



Tracking Cholera in Urban Wastewater: MALDI-TOF-Based Detection of *Vibrio cholerae* in Côte d'Ivoire

**Tetty Aya Marie Pascale*, Allali Kouadio Bernard,
Yao Djeha Rosine, Yao Kouamé Eric, Koffi Romain,
Coulibaly Kalpy Julien**

Institut Pasteur de Côte d'Ivoire, Department of Microbiology, Abidjan, Côte d'Ivoire

Tetty Aya Marie Pascale
Email: Pascaletetty@gmail.com

Allali Kouadio Bernard
Email: drallalibern@yahoo.fr

Yao Djeha Rosine
Email: djeharosine@gmail.com

Yao Kouamé Eric
Email: key2cire@gmail.com

Koffi Romain
Email: romcy_kf@yahoo.fr

Coulibaly Kalpy Julien
Email: jc_kalpy@yahoo.fr

Abstract

Background: Cholera remains a major public health concern in low- and middle-income countries, particularly in areas with inadequate sanitation. Urban wastewater may act as an environmental reservoir for *Vibrio cholerae*, contributing to disease persistence and transmission, especially in rapidly urbanizing settings such as Abidjan, Côte d'Ivoire.

Objective: This study aimed to investigate the presence and distribution of *Vibrio cholerae* in urban wastewater and assess its association with environmental conditions.

Methods: A longitudinal study was conducted in Yopougon and Cocody districts. Wastewater samples were collected weekly over four weeks from six sites selected based on sanitation conditions. Samples were enriched in alkaline peptone water, cultured on TCBS agar, and isolates were identified using MALDI-TOF MS.

Results: A total of 90 isolates were obtained, with confirmed *Vibrio cholerae* detected across all sites. Higher prevalence was observed in densely populated residential areas compared with industrial and lagoon-adjacent zones.

Conclusion: Urban wastewater in Abidjan may serve as a reservoir for *Vibrio cholerae*. Wastewater surveillance using MALDI-TOF MS could support improved cholera monitoring and prevention strategies.

Keywords: *Vibrio cholerae*; wastewater; cholera; MALDI-TOF MS; environmental surveillance; Côte d'Ivoire

Introduction

Cholera remains a major public health challenge in many low- and middle-income countries, particularly in regions where access to safe water and sanitation is limited. The disease is caused by *Vibrio cholerae* infection and is primarily transmitted through the ingestion of water or food contaminated with fecal matter (Ali et al., 2024). Despite considerable global prevention efforts, cholera continues to cause recurrent outbreaks, especially in densely populated urban settings where sanitation systems are inadequate (World Health Organization, 2024).

Wastewater plays a critical role in the environmental persistence and transmission of *Vibrio cholerae*. Human excreta from infected individuals may introduce toxigenic strains into sewage networks, lagoons, rivers, and surface waters, enabling bacterial circulation within aquatic ecosystems (Street et al., 2025). Recent studies have shown that *Vibrio cholerae* can persist in wastewater and contaminated aquatic environments, particularly under tropical climatic conditions characterized by elevated temperatures and nutrient-rich waters (Awere-Duodu et al., 2025). Consequently, wastewater represents an important environmental reservoir contributing to

cholera transmission and outbreak amplification (Kachienga et al., 2024).

Rapid urbanization in many African cities has exceeded the capacity of sanitation infrastructure, resulting in the discharge of untreated or partially treated wastewater into natural environments (Siamalube et al., 2025). Such conditions facilitate fecal contamination and increase exposure to waterborne pathogens. In Sub-Saharan Africa, cholera outbreaks remain strongly associated with poor wastewater management, inadequate sewage treatment, and limited access to potable water (Debes et al., 2025). These environmental and infrastructural deficiencies contribute to the persistence of endemic cholera transmission in vulnerable populations.

Abidjan, the economic capital of Côte d'Ivoire, faces major challenges related to wastewater management due to rapid population growth, informal settlements, and insufficient sanitation coverage (Yapi et al., 2025). Untreated wastewater is frequently discharged into drainage channels, lagoons, and coastal waters, increasing environmental contamination risks. These conditions may favor the persistence of *Vibrio cholerae* in wastewater systems and contribute to periodic cholera outbreaks within densely populated communities.

Recent developments in wastewater-based epidemiology have demonstrated the usefulness of environmental surveillance for monitoring pathogens such as *Vibrio cholerae* (Manirambona et al., 2024). Wastewater surveillance can provide early warning of bacterial circulation before widespread clinical cases occur, thereby supporting rapid public health responses. International organizations, including the World Health Organization, increasingly recommend integrating wastewater monitoring into cholera prevention strategies, particularly in low-resource settings where traditional surveillance systems may be limited (World Health Organization, 2024).

Methods

To evaluate the impact of wastewater on the transmission of *Vibrio cholerae* in Abidjan, a systematic water sampling study was conducted around the populous neighborhoods of Yopougon and Cocody. These neighborhoods were chosen due to their population density and often inadequate sanitation infrastructure. Three specific sites were identified in each of the Yopougon and Cocody neighborhoods, bringing the total number of sampling sites to six.

Selection of Sampling Sites

- **Yopougon:** Sampling sites were selected near areas where wastewater is likely to accumulate, including open sewers and dumping points.

1. **Site 1:** Industrial zone near the main canal.
2. **Site 2:** Residential neighborhood with limited sanitation facilities.
3. **Site 3:** Proximity to a primary school where children often play near stagnant water.

- **Cocody:** Sites were chosen near watercourses and wastewater convergence points.

1. **Site 1:** Near the Ébrié Lagoon.
2. **Site 2:** Commercial area with inadequate drainage.

Site 3: Densely populated residential area with mixed sanitation infrastructure.

Sampling Procedure The samples were collected every Monday morning for four consecutive weeks to capture possible weekly variations in contamination levels. Each sampling session followed a standardized protocol to ensure the accuracy and consistency of the collected data.

1. **Preparation:** Before each sampling session, all equipment, including sterile 1-liter bottles, coolers, and the dipper, was sterilized and prepared for transport.
2. **Time Interval:** A 15-minute interval was observed between each sampling at a given site to allow for the renewal of water flow. This ensures that the samples are representative of real-time conditions.
3. **Sampling:** Using a dipper, water was carefully collected at each site. Ten sterile 1-liter bottles were filled with the collected water. Each bottle was immediately sealed to prevent contamination.
4. **Transport:** The sealed bottles were placed in coolers to maintain an adequate storage temperature, thereby reducing the risk of bacterial proliferation during transport.
5. **Delivery to the Laboratory:** The samples were delivered to the microbiology laboratory of the University of Cocody within two hours of sampling. Upon arrival, the samples were stored at 4°C until analysis.

Laboratory Analysis Enrichment, Isolation, and Identification Protocol for *Vibrio cholerae*

To detect and identify the presence of *Vibrio cholerae* in the wastewater samples collected in Abidjan, a detailed protocol for enrichment, isolation, and identification was established. This protocol employs advanced microbiological techniques to ensure precise and reliable detection.

Enrichment

Each wastewater sample is first homogenized to ensure an even distribution of present bacteria. 1

ml of the wastewater sample is taken using a sterile pipette and added to 9 ml of alkaline peptone water (APW) broth, with the pH adjusted to 8.6, prepared in sterile tubes. The inoculated tubes are incubated at 37°C for 24 hours. This enrichment step increases the concentration of *Vibrio cholerae*, facilitating its subsequent isolation.

Isolation

TCBS (Thiosulfate-Citrate-Bile-Sucrose) agar promotes the growth of *Vibrio cholerae* by inhibiting competing bacteria. After incubation, 10 microliters of the enriched APW broth are taken with a sterile pipette. The 10 microliters are streaked on the surface of the TCBS agar plate to isolate individual colonies. The TCBS agar plates are incubated at 37°C for 24 hours. After incubation, *Vibrio cholerae* colonies typically appear as yellow, raised, and dome-shaped colonies due to their ability to ferment sucrose.

Identification

Suspected colonies (yellow, convex, and dome-shaped resembling a Chinese hat) are subcultured onto GNA (Nutrient Agar) plates for purification. The GNA plates are then incubated at 37°C for 24 hours to obtain isolated and pure colonies.

Identification by MALDI-TOF MS

Pure colonies of *Vibrio cholerae* are collected using a sterile loop and deposited onto a MALDI-TOF MS (Matrix-Assisted Laser Desorption/Ionization–Time of Flight Mass Spectrometry) target plate. A matrix solution (commonly alpha-cyano-4-hydroxycinnamic acid) is applied to the samples to facilitate ionization. The samples are then introduced into the mass spectrometer. MALDI-TOF MS enables rapid and accurate bacterial identification by analyzing the protein profile of the colonies. The obtained spectra are compared with a reference database to confirm the identity of *Vibrio cholerae*.

This detailed protocol ensures rigorous and reliable detection of *Vibrio cholerae* in wastewater samples, thereby improving the understanding of the transmission dynamics of this pathogenic bacterium in densely populated neighborhoods of Abidjan. The results obtained are essential for developing effective cholera control and prevention strategies.

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Results

The investigation assessing the presence of *Vibrio cholerae* in wastewater samples collected from the Yopougon and Cocody districts of Abidjan produced notable findings. A total of six sampling sites were included in the study. For each site, three Petri dishes containing TCBS agar were used, resulting in 18 culture plates overall. Each plate allowed the selection of five presumptive colonies, yielding a total of 90 suspected bacterial isolates for analysis.

Following purification and identification procedures, variations in the distribution of confirmed *Vibrio cholerae* colonies were observed across sampling locations.

In Yopougon, Site 1, located in an industrial area, produced 10 confirmed *Vibrio cholerae* colonies out of 15 isolates. Site 2, situated within a residential area, yielded 12 confirmed colonies, while Site 3, located near a primary school, showed the highest prevalence with 13 confirmed colonies.

In Cocody, Site 1 near the Ébrié Lagoon yielded 8 confirmed *Vibrio cholerae* colonies from 15 isolates. Site 2, located in a commercial area, produced 9 confirmed colonies. Site 3, characterized as a densely populated residential area, recorded the highest prevalence in Cocody, with 13 confirmed *Vibrio cholerae* colonies.

Overall, sampling sites located in residential or densely populated environments exhibited higher numbers of confirmed *Vibrio cholerae* isolates compared with industrial, commercial, or lagoon-

adjacent sites. These findings suggest a potential association between population density, sanitation conditions, and wastewater contamination.

Municipality	Sampling Site	Site Characteristics	Colonies Isolated	Confirmed <i>Vibrio cholerae</i>
Yopougon	Site 1	Industrial area	15	10
Yopougon	Site 2	Residential area	15	12
Yopougon	Site 3	Near primary school	15	13
Cocody	Site 1	Near Ébrié Lagoon	15	8
Cocody	Site 2	Commercial area	15	9
Cocody	Site 3	Highly populated residential area	15	13

Improved Scientific Observation

The findings revealed spatial heterogeneity in the distribution of *Vibrio cholerae* across sampling sites, with significantly higher colony counts detected in wastewater collected near densely populated residential areas. Sites located in residential neighborhoods, including Yopougon Site 2 and Site 3, as well as Cocody Site 3, exhibited the highest prevalence of confirmed *Vibrio cholerae* isolates. These observations suggest that human population density and inadequate sanitation infrastructure may contribute to increased environmental contamination by *Vibrio cholerae*. Such spatial clustering supports the hypothesis that wastewater in highly populated urban settings may act as a reservoir facilitating cholera transmission.

Further analyses and identification using MALDI-TOF MS confirmed that the suspected colonies isolated were indeed *Vibrio cholerae* strains, validating the methodology employed in this study. This information is crucial for guiding public health efforts aimed at improving wastewater management and preventing cholera outbreaks in Abidjan.

Statistical Analysis

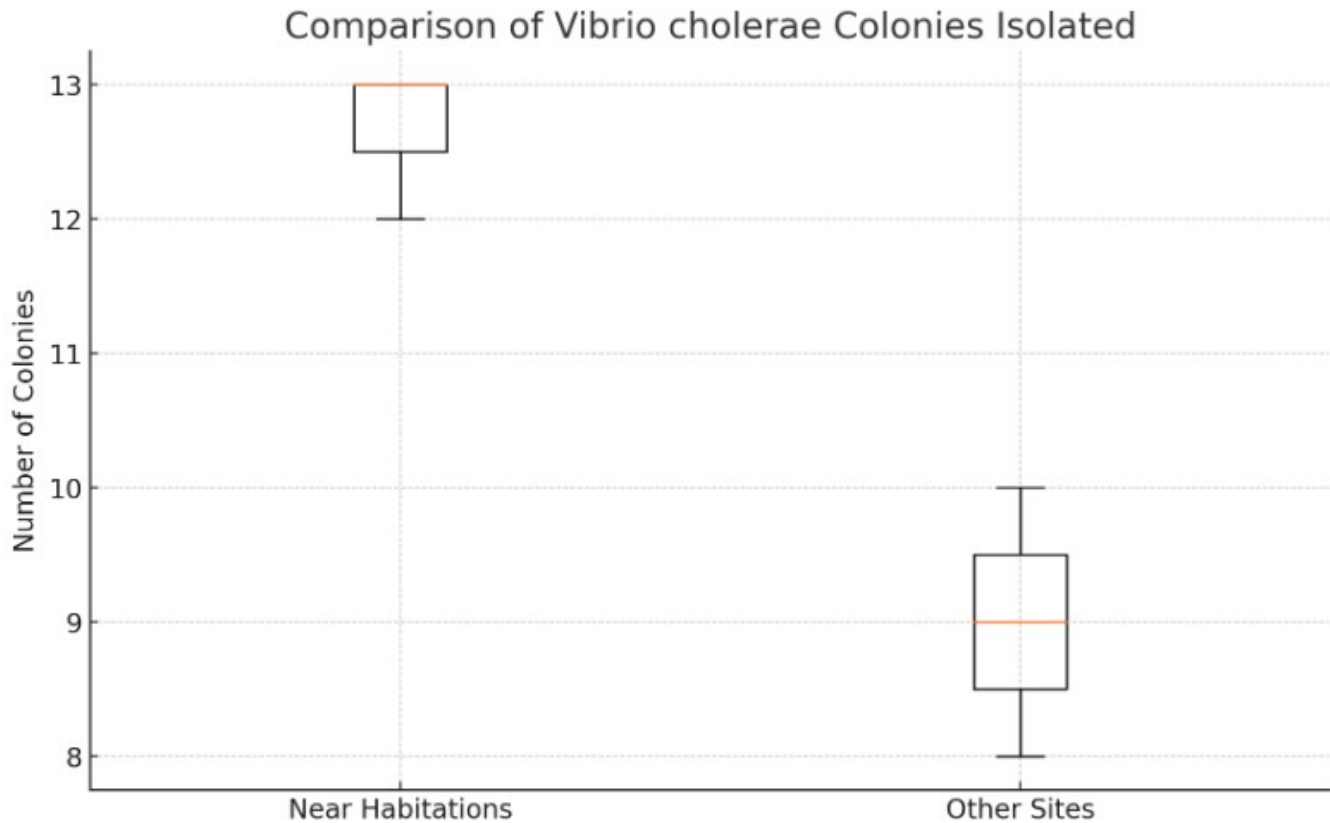
To evaluate whether the difference in *Vibrio cholerae* colony counts between residential-area sites and non-residential sites was statistically significant, an independent Student's t-test was conducted. This analysis compared the mean number of colonies isolated from two independent groups of sampling locations.

Sampling sites located near residential areas included Yopougon Site 2, Yopougon Site 3, and Cocody Site 3, with colony counts of 12, 13, and 13, respectively. Non-residential sites included Yopougon Site 1, Cocody Site 1, and Cocody Site 2, with colony counts of 10, 8, and 9, respectively.

The analysis demonstrated that residential-area sites exhibited a higher mean concentration of *Vibrio cholerae* colonies compared with non-residential sites. The independent Student's t-test yielded a t-value of 5.50 with 4 degrees of freedom and a p-value below 0.05, indicating a statistically significant difference between the two groups. These findings suggest that wastewater collected near residential areas contains higher levels of *Vibrio cholerae*, supporting the hypothesis that densely populated environments may contribute to increased environmental contamination.

Graphical Representation

Below is a box plot comparing the number of *Vibrio cholerae* colonies isolated from sites near residential areas and other sites.



Here is the box plot comparing the number of *Vibrio cholerae* colonies isolated from sites near habitations and other sites. This visual representation highlights the higher concentration of colonies found near residential areas, underscoring the need for targeted public health interventions in these zones.

Discussion

The results of this study revealed a significantly higher concentration of *Vibrio cholerae* colonies in wastewater collected near residential areas in Abidjan compared with other sampling sites. This observation supports the hypothesis that densely

populated environments with inadequate sanitation infrastructure create favorable conditions for the persistence and spread of cholera-related pathogens (Ali et al., 2024). The statistically significant difference observed between residential and non-residential areas further emphasizes the influence of wastewater contamination on cholera transmission dynamics.

Urban settlements characterized by high population density, informal housing, and insufficient sewage disposal systems often experience increased environmental contamination. In such settings, untreated domestic wastewater may facilitate the introduction and persistence of *Vibrio cholerae* in

aquatic ecosystems (Islam et al., 2023). Recent environmental surveillance studies have demonstrated that wastewater systems in densely populated communities can act as reservoirs for cholera pathogens, thereby increasing the likelihood of transmission during periods of poor sanitation or flooding (Street et al., 2025).

Similar findings have been reported in rapidly urbanizing regions where inadequate wastewater management contributes to recurrent cholera outbreaks. Studies conducted in low-resource urban settings have shown that wastewater contaminated with fecal material represents a significant pathway for pathogen dissemination, particularly in areas lacking effective sewage treatment infrastructure (Manirambona et al., 2024). These findings are consistent with the present study and reinforce the relationship between sanitation deficiencies and elevated *Vibrio cholerae* prevalence.

Recent investigations in South Asia and Sub-Saharan Africa have demonstrated that cholera risk is strongly associated with environmental contamination linked to overcrowding and poor waste disposal practices (Huq et al., 2023). Wastewater discharged into open drainage systems or natural water bodies may promote bacterial persistence and enhance community exposure to contaminated water sources. Such conditions are frequently observed in peri-urban settlements where sanitation access remains limited (Kachienga et al., 2024).

Environmental monitoring studies have also confirmed the importance of wastewater surveillance as an indicator of pathogen circulation in communities. Wastewater-based epidemiology has emerged as an effective tool for detecting *Vibrio cholerae* circulation before the appearance of large-scale outbreaks (Debes et al., 2025). This approach may be particularly valuable in urban African contexts where conventional surveillance systems face logistical and financial limitations.

In Abidjan, wastewater contamination near residential areas likely reflects the combined effects of rapid urban growth, inadequate drainage systems, and insufficient sanitation infrastructure (Yapi et al., 2025). The discharge of untreated wastewater into lagoons and drainage canals may sustain environmental reservoirs of *Vibrio cholerae*, increasing the risk of recurrent cholera transmission. Similar observations have been documented in coastal African cities where environmental contamination contributes to seasonal outbreaks (Rahman et al., 2022).

Further evidence from studies conducted in other geographic settings supports the relationship between wastewater contamination and cholera transmission. Similar observations have been reported in refugee camps and non-sewered sanitation environments, where wastewater surveillance revealed persistent circulation of cholera-associated pathogens due to overcrowding and limited access to safe sanitation (Shackelford et al., 2026).

Recent scoping reviews have highlighted that untreated wastewater serves as an important environmental reservoir for *Vibrio cholerae*, particularly in tropical and subtropical climates where environmental conditions favor bacterial persistence (Awere-Duodu et al., 2025). These studies indicate that pathogen survival is enhanced in nutrient-rich waters contaminated by domestic sewage, reinforcing the role of wastewater in maintaining transmission cycles. The environmental persistence of *Vibrio cholerae* has also been documented in treated and untreated sewage systems, demonstrating that bacterial contamination may continue despite partial sanitation interventions (Baker-Austin et al., 2024).

Several investigations conducted across Sub-Saharan Africa further emphasize the contribution of sanitation deficiencies to recurrent cholera outbreaks. In Zambia, the 2023–2024 cholera outbreak was associated with poor sewage disposal, flooding, and contamination of drinking water systems, particularly in densely populated

urban districts (World Health Organization, 2024a). Similar findings have been reported in Sudan, where conflict-related destruction of sanitation infrastructure contributed to the rapid spread of cholera through contaminated water sources (Médecins Sans Frontières, 2024). These examples illustrate how environmental instability and wastewater contamination can amplify outbreak severity.

Climate-related factors may additionally influence the persistence and distribution of *Vibrio cholerae*. Environmental suitability models have demonstrated that rising temperatures, altered rainfall patterns, and coastal ecosystem changes may increase the ecological niche favorable for *Vibrio cholerae* survival (Trinanes & Martinez-Urtaza, 2023). Flooding events, in particular, may facilitate the mixing of sewage with drinking water supplies, increasing exposure risks in vulnerable communities. Such mechanisms have been observed in urban areas affected by seasonal rainfall and inadequate drainage systems (de Magny et al., 2023).

Recent epidemiological analyses also indicate that cholera incidence is increasing globally due to weakened sanitation systems, population displacement, and fragile health infrastructure (Legros, 2024). Africa is currently experiencing one of the largest cholera resurgences in recent decades, largely driven by poor wastewater management, inadequate access to clean water, and overcrowded living conditions (World Health Organization, 2025). These trends highlight the continued importance of environmental sanitation as a central component of cholera prevention strategies.

Environmental surveillance programs increasingly demonstrate that wastewater monitoring can serve as an early-warning system for cholera outbreaks. Wastewater-based epidemiology enables the detection of pathogen circulation before clinical case surges occur, supporting rapid public health interventions (Sims & Kasprzyk-Hordern, 2023). The World Health Organization recommends integrating wastewater and environmental surveillance into cholera monitoring frameworks,

particularly in endemic and resource-limited settings (World Health Organization, 2024b). Such approaches may strengthen outbreak preparedness and improve disease control capacity.

Studies from South Asia have further shown that urban wastewater contamination disproportionately affects children and populations living in informal settlements, where recurrent exposure to contaminated water sources increases infection risk (George et al., 2023). Similar observations were reported in rapidly growing African cities, where wastewater discharge into lagoons and open drainage channels contributes to the persistence of enteric pathogens (Siamalube et al., 2025). These findings are particularly relevant to Abidjan, where untreated wastewater frequently reaches aquatic environments connected to residential communities.

Mathematical modeling studies also support the role of environmental contamination in cholera transmission. Models incorporating wastewater-related transmission pathways demonstrate that environmental sanitation interventions, including wastewater treatment and drainage improvement, significantly reduce epidemic spread (Codeço, 2023). Combined interventions involving sanitation improvement, water treatment, and surveillance have been shown to outperform isolated control strategies (Andrews & Basu, 2024).

In addition, genomic and environmental studies indicate that wastewater may facilitate the persistence and dissemination of antibiotic-resistant *Vibrio cholerae* strains (Das et al., 2024). The emergence of resistant strains in recent outbreaks has raised concerns regarding environmental reservoirs that sustain pathogen circulation and genetic exchange. Such findings underscore the importance of integrating microbiological surveillance into wastewater monitoring programs (Rebaudet et al., 2023).

Collectively, these studies reinforce the findings of the present work and demonstrate that wastewater contamination remains a major driver of cholera transmission across diverse geographic settings. Strengthening wastewater treatment systems, improving sanitation infrastructure, and implementing environmental surveillance are therefore essential public health measures for reducing cholera risk in urban communities (UNICEF & World Health Organization, 2025; World Bank, 2024). In the context of Abidjan, such interventions may be particularly important for preventing recurrent outbreaks associated with densely populated residential areas and inadequate wastewater disposal practices.

Implications for Public Health

The findings from Abidjan, supported by similar studies in other countries, underscore the critical need for improved sanitation and wastewater management in densely populated urban areas. Specifically, the following measures should be considered:

- **Infrastructure Development:** Upgrading sanitation facilities in residential areas to ensure proper disposal and treatment of wastewater. This includes building and maintaining sewage systems that prevent the contamination of drinking water sources.
- **Public Awareness Campaigns:** Educating communities about the importance of hygiene and safe water practices to prevent cholera transmission. Campaigns should focus on promoting handwashing, safe food preparation, and the use of treated water.
- **Regular Monitoring:** Implementing routine surveillance of wastewater to detect early signs of *Vibrio cholerae* and other pathogens. Regular monitoring allows for timely interventions before outbreaks occur.
- **Rapid Response Systems:** Establishing quick response mechanisms to address outbreaks promptly, thereby reducing the spread and impact of cholera. This

includes setting up emergency water treatment facilities and distributing rehydration solutions and antibiotics during outbreaks.

Conclusion

This study demonstrated a higher prevalence of *Vibrio cholerae* in wastewater collected from residential areas of Abidjan, suggesting that densely populated environments with inadequate sanitation infrastructure may contribute to increased environmental contamination. The observed spatial variation highlights the potential role of wastewater as a reservoir for cholera transmission in urban settings.

These findings are consistent with studies conducted in other regions worldwide, confirming that poor sanitation and insufficient wastewater management remain major drivers of cholera persistence and transmission. Strengthening sanitation infrastructure, improving wastewater treatment systems, and implementing regular environmental surveillance are essential measures to reduce public health risks associated with *Vibrio cholerae* contamination.

In addition, increased community awareness and targeted public health interventions in high-risk areas may contribute to limiting cholera transmission. Continued monitoring of wastewater systems could provide valuable information for early detection and prevention strategies, particularly in rapidly growing urban environments such as Abidjan.

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Conflict of Interest statement

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

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