Microbiological surveillance of *Salmonella* strains in the effluents of the Teaching Hospital (CHU) of Yopougon and Cocody, Côte d'Ivoire

ABSTRACT

Diarrhoeal diseases are a major public health problem worldwide, with salmonellosis being a leading cause of foodborne illness in humans. Environmental pollution from healthcare activities, particularly from the discharge of hospital effluent into urban sewer systems, poses a significant threat. These effluents can carry microorganisms, antibiotic residues and detergents that contribute to the development and spread of bacterial multiresistance.In Abidjan, the capital of Côte d'Ivoire, hospital wastewater is not treated before being discharged into the sewer system. This untreated wastewater eventually flows into the Ebrié lagoon, a critical water source for many local communities.

The dependence of the lagoon's population on these waters raises significant public health concerns, particularly with regard to potential contamination with pathogenic bacteria such as *Salmonella*.The aim of this study was to assess the presence of *Salmonella* in hospital wastewater to understand the risk of contamination and to inform potential mitigation strategies. A total of 60 wastewater samples were collected: 30 from the teaching hospital (CHU) of Yopougon and 30 from Cocody.

Upon analysis, no *Salmonella sp* strains were detected in any of the samples. However, 28 bacterial strains exhibiting characteristics similar to *Salmonella* were isolated, suggesting the presence of potentially virulent pathogens. In Yopougon's samples, 14 strains of *Proteus mirabilis* and 2 strains of *Klebsiellasp* were identified. In Cocody 's samples, 12 strains of *Citrobacterfreundiisp* were isolated.

These findings highlight the importance of monitoring and treating hospital wastewater to prevent the spread of potentially harmful bacteria into the environment.

KEYWARDS: *Salmonella*, wasted water, microbiological surveillance, waterborne bacteria

INTRODUCTION

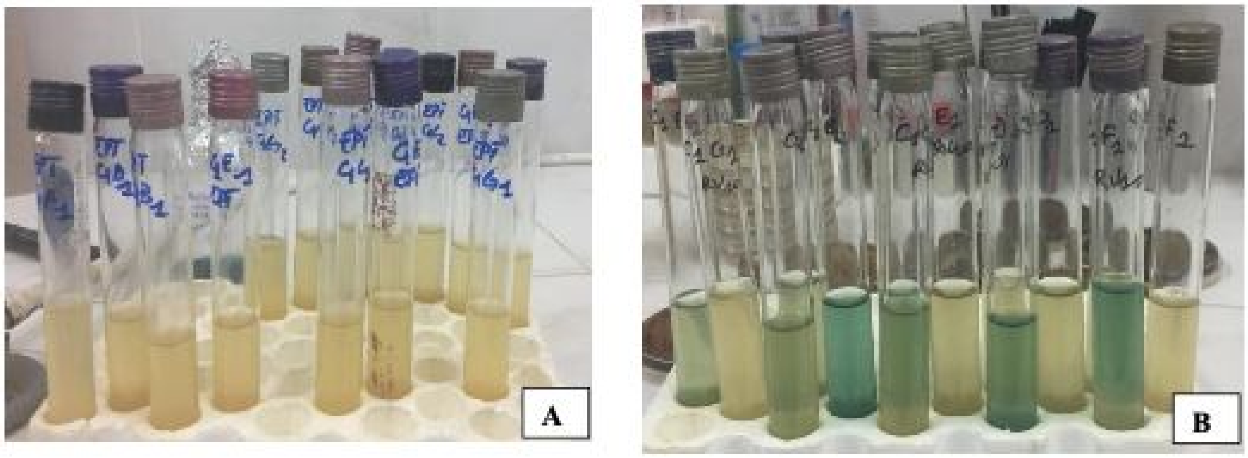
Water intended for human consumption and typical domestic use must be safe to drink. For water used for food preparation or hygiene, excellent physicochemical and bacteriological quality is essential[8]. The World Health Organization (WHO) estimates that 80% of diseases affecting the global population are directly related to poor water quality, inadequate sanitation and poor hygiene [3, 11, 22]. As a result of these problems, monitoring water quality has become a global public health priority[18,24,25].Abidjan is the most populous city in Côte d'Ivoire. The inadequacy of sanitation networks and the fact that over 30% of the population of Abidjan is not connected to the sewerage system results in untreated wastewater being discharged into the natural environment [14, 26,27,28,29,30,31].Wastewater from healthcare facilities can serve as a vector for infection transmission. Although most bacteria in wastewater are not pathogenic, certain pathogens such as Salmonella, likely introduced by human activities, have long been implicated in waterborne disease, particularly in developing countries[6]. In sub-Saharan Africa, these bacteria, which contaminate water and food through faeces, cause a mortality rate of 22-45% among infected individuals[13].Given the significant threat posed by salmonella worldwide, it is vital for every country to monitor salmonellosis. This study, entitled 'Microbiological surveillance of *Salmonella* strains in the effluents of Teaching Hospital (CHU) of Yopougon and Cocody, Côte d'Ivoire,' aims to address several critical issues. These include identifying the prevalence of *Salmonella* strains in Teaching Hospital effluents, understanding the patterns of antibiotic resistance, and evaluating the effectiveness of current disinfection protocols. By tackling these problems, the study seeks to enhance public health strategies, improve infection control measures, and reduce the incidence of salmonellosis in the region.

I-METHODS

The study involved sampling wastewater from two major hospitals in Abidjan: the teaching hospital (CHU) of Yopougon and Cocody. using a scoop attached to a rope, the wastewater from the manholes of the central collecting pipes of the different hospital structures was transferred into 10 sterile bottles, each with a one-liter capacity, at approximately ten-minute intervals. This method ensures that the continually renewed flow of water is adequately sampled. The samples were then stored in a cooler with cold packs and transported to the laboratory for analysis.
A total of 60 samples were collected, with 30 samples from each hospital. Sampling focused on water collected from the central collection pipes. Each sample was subjected to microbiological analysis to isolate and identify *Salmonella* strains.

1. Sample preparation

Samples were pre-enriched in EPT broth at 37°C for 24 hours. Then selective enrichment was performed in RV10 broth at 42°C for 24 hours.



A: Pre-enriched in EPT **B**: selective enrichment in RV10

Figure 1: Pre-enrichment and enrichment processes

2. Selective isolation

Enriched samples were plated on Hektoen agar and incubated at 37°C for 24 hours.Suspected *Salmonella*colonies were further identified using biochemical tests and the API 20 E system.The search for biochemical characteristics using the reduced Le Minor rack involved inoculating five culture media: urea-indole, Kligler-Hajna, Mannitol-mobility, iron lysine, and Simmons citrate media. Every strains with Urease and Indole negative were suspected *Salmonella*. (Figure 2 and 3). Typical Salmonella colonies are characterized by a slope without color change (indicating no lactose fermentation), a change in the pellet to yellow (indicating the absence of glucose fermentation), the presence of air bubbles (indicating gas formation), and blackening of the medium (indicating the presence of H_2S). (Figure 4).The mannitol-motility medium allowed for the detection of mannitol fermentation and the mobility of the strains. The inoculated tubes were incubated for 24 hours at 37°C. Mannitol fermentation is indicated by a color change in the medium from red to yellow. Mobile bacteria like *salmonella*diffuse from the inoculation line, while immobile bacteria grow only along the inoculation streak (Figure 5).

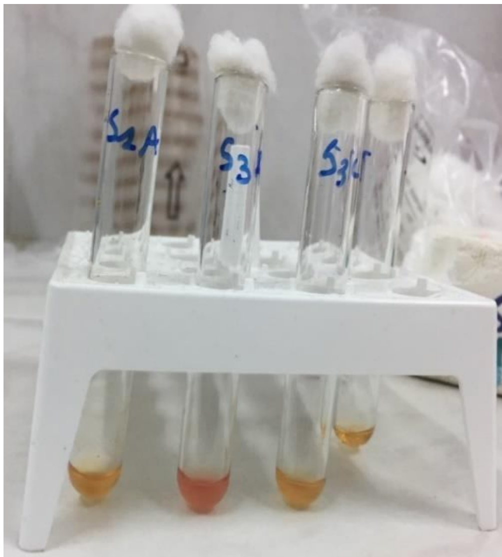


Figure 2: Urease test

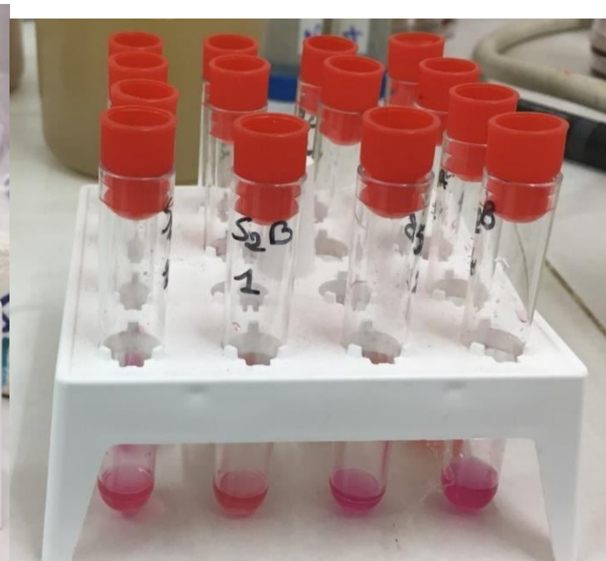


Figure 3: Indole test

Figure 4: inoculatingKligler-Hajna

Figure 5: Method Used to Assess Motility and Mannitol Fermentation

3. Antibiotic resistance profile (antibiogram)

The sensitivity of the strains to different antibiotics was determined using the disk diffusion method on agar medium, as described by Bauer et al. (1966), and interpretedaccording to the Comité d'Antibiogramme de la Société Française de Microbiologie (CA-SFM, 2019). The antibioticsusedincluded: Ciprofloxacin (30 µg), Ceftriaxone (30 µg), Cotrimoxazole (25 µg), Nalidixicacid (30 µg), Cefepime (5 µg), Amikacin (30 µg), Tobramycin (10 µg), Ticarcillin (75 µg), Chloramphenicol (30 µg), Cefoxitin (30 µg), Amoxicillin (20 µg), Amoxicillin + Clavulanicacid (20+10 µg), Imipenem (10 µg), and Gentamicin (10 µg).A 24-hour colony was collected from the ordinary agar using a Pasteur pipette and mixed into 5 mL of 0.85% NaCl saline solution. The suspension was then homogenized and adjusted using a densitometer to a turbidity equivalent to the 0.5 McFarland standard, corresponding to an inoculum of approximately 10^8 CFU/mL (CA-SFM, 2019). A swab was taken from the suspension and streaked across the entire surface of the pre-poured Mueller-Hinton agar in 90 mm diameter Petri dishes. Antibiotic disks were then placed on the agar surface using a disk applicator. After placing the disks, the Petri dishes were incubated for 24 hours at 37°C (Figure 6).The antibiotic diffused radially in the agar from the point where the disk was placed, creating a concentration gradient. The area of the agar where the antibiotic concentration was sufficient, i.e., equal to or greater than the minimum inhibitory concentration (MIC), remained free of bacterial growth after incubation. This area defined the zone of inhibition (Figure 7). The diameters of the inhibition zones were measured manually using a caliper. The results were then interpreted according to the CA-SFM 2019 recommendations, and the categories of sensitivity (S) and resistance (R) to the antibiotics were determined based on the inhibition zone diameters (in millimeters).

II- RESULTS

In this study, 60 wastewater samples were collected. Of these, 28 suspected *Salmonella* strains were isolated. Out of these 28 isolated strains, 12 (42.85%) were from the Cocody site and 16 (57.15%) were from the Yopougon site. After culture and biochemical testing, none of these strains was phenotypically confirmed as *Salmonella*.

Table 1: Distribution of bacterial strains isolated from hospital effluents

Bacteria	Number of strains	Percentage
Salmonella sp	0	0
Proteus mirabilis	14	50
Citrobacterfreundii	12	42,85
Klebsiellasp	02	7,15
Total	28	100

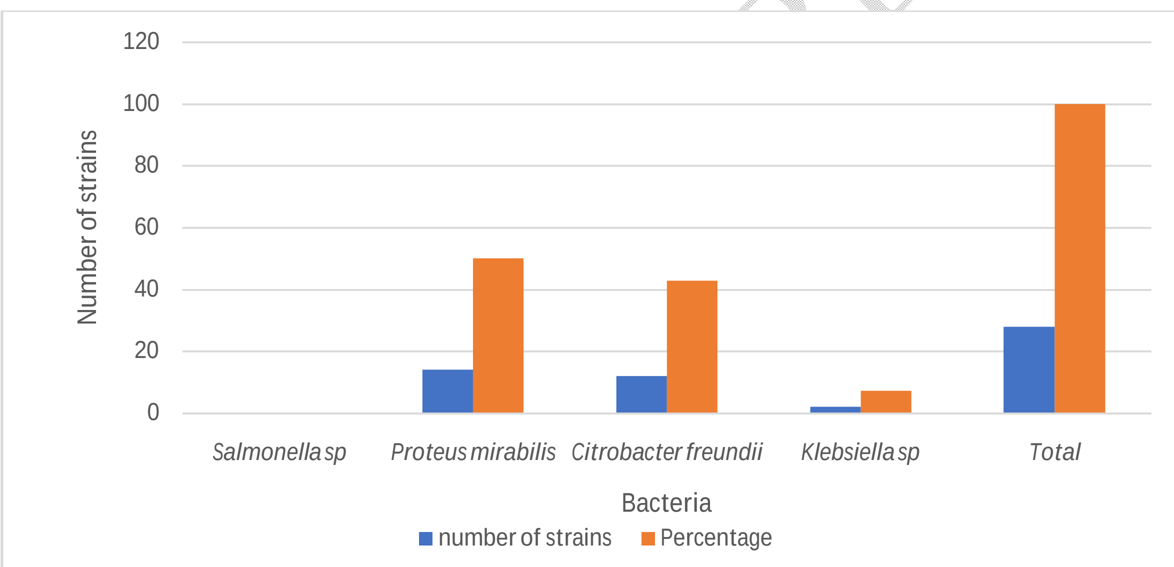


Fig 6: Graphical presentation of Distribution of bacterial strains isolated from hospital effluents

Figure 7: Identification of Isolated Strains Using the API 20 E Gallery

A : *Citrobacter freundii*

ONPG	ADH	LDC	ODC	CIT	H2S	UREE	TDA	IND	VP	GEL	GLU	MAN	INO	SOR	RHA	SAC	MEL	AMY	ARA
+	+	-	-	+	+	-	-	-	-	+	+	+	-	+	+	-	-	-	+

B : *Klebsiella sp*

ONPG	ADH	LDC	ODC	CIT	H2S	UREE	TDA	IND	VP	GEL	GLU	MAN	INO	SOR	RHA	SAC	MEL	AMY	ARA
+	-	+	+	+	-	+	-	+	+	-	+	+	+	+	+	+	+	+	+

C : *Proteus Mirabilis*

ONPG	ADH	LDC	ODC	CIT	H2S	UREE	TDA	IND	VP	GEL	GLU	MAN	INO	SOR	RHA	SAC	MEL	AMY	ARA
-	-	-	+	+	+	+	+	-	+	+	+	-	-	-	-	-	-	-	-

ONPG : Ortho-Nitro-Phenyl-Galactoside, ADH : Arginine Dihydrolase, LDC : Lysine Décarboxylase, ODC : Ornithine Décarboxylase, CIT : Citrate, H2S : Sulfure d'Hydrogène, TDA : Tryptophane Désaminanse, IND : Indole, VP : Acétoïne, GEL : Gelatinase, GLU : Glucose, MAN : Mannitol, INO : Inositol, SOR : Sorbitol, RHA : Rhamnose, SAC : Saccharose, MEL : Melibiose, AMY : Amygdaline, ARA : Arabinose.

Table 2. Identification of Isolated Strains Using the API 20 E Gallery

Antibiotic resistance of isolated strains

In this study, antibiograms were performed on the twelve (12) Citrobacterfreundii strains isolated from Cocody. As these bacteria exhibit multiple antibiotic resistance, it was important to subject these strains to an antibiogram to understand their significant presence and to establish their resistance profiles.Figure 8and 9 show the general susceptibility and resistance profile to different antibiotics.

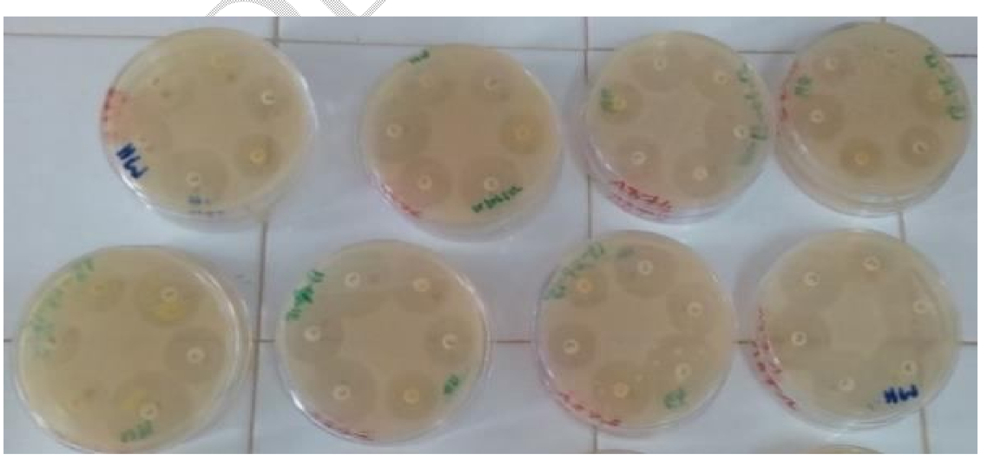


Figure 8: General susceptibility and resistance profile to different antibiotics

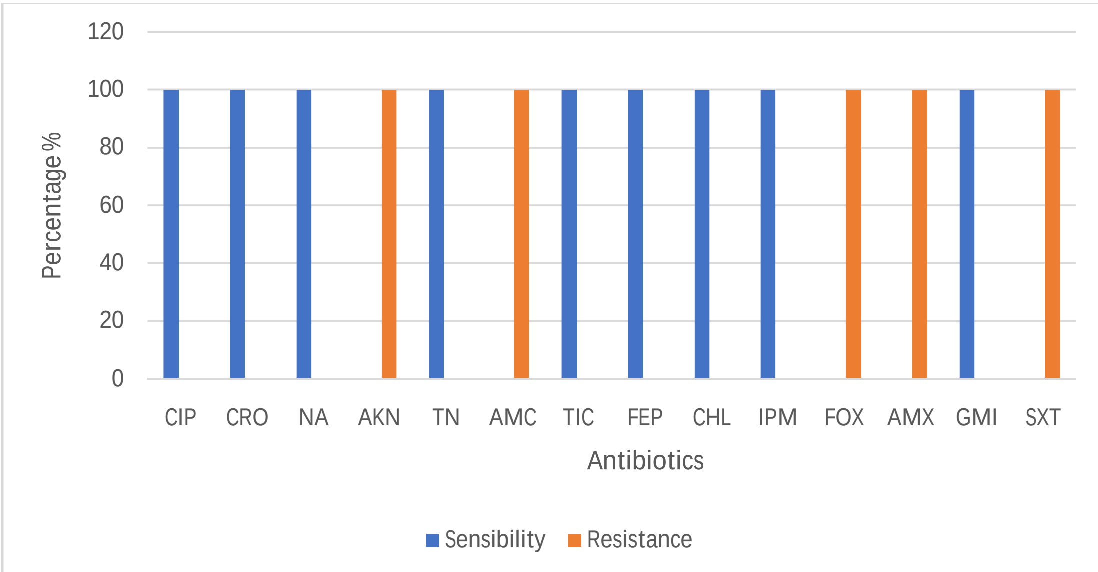


Fig 9: Antibiotics resistance and sensitivity test results

Based on this antibiogram, Citrobacterfreundii is sensitive to fluoroquinolones, cephalosporins, aminoglycosides, penicillins, and carbapenems, but resistant to amoxicillin, amoxicillin/clavulanic acid, trimethoprim/sulfamethoxazole, and cefoxitin.

Abréviations:•Ciprofloxacin (CIP) •Ceftriaxone (CRO)•Nalidixic Acid (NA)•Amikacin (AKN)•Tobramycin (TN)• Amoxicillin + Clavulanic Acid (AMC)•Ticarcillin (TIC)•Cefepime (FEP)• Chloramphenicol (CHL) •Imipenem (IMP)•Cefoxitin (FOX)• Amoxicillin (AMX)• Gentamicin (GMI) •Cotrimoxazole (SXT)

III- DISCUSSION

The absence of Salmonella strains in the hospital effluent samples from Yopougon and Cocody can be attributed to the number of samples collected during our study: 30 samples per health centre. A study of Salmonella genes in the wastewater of OuedKhoumaneMeknès in Morocco showed that only 2 samples (2.38%) out of 84 samples collected from a single site were identified as Salmonella [10]. This could explain the absence of *Salmonella*strains due to insufficient sampling in each area.

Teaching hospitals use large quantities of antiseptics such as sodium hypochlorite, iodine derivatives, chlorhexidine, and quaternary ammonium for surface and floor cleaning, to which *Salmonella*is sensitive [9].These disinfectants are commonly used in hospital cleaning, and bacteria present in hospitals are discharged through the sewage system.The sensitivity of *Salmonella* to these disinfectants may explain its absence in the test samples.

The 100% prevalence of *Citrobacterfreundii* in Cocody CHU can be explained by its natural resistance to several broad-spectrum antibiotic families commonly used, such as aminoglycosides, penicillins, cephalosporins, and sulfonamides [15]. These antibiotics are widely used by the population and hospitals due to their efficacy in treating various conditions. However, antibiotics and health-related waste are disposed of through the same sanitary waste channels. The coexistence of these bacteria with antibiotics in the environment could explain the inability to prevent the proliferation of *Citrobacterfreundii*, resulting in their significant presence in the analyzed Cocody effluent.

Proteus mirabilis also showed a significant presence in the Yopougon effluent. It's a versatile pathogen known for its ability to thrive in a variety of environments, including soil, water and sewage. It plays an important role in the decomposition of organic matter and is also part of the faecal flora in the digestive systems of humans and animals. The ability of this bacterium to adapt to different environments, together with its urease production and swarming motility, contribute to its pathogenic potential and environmental persistence[1,14]. These enterobacteria contribute to the maintenance of the commensal flora and can cause opportunistic infections when this flora is imbalanced[13].Their significant presence in the effluents of the Yopougon CHU (87.5%) can be justified by the fact that these bacteria live as saprophytes and contribute to the balance of the human digestive flora. *Proteus mirabilis* is also a nosocomial uropathogen which is prevalent in hospital settings [14].The coupling of health-related and human activity waste disposal channels could justify the presence of these bacteria in the waters of this CHU. Studies in Spain have shown that Proteus mirabilis has excellent survival in the environment and wastewater[7].
The coupling of health-related and human activity waste disposal channels could justify the presence of these bacteria in the waters of this CHU.
Klebsiellapneumoniae is similar to Proteus mirabilis in that both are nosocomial uropathogens commonly found in hospital settings. These pathogens are responsible for a significant number of urinary tract infections in hospitalized patients, posing a challenge due to their resistance to multiple antibiotics [23].Infection and carriage rates of Klebsiellapneumoniae significantly increase with the extensive use of antimicrobial agents, as these agents contribute to the development and spread of antibiotic resistance[12,24].Hospitals use antimicrobials and antibiotics, which enter the sewers with microorganisms and are directed to a single destination without prior treatment. Their resistance to antibiotics could be explained by the ineffectiveness of these substances against these bacteria.

CONCLUSION & PERSPECTIVES

The investigation of two sampling sites in Abidjan allowed us to collect sixty (60) wastewater samples from the hospital effluents of Cocody and Yopougon to test for Salmonella. After analysis, 28 samples were found to have *Salmonella*-like characteristics. Using the API 20 E gallery, the results were as follows: 12 samples of *Citrobacterfreundii* from Cocody, 14 strains of *Proteus mirabilis*, and 2 strains of *Klebsiella sp*. from Yopougon. These are all class 2 bacteria, much important as *Salmonella*. The strains of *Citrobacterfreundii* isolated were resistant to the families of antibiotics tested (aminopenicillins, cephalosporins, and sulfonamides), confirming their natural resistance profile to these antibiotics. *Proteus mirabilis* are invasive bacteria with a significant lifespan in wastewater. They are also resistant to common disinfectants.

Although *Salmonella* was not isolated in this study, the high prevalence of these pathogenic bacteria should lead to increased monitoring, revision of hygiene practices, and treatment of hospital wastewater before discharge into the Ebrié lagoon. The constant exposure of bacteria to antibiotics in hospital wastewater could result in these bacteria becoming less sensitive to these therapeutic substances, in addition to their natural resistance profiles. Looking ahead, we plan to increase the sample size and then perform amplification and sequencing to investigate the genomic biodiversity of *Citrobacterfreundii* in wastewater samples.

Disclaimer (Artificial intelligence)

Option 1:

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of manuscripts.

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