Detection and Enumeration of Coliforms in Ganga Water Collected from Different Ghats

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Abstract

The current investigation was done to detect and enumerate coliforms at different ghats of Ganga River. The project aims to check the bacteriological quality of water and to check resistant profile of most prevalent isolates. These samples were processed and analysed for their quality with respect to various microbiological aspects. Methods used for the investigation of coliform are MPN method. According to the method all water samples collected from different Ghats were unsafe. The water was mainly contaminated by coliform bacteria Escherichia, followed by Klebsiella, Enterobacter and Citrobacter. 29 different species were identified from ten different ghats. Drug susceptibility of isolates revealed that highest susceptibility was seen in Tetracycline and ciprofloxacin.

Keywords: Klebsiella; Enterobacter; Coliform; Drug susceptibility

Introduction

Water is elixir of life. Quality water is vital to the health, social and economic wellbeing of people. Although contaminants in water are divided roughly into three categories viz, physical, chemical and biological. Environmental risk assessment today reveals that the exposure to biological contaminants especially water-borne pathogens needs to be given higher priority in treatment and regulatory programs for domestic water supplies. Diseases that spread through the contaminated water principally in areas of poor sanitation are Hepatitis-A, Hepatitis-E, Typhoid fever, diarrhea and dysentery etc [1]. Water which looks and tastes good may not necessarily be safe to drink it may be polluted with harmful bacteria, parasites and viruses [2]. These microbes can exist in surface and ground water supplies and can cause immediate sickness in humans if not properly treated.

In India, the River Ganga is the longest and most important river that passes along 29 cities, 23 class II cities and approximately 50 towns because of which wastes of different types such as industrial, sewage etc. are discharged into this mighty river eco-system. Ganga, the mighty Indian River originates from the snowed peaks of Himalayas, is the lifeline of millions of Indians. From its source to its entry in to the Bay of Bengal, it travels a distance of around 2525 Km [3]. The river with its well-knit tributaries drains the Ganga Basin which encompasses an area of more than a million square kilometers (1060, 00 sq km) spread over four countries- India, Nepal, Bangladesh and China. Haridwar is a city in Northern India on the bank of the Ganga River north east of Delhi. It is a Hindu pilgrimage center. Haridwar lies along the Ganga River at the boundary between the Indo-Gangetic plain (South) and the Himalayan foothills (North). The water supply of the Ganga system is partly dependent on the rains brought by the monsoon winds from July to October as well as on the flow from melting Himalayan glaciers in the hot season from April to June.

The religious importance of Ganga may exceed that of any other river in the world. During festivals people take a holy dip in the river and also use it for drinking, irrespective of its water quality [4]. The root of the problem lies in the fact that there is a conflict between the belief systems of people, and the strategies applied to de-pollute the river.

The organic matter contribution during mass bathing is normally significant, as revealed by earlier studies. Traditionally, indicator micro-organisms have been used to suggest the presence of pathogens. Coliform bacteria are used as indicators for water quality. Freedom from contamination with fecal matter is the most important parameter of water quality because it is generally considered to be a greater risk to human health as it is more likely to contain dangerous enteric pathogens [5]. Presence of indicator bacteria in water bodies as a sign of fecal contamination suggests the potential danger of health risk. The higher the level of indicator bacteria, the higher the level of fecal contamination and the greater will be the risks of water borne diseases. A previous study made on the impact of mass bathing on river water quality at Haridwar revealed that bacteriological parameters such as total coliforms and faecal coliform bacteria were found to be exceptionally high in comparison to their standard values, during Ardh Kumbh Mela.

Population pressures, lack of proper investment in water quality infrastructure, limited governmental initiatives, and a lack of empowerment of the people all continue to contribute to the deteriorating state of the Ganga. Till date, there have been various studies on river water quality with reference to geochemistry and pollution, and suitability assessment of groundwater for different uses.

The water from River Ganga is used for irrigation primarily; and as a source for drinking water and industrial applications. It, therefore, becomes imperative to assess the suitability of water for different uses. The present study has been undertaken to assess the water quality of Ganga at different Ghats of Rishikesh town and Haridwar for its suitability for anthropogenic uses. Rishikesh, surrounded by virgin forests at the toe of the Himalayas, is the first town on River Ganga...
taken up under the Ganga Action Plan (GAP) Phase-I for pollution abatement of the river.

Rishikesh Located in the foothills of the Himalayas in northern India, it is known as The Gateway to the Garhwal Himalayas. It is located approximately 25 km north of the city Haridwar.

Rishikesh, unlike other cities, is the first pilgrimage-cum-tourist destination after the river enters plains, and upstream-located industrial/anthropogenic sources of pollution which may affect the water quality are absent. Any addition upstream of Rishikesh may be treated as natural [6]. Since natural flow of water in winters is lean; and tourist activity during December to April is more, it may affect water quality adversely particularly during this period [7]. The present study was, therefore, carried out from January 2017 to April 2017 to study the designated best use of water, and its suitability for different usages based on various indices of water quality. The Himalayan Rivers have an important place in Indian culture and tradition [8]. They are the lifeline of majority of population in cities, towns and villages and are considered sacred. Haridwar is a city in Northern India on the bank of the Ganga River. It is a Hindu pilgrimage center [9]; Haridwar lies along the Ganges River at the boundary between the Indo-gangetic plain and the Himalayan foothills. The water supply of the Ganga system is partly dependent on the rains brought by the monsoon winds from July to October as well as on the flow from melting Himalayan glaciers in the hot season from April to June [10]. Har ki pauri in Haridwar is the area where thousands of pilgrims congregate, and the festivities commence. Bathing activities continue for most part of the year but peak in summer. Near the Harki-Pauri faecal matter, decaying leaves, flowers, wooden parts, waste cloths, food material, ashes, and charcoal can be observed in the Ganga at this point which certainly affects the microbiological parameters of Ganga water. The main objective of the present investigation was to estimate coliforms (Indicators of biological pollutions) at different Ghats (bathing place of pilgrims) during winters when water comparatively remains clear than the warmer part of the river. Secondly the Ghats were located after the water falling from local tributaries of Ganga River. The coliform count is being estimated by Rawat, 2017 [11-16].

Materials and Methods

Study area

The River Ganga represents a highly polluted aquatic environment which receives direct discharges of domestic, commercial, industrial and agricultural wastewaters. The river water samples were collected from different Ghats of Haridwar (29°C 58’ 0” N 78°C 10’ 0” E) and Rishikesh (30°C 7’ 0” N 78°C 19’ 0” E), Uttarakhand, India. Depending on the religious importance and pilgrim pressure, ten Ghats of the river Ganga, were selected for the present study mentioned in given Table 1.

Sample collection

Sampling locations were identified on the basis of prominent activities of bathing, washing, and addition of sewage/wastewater into the river. The water samples were taken from ten different Ghats (Table 1). The sampling was done by immersion type of sampling method from 30 cm depth of water body and in pre-rinsed sterilized glass bottles. 200 ml of water sample is collected in glass bottles. During collection of samples, extreme care was exercised to avoid contamination.

Table 1: Brief description of places (Ghats) from where the samples were collected.

| Sample No. | Sampling point       | Sampling code |
|------------|----------------------|---------------|
| 1          | Ganesh Ghat (Haridwar)| G1            |
| 2          | Triveni Ghat (Rishikesh)| G2          |
| 3          | Ram Ghat (Rishikesh)  | G3            |
| 4          | Laxman Ghat (Rishikesh)| G4          |
| 5          | Saptrishi Ghat (Haridwar)| G5        |
| 6          | Kankhal Ghat (Haridwar) | G6            |

Microbiological analysis of water samples

Most probable number (MPN) technique: The number of total coliforms (Enterobacter, Citrobacter, Klebsiella, and Escherichia) in a water sample can be determined by statistical estimation called the most probable number (MPN) test. This test involves a multiple series of Durham Fermentation tubes and is divided into three parts: The presumptive, confirmed and completed tests.

Presumptive test: This test was performed according to the method given in APHA. In addition to determining the presence or absence of coliforms, the presumptive test was also used to determine the most probable number of coliforms present in the sample

Procedure: Fermentation tubes containing 10 ml of single strength lactose broth (SSLB) and double strength broth (DSLB) were prepared. A Durham's tube was placed in each tube in an inverted position. The tubes were labelled according to the amount of water sample to be dispensed into it i.e., 10 ml, 1 ml and 0.1 ml. The water sample was homogenized by shaking the bottle containing the water vigorously several times. Aliquots of 10 ml of the water sample were transferred to each of the 5 DSLB tubes using a 10 ml pipette, with a micropipette, 1 ml and 0.1 ml of the water sample were transferred to batches of 5 ml SSLB tubes each [17-20]. The tubes were incubated at 37 ± 1°C for 24 hrs and the number of tubes in each set that produced 10% or more gas with a change in colour from purple to yellow was recorded. MPN was determined by referring to the MPN table by Mackie and McCartney.

Confirmatory test: Presumptive test was confirmed by the method described by Benson. The confirmed stage is performed on all primary fermentation tubes which results in gas formation after the 24-hr and 48-hr periods. Fermentation tubes containing brilliant green lactose bile broth were inoculated with medium from the tubes showing a positive result in the presumptive test [21-27]. The inoculated tubes were incubated for 48+3 hr at 35±0.5°C.  

Procedure: A loopful of culture from positive tubes was transferred to a fermentation tube containing brilliant green lactose bile broth. The inoculated brilliant green lactose bile broth tubes were incubated for 48+3 hrs. at 35 ± 0.5°C. Number of tubes that produced gas was recorded.

Complete test: The completed test is performed on all samples showing a positive result in the confirmed test [28-30]. Plates of eosin methylene blue are streaked with sample to be analyzed. The streaked plates are incubated for 24+2 hrs. at 35 ± 0.5°C. After incubation colonies (nucleated, with or without metallic sheen) were transferred.
to a nutrient agar slants which were incubated at 35 ± 0.5°C for 24±2 hr. From the agar slants gram staining was done and examined microscopically [31-35]. The presence of gram-negative, non-sporforming, rod-shaped bacteria in the agar culture were considered as a satisfactorily completed test, demonstrating the positive presence of coliform bacteria in the analyzed sample.

Procedure: Eosin methylene blue plates from each tube of brilliant green lactose bile broth showing were streaked. Plates were incubated at 35±0.5°C for 24±2 hr. After incubation well-isolated coliform colonies were observed [36-38]. The colonies developed on eosin methylene blue agar were called: typical (nucleated, with or without atmospheric oxygen). The colonies developed on eosin methylene blue agar were: atypical (opaque, nucleated, mucoid, pink after 24-hr incubation); or negative (all others).

Identification of bacterial isolates

Different bacterial isolates were characterized by cultural, morphological and further identified by biochemical examination in accordance with Bergey's Manual of Determinative Bacteriology [39-41].

Culture purification: A pure culture of bacteria was obtained. With the help of an inoculating needle a small portion from the top of a well isolated colony was carefully picked which was grown on EMB agar and later streaked on nutrient agar plates. The plates were incubated at 37 ± 1°C for 24 hr and a single well isolated colony was tested by staining.

Morphological characterization of isolated strains: For morphological characterization of isolates gram staining was performed and motility testing of the isolated strains of bacteria was performed as per given in APHA.

Biochemical characterization of isolated bacterial strains

IMViC tests: Standard methods to perform IMViC test were given in APHA. The traditional IMViC test i.e., Indole, Methyl red, Voges-Proskauer and Citrate utilization are biochemical tests and were used to differentiate the coliforms.

Indole test: Indole test determines the ability of bacteria to hydrolyze tryptophan to its metabolic product namely Indole. The source of tryptophan in the culture medium is the peptone. The indole thus formed accumulates in the medium and is detected by a reagent producing cherry red color.

Procedure: The media was dispensed in 5 ml portion in test tubes and sterilized by autoclaving at 121°C for 15-20 minutes. Then 5 ml of medium were inoculated from a pure culture and incubated at 37°C for 24-48 hours. To this 0.5 ml of Kovac's reagent were added and results were observed. A distinct red colour was methyl red positive and distinct yellow colour was methyl red negative.

Voges-Proskauer test: The Voges-Proskauer (VP) test is used to determine if an organism produces acetyl methyl carbinol from glucose fermentation. If present, acetyl methyl carbinol is converted to diacetyl in the presence of α- naphthol, strong alkali (40% KOH), and atmospheric oxygen. The α-naphthol was not part of the original procedure but was found to act as a color intensifier by Barritt and must be added first. The diacetyl and quaniidine-containing compounds found in the peptones of the broth then condense to form a pinkish red polymer.

Procedure: The media was dispensed in 5 ml portion in test tubes and sterilized by autoclaving at 121°C for 15-20 minutes. Then 5 ml of medium were inoculated from a pure culture and incubated at 37°C for 24-48 hours. Then 0.6 ml of α-naphthol and 0.2 ml of potassium hydroxide solution was added. The tubes were kept standing for 5-10 minutes and the results were observed. Development of red constituted a positive test.

Citrate utilization test: Citrate test is used to determine if the bacteria is capable of utilizing citrate as the sole source of carbon for metabolism resulting alkalinity.

Procedure: The Simmons's Citrate Agar slants were streaked with a pure culture with the help of inoculation loop. The slants were incubated for 48 hrs at 37 ± 1°C and the results were observed. Growth on the medium with a change in colour of the medium from green to blue constituted a positive test. No change in colour showed a negative test.

Triple Sugar Iron Agar (TSIA) test:

This test is used to determine the ability of bacteria to ferment sugars and to produce hydrogen sulphide.

Procedure: Triple sugar iron agar slants were prepared having thick butt. Thereafter, the isolated organism was inoculated with the help of inoculating needle to a TSIA by first streaking the surface of the slant and then stabbing the medium in the butt region. Slants were incubated at 37°C for 24 hrs. After 24 hrs slants were observed for production of acid and gas. Yellow color both in butt and in the slant means lactose is fermented. Some organisms generate gases, which results in bubbles/cracks on the medium. Yellow butt and red slants demonstrate that lactose is not fermented only glucose is fermented. If H2S is produced, the black color of ferrous sulphide is seen. Absence of color change means negative results.

Mannitol motility test: This test is used to detect the ability of bacteria to ferment mannitol and produce nitrogen gas; and to indicate the motility of the organism.

Procedure: Mannitol motility medium was prepared with a thick butt only. Thereafter, the isolated organism was inoculated with the help of inoculating needle and incubation is given at 37°C for 24-48 hrs and the results were observed. Growth on the medium with a change in colour of the medium from red to yellow constituted a positive test. No change in colour showed a negative test.

Antibiotic susceptibility test

Antibiotic resistance of bacteria of Enterobacteriaceae family was determined by the disc diffusion method. The isolates were screened against five antibiotics such as Gentamicin, Ciprofloxacin,
Tetracycline, Polymyxin, and Penicillin. A bacterial suspension of overnight grown cultures was prepared and turbidity was adjusted to a 0.5 McFarland standard. A sterile cotton swab was used to inoculate the bacterial suspension on the surface of a Mueller Hinton Agar plate. The bacterial isolates were scored as susceptible, intermediate or resistant to a given antibiotic by the inhibition zone diameter around the antibiotic disc.

**Antibiotic sensitivity test:** After confirming the identity of isolates as coliforms by tested for susceptibility resistance to number of antibiotics by disc diffusion method, using Muller-Hinton agar assay medium.

**Preparation of 0.5% McFarland standard:** Solution A were prepared by adding 1 ml of Barium chloride (BaCl₂·2H₂O) to 100 ml distilled water and solution B by adding 1 ml of Sulphuric acid H₂SO₄ (0.3N) to 100 ml of distilled water. Then 0.5 of solution A was added to 99.5 ml of solution B mixed well and distributed in test tubes with screw cap.

**Results and Discussion**

The present study was undertaken to gather information regarding the impact of human activities, particularly bathing on water of the river Ganga. Study on microbiological characteristics of the water of the river was done.

Water bodies play an important role in human development and river is one of the important potable water supply sources. Also, rivers provide land fertility and transportation medium [42]. A considerable amount of pollution in the river is caused by the domestic sources.

**Most Probable Number (MPN) of coliforms**

Coliforms count performed by multiple tube fermentation to determine the most probable number (MPN) is commonly used as indicator of potability of water. It gives us statistical estimate of coliforms population and measured the extent of coliforms in 100 ml water. Coliforms produces gas and acid hence the production of gas in Durham tube, constituted positive test [43].

It is generally believed that more is the MPN of coliforms, higher is the extent of pollution in a given sample indicating heavy bacterial load and contamination.

The main sources of organic burden of sewage in most communities are human body waste or faeces and microorganisms themselves are a part of human faeces. When water sample is tested, it is not feasible to identify all water borne pathogens. The solution is to look for indicator organism i.e., coliforms group of bacteria which contain *E. coli*, *Enterobacter aerogenes*, *Klebsiella pneumoniae*. During the study plates were incubated at 37°C and 44.5°C.

All the water samples collected during the study were positive with respect to the coliforms occurrence well above the permissible limits, though the counts were variable. It is generally believed that more is the MPN of coliforms, higher is the extent of pollution in a given sample.

Distribution of coliforms was highly variable of different ghats of Haridwar and Rishikesh. For the Ghat1, coliform count 1600 MPN/100 ml was observed. For Ghat 2 coliform count 350 MPN/100 ml was observed (Figure 1). For Ghat 3 coliform count 1600 MPN/100 ml was observed. For Ghat 4 coliform count 350 MPN/100 ml was observed. For Ghat 5 coliform count 350 MPN/100 ml was observed. For Ghat 6 coliform count 280 MPN/100 ml was observed. For Ghat 7 coliform count 280 MPN/100 ml was observed. For Ghat 8 coliform count 280 MPN/100 ml was observed. For Ghat 9 coliform count 540 MPN/100 ml was observed. For Ghat 10 coliform count (1600 MPN/100 ml) was observed (Table 3).

### Table 2: List of antibiotics Octodiscs for antimicrobial drug susceptibility Himedia testing.

| Antibiotics | Symbol | Disc contents | Manufacturer |
|-------------|--------|---------------|--------------|
| Tetracycline | TE     | 30 mcg/disc    | Hi media     |
| Gentamicin   | GEN    | 10 mcg/disc    | Hi media     |
| Penicillin   | P      | 2 units/disc   | Hi media     |
| Ciprofloxacin| CIP    | 5 mcg/disc     | Hi media     |
| Polymyxin   | PB     | 50 units/disc  | Hi media     |

**Procedure:** The inoculum was prepared by adding a saline suspension of isolated colonies selected from an 18-24 hour agar plate. The suspension is adjusted to match the 0.5 McFarland turbidity standards, using saline and a vortex mixer. Then it was spread on Petriplates on Muller-Hinton agar and after 10-15 min, discs of different antibiotics (Hi media) to be tested were placed on surface of seeded petriplates. Plates were observed for zones of inhibition, after 24 hrs of incubation at 37°C. Zone diameter was measured in mm.

The organisms are inoculated into nutrient broth and incubated at 37°C overnight. Broth culture was then spread on Petri plates on Muller-Hinton agar and after 10-15 min, discs of different antibiotics (Table 2) to be tested were placed on surface of seeded petriplates [41]. Plates were observed for zones of inhibition, after 24 hrs of incubation at 37°C. Zone diameters were measured in mm.
Table 3: Most Probable Number (MPN) of total coliforms in Ganga Water Samples (per 100 ml water) taken from Haridwar and Rishikesh.

|   |   |   |   |   |
|---|---|---|---|---|
|2  | G2 | Triveni Ghat (Rishikesh) | 31th Jan 2017 | 08:13 | 350 |
|3  | G3 | Ram Ghat (Rishikesh)     | 7th Feb 2017   | 08:27 | 1600 |
|4  | G4 | Laxman Ghat (Rishikesh)  | 15th Feb 2017  | 09:17 | 350  |
|5  | G5 | Saptarshi Ghat (Haridwar)| 21th Feb 2017  | 08:04 | 350  |
|6  | G6 | Kankhal Ghat (Haridwar)  | 5th Mar 2017   | 09:27 | 280  |
|7  | G7 | Luvkush Ghat (Haridwar)  | 11th Mar 2017  | 08:49 | 920  |
|8  | G8 | Sai Ghat (Rishikesh)     | 29th Mar 2017  | 07:30 | 280  |
|9  | G9 | Vishnu Ghat (Haridwar)   | 5th Apr 2017   | 07:47 | 540  |
|10 | G10| Har ki Pauri (Haridwar)  | 11th Apr 2017  | 07:36 | 1600 |

Bacterial morphology on selective media (EMB agar)

_E. coli_ colonies were identified as those that showed green metallic sheen on EMB agar while colonies that form colonies other than green metallic sheen were identified as other coliforms. The morphology of _E. coli_ colonies on EMB agar is shown on Figure 2 and morphology of _Klebsiella pneumoniae_ colonies on EMB agar is shown on Figures 3 and 4.
Morphological studies

Gram’s staining: Gram staining reactions were studied with all the isolated culture to determine their morphology; all isolates were Gram negative bacilli (Figure 5).

Biochemical characterization of isolates from various Ganga water samples

The water samples were spread on the plates containing EMB medium or MacConkey Medium and Nutrient Agar medium according to the test required and the colonies that appeared after 24-48 hours were picked up based on their characteristics. Biochemical tests like IMVC, TSIA test, motility test and test for production of Urease, Catalase, and Oxidase etc. were performed (Table 4).

Out of the different isolates from samples including control, most predominant genera were Escherichia, Citrobacter, Enterobacter, Klebsiella. There was not much variation in the type of organism in the test sample (Figure 6). The predominant organisms were mostly members of Enterobacteriaceae family which constitute the normal flora of human enter on.
| Sample No. | Gram staining | Catalase | Oxidase | Indole test | Methyl Red test | Voges Proskauer test | Citrate test | Urease test | Probable organism |
|------------|---------------|----------|---------|-------------|-----------------|----------------------|--------------|-------------|------------------|
| G1         | -             | +        | -       | +           | +               | -                    | -            | -           | E. coli          |
|            | -             | +        | -       | -           | +               | +                    | +            | +           | Klebsiella       |
| G2         | -             | +        | -       | -           | *               | +                    | +            | +           | E. coli          |
| G3         | -             | +        | -       | +           | +               | -                    | -            | -           | E. coli          |
|            | -             | +        | -       | -           | +               | +                    | V            | +           | Enterobacter      |
| G4         | -             | +        | -       | -           | +               | V                    | E. coli      |             |                  |
| G5         | -             | +        | -       | +           | +               | -                    | -            | -           | E. coli          |
| G6         | -             | +        | -       | +           | +               | -                    | -            | -           | E. coli          |
|            | -             | +        | -       | -           | +               | +                    | V            | +           | Klebsiella       |
| G7         | -             | +        | -       | +           | +               | -                    | -            | -           | E. coli          |
|            | -             | +        | -       | -           | +               | +                    | Klebsiella   |             | Citrobacter       |
| G8         | -             | +        | -       | -           | +               | +                    | Klebsiella   |             |                  |
| G9         | -             | +        | -       | -           | +               | V                    | E. coli      |             | Klebsiella       |
| G10        | -             | +        | -       | +           | +               | -                    | -            | -           | E. coli          |

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In vitro antimicrobial susceptibility testing

In vitro antibiotic susceptibility of isolates was performed against seven different antibiotics such as Tetracycline, Gentamicin, Penicillin, Ciprofloxacin, Polymyxin, Cefepime. The results of the susceptibility testing are given in Table 5.

| S.No | Sample No. | Antibiotics |
|------|------------|-------------|
|      |            | TE | GEN | P | CIP | IPM | CPM |
| 1    | G1         | 19 | 22  | - | 27  | 26  | 16  |
| 2    | G2         | 18 | 20  | - | 28  | 27  | 15  |
| 3    | G3         | 19 | 19  | - | 32  | 24  | 16  |
| 4    | G4         | 22 | 20  | - | 29  | 20  | 14  |
| 5    | G5         | 21 | 22  | - | 25  | 19  | 13  |
| 6    | G6         | 22 | 23  | - | 27  | 29  | 16  |
| 7    | G7         | 19 | 13  | - | 29  | 22  | 16  |
| 8    | G9         | 20 | 20  | - | 24  | 24  | 16  |

Table 5: Zones of inhibition (mm) for various antibiotics against *E. coli* spp.

For isolates of *E. coli*, Tetracycline and ciprofloxacin showed 100% sensitivity. Gentamycin showed 87.5% sensitivity. 100% of isolates were resistant to Penicillin. 75% of isolates were intermediate to cefepime. 87.5% of isolates were resistant to Polymyxin (Figure 7).

Summary and Conclusions

The present study entitled, “Detection and Enumeration of coliforms in Ganga water collected from different Ghats” was carried out in the Department of Microbiology, Sardar Bhagwan Singh (PG) Institute of Biomedical Science and Research, Balawala, Dehradun (UK) during 2016-2017.

Various samples were collected from the river at different Ghats of Haridwar and Rishikesh. These samples were collected when innumerable people take bath in the holy river. These samples were processed and analyzed for their quality with respect to various microbiological aspects and compared with prescribed standards. About 10 total different samples were taken and subjected to microbiological and biochemical tests for following objectives:

- To check the bacteriological quality of Ganga water at different Ghats;
- To check the resistant profile of most prevalent isolates;
- To differentiate various coliforms in water sample.

Method used for the investigation of coliforms is MPN method. It is based on the bacteria ability to produce gas when grown in Lactose broth medium at 37°C. The MPN values for coliforms ranged between 280 and 1600/100ml. IMV iC tests were carried out to differentiate the source of contamination in Ganga water. The results of present investigation are summarized below:

- According to MPN for detection of coliforms, all water samples collected from different Ghats were unsafe.
• The water was mainly contaminated by coliform bacteria Escherichia, followed by Klebsiella, Enterobacter and Citrobacter.

• Twenty nine different species were identified from ten different samples.

• Reduced MPN index was recorded in G6(Kankhal Ghat) and G8(Sai Ghat).

Thus, it can be concluded that water sample collected from different ghats of Haridwar and Rishikesh were unsafe. Variation of coliforms occurrence is entirely dependent on temperature.

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