2.2 Isolation of Bacteria

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

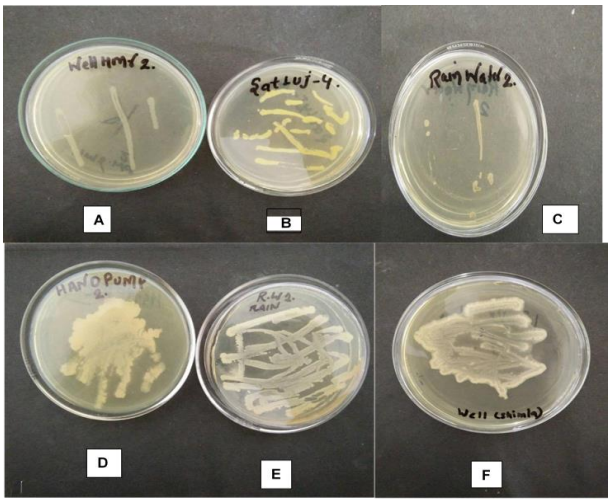


Fig.2. Nutrient agar plates (A-Well Hamirpur, B-Sutlej, C-Rain Water 2, D- Handpump Water, E-Rain Water 1, F-Well Shimla)

Different colonies grow in different manner on agar plates. Colony of well Hamirpur is smooth, light cream colour. Colony of Sutlej water is yellowish. Colony of rain water is also

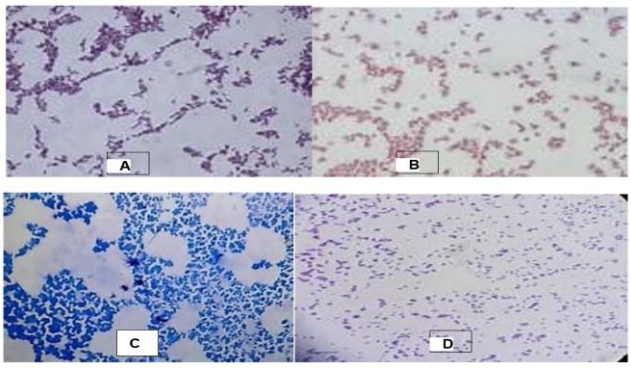
creamish. Colony of handpump water grows vigorously. Colonies of rain water and well Shimla is also creamish.

- Identification of bacteria
- Gram's staining (Table 2)

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
FERMANTATI- ON 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*

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



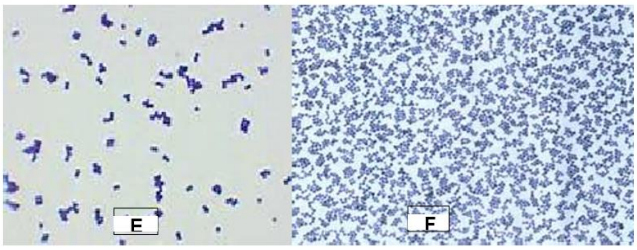


Fig.3. Gram's Staining (A-Bacillus subtilis, B-Enterobacter sp., C-Staphylococcus aureus, D-Streptococcus sp., E-Micrococcus luteus, F- Staphylococcus epidermidis).

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*

The result reveals that nine water sample may contain different kind of bacteria that are *Bacillus subtilis*, *Streptococcus sp. staphylococcus epidermidis*, *staphylococcus aureus*, *Micrococcus lutes*, *Enterobacter sp. Bacillus subtilis* is found 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 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, resulting from 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 immunocompromised patients.

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

2.3 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 (Fig.4).

After 48 hours, there is production of acid and gas in three test tubes of double strength and five test tubes of single strength. It gave unsatisfactory grade to sewage water. The MPN count for sewage water is 1100. This show that sewage water has high quantity of *E.coli* and highly contaminated with faecal matter.

Fig.4. Control for MPN.

Fig.5. Result for MPN after 48 hrs.

Cooke 1995, have examined the drinking water for the presence or absence of *Salmonella*, *Citrobacter*, *E. coli* and *Vibrio* species in the Indira Sagar/Omkeshwar project affected areas and rehabilitation/ resettlement colonies of Sardar project in Madhya Pradesh. Tambekar *et al* (2008) revalidated the testing methods for assessing microbial safety of drinking water in the villages of Amrawati district of Maharashtra for using bacteriological analysis with the help of Multiple Tube fermentation technique to determine most probable number (MPN), Membrane filter techniques, Eijekamn's test for thermotolerant coliform and Manja's Rapid hydrogen sulphide test for detection of faecal contaminations in drinking water (Fig 5).

III. CONCLUSIONS

A total of 10 sample 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 Enterobactor sp. Staphylococcus epidermidis* and *Micrococcus luteus* were found in 9 samples. The sample which was collected from the Grassland was contaminated with the coliform. The sample which was collected from the sewage, was highly contaminated with the *E. coli*. Thus, the present study suggested that sources and water samples from Tap water, Handpump, well were suitable for drinking. Other sample except Grass land 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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