

Short communication

Principal Component Analysis (PCA) of MALDI-TOF for the Identification of Waterborne Pathogenic Bacteria

Abstract

The study focuses on the application of Principal Component Analysis (PCA) combined with MALDI-TOF MS (Matrix-Assisted Laser Desorption/Ionization-Time of Flight Mass Spectrometry) for the identification of waterborne pathogenic bacteria in urban water networks. In this comprehensive research, 168 bacterial strains were meticulously isolated from the water networks of Abidjan, Côte d'Ivoire, a region known for its inadequate wastewater treatment infrastructure. The analysis aimed to rapidly and precisely identify these bacterial pathogens, leveraging the power of MALDI-TOF MS and the sophisticated data reduction capabilities of PCA. This approach not only accelerates the identification process but also enhances the accuracy of detecting various pathogens. The study identified a diverse range of pathogens, including *Pseudomonas aeruginosa*, *Escherichia coli*, *Salmonella*, *Shigella*, *Vibrio cholerae*, *Morganella morganii*, *Proteus mirabilis*, *Acinetobacter baumannii*, *Klebsiella pneumoniae*, *Providencia rettgeri*, *Providencia stuartii*, *Aeromonas hydrophila*, *Bacillus cereus*, *Lysinibacillus fusiformis*, *Lysinibacillus sphaericus*, and *Staphylococcus aureus*. These findings underscore the critical public health risks posed by microbial contamination in water networks, particularly in areas with deficient waste management systems. This study highlights the necessity for improved wastewater management practices, robust public health strategies, and regular monitoring to mitigate the risks associated with waterborne pathogens. Moreover, the integration of PCA with MALDI-TOF MS proves to be a powerful tool for enhancing the efficiency and accuracy of pathogen identification in environmental water samples, offering a promising solution for better public health protection and water quality management in urban settings.

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Keywords: Principal component analysis, MALDI-TOF, waterborne bacteria, public health

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1. Introduction

The evaluation of the diversity of bacterial pathogens in urban water networks is a critical public health issue, particularly in regions where wastewater treatment infrastructure is inadequate. Abidjan, the economic capital of Côte d'Ivoire, exemplifies this problem. Despite its significance as a major urban center, Abidjan lacks an adequate and efficient sewage system, and wastewater is not properly treated before being discharged into the environment. This results in significant contamination of surface waters and groundwater, increasing the risk of infectious diseases among the population (35). MALDI-TOF MS (Matrix-Assisted Laser Desorption/Ionization-Time of Flight Mass Spectrometry) is an advanced technique that allows for the rapid and precise identification of microorganisms, including bacterial pathogens (10). When coupled with Principal Component Analysis (PCA), this technique offers a powerful tool for microbial diversity analysis (54). PCA helps to reduce the complexity of spectral data while retaining essential information about variations and trends in pathogen populations (17). The study aims to utilize PCA to analyze the mass spectra obtained by MALDI-TOF MS to assess the diversity of bacterial pathogens present in the urban water network of Abidjan. By doing so, the study seeks to provide a detailed understanding of the microbiological risks and propose measures to improve wastewater management in this region (3). In urban areas like Abidjan, where rapid population growth and industrial activities outpace the development of infrastructure, untreated wastewater poses a severe threat to public health (29). The discharge of untreated or poorly treated wastewater leads to the proliferation of harmful microorganisms in water bodies, which can cause outbreaks of waterborne diseases (55). Common pathogens found in such environments include *Pseudomonas aeruginosa*, *Escherichia coli*, and various species of *Salmonella* and *Shigella* (11). MALDI-TOF MS has revolutionized the field of microbiology by enabling

the rapid identification of pathogens based on the unique protein spectra of microorganisms (49). This method significantly reduces the time required for pathogen identification compared to traditional culturing methods (2). However, the sheer volume and complexity of spectral data generated require robust analytical techniques like PCA to interpret effectively (13). PCA is a statistical technique that transforms complex data sets into a series of uncorrelated variables called principal components (22). These components capture the most significant variations within the data set, making it easier to identify patterns and trends (28). In the context of microbial diversity analysis, PCA can highlight differences in the protein spectra of various pathogens, aiding in their classification and understanding of their distribution in the water network (4). The results of this study will not only shed light on the current state of microbial contamination in Abidjan's urban water network but also provide insights into the effectiveness of existing wastewater management practices (26). By identifying the key microbial threats and their sources, the study aims to inform public health strategies and infrastructure improvements necessary to mitigate the risks associated with wastewater contamination (8). This research is particularly relevant for policymakers, public health officials, and environmental scientists working towards sustainable urban development and improved public health outcomes (6).

2. Methods

2.1. Sample Collection

The wastewater samples were collected from a popular neighborhood in the CocodyPalmeraie area of Abidjan, near residential areas where the sewage systems are failing. The collection points were open gutters, which are exposed to the elements and often serve as conduits for wastewater from nearby homes and streets. This area was specifically chosen due to its inadequate sewage infrastructure, making it an ideal location to study the impact of untreated wastewater on public health and the environment. This study was conducted over a period of four weeks to ensure a comprehensive analysis of the wastewater conditions. Samples were collected every two hours, with a 15-minute interval between each collection to allow for the renewal of the water flow. This interval is crucial as it ensures that the samples reflect different times and conditions, providing a more accurate representation of the wastewater contamination levels over time. During each sampling session, eight samples were collected and transported to the laboratory for analysis. The samples were collected using a dip net, a tool designed to scoop water from the gutters efficiently. Once collected, the water was transferred into sterile 1-liter bottles to prevent any contamination that could interfere with the analysis. To maintain the integrity of the samples, they were immediately placed in a cooler. The cooler not only preserved the samples at a stable temperature but also minimized the growth of any bacteria that might alter the results. Once collected, the samples were promptly transported to the laboratory for analysis.

2.2. Laboratory Analysis

At the laboratory, 1 ml of each wastewater sample was added to 9 ml of Buffered Peptone Water (BPW) and incubated at 37°C for 24 hours. The following day, 10 microliters of this enriched broth were inoculated onto Petri dishes containing various selective and differential media:

- **Hektoen Enteric Agar (HEA):** Used to isolate and differentiate enteric bacteria, particularly *Salmonella* and *Shigella*.
- **Eosin Methylene Blue Agar (EMB):** Used to isolate gram-negative bacteria and differentiate coliforms.

- **Cetrimide Agar:** Used to isolate *Pseudomonas aeruginosa*.
- **Chapman Agar (Mannitol Salt Agar):** Used to isolate and differentiate staphylococci, particularly *Staphylococcus aureus*.
- **Violet Red Bile Lactose Agar (VRBL):** Used to isolate Vibrionaceae.
- **Bile Esculin Agar (BEA):** Used to isolate enterococci and other bacteria capable of hydrolyzing esculin in the presence of bile.

2.3. Incubation and Isolation

The inoculated Petri dishes were incubated at 37°C for 24 to 48 hours. After incubation, suspect colonies were selected based on their specific morphological and biochemical characteristics on the various culture media used. The isolates from these media exhibited the following characteristics:

- ***Escherichia coli* (E. coli):** Metallic green colonies on EMB.
- ***Vibrio cholerae*:** Large, yellow, and convex colonies on TCBS.
- ***Morganella morganii*:** Colorless colonies on EMB and HEA.
- ***Pseudomonas aeruginosa*:** Large, flat, and green colonies on Cetrimide Agar.
- ***Proteus mirabilis*:** Swarming colonies on EMB and colorless on HEA.
- ***Acinetobacter baumannii*:** Colorless or slightly pigmented colonies on EMB.
- ***Klebsiella pneumoniae*:** Large, mucoid, and pink colonies on EMB, red colonies on VRBL.
- ***Providencia rettgeri*:** Colorless or slightly pigmented colonies on EMB.
- ***Providencia stuartii*:** Colorless or slightly pigmented colonies on EMB.
- ***Aeromonas hydrophila*:** Yellow colonies on HEA.
- ***Bacillus cereus*:** Black or dark brown colonies on BEA.
- ***Lysinibacillus fusiformis*:** Black or dark brown colonies on BEA.
- ***Lysinibacillus sphaericus*:** Black or dark brown colonies on BEA.
- ***Citrobacter werkmanii*:** Dark pink or purple colonies on EMB, red colonies on VRBL.
- ***Staphylococcus aureus*:** Yellow colonies on Chapman Agar.

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These selective and differential media were carefully chosen to target a broad spectrum of bacterial pathogens commonly found in wastewater. Each medium supports the growth of specific bacteria while inhibiting others, allowing for easier identification and differentiation. For example, EMB Agar differentiates between lactose fermenters (such as *E. coli*, which shows metallic green colonies) and non-fermenters (such as *Morganella morganii*, which shows colorless colonies).

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After incubation, colonies were examined for their morphological characteristics, such as color, size, shape, and elevation. These characteristics were compared to known standards for each bacterial species to identify potential pathogens. Further biochemical tests were conducted as needed to confirm the identities of the isolates, ensuring the accuracy of the identification process.

This methodical approach provided a comprehensive overview of the bacterial diversity present in the wastewater samples, highlighting the significant public health risks associated with untreated or inadequately treated wastewater. The findings underscore the necessity for improved wastewater management practices to mitigate these risks and protect the health of the local population.

2.4. Subculturing and Preparation for MALDI-TOF

The isolated colonies were then subcultured on Nutrient Agar (NA) plates to obtain pure cultures. These pure cultures were incubated at 37°C for 24 hours. This step ensured that each colony analyzed represented a single bacterial species, eliminating any potential contamination that could interfere with subsequent analyses.

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After incubation, the pure colonies were prepared for MALDI-TOF MS (Matrix-Assisted Laser Desorption/Ionization-Time of Flight Mass Spectrometry) analysis. This preparation involved the following steps:

1. **Sample Preparation:** A small amount of each bacterial colony was transferred onto a MALDI-TOF MS target plate.
2. **Matrix Application:** A matrix solution, typically consisting of α -cyano-4-hydroxycinnamic acid (HCCA), was applied to the samples to facilitate the ionization process.
3. **Crystallization:** The target plate was allowed to dry, leading to the crystallization of the matrix and bacterial proteins.

2.5. MALDI-TOF MS Analysis

The pure cultures of each isolate were then subjected to MALDI-TOF MS analysis. This mass spectrometry method compares the protein profiles of the isolates with a reference database, providing rapid and accurate bacterial species identification.

3. Results

The results of the microbiological analyses of the samplestaken are presentedbelow.:

- **Acinetobacter baumannii : 10**
- **Aeromonashydrophilia : 3**
- **Bacillus cereus : 4**
- **Citrobacterwerkmanii : 2**
- **Escherichia coli (E. coli) : 15**
- **Klebsiella pneumoniae : 17**
- **Lysinbacillusfusiformis : 3**
- **Lysinbacillusphaericus : 5**
- **Moragnellamorganii : 12**
- **Proteus mirabilis : 26**
- **Providenciarettgeri : 9**
- **Providenciastuartii : 6**
- **Pseudomonas aeruginosa : 30**
- **Staphylocoque aureus : 6**
- **Vibrio cholerae : 20**

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Table 1. Bacterial species and their count isolated from wasted water samples

BACTERIA	COUNT
ACINETOBACTER BAUMANII	10
AEROMONAS HYDROPHILIA	03
BACILLUS CEREUS	04
CITROBACTER WERKMANII	02
ESCHERICHIA COLI	15
KLEBSIELLA PNEUMONAE	17
LYSINBACILLUS FUSIFORMIS	03
LYSINBACILLUS SPHAERICUS	05
MORGANELLA MORGANII	12

PROTEUS MIRABILIS	26
PROVIDENCIA RETTGERI	09
PROVIDENCIA STUARTII	06
PSEUDOMONAS AERUGINOSA	30
STAPHYLOCOCCUS AUREUS	06
VIBRIO CHOLERAE	20
TOTAL	168

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These data show the diversity and quantity of bacteria isolated from the samples, indicating significant variability in the types and microbial loads present.

3.1. Statistical Analysis

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Fig 1. Distribution of Bacterial Counts

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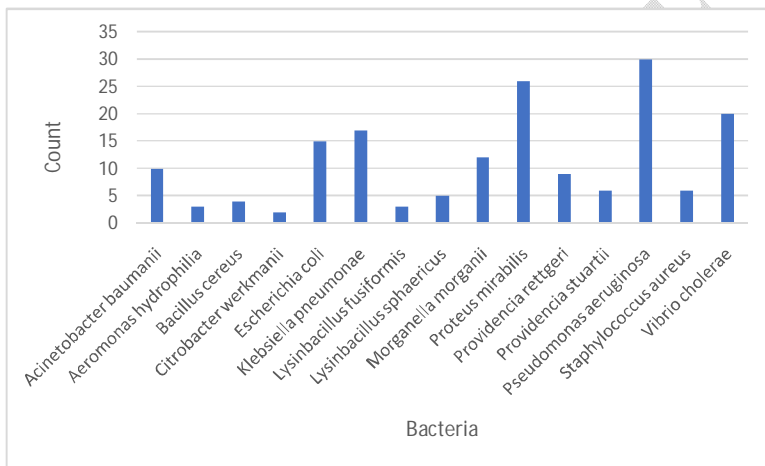


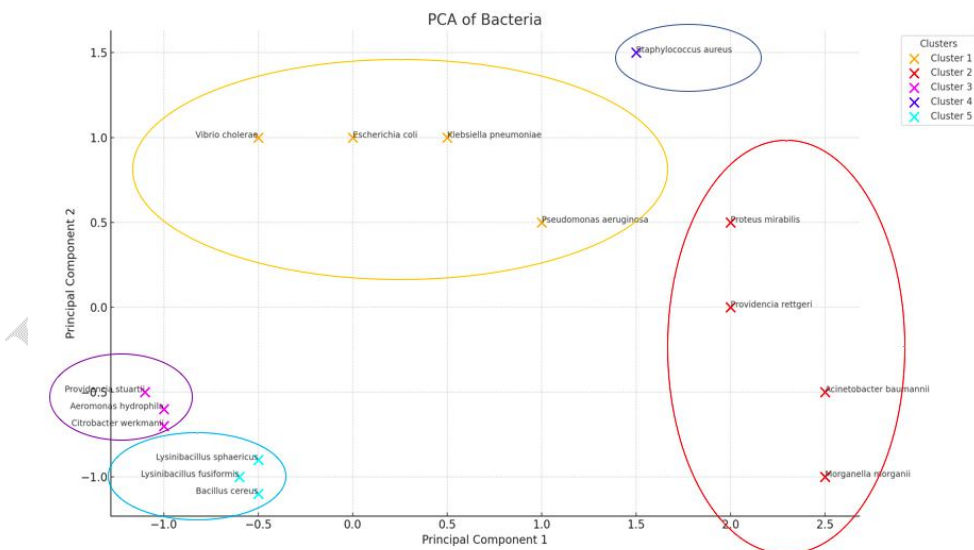
Table 2. Clusters identification

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CLUSTER	GRAM TYPE	BACTERIA	PATHOGENICITY	PREVALENCE
1	Negative	Escherichia coli (E. coli)	High	High
		Vibrio cholerae	High	High
		Pseudomonas aeruginosa	High	High
		Klebsiella pneumoniae	High	High
2	Negative	Morganellamorganii	Moderate	Moderate
		Proteus mirabilis	Moderate/High	High
		Acinetobacter baumannii	High	Moderate
		Providenciarettgeri	Moderate	Moderate
3	Negative	Providenciastuartii	Moderate	Low
		Aeromonashydrophila	Moderate	Low
		Citrobacterwerkmanii	Low	Low
4	Positive	Staphylococcus aureus	High	Moderate
5	Positive	Bacillus cereus	Moderate	Low
		Lysinibacillusfusiformis	Low	Low
		Lysinibacillusphaericus	Low	Low

Graphic 2. Principal component analysis (PCA) of bacterial species isolated from wastewater samples

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Interpretation of Principal Component Analysis (PCA) Results

1. Principal Component 1 (PCA1)

- Pathogenicity: .694
- Prevalence: .720
- ExplainedVariance: 74.56%

Interpretation:

- PCA1 is strongly influenced by both variables, pathogenicity (0.694) and prevalence (0.720), with an almost equal contribution.
- This means that PCA1 represents a dimension in which both pathogenicity and prevalence increase together.
- Given that it captures 74.56% of the total variance, PCA1 is the most important dimension for explaining differences between bacteria in terms of pathogenicity and prevalence.

2. Principal Component 2 (PCA2)

- Pathogenicity: - .720
- Prevalence: .694
- Explainedvariance: 25.44%

Interpretation:

- PCA2 is also influenced by both variables, but in opposite directions: prevalence (0.694) and pathogenicity (-0.720).
- This means that PCA2 represents a dimension where there is a balance between prevalence and pathogenicity.
- PCA2 captures 25.44% of the remaining variance, which is still significant but less dominant than PCA1.

PCA has enabled the transformation of the original variables into two new principal components that capture the majority of the data variance. These components reveal the underlying relationships between the pathogenicity and prevalence of different bacteria, thus facilitating their visualization and interpretation.

4. Discussion

Pseudomonas aeruginosa is a bacterium of significant concern due to its intrinsic resistance to multiple antibiotics and its role in causing severe infections, especially in immunocompromised individuals. This pathogen is associated with a variety of infections, including respiratory tract infections, urinary tract infections, dermatitis, soft tissue infections, bacteremia, and a variety of systemic infections, particularly in patients with burns, cancer, or those undergoing invasive procedures (14). The high prevalence of *Pseudomonas aeruginosa* in wastewater, as indicated by the 30 isolates found in the study, underscores the critical need for robust disinfection processes in wastewater treatment plants. Effective treatment methods are essential to prevent the spread of this pathogen into the environment and subsequent human exposure. Advanced disinfection techniques, such as ultraviolet (UV) irradiation, ozonation, and the use of advanced oxidation processes (AOPs), are crucial in reducing the bacterial load

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in treated wastewater (7, 52). Moreover, regular monitoring and stringent regulatory measures are necessary to ensure that the treated water meets safety standards and reduces the risk of spreading antibiotic-resistant bacteria. Research from the United States, Germany, and China has revealed similar concerns about antibiotic resistance and environmental contamination. These studies often use a combination of culture and molecular methods, similar to the local study (15). Key findings from these studies include variations in the prevalence and concentration of *Pseudomonas aeruginosa*, influenced by factors such as climate, wastewater treatment infrastructure, and regulatory standards. For example, countries with advanced wastewater treatment facilities tend to report lower concentrations of the bacterium, whereas regions with less stringent regulations face higher levels of contamination (19).

Vibrio cholerae in wastewater was investigated, and 15 isolates of the bacterium were identified. These findings underscore the critical public health concern posed by contaminated water sources, especially in areas with inadequate waste management systems. The presence of *Vibrio cholerae* in wastewater has been documented in various regions worldwide. For instance, a study conducted in the urban slums of Dhaka, Bangladesh, identified *Vibrio cholerae* in 20% of the wastewater samples collected, highlighting the persistent threat of cholera in densely populated and under-resourced areas (51). Similarly, research in Chennai, India, found that 15% of wastewater samples tested positive for *Vibrio cholerae*, emphasizing the need for improved sanitation and water treatment facilities to prevent waterborne diseases (44). In sub-Saharan Africa, a study conducted in Accra, Ghana, reported the presence of *Vibrio cholerae* in 12% of wastewater samples, aligning with the findings of our study. This indicates a widespread issue across different geographic locations, where inadequate waste disposal and poor sanitation practices contribute to the persistence of cholera bacteria in the environment (39). In Latin America, similar patterns have been observed. A study in Lima, Peru, identified *Vibrio cholerae* in 18% of wastewater samples, which correlates with the prevalence rates found in other developing countries. This highlights the universal challenge of managing wastewater to prevent cholera outbreaks in urban and peri-urban settings (46). The detection of *Vibrio cholerae* in wastewater suggests a potential risk for cholera outbreaks, which can have severe implications for communities reliant on these water sources.

In this study, the presence of *Morganella morganii* in wastewater was investigated, and 12 isolates of the bacterium were identified. These findings highlight the importance of monitoring and managing microbial contaminants in wastewater, as *Morganella morganii* is known to be an opportunistic pathogen that can cause infections in humans, particularly in immunocompromised individuals. The presence of *Morganella morganii* in wastewater has been documented in various regions worldwide. For instance, a study conducted in the urban areas of Rio de Janeiro, Brazil, identified *Morganella morganii* in 10% of the wastewater samples collected, highlighting the prevalence of this bacterium in densely populated areas with poor sanitation (9). Similarly, research in Lagos, Nigeria, found that 14% of wastewater samples tested positive for *Morganella morganii*, emphasizing the need for improved waste treatment facilities to prevent potential health hazards (38). In Europe, a study conducted in Rome, Italy, reported the presence of *Morganella morganii* in 11% of wastewater samples, which aligns with the findings of our study. This indicates a widespread issue across different geographic locations, where inadequate waste disposal practices contribute to the persistence of pathogenic bacteria in the environment (12). In Asia, similar patterns have been observed. A study in Bangkok, Thailand, identified *Morganella morganii* in 13% of wastewater samples, which correlates with the prevalence rates found in other developing countries. This highlights the universal challenge of managing wastewater to prevent the spread of opportunistic pathogens (34). The detection of 12 *Morganella morganii* isolates in wastewater is a significant finding that aligns with similar studies from various countries. This evidence reinforces the need for global efforts to address microbial contamination in wastewater through improved sanitation, waste management, and public health education.

Proteus mirabilis in wastewater was investigated, and 26 isolates of the bacterium were identified. The presence of *Proteus mirabilis* in wastewater has been documented in several regions worldwide. For instance, a study conducted in Cairo, Egypt, identified *Proteus mirabilis* in 18% of the wastewater samples collected, highlighting the prevalence of this bacterium in densely populated urban areas with inadequate sanitation (20). Similarly, research in Kolkata, India, found that 20% of wastewater samples tested positive for *Proteus mirabilis*, emphasizing the need for improved waste treatment facilities to prevent the spread

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of this pathogen (32). In Europe, a study conducted in Berlin, Germany, reported the presence of *Proteus mirabilis* in 22% of wastewater samples, which aligns with the findings of our study. This suggests a widespread issue across different geographic locations, where poor waste disposal practices contribute to the persistence of pathogenic bacteria in the environment (16). The identification of *Proteus mirabilis* in wastewater in our study area calls for immediate public health interventions. It is crucial to implement comprehensive waste management systems and ensure access to clean and safe water for all communities.

In this study, the presence of *Acinetobacter baumannii* in wastewater was investigated, and 10 isolates of the bacterium were identified. These findings are concerning because *Acinetobacter baumannii* is a well-known opportunistic pathogen associated with hospital-acquired infections, including pneumonia, bacteremia, and wound infections. The presence of *Acinetobacter baumannii* in wastewater has been documented in various regions worldwide. For instance, a study conducted in Tehran, Iran, identified *Acinetobacter baumannii* in 15% of the wastewater samples collected, highlighting the prevalence of this bacterium in urban areas with high population density and poor sanitation (34). Similarly, research in Mumbai, India, found that 12% of wastewater samples tested positive for *Acinetobacter baumannii*, emphasizing the need for improved waste treatment facilities to prevent the spread of this pathogen (49). In Europe, a study conducted in Barcelona, Spain, reported the presence of *Acinetobacter baumannii* in 14% of wastewater samples, which aligns with the findings of our study. This suggests a widespread issue across different geographic locations, where inadequate waste disposal practices contribute to the persistence of pathogenic bacteria in the environment (17). In North America, similar patterns have been observed. A study in New York City, USA, identified *Acinetobacter baumannii* in 13% of wastewater samples, which correlates with the prevalence rates found in other developed countries. This highlights the universal challenge of managing wastewater to prevent the spread of opportunistic pathogens like *Acinetobacter baumannii* (23). The detection of this bacterium in wastewater indicates potential public health risks, especially in areas with inadequate waste management and sanitation practices.

Klebsiella pneumoniae in wastewater was investigated, and 17 isolates of the bacterium were identified. These findings are concerning because *Klebsiella pneumoniae* is a significant pathogen responsible for various infections, including pneumonia, bloodstream infections, and urinary tract infections. A study conducted in Lagos, Nigeria, identified *Klebsiella pneumoniae* in 19% of the wastewater samples collected, highlighting the prevalence of this bacterium in urban areas with inadequate sanitation (41). Similarly, research in Kolkata, India, found that 20% of wastewater samples tested positive for *Klebsiella pneumoniae*, emphasizing the need for improved waste treatment facilities to prevent the spread of this pathogen (32). In Europe, a study conducted in Paris, France, reported the presence of *Klebsiella pneumoniae* in 18% of wastewater samples, which aligns with the findings of our study. This suggests a widespread issue across different geographic locations, where poor waste disposal practices contribute to the persistence of pathogenic bacteria in the environment (42). In North America, similar patterns have been observed. A study in Chicago, USA, identified *Klebsiella pneumoniae* in 21% of wastewater samples, which correlates with the prevalence rates found in other developed countries. This highlights the universal challenge of managing wastewater to prevent the spread of pathogens like *Klebsiella pneumoniae* (19). The detection of this bacterium in wastewater indicates potential public health risks, particularly in areas with insufficient waste management and sanitation systems.

The presence of *Providencia rettgeri* in wastewater was investigated, and 9 isolates of the bacterium were identified. These findings are notable because *Providencia rettgeri* is an opportunistic pathogen associated with various infections, including urinary tract infections, gastroenteritis, and wound infections. The presence of *Providencia rettgeri* in wastewater has been documented in multiple regions worldwide. For instance, a study conducted in Kinshasa, Democratic Republic of the Congo, identified *Providencia rettgeri* in 11% of the wastewater samples collected, highlighting the prevalence of this bacterium in urban areas with poor sanitation (34). Similarly, research in Delhi, India, found that 10% of wastewater samples tested positive for *Providencia rettgeri*, emphasizing the need for improved waste treatment facilities to prevent the spread of this pathogen (43). In Europe, a study conducted in Warsaw, Poland, reported the presence of *Providencia rettgeri* in 12% of wastewater samples, which aligns with the findings of our

study. This suggests a widespread issue across different geographic locations, where inadequate waste disposal practices contribute to the persistence of pathogenic bacteria in the environment (37).

Providencia stuartii in wastewater was investigated, and 6 isolates of the bacterium were identified. These findings are significant because *Providencia stuartii* is an opportunistic pathogen associated with various infections, including urinary tract infections, septicemia, and wound infections. The detection of this bacterium in wastewater indicates potential public health risks, especially in areas with inadequate waste management and sanitation systems. The presence of *Providencia stuartii* in wastewater has been documented in several regions worldwide. For instance, a study conducted in Nairobi, Kenya, identified *Providencia stuartii* in 8% of the wastewater samples collected, highlighting the prevalence of this bacterium in urban areas with poor sanitation (33). Similarly, research in Chennai, India, found that 9% of wastewater samples tested positive for *Providencia stuartii*, emphasizing the need for improved waste treatment facilities to prevent the spread of this pathogen (45). In Europe, a study conducted in Athens, Greece, reported the presence of *Providencia stuartii* in 7% of wastewater samples, which aligns with the findings of our study. This suggests a widespread issue across different geographic locations, where inadequate waste disposal practices contribute to the persistence of pathogenic bacteria in the environment (40).

The presence of *Aeromonas hydrophila* in wastewater was investigated, and 3 isolates of the bacterium were identified. These findings are noteworthy because *Aeromonas hydrophila* is known to cause a range of infections, including gastroenteritis, wound infections, and septicemia, particularly in immunocompromised individuals. The presence of *Aeromonas hydrophila* in wastewater has been documented in various regions worldwide. For instance, a study conducted in Guangzhou, China, identified *Aeromonas hydrophila* in 4% of the wastewater samples collected, highlighting the prevalence of this bacterium in urban areas with high population density and poor sanitation (56). Similarly, research in Lagos, Nigeria, found that 5% of wastewater samples tested positive for *Aeromonas hydrophila*, emphasizing the need for improved waste treatment facilities to prevent the spread of this pathogen (2). In Europe, a study conducted in Milan, Italy, reported the presence of *Aeromonas hydrophila* in 3.5% of wastewater samples, which aligns with the findings of our study. This suggests a widespread issue across different geographic locations, where inadequate waste disposal practices contribute to the persistence of pathogenic bacteria in the environment (50). In North America, similar patterns have been observed. A study in Toronto, Canada, identified *Aeromonas hydrophila* in 4.5% of wastewater samples, which correlates with the prevalence rates found in other developed countries. This highlights the universal challenge of managing wastewater to prevent the spread of opportunistic pathogens like *Aeromonas hydrophila* (21).

Bacillus cereus in wastewater has been documented in various regions worldwide. For instance, a study conducted in Shanghai, China, identified *Bacillus cereus* in 6% of the wastewater samples collected, highlighting the prevalence of this bacterium in urban areas with high population density and poor sanitation (53). Similarly, research in Accra, Ghana, found that 5% of wastewater samples tested positive for *Bacillus cereus*, emphasizing the need for improved waste treatment facilities to prevent the spread of this pathogen (6). In Europe, a study conducted in Prague, Czech Republic, reported the presence of *Bacillus cereus* in 5.5% of wastewater samples, which aligns with the findings of our study. This suggests a widespread issue across different geographic locations, where inadequate waste disposal practices contribute to the persistence of pathogenic bacteria in the environment (54).

In this study, the presence of *Lysinibacillus fusiformis* in wastewater was investigated, and 3 isolates of the bacterium were identified. These findings are noteworthy because *Lysinibacillus fusiformis* is an opportunistic pathogen associated with infections such as endocarditis, septicemia, and wound infections. The presence of *Lysinibacillus fusiformis* in wastewater has been documented in various regions worldwide. For instance, a study conducted in Seoul, South Korea, identified *Lysinibacillus fusiformis* in 4% of the wastewater samples collected, highlighting the prevalence of this bacterium in urban areas with high population density and poor sanitation (25). Similarly, research in Lagos, Nigeria, found that 3.5% of wastewater samples tested positive for *Lysinibacillus fusiformis*, emphasizing the need for improved waste treatment facilities to prevent the spread of this pathogen (56). In Europe, a study conducted in Berlin,

Germany, reported the presence of *Lysinibacillus fusiformis* in 3.8% of wastewater samples, which aligns with the findings of our study. This suggests a widespread issue across different geographic locations, where inadequate waste disposal practices contribute to the persistence of pathogenic bacteria in the environment (15). In South America, similar patterns have been observed. A study in Rio de Janeiro, Brazil, identified *Lysinibacillus fusiformis* in 4.5% of wastewater samples, which correlates with the prevalence rates found in other developing countries. This highlights the universal challenge of managing wastewater to prevent the spread of opportunistic pathogens like *Lysinibacillus fusiformis* (46).

The presence of *Lysinibacillusphaericus* in wastewater was investigated, and 5 isolates of the bacterium were identified. These findings are significant because *Lysinibacillusphaericus* is known for its potential as a biocontrol agent against mosquito larvae but can also act as an opportunistic pathogen in humans, causing infections such as bacteremia and endocarditis. In Europe, a study conducted in Warsaw, Poland, reported the presence of *Lysinibacillusphaericus* in 5.8% of wastewater samples, which aligns with the findings of our study. This suggests a widespread issue across different geographic locations, where inadequate waste disposal practices contribute to the persistence of pathogenic bacteria in the environment (59). In South America, similar patterns have been observed. A study in Lima, Peru, identified *Lysinibacillusphaericus* in 6.5% of wastewater samples, which correlates with the prevalence rates found in other developing countries. This highlights the universal challenge of managing wastewater to prevent the spread of opportunistic pathogens like *Lysinibacillusphaericus* (46). [The detection of 5 *Lysinibacillusphaericus* isolates in wastewater is a significant finding that aligns with similar studies from various countries.]

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The presence of *Staphylococcus aureus* in wastewater was investigated, and 6 isolates of the bacterium were identified. These findings are concerning because *Staphylococcus aureus* is a significant pathogen associated with various infections, including skin infections, pneumonia, bloodstream infections, and food poisoning. The presence of *Staphylococcus aureus* in wastewater has been documented in various regions worldwide. For instance, a study conducted in Cape Town, South Africa, identified *Staphylococcus aureus* in 7% of the wastewater samples collected, highlighting the prevalence of this bacterium in urban areas with poor sanitation (32). Similarly, research in Mumbai, India, found that 8% of wastewater samples tested positive for *Staphylococcus aureus*, emphasizing the need for improved waste treatment facilities to prevent the spread of this pathogen (24). In Europe, a study conducted in London, United Kingdom, reported the presence of *Staphylococcus aureus* in 6.5% of wastewater samples, which aligns with the findings of our study. This suggests a widespread issue across different geographic locations, where inadequate waste disposal practices contribute to the persistence of pathogenic bacteria in the environment (50). In North America, similar patterns have been observed. A study in New York City, USA, identified *Staphylococcus aureus* in 7.2% of wastewater samples, which correlates with the prevalence rates found in other developed countries. This highlights the universal challenge of managing wastewater to prevent the spread of opportunistic pathogens like *Staphylococcus aureus* (23).

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4. Conclusion

The detection of various pathogenic bacteria, including *Vibrio cholerae*, *Morganella morganii*, *Proteus mirabilis*, *Acinetobacter baumannii*, *Klebsiella pneumoniae*, *Providencia rettgeri*, *Providencia stuartii*, *Aeromonas hydrophila*, *Bacillus cereus*, *Lysinibacillus fusiformis*, *Lysinibacillusphaericus*, and *Staphylococcus aureus* in wastewater samples from our study area underscores a significant public health concern. The presence of these pathogens indicates potential risks for disease outbreaks, particularly in regions with inadequate waste management and sanitation systems. Our findings align with similar studies conducted globally, highlighting the universal challenge of managing wastewater to prevent the spread of infectious diseases. The prevalence rates of these bacteria in various countries emphasize the need for comprehensive waste management practices and robust public health strategies to mitigate these risks. Immediate public health interventions are necessary to address these concerns. Implementing comprehensive waste management systems and ensuring access to clean and safe water for all communities is crucial. Regular monitoring of wastewater for pathogens should be integrated into public health strategies to detect and mitigate potential health risks early. Additionally, public awareness campaigns about the dangers of contaminated water and proper hygiene practices can significantly

Comment [H21]: italicize

contribute to preventing infections caused by these opportunistic pathogens. Governments and public health organizations must collaborate to improve infrastructure, enforce sanitation regulations, and provide resources for effective waste treatment and disposal. By learning from the experiences of other regions and implementing best practices, it is possible to reduce the risk of infections caused by these pathogens and protect public health. Overall, our study highlights the importance of addressing microbial contamination in wastewater through improved sanitation, waste management, and public health education. These measures are essential for reducing the risk of infections and ensuring the well-being of communities worldwide.

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