

Original Article

Microbial Contamination of Water Bodies: Isolation and Biochemical Identification of *Salmonella* and *Vibrio spp.* From Kamori river Bhiwandi

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Abstract

Water-borne pathogens such as *Salmonella*, *Vibrio cholerae*, and *Vibrio parahaemolyticus* pose significant public health risks. Contamination of water bodies due to industrial, agricultural, and domestic waste, along with improper disposal practices, facilitates the introduction of these harmful bacteria. This study aimed to isolate, identify, and characterize these pathogens from a single river water source, the Kamori River in Bhiwandi. Water samples were collected from the Kamori River and analyzed using culture techniques, selective media, and biochemical assays for preliminary identification. *Salmonella*, *Vibrio cholerae*, and *Vibrio parahaemolyticus* were successfully isolated and confirmed through biochemical assays, highlighting the potential health hazards associated with microbial contamination in river water.

Keywords: Waterborne pathogens, *Vibrio cholerae*, *Vibrio parahaemolyticus*, *Salmonella spp.*, Water contamination, Microbial water quality, Bacterial isolation, Biochemical characterization

Introduction

Chief among the necessary resources for survival are water, which sustains both physical environments and human Communities. But this can likewise be a avenue for bacterial infection that may incite quite serious myslisease. Some notorious bacteria are known to be part of a dreadful family; for instance, *Salmonella* or *Vibrio cholerae*, well known in pathology. Meanwhile, of the less-known, but toxin-producing bacteria is a *Vibrio parahaemolyticus*, it is the one which up till recently raising its first media coverage and start doubting its importance in the health world. *Vibrio parahaemolyticus* is one of those species of pathogenic bacteria that deserve scientific attention due to pollution, global warming, and human activities affecting water sources.

Consequently, the knowledge of how *Vibrio parahaemolyticus* is transmitted in various waters is one of the factors that feed into the operation of surveillance systems, preparation of water treatment practices, and safeguarding of populations from waterborne diseases.

This bacterium is living in saltwater and can bring about diseases which can be as small as stomach discomfort or as perilous as the recurrently fatal infection. This Micro organism survives in salt water and can cause diseases ranging from mild stomach upset to very serious and often life-threatening infections. As pollution, global warming, and human activity impact sources of water, *Vibrio parahaemolyticus* needs to be studied.

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Knowing how it propagates in different waters is hence necessary to bolster monitoring systems, improve water treatment methods, and safeguard the community against waterborne diseases. The interest in this research is not merely theoretical, but rather the foundation for outbreak prevention and public health around the globe.

Water Pollution and Its Impact:

According to the Centre for Science and Environment, nearly 75–80% of river pollution is caused by raw sewage, industrial waste, and garbage, amounting to over 3 billion liters of waste daily (Mishra, 2010; Martínez et al., 2009). The United Nations has also reported that access to clean freshwater is becoming a major concern, with nearly 900 million people suffering from diarrhea each year due to contaminated water (Goel & Grad, 2008).

Industrial waste introduces harmful chemicals into water bodies, affecting water quality. Heavy metals, trace elements, and suspended solids impact both human health and the environment (Radha et al., 2007). While some trace elements are needed for metabolism, others can be toxic when consumed in large amounts. The presence of major ions in drinking water also affects its taste and safety (Delpla et al., 2009). Waterborne diseases are a major concern worldwide. Pathogens like *Salmonella*, *Vibrio cholerae*, and *E. coli* enter water bodies through fecal contamination, causing diseases like typhoid and Dysentery (Faparuset al., 2011). Other harmful microorganisms include *Entamoeba histolytica*, *Giardia lamblia*, and *Cryptosporidium*, which cause diarrhea (Kelly et al., 1997).

Water Scarcity and Disease Transmission:

Currently, one-third of the world's population lives in areas facing water shortages. With growing populations and increased water consumption, clean water access is becoming even more limited (Asano et al., 2007). As a result, the risk of waterborne diseases is expected to rise (Suresh & Smith, 2004).

Salmonella is one of the most widespread waterborne pathogens. It consists of many different strains that cause severe intestinal infections. It spreads mainly through contaminated food and water, and in some cases, can enter the

bloodstream, leading to serious illnesses like typhoid fever (Pond, 2005).

Natural water sources such as rivers, lakes, and groundwater are common carriers of *Salmonella*, which enters these environments through human and animal waste or agricultural runoff (Ashbolt, 2004; Leclerc et al., 2002).

Research shows that *Salmonella* can survive in raw sewage and even remain in treated wastewater, posing long-term risks to human health (Maier et al., 2000; Wéry et al., 2008).

Major Bacterial Pathogens: *Salmonella* and *Vibrio*

1. *Salmonella* and Its Impact:

Salmonella consists of a broad range of bacteria, which is divided into more than 2,500 serotypes. It is typically associated with foodborne illness that results from consumption of contaminated foods, such as poultry, eggs, and meat, but it can also be spread through contaminated water. Some strains cause mild gastrointestinal distress but other strains can result in more serious diseases like typhoid fever. The bacteria can thrive in different environments including water, soil, and on plants. Infected animals, including poultry, pigs, and wild birds can act as carriers of the bacteria (Dolejská et al., 2009; Lightfoot, 2004; Wray & Wray, 2000).

2. *Vibrio* Bacteria and Their Effects:

This genus comprises multiple pathogenic bacteria mostly found in water, regularly vast in coastal and marine environments.

***Vibrio cholerae*:** Causes *cholera*, one of the most active diarrhetic diseases; threat to life when alarming dehydration and proper remedies are not available.

***Vibrio parahaemolyticus*:** One of the major causes of food-borne illness from seafood, usually through undercooked or raw shellfish, particularly oysters. This bacteria forms toxin disrupting normal body activities which lead to infections. Their ability to grow within warm waters makes them of greater concern in a climate-changed world.

Collection of Water Samples:

Sterile 500 mL plastic bottles were employed and Water samples were collected from Kamori river Bhiwa locations in February 2025. Samples were labeled by date, time, and location,

stored in ice-filled coolers, and brought directly to the lab for processing.

Morphological Analysis:

The purified bacterial colonies were observed for their shape, size, and other physical characteristics. Gram staining was performed to determine their cellular structure.

Biochemical Analysis:

The following biochemical tests were performed to confirm the identity of the bacteria:

- Gram Staining
- Catalase Test
- Oxidase Test
- Indole Production Test
- Methyl Red Test (for *Salmonella* spp., *Vibrio cholerae*, and *Vibrio parahaemolyticus*)
- Voges-Proskauer Test
- Citrate Utilization Test
- Hydrogen Sulfide (H₂S) Production Test
- TSI (Triple sugar iron Test)

The biochemical tests were performed following the guidelines in *Bergey's Manual of Systematic Bacteriology*.

Pre-enrichment For *Salmonella* and *vibrio*:

Pre-enrichment of *Salmonella* and *Vibrio*.

Pre-enrichment is important for the recovery of pathogens such as *Salmonella* and *Vibrio* from food and environments, enrichment of the target bacteria, particularly when stressed or in small numbers.

Salmonella pre-enrichment reactivates the damaged cells and stimulates growth to a detectable level. The samples are incubated in a

non-selective medium, such as Buffered Peptone Water (BPW), to allow recovery prior to selective plating.

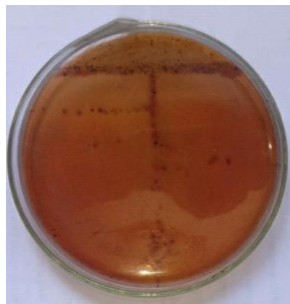
Pre-enrichment supports growth of *Vibrio* species in low-abundance environments. It occurs in Alkaline Peptone Water (APW) pH 8.6, in which growth of *Vibrio* spp. is favored and that of others is suppressed by sodium chloride. The incubation temperature may be set specifically to the selected *Vibrio* species.

Isolation of *Salmonella*: Combine 1 mL of the sample with 9 mL of Buffered Peptone Water and incubate at 37°C for 18 hours. Take one loopful and inoculate on *Salmonella-Shigella* Agar and incubate at 37°C for 24 hours. The likely colonies of *Salmonella* are pale pink-red with black center on XLD Agar. Pick these colonies, streak on fresh nutrient agar, and perform further identification. Pure colony of each of the samples is obtained by Gram staining and biochemical tests.

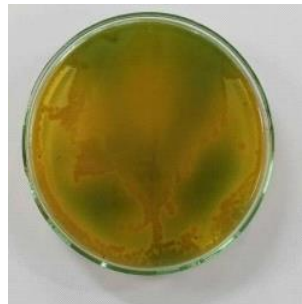
Isolation of *Vibrio cholerae*: Combine 1 mL sample with 9 mL Alkaline Peptone Water; incubate at 37°C for 18 hours. Streak on TCBS agar and incubate at 37°C for 24 hours. Yellow colony with hard center and clear edges shows *Vibrio* spp. Pure culture on nutrient agar for Gram stain and biochemical testing.

Isolation of *V. parahaemolyticus*: Similar to *Vibrio cholerae*, cultures are transferred to Alkaline Peptone Water and grown on TCBS agar. Green mucoid colonies form in 24 hours. A colony is subculture to a new culture, kept on nutrient agar, and examined by Gram stain and chemical tests.

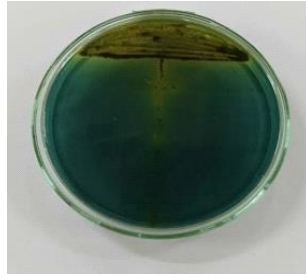
Isolation of *Salmonella*:



Isolation of *Vibrio cholerae*



Isolation of *Vibrio parahaemolyticus*:



Oxidase Test

Salmonella



Vibrio parahaemolyticus



Vibrio cholerae



Catalase Test

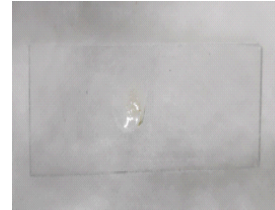
Salmonella



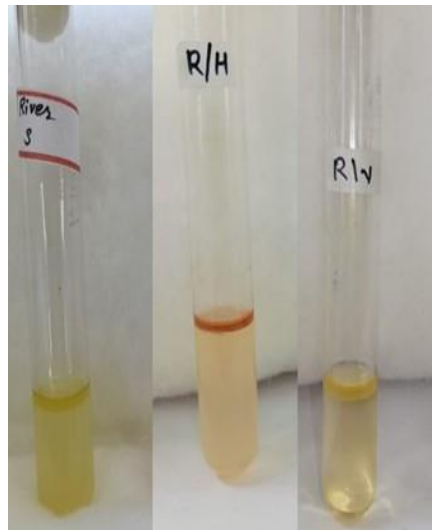
Vibrio parahaemolyticus



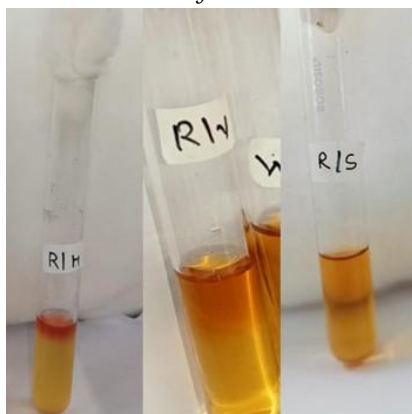
Vibrio cholerae



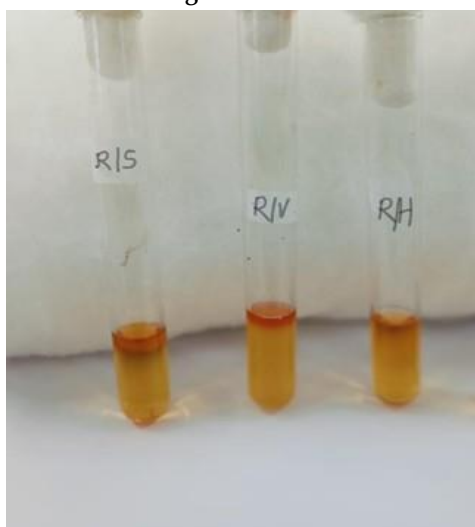
Indol test:



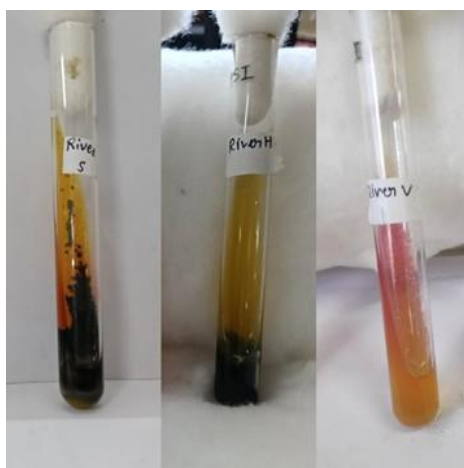
Methly red test



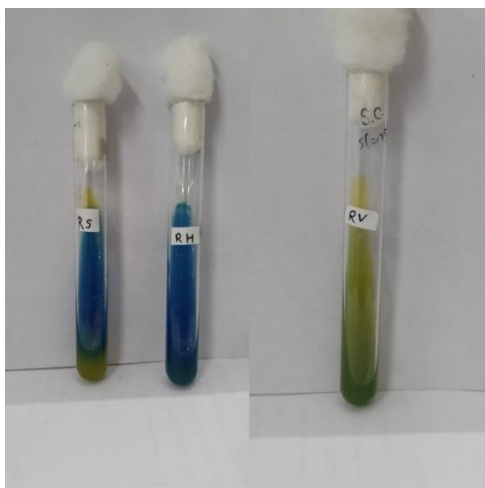
Voges Proskauer



TSI Test:



Citrate Utilization:



Result:

Morphology Characteristics

Characteristic	<i>Salmonella Typhi</i> (SS Agar)	<i>Vibrio cholerae</i> (TCBS Agar)	<i>Vibrio parahaemolyticus</i> (TS Agar)
Colony colour	Colorless with black centers (H ₂ S +)	Yellow	Green/blue-green
Shape	Smooth, round	Smooth, round	Smooth, mucoid
Size (mm)	2.0 – 3.0	1.0 – 2.0	1.0 – 2.0
Gram Staining	Gram – negative rods	Gram-negative curved rods	Gram-negative curved rods

These observations support the preliminary identification of the colonies. *Salmonella Typhi* produced characteristic black-centered colonies on SS agar due to hydrogen sulfide production. *Vibrio*

cholerae and *V. parahaemolyticus* showed distinct yellow and green colonies, respectively, on TCBS agar.

Biochemical Test

Test	<i>Salmonella Typhi</i>	<i>Vibrio Cholerae</i>	<i>Vibrio Parahaemolyticus</i>
Catalase	+	+	+
Oxidase	-	+	+
Indole Test	-	-	+
Methyl Red	+	+	+
Voges-Proskauer	+	+	+
TSI Test	Alkaline slant/ Acidic butt, H ₂ S production	Acidic slant/ Acidic butt, no H ₂ S	Alkaline slant/ Acidic butt, H ₂ S production
Citrate Utilization	+	+	+

- The *Salmonella* strain displayed H₂S production on TSI, catalase positivity, and MR+/VP+ profile—hallmarks of *Salmonella Typhi*.
- Vibrio cholerae* showed an acid slant and butt on TSI (unusual but possible for some strains), oxidase positivity, and a strong VP response.

- Vibrio parahaemolyticus* was positive for catalase, oxidase, indole, citrate, and produced H₂S—matching its known biochemical behavior.

Discussion

The primary objective of this study was to test for pathogenic bacteria called *Vibrio cholerae*, *Vibrio parahaemolyticus*, and *Salmonella* from water samples obtained from Kamori river in Mumbai.

Water samples were collected from various points along the beach with great caution and processed as per standard microbiological procedures. Following careful testing, suspected colonies of these pathogenic bacteria were isolated and put through various tests.

The results may confirm the presence of three major waterborne pathogens in Kamori River:

- *Salmonella Typhi* can lead to typhoid fever and other severe intestinal conditions.
- *Vibrio cholerae* is known for causing cholera outbreaks, especially in unsanitary water conditions.
- *Vibrio parahaemolyticus* causes gastrointestinal illness, often linked to contaminated seafood.

The identification of these bacteria highlights significant fecal and industrial pollution in Kamori River, putting the local population at risk, especially those using river water for domestic purposes.

Public Health & Environmental Impact

- The presence of all three pathogens indicates extreme contamination and unsafe conditions for direct human contact or consumption.
- Industrial runoff, poor sewage management, and lack of water treatment appear to be the primary pollution sources.
- The confirmed growth of these organisms despite river flow suggests that pollutants are continuously entering the water body, requiring immediate intervention.

Recommendations

Immediate Steps

- Post warnings and restrict use of river water for bathing or cooking.
- Conduct frequent microbiological assessments of river water.
- Launch public health campaigns on safe water practices and disease prevention.

Long-Term Solutions

- Modernize and monitor sewage systems in Bhiwandi.
- Enforce stricter environmental regulations for industrial waste disposal.
- Introduce advanced disinfection methods (e.g., ozone, membrane filtration, UV).

Conclusion and Summary

The study confirms the contamination of Kamori River waters with hazardous pathogens, emphasizing the need for immediate action to safeguard public health. Addressing water pollution through sustainable practices and innovative treatment solutions is critical to preventing waterborne disease outbreaks.

Emerging methods will be the new technologies that are likely to dominate the future of water purification concerning the removal of pathogenic bacteria, e.g., *Vibrio* and *Salmonella*. Hence, it becomes important that researches aim to develop these promising technologies in a manner that not only makes them affordable and scalable but also sustainable, thereby making them accessible to the masses. Advanced water treatment methods will significantly limit the possibility of waterborne disease, ultimately toward ensuring safe drinking water for all.

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Conflicts of interest

The authors declare that there are no conflicts of interest regarding the publication of this paper

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