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ANALYSIS OF THE SOURCE WATER OF THE VRISHABHAVATHI RIVER IN BENGALURU CITY: COMPARISON WITH BOTTLED GANGA WATER

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Abstract
The Vrishabhavathi is the only river in urban Bengaluru that originates and flows through the western - southwestern part of the city. This study compared the source water of the Vrishabhavathi River at Sri Dakshinamukhi Nanditeertha temple with bottled Ganga water. Vrishabhavathi water showed neutral pH, low BOD/COD, and only Gram-positive soil bacteria, with no coliforms, bacteriocins, or bacteriophages, indicating that it is microbiologically clean. In contrast, Ganga water contained extremely high bacterial and coliphage counts. Neither sample exhibited antibacterial activity against E. coli or S. aureus. The results highlight the purity of Vrishabhavathi water at its origin and provide a baseline for river quality monitoring and conservation efforts.

Keywords: Water pollution, Vrishabhavathi, Ganga river, bacteriophage

1. INTRODUCTION

Water is essential for life but increasing urbanization, pollution and poor wastewater management have severely degraded river systems of major rivers as well as smaller urban rivers in India. Contamination from sewage, heavy metals and pesticides render such water unpotable (Singh *et al*., 2022). Interestingly, the Ganga shows unique self-cleaning due to the presence of bacteriophages and bacteriocins (Tyagi and Dubey, 2020). Despite the presence of coliforms even in the headwaters, this water is comparatively safe for consumption due to a high coliphage count. Bengaluru city's only river – the Vrishabhavathi – is heavily polluted all along its course and has been studied by various groups (Ramachandra *et al*., 2017; Madhukar and Srikantaswamy, 2013). Yet, the source water at the Sri Dakshinamukha Nanditeertha temple in Malleswaram which is distributed as *teertha* to devotees, has no microbiological documentation of the water quality.
In this study, source water from Sri Dakshinamukha Nanditeertha temple and bottled Ganga water were analysed for physicochemical properties, bacterial profile, bacteriocins and bacteriophages using standard microbiological and biochemical methods.

2. MATERIALS AND METHODS

2.1 Sample collection

The bottled *teertha* available at the Sri Dakshinamukha Nandi teertha temple was used in the study. This water is collected straight from the mouth of the Nandi idol before it flows into the *kalyani*. Two samples – one in January-February and the other in April-May – were used in the study. Ganga water samples were sourced from Kashi (Varanasi).

2.2 Physico-chemical properties of water samples

The pH, conductivity, total dissolved solids and temperature were measured using a hand – held Hanna 1500 meter.

2.3 Biochemical oxygen demand (BOD):

(Modified from Sawyer and McCarty, 1978)
Water samples were tested for 0^{th} and 5^{th} day after incubation in the dark at 20°C. Briefly, dilutions were made by

adding 5 ml of sample water to 295 ml of 2mL/L of phosphate buffer (pH 7.2), $MgSO_4.7H_2O$, $CaCl_2$, $FeCl_3$ and $NaSO_3$ solutions. The oxygen demand in the water was determined by titration against sodium thiosulfate solution (0.25N) using starch as an indicator. The dissolved oxygen (DO) was calculated from the formula that 1 mL of sodium thiosulfate (0.025N) equals to 1 mg/L dissolved oxygen. Therefore, Dissolved oxygen (DO) (in mg/L) = mL of sodium thiosulfate (0.025N) consumed. BOD was calculated as:

BOD = [DO (day 5) – DO (day 0)] / Volume of sample

2.4 Chemical oxygen demand (COD): (Modified from Sawyer *et al*., 2000).

10mL of water sample was refluxed with 1 mL of potassium dichromate in sulphuric acid medium and the excess of dichromate was titrated against ferrous ammonium sulphate (FAS) using ferroin as an indicator. COD was calculated as:

$$COD\ (mg\ O_2\ /L) = [(A-B) \times M \times 8000)] / (V\ sample)$$

Where: A= volume of FAS used for blank (mL); B = volume of FAS used for sample (mL) M = molarity of FAS and 8000 = milli equivalent weight of oxygen (8) ×1000 mL/L.

2.5 Isolation of bacteria

Dilutions of the water samples were made in normal saline (0.9% NaCl w/v). 100µL of the 10^7 and 10^8 dilution was plated on both Luria agar and MacConkey agar. Lactose fermenters were identified by the appearance of red colonies on MacConkey agar.

2.6 Colony characterization of bacteria

Bacterial colonies were characterized by their size, shape, colour, elevation, margin and texture (Cappuccino and Sherman, 2014). Gram staining was done as per the protocol using crystal violet as a stain and safranin as a counter stain.

2.7 Biochemical characterization of the bacteria:

The biochemical tests carried out to characterise the bacteria are listed in Table 1.

Table 1. Biochemical test for the characterisation of bacteria in the water samples.

Test	Procedure	Interpretation
idase test	cterial suspension was streaked on to Whatman No 1 filter paper soaked with α-naphthol in ethanol.	velopment of red colour indicates a positive test.
alase test	cterial suspension was taken on a clean slide and a few drops hydrogen peroxide added to it.	ervescence indicates a positive test.
tease test	cterial suspension was streaked on Luria agar plates containing milk powder.	arance zone around the streak indicates a positive test.

2.8 Anti-microbial activity: Bacteriocin screening

E. coli (Gram negative) and *Staphylococcus aureus* (Gram positive) cultures were used to test the anti-microbial activity. The cultures were spread on nutrient agar with a central well and 20µL of the dilution was added. The plates were incubated in an upright position to enable diffusion and the anti-microbial activity determined by a zone of clearance around the well. Ampicillin (1mg/mL) was used as a control.

2.9 Detection of bacteriophage

Screening of coliphages was performed with *E. coli* (DH5α) as the host strain using the double layer agar method with dilutions of water samples. Phages were classified based on plaque morphology.

3. RESULTS AND DISCUSSION

Most river water carries some microflora even at their source. Even glacial melts which are the source of many rivers have shown the presence of both bacteria and viruses (Baghel *et al*., 2004; Kumar *et al*., 2021).

The Vrishabhavathi river in Bengaluru city was first reported to originate at the bull temple in Basavanagudi

(Ramachandra *et al*., 2017), then linked to a place in Peenya (Basha and Ramya, 2021). One of the other places associated with the source of the river is the Sri Dakshinamukha Nanditeertha temple, Malleswaram, a temple excavated in 1998. Here, water flows out of the mouth of the Nandi idol placed above a Shivaling. The same water is distributed as *teertha* to the devotees and then flows into the temple *kalyani*. An underground stream from this location flows out into what becomes a major tributary of the Vrishabhavathi river.

It is interesting to note that the *kalyani* water is clear is inhabited by a number of fishes and freshwater tortoise. This indicates that the water has a Biological Oxygen Demand (BOD) and Chemical Oxygen Demand (COD) well within the accepted range to support life.

Our study therefore concentrated on the analysis of the bacterial microflora of the water from the mouth of the Nandi, which is a source of the Vrishabhavathi river. Two samples – one in January-February and the other in April-May – were used in the study.

3.1 Physico-chemical characteristics of Vrishabhavathi source water

Water collected at the source was clear and potable. The physico-chemical properties of the water are given in the Table 2.

Table 2. Physico-chemical characteristics of Vrishabhavathi source water.

Parameter	Jan/Feb sample	Apr/May sample
pH	7.6	7.86
Temperature (ºC)	26.8	26.8
Conductivity (µS/cm)	1478	1219
Total dissolved solids (ppm)	722	610
BOD	1.7	1.6
COD	0	0

The near-neutral pH of the water suggests the absence of alkaline earth metals like calcium and magnesium. The conductivity of the water is well within the acceptable limits for drinking water which is 1000 – 15,000 microSiemens per centimeter (µS/cm). The low biological oxygen demand (BOD) (< 5mg/ml) indicates the absence of organic pollutants. Similarly, the undetectable chemical oxygen demand (COD) emphasizes the purity of the water.

3.2 Bacterial load: Jan-Feb sample

In order to determine the bacterial load in a given water sample, the total number of living bacteria can be as curtained by simple biological methods this is referred to as total viable count (TVC) of the water.

Dilution of the water sample were made in normal saline and plated on simple nutrient agar, a non-selective complex medium that supports the growth of all micro-organisms. The TVC of the Sri Dakshinamukha Nanditeertha water, Jan-Feb water sample is given in Table 3.

Table 3. Total viable count of the Jan-Feb sample of the Vrishabhavathi source water.

MEDIUM	DILUTION	COLONY FORMING UNITS (cfu)	TOTAL PLATE COUNT (cfu/ml)
Nutrient Agar	10^9	13	$13x10^9$
Nutrient Agar	10^8	6	$6x10^8$
Mac Conkey Agar	10^9	2	$2x10^9$
Mac Conkey Agar	10^8	1	$1x10^8$

Around 13 and 6 colony forming units (CFU) were obtained when 100µL of the 10^9 and 10^8 dilution was plated on nutrient agar. The total viable count is therefore ~ 13 x 10^9CFU/ml. Out of these, there were a few bacteria that grew on MacConkey agar as well. The total plate count on Mac Conkey Agar was ~ 10^8 CFU/ml.

The colonies from the nutrient agar plate were transferred to a master plate for the determination of colony characteristics (see Fig 1).

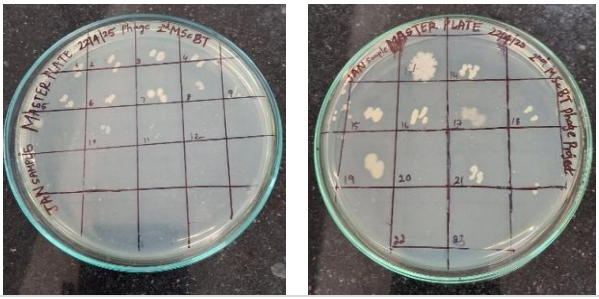

Fig 1. Master plate of Jan-Feb sample with bacterial colonies obtained by plating the water dilutions on nutrient agar.

3.3 Bacterial profile of the Jan-Feb water sample

The broad identification of bacteria can be done by observing the appearance of the colonies they form on solid media. As the first step in characterization of the bacteria in the water samples, 21 different types of bacterial colonies were identified and selected for further study. The colony characters of these bacterial colonies are given in Table 4.

Table 4. Colony characteristics of the bacteria in the Jan- Feb sample of Vrishabhavathi source water.

COLONY	1,2	3,4,5,6,8 & 10	7& 12	9&11	13	14,15 &16	17	18, 20 &21	19
Size	3mm	4mm	4mm	3mm	1.2mm	7mm	2.4cm	7mm	9mm
Shape	Concentric	Irregular	Circular	Irregular	**Irregular spreading**	Irregular	**Irregular spreading**	Irregular	Concentric
Elevation	Flat	Flat	Raised	Raised	Flat	Raised	Flat Filiform	Flat	Flat
Colour	Cream	Cream	Buff	Buff	Cream	Cream	Buff	Cream	Cream
Margin	Undulate	Undulate	Entire	Wavy	Lobate	Wavy	Erose	Wavy	Entire
Surface/ Submerged	Surface	Surface	Surface	Surface	Surface	Surface	Surface	Surface	Surface
Texture	Smooth	Smooth	Smooth	Glittering	Rough	Smooth	Smooth	Smooth	Smooth
Gram Staining	Gram Positive rods	Gram Positive rods	Gram Positive rods	Gram Positive rods	Gram Positive coccobacilli	Gram Positive rods	Gram Positive coccobacilli	Gram Positive rods	Gram Positive rods

All the bacteria were Gram positive rods with two colonies (13 and 17) showing the presence of coccobacilli. This indicates the complete absence of members of the Enterobacteriaceae family.

3.4 Bacterial load and profile of the April-May water sample

For the April-May sample, even at 10^8 dilution, a smear with numerous merged colonies were obtained on the agar plate (Fig 2). It appears that this irregular spreading could be due to motility. The bacterial load was estimated to be $>10^8$ CFU/ml. Lactose fermenters were not observed on MacConkey agar. When compared with the Jan-Feb water sample, the two sets of colonies (13 and 17) which showed an irregular spreading edge, appear to be predominant in the April-May sample while the others were not detected.

Fig 2. Bacterial profile of the April-May water sample from the Nanditeertha temple. Irregular spreading could be due to motility of the bacteria.

The colony characters of the growth in the April-May sample were similar to the colonies 13 and 17 of the Jan-Feb water sample (Table 5).

Table 5. Colony characteristics of the bacteria from the April-May water sample.

COLONY	SIZE	SHAPE	ELEVATION	COLOUR	MARGIN	SURFACE/ SUBMERGED	TEXTURE	MOTILITY	GRAM STAINING
1	Varied	Irregular	Flat	White	Entire	Surface	Smooth	Gliding	Gram positive coccobacilli

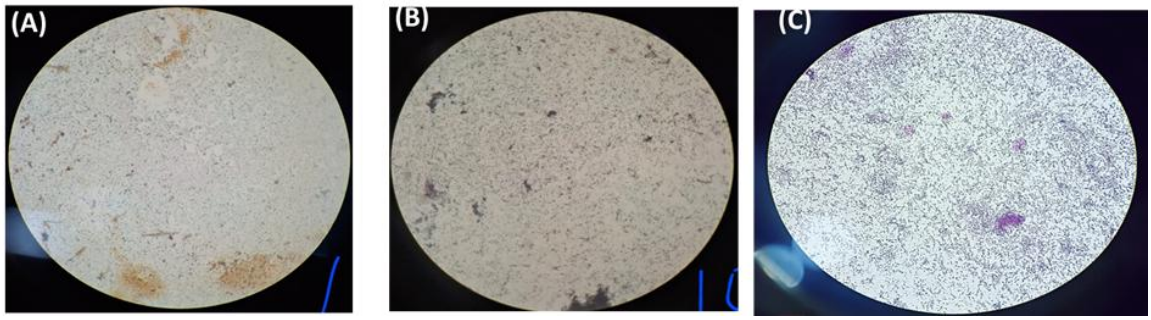

Fig 3. Gram stain of the bacteria from the Jan-Feb water sample (A) and (B) and the April-May water sample (C).

All the bacterial colonies showed a Gram-positive stain indicating the absence of Proteobacteria, including pathogenic coliforms of the family Enterobacteriaceae, such as *E. coli, S. typhi, S. dysenteriae* etc. The Gram - positive rods suggest the presence of genera such as *Bacillus spp.* as shown in Fig 3, which are common soil bacteria. No fungal colonies were observed.

3.5 Biochemical characteristics of the bacterial isolates

Several biochemical tests are used to characterize bacterial isolates. These include IMViC (indole, methyl red, Voges-Proskauer, citrate) tests and tests for oxidase, catalase, and protease. For this study, we chose to perform the oxidase, catalase and protease tests (Table 6), since the Gram stain showed the absence of Gram negative bacteria.

Table 6. Biochemical test results for the bacterial isolates.

COLONY	1&2	3,4,5,6,8 & 10	7&12	9&11	13	14,15 & 16	17	18,20 & 21	19
CATALASE	–	–	–	+	–	–	+	–	–
PROTEASE	–	–	–	–	+	+	–	–	+
OXIDASE	–	–	–	–	–	–	–	–	–

The oxidase test is basically a test for cytochrome c oxidase in aerobes. This test involves using a reagent that can act as an electron acceptor from the mitochondrial electron transport chain. While Kovacs reagent (N, N, N', N'-tetramethyl phenylene diamine dihydrochloride) is generally preferred as the artificial terminal electron acceptor in this test, α- naphthol in ethanol can also be used. The reagent turns red when aerobic organisms are streaked on it (Cappuccino and Sherman, 2014). None of the bacterial isolates showed a positive oxidase test. The consistent oxidase negative result again suggests that the bacteria present are facultative anaerobes that reside in lower layers of soil.

The catalase test is also a test for aerobes. When molecular oxygen is present in the system, it can get converted into harmful peroxide (O_2^-) which is broken down by the enzyme catalase. The bubbling of oxygen gas from hydrogen peroxide can be observed with the naked eye. Catalase positive and oxidase negative colonies 9, 11 and 17 suggest that they could be *Bacillus spp.*

The presence of at least three protease positive bacteria (colonies 13, 14/15/16 and 19) indicates that there could be some runoff from nearby anthropogenic activity into the feeder aquifer.

For the further characterization of the Vrishabhavathi source water, we needed a water sample that has been shown to have bacteriocins and bacteriophages to use as a control. Hence, bottled Ganga water procured in the month of January, 2025 from Kashi (Varanasi) was also used in the study.

3.6 Preliminary observations on the bottled Ganga water

In comparison with the Vrishabhavathi source water, the Ganga water showed lower conductivity (Table 7). This could be because bottled Ganga water is actually collected at Uttarkashi, Rishikesh or Haridwar (considered to be head waters), filtered (sometimes) and then distributed to Prayagraj, Kashi etc. (https://gangajal.online/).

Table 7. Physico-chemical characteristics of bottled Ganga water.

Parameter	Value
pH	7.34
Temperature	26.8°C
Conductivity	613 µs/cm
Total dissolved solids	307 ppm

The value of the parameters measured in Table 7 show that the physical parameters of the Ganga water is within the limits allowed for potability.

3.7 Bacterial profile and plate count: bottled Ganga water

While the Ganga water showed positive physico-chemical characteristics, it was imperative to test for the bacterial count and profile in order to know the microbiological quality of the water. The total viable count for the dilutions of the Ganga water was done on nutrient agar (Table 8).

Table 8. Total viable count on nutrient agar for the bottled Ganga water.

MEDIUM	DILUTION	TOTAL PLATE COUNT (cfu/ml)
Nutrient agar	10^{9}	Innumerable
Nutrient agar	10^{16}	170 X 10^{16}

When dilutions of the Ganga water were plated on nutrient agar, even at 10^{9} dilution numerous colonies were observed (Table 8). 10^{16} dilution showed 170 x 10^{16} colonies on nutrient agar plate, thus giving a total viable count of 170 x 10^{17}CFU/ ml.

These colonies were then subjected to Gram stain. As can be seen in Fig. 4, Gram negative bacteria were observed, stain indicating the presence of Proteobacteria, including pathogenic coliforms of the family Enterobacteriaceae, such as *E. coli, S. typhi, S. dysenteriae* etc.

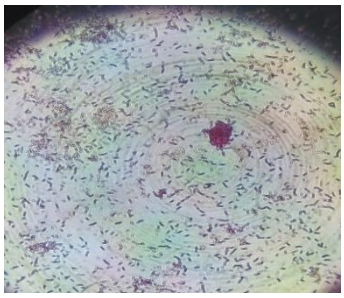

Fig 4. Gram staining of Ganga water sample.

The presence of coliforms in the Ganga water thus made it suitable to be used as a control to check for the presence of bacteriocins and coliphages.

3.8 Vrishabhavathi source water – presence of bacteriocins

Bacteriocins are small peptides that have membrane disrupting properties. They are produced by nearly 50-60% of bacteria including members of the Enterobacteriaceae family. Bacteriocins help in securing an environment especially when nutritional factors are limiting. They ensure that the host bacterium is not outnumbered by other bacteria and is thus a survival tactic evolved by bacteria. They also offer an alternative to antibiotics (Kumariya *et al*., 2019).

In a recent study, Tyagi and Dubey (2020) reported the presence of bacteriocins in fresh as well as stored Ganga water. Hence, the sample water procured in January from Kashi was used to test for antibacterial activity using the agar-well method. The two target bacteria used were *E. coli* (Gram negative) and *S. aureus* (Gram positive), with ampicillin as a positive control. The results of this experiment are shown in Fig 5.

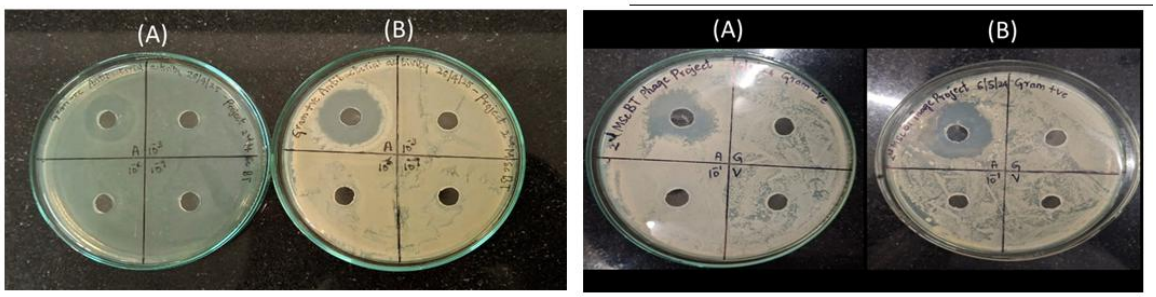

Fig 5. Antibacterial activity of Jan-Feb water (left) and Ganga water (right) against (A) *E. coli* and (B) *S. aureus* with ampicillin as a control.

As is evident from Fig 5, the Vrishabhavathi water showed no antibacterial activity against either *E. coli* or *S. aureus*. More surprisingly, even the Ganga water (Fig 5, right) showed no antibacterial activity. While it is possible that the antibacterial activity is not effective against *E. coli* (which could be the producer organism), we expected to find some antibacterial activity against *S. aureus.* Nevertheless, since no zones of clearance were seen in both Vrishabhavathi and Ganga water, this test was not conclusive.

3.9 Vrishabhavathi source water- presence of bacteriophages

Ganga river water was source of the very first discovery of bacteriophages (Hankin, 1896). The unique feature of this river is that, even at the source – the head waters near Gaumukh, a significant number of bacteriophages have been isolated (Kairnar, 2016). It was therefore logical to use dilutions of Ganga water (which is known to harbour bacteriophages) as a positive control, to test for phages in the Vrishabhavathi source water. Serial dilutions up to 10^{16} of both water samples were made and plated with *E. coli* DH5α as a host.

Fig 6. Detection of bacteriophage plaques in Vrishabhavathi (extreme left) and bottled Ganga water (right).

As can be observed from Fig 6, there were no plaques even in undiluted Vrishabhavathi source water. Clear plaques were seen in the Ganga water sample at the 10^{14} dilution indicating the presence of phages. At least 3 different plaque morphologies were seen – small, large and slightly turbid. The *E. coli* strain DH5α used for this experiment is a *recA*$^-$ strain, hence lysogenic phages have probably not been selected. The lytic phage count in the Ganga water sample is > 10^{14} pfu/ml, but the total phage count could be much higher.

This study found the Vrishabhavathi source water to have neutral pH, low BOD and COD. Gram positive facultative anaerobic bacteria were present but coliforms, bacteriocins or coliphages were not found. In contrast, Ganga water had very high bacterial (>10^{16} CFU/ml) and phage (> 10^{14} PFU/ml) counts. Both lacked antibacterial activity.

The study demonstrates that the Vrishabhavathi source water at Sri Dakshinamukhi Nanditeertha temple is microbiologically clean and its neutral pH, low BOD/COD and absence of coliforms makes it safe for consumption. This study will be used as a pilot reference for the systematic documentation of the Vrishabhavathi river water.

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5. AUTHOR CONTRIBUTIONS AND STATEMENTS

VB conceptualized the project; AB executed the experiments; both AB and VB were involved in manuscript preparation.

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Informed Consent / Ethical Compliance: Authors state that there is no informed consent/human or animal involvement in the article.

Data Availability: All data included in this research article will be provided on request.

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