Assessing the Microbial Ecology of Uppalapadu Lake: Prevalence and Diversity of Bacterial Species in Soil and Water

Isarapu VeeraVenkata Satyavathi[1*] K. Usha Rani[2],

[*1]Lecturer in Zoology, Govt. Degree College, Madugula, Andhra Pradesh India.

[2]Lecturer in Zoology, DNR (A) College, Bhimavaram, Andhra Pradesh India.

Abstract:

This study investigated the presence of bacterial species in soil, water, and crab samples from Uppalapadu Lake, Andhra Pradesh. Biochemical tests revealed five distinct bacterial species: *Pseudomonas, Vibrio, Klebsiella, Escherichia coli (E. coli),* and *Staphylococcus. Pseudomonas* species were the most prevalent (40%) in *Labeo rohita* intestines, followed by Vibrio sp. (27%), *E. coli* (20%), *Klebsiella sp.* (16%), and Staphylococcus sp. (12%). Similarly, Pseudomonas spp. predominated in soil and water samples, while Staphylococcus spp. had the lowest prevalence. The biochemical analysis of bacterial cultures is presented in table. The study highlights the importance of monitoring bacterial populations in aquatic environments to ensure sustainable aquaculture and prevent the spread of diseases. The findings have implications for the development of targeted interventions to mitigate the impact of bacterial populations on environmental health and aquaculture productivity.

Key words;- Bacterial, Aquatic, *Pseudomonas, Vibrio,* Aquaculture

1.0 Introduction:

Labeo rohita, commonly known as rohu, is a key species within the carp family (Cyprinidae) and holds significant ecological and economic importance in South Asia. Native to the freshwater rivers of northern, central, and eastern India, as well as extending into Pakistan, Bangladesh, Nepal, Myanmar, and Vietnam, rohu is an omnivorous fish that plays a crucial role in local aquaculture and fishing industries (Jhingran, 1991; Reddy et al., 2001). It is renowned for its rapid growth rate, which allows it to reach lengths of up to 200 cm and weights of up to 45 kg, and for its desirable taste, making it a popular choice for consumption (Mishra et al., 2006). Rohu can live up to a decade, which contributes to its sustainability and significance in aquaculture practices (Jhingran & Pullin, 1985).

Rohu's extensive use in aquaculture is largely attributed to its high growth rate, adaptability to a variety of environments, and consumer preference for its mild flavor and nutritional value (Kumar et al., 2013). In many regions, particularly in South Asia, rohu is a staple food source and supports local economies through both small-scale and commercial fisheries (Pillay & Kutty, 2005).

However, the health and productivity of rohu populations can be adversely affected by a range of infectious microorganisms, including bacteria, fungi, viruses, and protozoa (Austin & Austin, 1999). Among these, bacterial pathogens are particularly concerning due to their potential to cause severe diseases in fish, which can lead to significant economic losses in aquaculture and fishing industries (Ellis, 1988). Pathogenic bacteria such as *Aeromonas hydrophila*, *Pseudomonas aeruginosa*, and *Flavobacterium columnare* have been documented to cause a variety of diseases in fish, manifesting in symptoms such as fin rot, septicemia, and ulcerative conditions (Austin & Austin, 1999; Plumb, 1999).

The transmission of these pathogens can occur through direct contact with contaminated water, soil, or through ingestion of infected fish (Noga, 2000). Moreover, bacterial infections can also pose risks to human health, particularly when consuming inadequately processed or contaminated fish products (Wang et al., 2015). This dual threat underscores the importance of understanding and managing bacterial infections in aquaculture settings.

This study aims to investigate the relationship between bacterial pathogens and Labeo rohita specimens collected from Uppalapadu Lake in Andhra Pradesh, India. By employing biochemical tests to identify bacterial pathogens and analyzing environmental conditions through water and soil sample analyses, the study seeks to elucidate the potential sources and impacts of bacterial contamination in this specific aquatic environment. Understanding these factors is critical for implementing effective disease management strategies and ensuring both fish health and public safety.

2.0 Materials and Methods:

2.1 Study Area:

The study was conducted at Uppalapadu Lake, which is located in the Guntur district of Andhra Pradesh, India. Specifically, the lake is situated within the Pedakakani Mandal and is geographically positioned at 16°18'24.5" N latitude and 80°30'36" E longitude.

2.2 Sample Collection:

Two species of fish samples were collected from the Fishery Ghat (Landing Centre) in zip-locked bags for analysis. In addition, water and soil samples were collected from Uppalapadu Lake in October 2023. All samples were transported to the laboratory for subsequent analysis.

2.3 Microbiological Analysis:

Sterilization of materials was carried out following the procedures outlined by Adibe and Eze (2004). Culture media were freshly prepared and sterilized using an autoclave at 121°C for 15 minutes. For sample preparation,

the method described by Obi and Krakowiak (1983) was employed. Additionally, serial dilution was performed to prepare samples for microbiological analysis.

2.4 Inoculation of Sample in Agar Plate:

Duplicate plates of nutrient agar were inoculated with 0.1 ml of the diluted solution (10^{-2} to 10^{-6}) using the glass spreader technique. All plates were incubated at 37°C for 24 h before colony enumeration and isolation.

2.5 Counting of Bacterial Load:

The method described by Collins et al. (1989) was used to estimate the total viable count of the isolates. Countable plates showing 1 to 32 colonies were selected and counted. The mean colony count on the nutrient agar plates of each given dilution was used to estimate the total viable count for the samples in colony-forming units per gram (CFU mL-1).

2.6 Identification of Pathogenic Bacteria:

This study confirmed several pathogenic bacteria, including Escherichia coli, Vibrio cholera, Salmonella, and Shigella, which can cause foodborne illness in humans.

2.7 Biochemical Tests:

2.7 (i) Gram Stain:

The Gram stain reaction is based on the difference in the chemical composition of bacterial cell walls. Gram-positive cells have a thick peptidoglycan layer, whereas in Gram-negative cells, it is much thinner and surrounded by outer lipid-containing layers.

2.7 (ii) Motility:

The motility test was performed using motility test medium. If using Motility Test Medium with TTC, observe for a pink color diffusing from the line of inoculation.

2.7 (iii) Oxidase Test:

The oxidase test is a biochemical reaction that assays for the presence of cytochrome oxidase. A small piece of filter paper was soaked in 1% Kovács oxidase reagent and dried. A well-isolated colony was picked from a fresh bacterial plate and rubbed into the filter paper soaked with Kovács oxidase reagent. Color change indicates the result of this test.

2.7 (iv) Catalase Test:

The catalase test is used to identify organisms that produce the enzyme catalase. This enzyme detoxifies hydrogen peroxide by breaking it down into water and oxygen gas.

2.7 (v) Starch Hydrolysis:

This test is used to identify bacteria that can hydrolyze starch using the enzymes α-amylase and oligo-1,6-glucosidase.

2.7 (vi) Gelatin Hydrolysis:

The gelatin hydrolysis test detects the ability of bacteria to produce gelatinases.

2.7 (vii) Indole Test:

The indole test screens for the ability of an organism to degrade the amino acid tryptophan and produce indole.

2.7 (viii) Methyl Red Test:

The methyl red test tests for the ability to perform mixed acid fermentation.

2.7 (ix) Voges-Proskauer Test:

Using a sterile inoculating loop, pick up well-isolated colonies of sample bacteria from 18 to 24 hours old culture and inoculate the broth.

2.7 (x) Citrate Utilization Test:

The citrate utilization test is used to determine the ability of a bacterium to utilize citrate as its only source of carbon.

2.7 (xi) Urease Test:

The urease test identifies those organisms capable of hydrolyzing urea to produce ammonia and carbon dioxide.

3.0 Results and Discussion:

3.1 Sample collection

Klebsiella pneumonia*: Klebsiella pneumonia* is a Gram-negative bacterium that can cause a range of infections, including pneumonia, urinary tract infections, and other healthcare-associated infections. It is known for its ability to form biofilms and its potential to acquire antibiotic resistance.

Escherichia coli: *Escherichia coli* (E. coli) is a Gram-negative bacterium that is commonly found in the intestines of humans and other warm-blooded animals. While many strains of E. coli are harmless and part of the normal gut flora, some pathogenic strains can cause gastrointestinal infections, urinary tract infections, and other conditions.

Staphylococcus species: *Staphylococcus* species are Gram-positive cocci that often form clusters resembling grapes. Staphylococcus aureus is the most well-known species within this genus and can cause a variety of infections, including skin infections, abscesses, and more severe conditions such as sepsis. Other species, such as *Staphylococcus* epidermidis, are typically part of the skin flora but can also be opportunistic pathogens, particularly in immunocompromised individuals or those with indwelling medical devices.

Image 01 sample collection

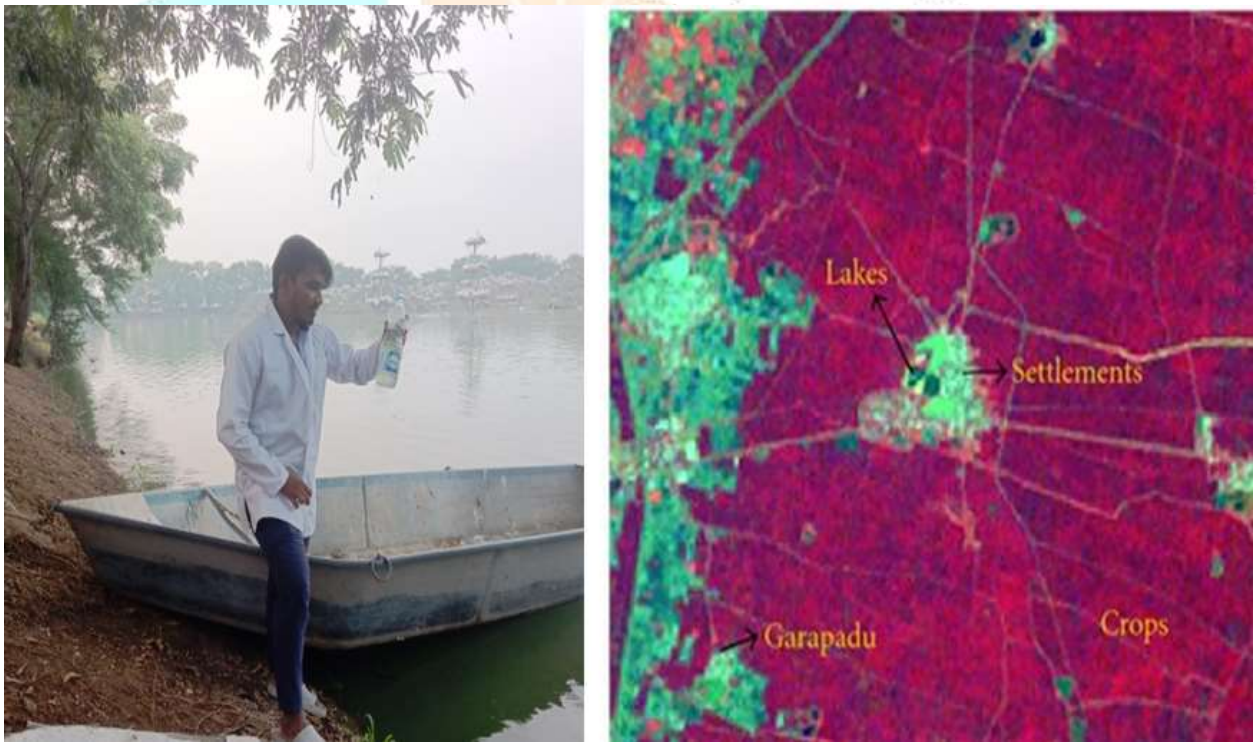
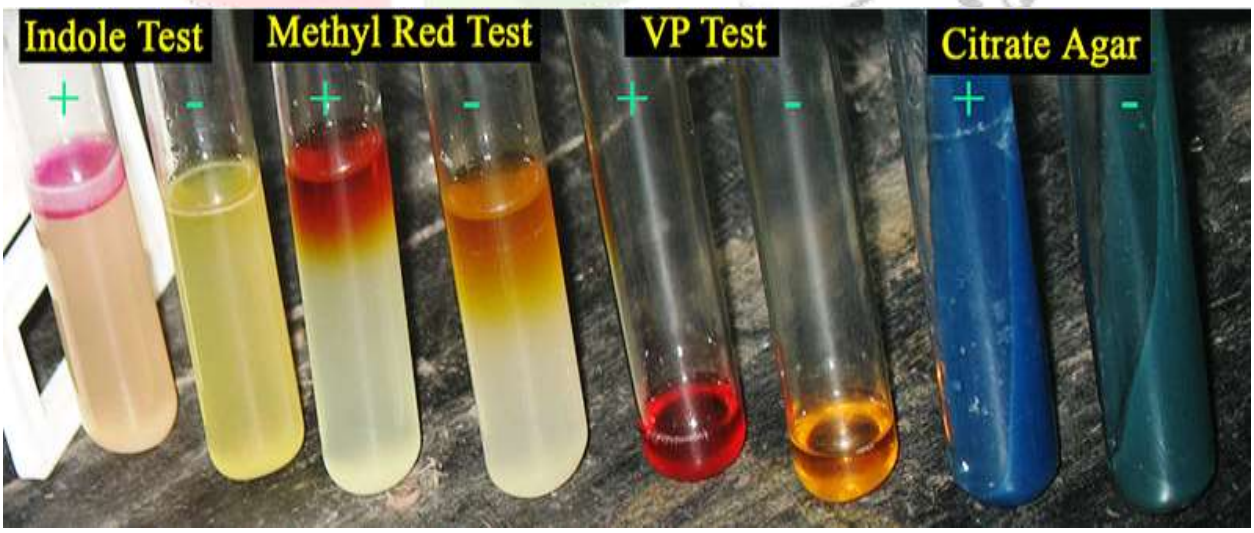


Table 1: Biochemical analysis of bacteria cultures from soil, water and crab samples.

S.no	Biochemical test	*Pseudomonas sp*	*Vibrio sp*	*Klebsiella sp*	*Staphylococcus sp*	*E coli*
1	**Gram staining**	Negative	Negative	Negative	Positive	Negative
2	**Motility**	Motile	Motile	non-motile	non motile	Motile
3	**Oxidase test**	P	P	N	N	N
4	**Catalase test**	N	P	P	P	P
5	**Starch hydrolysis**	N	N	N	P	N
6	**Gelatine hydrolysis**	P	P	N	P	P
7	**Indole**	**N**	**P**	**N**	**N**	**P**
8	**Methyl red test**	**N**	**N**	**N**	**P**	**P**
9	**Voges proskeur**	**N**	**P**	**P**	**P**	**N**
10	**Citrate utilization test**	**P**	**P**	**P**	**P**	**N**
11	**urease**	**P**	**N**	**P**	**P**	**N**

Pseudomonas species are Gram-negative bacteria known for their versatility and ability to thrive in various environments. They are often found in soil, water, and plant surfaces and some species can cause infections in humans, particularly in immunocompromised individuals.

Image 02 Biochemical test for soil, and water



Vibrio species are also Gram-negative and commonly found in aquatic environments, especially marine habitats. *Vibrio cholera*, a well-known Vibrio species, is responsible for causing cholera, a severe diarrheal disease.

Table 2: % of colonies in fish samples

		% of colonies in fish samples				
s.no	**Micro organisms**	**Head**	**Tail**	**Middle**	**Intestine**	**Total**
1	***Pseudomonas Sp***	8	9	8	15	40
2	***Vibrio Sp***	6	5	7	9	27
3	***Klebsiella sp***	7	2	3	4	16
4	***Staphylococcus sp***	6	3	2	1	12
5	***E coli***	6	4	3	7	20

Similarly, in line with the aforementioned findings, the predominant bacterial species observed was *Pseudomonas* spp., while the least prevalence was recorded for *Staphylococcus* spp. across both soil and water samples.

Klebsiella species are Gram-negative rods that can be found in soil, water, and the intestinal tracts of humans and animals. Some species, such as *Klebsiella pneumoniae*, can cause pneumonia, urinary tract infections, and other healthcare-associated infections. E. coli is a Gram-negative bacterium commonly found in the intestines of humans and warm-blooded animals.

Staphylococcus species are Gram-positive bacteria that often form clusters. *Staphylococcus* aureus is the most well-known species and can cause skin infections, abscesses, and even life-threatening conditions like sepsis.

Table 3 presents the biochemical analysis of bacteria cultures from soil, water, and crab samples.

S.No	**Microorganisms**	**% of colonies in soil and water**	
		Soil	water
1	***Pseudomonas sp***	9	6
2	***Vibrio sp***	5	4
3	***Klebsiella sp***	4	3
4	***Staphylococcus sp***	2	2
5	***E. coli***	5	3

Among the tested samples from *Labeo rohita* intestines, Pseudomonas sp. was the most prevalent bacterial species (40%), followed by Vibrio sp. (27%), E. coli (20%), Klebsiella sp. (16%), and *Staphylococcus sp.* (12%). Similarly, in soil and water samples, *Pseudomonas spp.* was the predominant bacterial species, while *Staphylococcus spp*. had the lowest prevalence.

4.0 Conclusion:

Labeo rohita, a prominent freshwater fish species native to South Asia, is extensively cultured for its high market demand and culinary preference. However, bacterial infections, such as Aeromonas and Pseudomonas species, pose significant threats to fish health and aquaculture production, leading to conditions like hemorrhagic septicemia, fin rot, and ulcerative lesions. The economic impact is substantial, causing losses in fish stocks and decreasing market value. Antibiotic resistance complicates treatment, necessitating preventive measures like maintaining water quality and implementing biosecurity protocols.

Our study investigated the correlation between different bacterial pathogens and Labeo rohita specimens from Uppalapadu Lake in Andhra Pradesh. Biochemical tests revealed the presence of five distinct bacterial species (E. coli, Pseudomonas spp., Klebsiella spp., Vibrio spp., and Staphylococcus spp.) in various samples, including the intestine, head, tail, and middle part of *Labeo rohita*. The most prevalent bacterial species was *Pseudomonas* spp., followed by *Vibrio* spp., E. coli, *Klebsiella* spp., and *Staphylococcus* spp.

Effective management practices, including prompt detection and treatment, are crucial to mitigate the dangers posed by bacterial infections in *Labeo rohita*. Our findings highlight the need for continued monitoring and research to ensure sustainable production and mitigate disease risks in aquaculture.

5.0 References

1. Austin, B., & Austin, D. A. (1999). Bacterial Fish Pathogens: Disease in Farmed and Wild Fish (2nd ed.). Springer.

2. Ellis, A. E. (1988). Fish Immunity and Pathology. Academic Press.

3. Jhingran, V. G. (1991). Fish and Fisheries of India. Hindustan Publishing Corporation.

4. Jhingran, V. G., & Pullin, R. S. V. (1985). The Biology, Ecology, and Culture of Indian Major Carps. International Center for Living Aquatic Resources Management.

5. Kumar, S., et al. (2013). Aquaculture and Fisheries Management. Springer.

6. Mishra, S., et al. (2006). Aquaculture and Fish Health. CRC Press.

7. Noga, E. J. (2000). Fish Disease: Diagnosis and Treatment. Mosby.

8. Pillay, T. V. R., & Kutty, M. N. (2005). Aquaculture: Principles and Practices. Blackwell Publishing.

9. Plumb, J. A. (1999). Health Maintenance and Principal Microbial Diseases of Cultured Fishes. Iowa State University Press.

10. Reddy, P. V. S., et al. (2001). Fisheries Management and Conservation. Academic Press.

11. Wang, X., et al. (2015). Food Safety and Human Health: The Implications of Fish Pathogens. Food Control, 50, 1-10.