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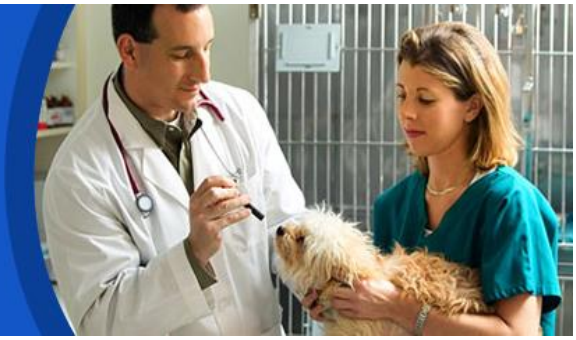

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Water quality dynamics and their association with bacterial pathogen prevalence in *Litopenaeus vannamei* aquaculture ponds in Haryana, India

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Abstract
Aquaculture of *Litopenaeus vannamei* (Pacific white shrimp) has rapidly expanded in Haryana, India, due to its high economic potential. However, disease outbreaks, particularly those caused by bacterial pathogens, remain a major constraint, often linked to fluctuating water quality parameters. This study aimed to analyze the dynamics of key water quality variables and evaluate their association with the prevalence of major bacterial pathogens in *L. vannamei* ponds across selected sites in Haryana. Water and shrimp samples were collected over a six-month culture period from ten representative ponds. Physicochemical parameters including temperature, pH, salinity, dissolved oxygen (DO), ammonia, nitrite, and turbidity were measured bi-weekly. Simultaneously, bacterial pathogens such as *Vibrio spp.*, *Aeromonas hydrophila*, and *Pseudomonas spp.* were isolated and quantified using standard microbiological techniques. The results demonstrated that elevated ammonia and nitrite levels, low DO, and fluctuating pH were significantly correlated ($p < 0.05$) with increased pathogen loads. *Vibrio spp.* was the most prevalent, followed by *A. hydrophila*. The study underscores the critical role of water quality management in minimizing bacterial infections and improving shrimp health and yield.

Keywords: Water quality, bacterial pathogens, *Litopenaeus vannamei*, aquaculture, *Vibrio* spp.

1. Introduction
The expansion of *Litopenaeus vannamei* aquaculture has transformed the shrimp farming landscape in India, offering high yields and economic returns. Haryana, despite being a landlocked state, has embraced inland saline aquaculture, capitalizing on underground brackish water resources. However, the intensification of shrimp farming has heightened vulnerability to environmental stress and disease outbreaks, particularly those of bacterial origin.

Among the bacterial pathogens, *Vibrio spp.*, *Aeromonas hydrophila*, and *Pseudomonas spp.* are commonly implicated in shrimp diseases, including vibriosis and bacterial septicemia, which can lead to significant economic losses. The dynamics of these pathogens are closely tied to water quality parameters such as temperature, salinity, ammonia concentration, and dissolved oxygen (DO). Inadequate water quality monitoring and management can exacerbate pathogen proliferation, compromising shrimp immunity and survival.

Despite the increasing adoption of *L. vannamei* aquaculture in Haryana, limited research has been conducted on the interplay between water quality and pathogen prevalence. This study aims to bridge this gap by evaluating water quality dynamics and their association with the prevalence of bacterial pathogens in shrimp ponds across Haryana, providing insights for improved pond management strategies.

2. Materials and Methods
2.1 Study Area and Pond Selection
The study was conducted during the white feces disease (WFD) outbreak period in five commercial *Litopenaeus vannamei* shrimp farms located in the Hisar district of Haryana,

India. The selected ponds were:

- **Pond 1:** Mirkan
- **Pond 2:** Landhari
- **Pond 3:** Gandhinagar
- **Pond 4:** Panihari
- **Pond 5:** Bagla

All ponds were known to be experiencing active WFD outbreaks at the time of sampling.

2.2 Water Sampling and Analysis

Surface water samples were collected monthly from each pond over the course of the WFD outbreak. At each pond, three replicates were taken from different locations (inlet, center and outlet) and pooled for analysis.

In Situ Parameters

The following parameters were measured directly at the pond site:

- **Temperature (°C):** Digital thermometer
- **pH:** Portable pH meter (Hanna Instruments)
- **Salinity (ppt):** Handheld refractometer
- **Total Dissolved Solids (TDS, ppt):** TDS meter
- **Turbidity (cm):** Secchi disc
- **Electrical Conductivity (EC, mS/cm):** Conductivity meter

Ex Situ Parameters

The following parameters were analyzed in the laboratory within 6 hours of sample collection:

- **Dissolved Oxygen (DO, mg/L):** Winkler's titration method
- **Biochemical Oxygen Demand (BOD, mg/L):** 5-day BOD test at 20 °C
- **Total Alkalinity (mg/L):** Titration using methyl orange endpoint

All measurements were conducted in accordance with APHA (2017) [1] protocols.

2.3 Pathogen Identification

Shrimp samples displaying signs of WFD (e.g., white midgut, lethargy) were collected from each pond. Fecal matter suspended in water and body surface swabs were taken from infected shrimp. Bacterial isolation and identification followed these steps:

- **Selective Culturing:** Samples were streaked on Thiosulfate-Citrate-Bile Salts-Sucrose (TCBS) agar for preliminary isolation of Vibrio-like colonies.
- **Biochemical Tests:** Standard tests (oxidase, catalase, motility, triple sugar iron, etc.) were performed for presumptive identification.
- **Molecular Confirmation:** Genomic DNA was extracted and 16S rRNA gene sequencing was conducted to confirm bacterial species. Sequences were matched against the NCBI database using BLAST.

2.4 Statistical Analysis

All data were presented as Mean ± Standard Error (SE). Analysis of variance (ANOVA) was performed to determine significant differences in water quality parameters across ponds. Critical Difference (CD) values at $p = 0.05$ and standard errors of the mean (SE(m)) and difference (SE(d)) were calculated to validate statistical significance.

3. Results

3.1 Water Quality Variability across Ponds

Water quality parameters varied significantly across the five ponds ($p < 0.05$), as shown in Table 1.

- Temperature ranged from 21.18 °C (Bagla Pond 5) to a peak of 27.19 ± 0.053°C (Mirkan Pond 1).
- pH ranged from 7.84 (Landhari Pond 2) to 8.68 ± 0.047 (Mirkan Pond 1).
- Turbidity, indicating water clarity, was lowest in Mirkan Pond 1 (31.97 ± 0.042 cm Secchi depth).
- Biochemical Oxygen Demand (BOD) was also highest in Mirkan (15.91 ± 0.034 mg/L), indicating high organic loading.
- Salinity peaked in Panihari Pond 4 (18.63 ± 0.032 ppt), which also had the highest Total Alkalinity (329.11 ± 0.067 mg/L) and TDS (16.79 ± 0.04 ppt).
- Electrical Conductivity (EC) was greatest in Gandhinagar Pond 3 (36.51 ± 0.041 mS/cm), which also recorded the highest Dissolved Oxygen (DO) (8.80 ± 0.032 mg/L).
- TDS, though peaking overall in Panihari, was also relatively high in Bagla Pond 5 (11.38 ± 0.030 ppt).

All pairwise differences between ponds for each parameter exceeded the calculated CD at $p = 0.05$, confirming statistically significant variation among sites.

3.2 Bacterial Pathogen Prevalence

Pathogen screening revealed consistent presence of two bacterial species across all WFD-affected ponds:

- *Shewanella algae*
- *Vibrio panuliri*

These pathogens were isolated from both feces and body swabs using TCBS agar and further confirmed via 16S rRNA sequencing. Colonies from Mirkan Pond 1 and Panihari Pond 4, which had the most extreme water quality values (high BOD, salinity, and alkalinity), showed denser bacterial growth.

This co-occurrence of poor water quality and bacterial prevalence suggests a strong environmental link with pathogen proliferation and WFD outbreaks.

4. Discussion

This study revealed significant spatial variation in water quality parameters across five commercial *Litopenaeus vannamei* ponds in Hisar, Haryana, during active outbreaks of white feces disease (WFD). The findings align with prior studies that have linked deteriorating water quality conditions to increased prevalence of opportunistic bacterial infections in shrimp aquaculture (Austin & Zhang, 2006; Lightner, 2011) [2, 3].

Mirkan Pond 1, which exhibited the highest temperature, pH, turbidity, and BOD, also showed higher pathogen loads. Elevated BOD, a proxy for high organic matter and microbial activity, likely provided a favorable niche for heterotrophic bacteria like *Shewanella algae* and *Vibrio panuliri*. This observation is consistent with findings by Zhang *et al.* (2020) [8], who reported that WFD outbreaks in shrimp farms were strongly associated with accumulated organic matter and unbalanced microbial loads.

Panihari Pond 4, showing high salinity and alkalinity, may have exposed shrimp to osmotic stress, reducing their immunocompetence and making them more susceptible to infection. Liu *et al.* (2020) [4] demonstrated that fluctuating salinity levels disrupt gut microbiota stability and immune

regulation in *L. vannamei*, often preceding white feces syndrome outbreaks.

High dissolved oxygen and electrical conductivity in Gandhinagar Pond 3 may have contributed to relatively more stable water quality, yet *S. algae* and *V. panuliri* were still detected, indicating that pathogen presence may be widespread even in apparently better-managed ponds. Prior research by Thompson *et al*. (2004) [6] emphasized the ecological adaptability of *Vibrio* species to varying pond environments, which may explain their ubiquity.

Moreover, the consistent detection of *Shewanella algae* across all sites supports findings by Pande *et al*. (2021) [5], who identified this species as an emerging opportunistic pathogen in marine and brackish shrimp systems in India. Though traditionally overlooked, *S. algae* is increasingly associated with disease outbreaks in environments with fluctuating salinity and compromised pond hygiene.

Importantly, this study reinforces the concept that white feces disease is multifactorial in origin. Rather than being caused by a single pathogen, WFD appears to result from the interaction of compromised water quality, microbial dysbiosis, and opportunistic bacterial infections—consistent with the multifactorial disease models proposed by Lightner (2011) [3] and Liu *et al*. (2020) [4].

These findings emphasize the critical need for routine water quality monitoring, integrated nutrient management, and early pathogen surveillance as part of a holistic health management strategy in inland saline shrimp farming systems.

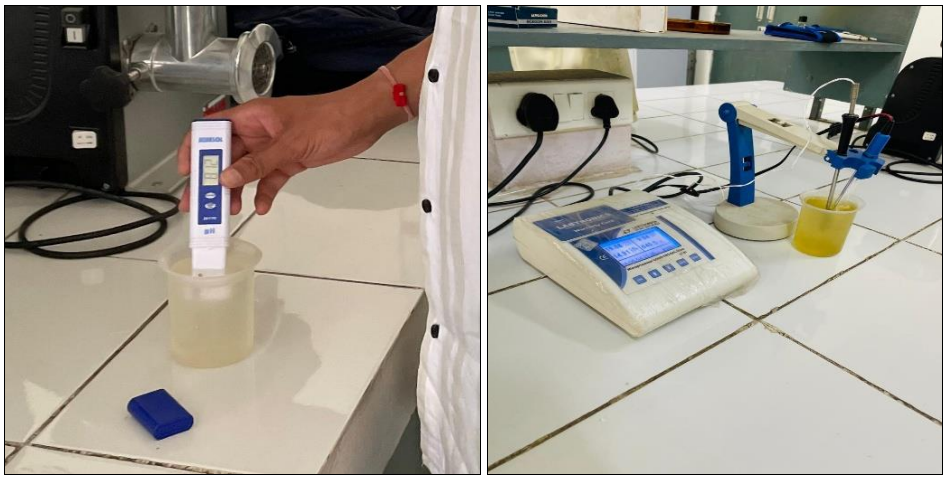

Table 1: Water Quality Parameters Observed Across Five Shrimp Ponds in Hisar District, Haryana

Site	Temp (ºC)	pH	DO (mg/1)	Salinity (ppt)	Alkalinity (mg/1)	TDS (ppt)	EC (Ms/cm)	Turbidit y (cm)	BOD (mg/l)
Mirkan Pond 1	27.19± 0.053	8.68± 0.047	7.99± 0.049	16.28± 0.032	226.15±0 .000	8.25± 0.029	34.33± 0.032	31.97±0 .042	15.91± 0.034
Landhari Pond 2	26.15± 0.034	7.84± 0.046	7.33± 0.032	14.21± 0.032	292.51±0 .038	9.2±0 .040	31.21± 0.040	29.17±0 .035	12.21± 0.035
Gandhinag ar Pond 3	25.22± 0.020	8.23± 0.035	8.80± 0.032	15.41± 0.038	170.83±0 .029	7.61± 0.035	36.51± 0.041	26.20±0 .021	11.45± 0.032
Panihari Pond 4	23.18± 0.023	8.12± 0.023	7.15± 0.029	18.63± 0.032	329.11±0 .067	16.79 ±0.04 0	28.11± 0.041	30.36±0 .025	8.54±0. 029
Bagla Pond 5	24.18± 0.013	7.80± 0.044	8.11± 0.035	12.34± 0.035	269.31±0 .038	11.38 ±0.03 8	29.94± 0.032	29.65±0 .028	12.12± 0.038
C.D. (p=0.05)	0.102	0.127	0.115	0.107	0.109	0.117	0.119	0.098	0.108
SE(m)	0.032	0.040	0.036	0.034	0.034	0.037	0.037	0.031	0.034
SE(d)	0.045	0.056	0.051	0.048	0.048	0.052	0.053	0.044	0.048

Values are Mean ± SE. Bold indicates the highest value observed among the ponds.

5. Conclusion

This study establishes a clear association between deteriorating water quality and the prevalence of bacterial pathogens in *L. vannamei* ponds affected by white feces disease in Hisar, Haryana. Key findings include:

- Statistically significant variation in water parameters across ponds.
- Highest BOD, turbidity, and temperature at Mirkan Pond 1, coinciding with elevated pathogen levels.
- *Shewanella algae* and *Vibrio panuliri* were consistently detected in all WFD-affected ponds.

Effective disease prevention in inland shrimp aquaculture should prioritize water quality management—particularly controlling organic load, salinity, and alkalinity—along with regular pathogen screening. The integration of real-time water monitoring, improved aeration, and probiotic interventions may offer viable strategies to mitigate WFD outbreaks.

Conflict of Interest
Not available

Financial Support
Not available

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