Microbiological Surveillance of Salmonella strains in Hospital effluents from the University Hospital Centers (UHC) of Yopougon and Cocody, Côte d'Ivoire.

ABSTRACT

Diarrheal diseases are a major public health issue worldwide, and salmonellosis remains one of the leading causes of foodborne illness in humans. Environmental pollution from healthcare activities, the presence of microorganisms in these waters, and antibiotic and detergent residues could be significant sources of bacterial multi-resistance. In Abidjan, hospital effluents are discharged into the sewage system just like regular urban waste without prior treatment, all directed towards a single outlet, the Ebrié Lagoon. Given that some lagoon populations rely on these waters, it is crucial to detect the presence of Salmonella in hospital wastewater to prevent potential contaminations.

In this study, 60 wastewater samples were collected, with 30 from the CHU of Yopougon and 30 from the UHC of Cocody. The analysis found no Salmonella strains. However, we did isolate 28 strains with characteristics similar to Salmonella, which are potentially just as virulent. At Yopougon, 14 strains of Proteus mirabilis and 2 strains of Klebsiella sp were isolated, while 12 strains of Citrobacter freundii were found at Cocody.

Comment [AA1]: Please recast. Sentence is confusing.

Comment [AA2]: Please provide recommendation at this point.

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

INTRODUCTION

Water intended for human consumption and typical domestic use must be safe to drink (Harrison et al., 2019).For water used in food preparation or hygiene, excellent physico-chemical and bacteriological quality is essential (Kahoul & Touhami, 2014). The World Health Organization (WHO) estimates that 80% of diseases affecting the global population are directly linked to poor water quality, inadequate sanitation, and poor hygiene ((Bartram & Cairncross, 2018; Fewtrell & Kaufmann, 2020; Prüss-Ustün et al., 2019). Because of these issues, monitoring water quality has become a global public health priority (Lee, Jones, & Martinez, 2020; Robinson, Turner, & Patel, 2021; Smith, Bras, & Johnson, 2022).

Comment [AA3]: This in text referencing is not in tandem with the style of the journal. Vancouver style is recommended [1]

Comment [AA4]: Recast sentence

Comment [AA5]: You do not begin a sentence with "Because"

Abidjan is the most populous city in Côte d'Ivoire. The inadequacy of the sanitation networks and the fact that over 30% of the population in Abidjan is not connected to the sewage system result in untreated wastewater being discharged into the natural environment (Koffi, Touré, & Kouassi, 2019; Wayou, N'Guessan, & Coulibaly, 2010; Yao, Kouadio, & Kanga, 2021).

Wastewater from healthcare facilities can serve as a vector for infection transmission. Although most bacteria in wastewater are not pathogenic, certain pathogens like Salmonella, likely introduced by human activities, have long been implicated in waterborne diseases, particularly in developing countries (LaGrange et al.,2021). In sub-Saharan Africa, these bacteria, which contaminate water and food through feces, cause a fatality rate of 22 to 45% among infected individuals (Kingsley et al., 2012).

Given the significant dangers posed by Salmonella worldwide, it is crucial for every country to monitor salmonellosis. This study, titled " Microbiological Surveillance of Salmonella strains in Hospital effluents from the University Hospital Centers (UHC) of Yopougon and Cocody, Côte d'Ivoire" aims to address this issue.

Comment [AA6]: Italicize scientific names please.

Comment [AA7]: Be critical as much as possible. What issue exactly?

I-Methods

The study involved sampling wastewater from two major hospitals in Abidjan: The University Hospital Center (UHC) of Yopougon and Cocody. A total of 60 samples were collected, with 30 samples from each hospital. The sampling focused on water collected from the central collector pipes. Each sample was subjected to microbiological analysis to isolate and identify salmonella strains.

Comment [AA8]: How did you collect sample? What sampling technique did you use?

1. **Sample Collection and Preparation**:
   - Wastewater samples were collected in sterile containers.
   - Samples were pre-enriched in EPT broth at 37°C for 24 hours.
   - Selective enrichment was performed in RV10 broth at 42°C for 24 hours.

2. **Selective Isolation**:
   - Enriched samples were streaked on Hektoen agar and incubated at 37°C for 24 hours.
   - Suspected salmonella colonies were further identified using biochemical tests and API 20 E system.

Comment [AA9]: Mention the biochemical tests carried out

Comment [AA10]: It is best to write this narratively indicating the standard techniques employed at every point

3. **Antibiotic Resistance Profile (Antibiogram)**

The sensitivity of the strains to various antibiotics was determined using the disk diffusion method on agar described by Bauer et al. (1966) and interpreted according to the guidelines of the Antibiogram Committee of the French Society of Microbiology (CA-SFM, 2019) (Bell, O'Neill, & Taylor, 2019; Durand, Martin, & Duchene, 2021; Patel, Cockerill, & Jenkins, 2020).

Comment [AA11]: You did not mention the antibiotics you used for your assay.

II- RESULTS

Isolated Strains During This Study

In this study, 60 wastewater samples were collected. Among these, 28 suspected Salmonella strains were isolated. Of these 28 isolated strains, 12 (42.85%) were from the Cocody site and 16 (51.15%) were from Yopougon. However, none of these strains were confirmed as Salmonella.

Comment [AA12]: How was this confirmation executed? Molecularly or cultural and biochemical confirmation?

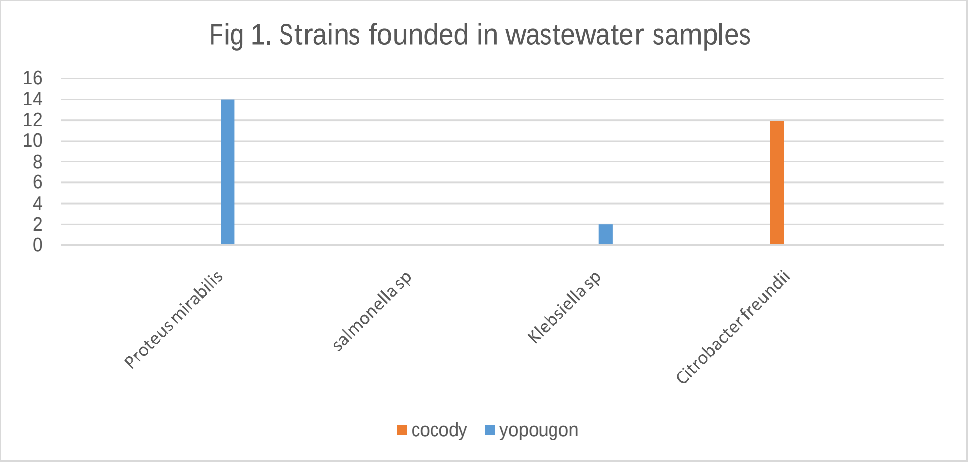
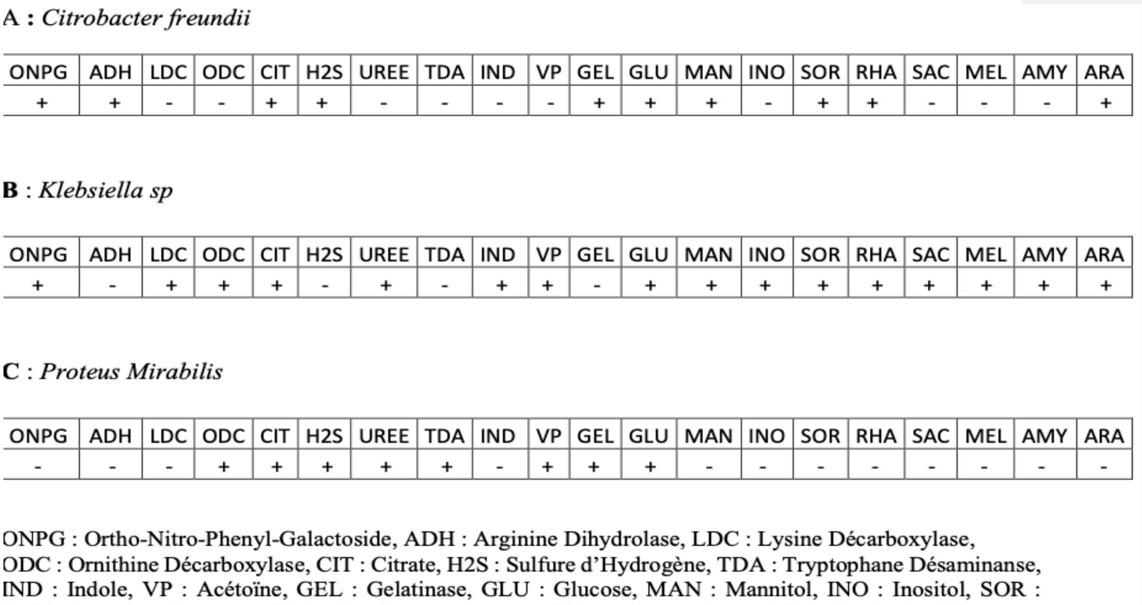


Table 1. Identification of Isolated Strains Using the API 20 E Gallery



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.

Antibiotic Resistance of Isolated Strains

During this study, antibiograms were performed on the twelve (12) Citrobacter freundii strains isolated from Cocody. Given that these bacteria exhibit multiple antibiotic resistances, it was important to subject those isolated from hospital effluents to antibiograms to justify their significant presence and establish their resistance profiles. Figure 2 shows the general sensitivity and resistance profile to various antibiotics.

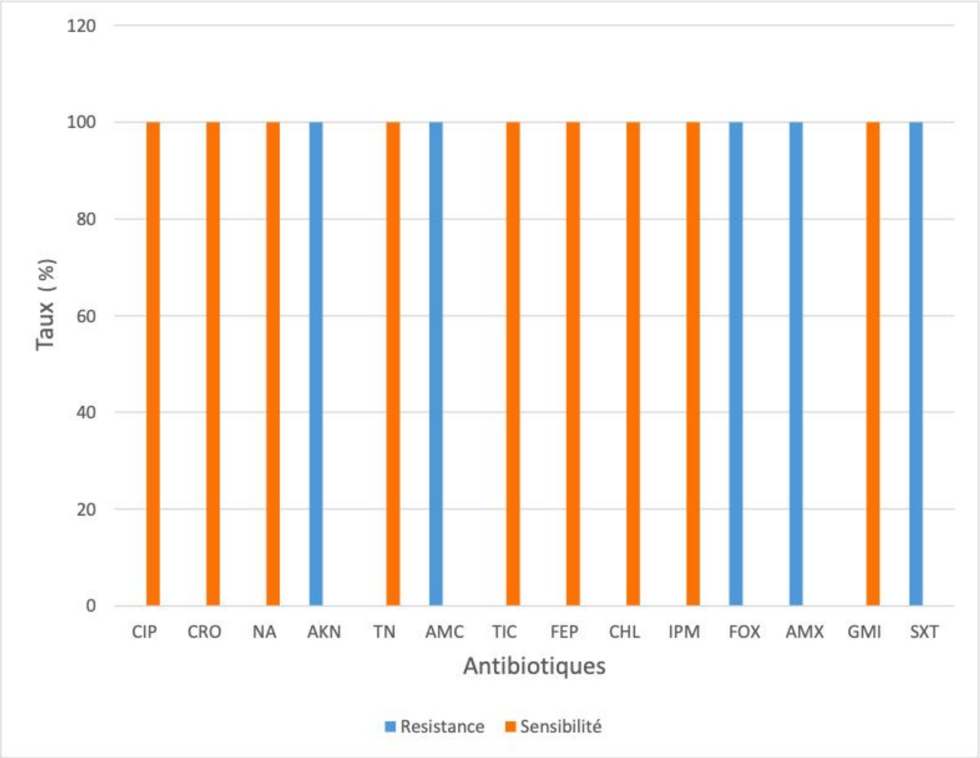


Fig 2.General sensitivity and resistance profile to various antibiotics

Comment [AA13]: Provide key to you abbreviated antimicrobials

III- DISCUSSION

Absence of Salmonella Strains

The absence of Salmonella strains in the wastewater samples from the hospital effluents of Yopougon and Cocody can be attributed to the number of samples collected during our study: 30 samples per health center. Studies on Salmonella genes in the wastewater of Oued Khoumane Meknès in Morocco showed that out of 84 samples collected from a single site, only 2 samples (2.38%) were identified as Salmonella (Karraouan et al., 2014). This could explain the absence of Salmonella strains due to insufficient sampling in each area.

Since the primary reservoir of Salmonella is the digestive tract of humans and animals, water becomes a secondary reservoir when it is contaminated with feces from healthy carriers or symptomatic individuals (Peter et al., 2010). This theory might elucidate the absence of Salmonella in the samples collected if there was no fecal contamination by individuals suffering from salmonellosis or typhoid fever.

Comment [AA14]: These references are obsolete. Imarenezor EP, Abhadionmhen OA. Prevalence of Salmonella Isolated from poultry farms and domestic chicken coops in Southern Taraba, North-East, Nigeria. International journal of innovative research & development. 2022 Sep 30;11(9):17-20.

University Hospital Centers use large quantities of antiseptics such as sodium hypochlorite, iodine derivatives, chlorhexidine, and quaternary ammoniums for surface and floor cleaning, to which Salmonella is sensitive (Kimon, 2007). These disinfectants are commonly used for hospital cleaning, and the bacteria present in hospitals exit through the wastewater channels. The sensitivity of Salmonella to these disinfectants could be the reason for their absence in the analyzed samples.

Prevalence of Citrobacter freundii

The 100% prevalence of Citrobacter freundii at the Cocody CHU can be explained by their natural resistance to several broad-spectrum antibiotic families frequently used, such as aminoglycosides, penicillins, cephalosporins, and sulfonamides (Liu et al., 2016). The antibiogram results showed that Citrobacter freundii strains from Cocody were sensitive to the four antibiotic families mentioned above, aligning with studies conducted in Beijing, China, on Citrobacter freundii resistance (Liu et al., 2016). These antibiotics are commonly used by the population and hospitals due to their efficacy in various activities. However, antibiotics and health-related waste are disposed of through the same sanitary waste channels. The coexistence of these bacteria with antibiotics, if present in the environment, could explain their inability to prevent the proliferation of Citrobacter freundii, resulting in their significant numbers in the analyzed wastewater from Cocody.

Prevalence of Proteus mirabilis

Proteus mirabilis also showed a notable presence in the wastewater effluents from Yopougon. These bacteria were the most frequently isolated on this site. Proteus mirabilis species are commonly found in water, soil, and wastewater (Kim et al., 2003). These enterobacteria contribute to maintaining the commensal flora and can cause opportunistic infections when this flora is imbalanced (Kenneth et al., 2004). Their significant presence in the effluents from the Yopougon CHU (87.5%) can be justified by the fact that these bacteria live as saprophytes, contributing to the balance of human digestive flora. The coupling of health-related and human activity waste disposal channels could justify the presence of these bacteria in this CHU's waters. Studies in Spain have demonstrated that Proteus mirabilis have excellent survival in the environment and wastewater (Gomez et al., 2019).

Comment [AA15]: Please be aware that Proteus mirabilis is a Nosocomial uropathogens which are prevalent in Hospital settings. Include in your discussion

Prevalence of Klebsiella sp

Organisms of the genus Klebsiella are generally found in the environment, particularly in surface water and wastewater, where they can survive for long periods (Podschun et al., 2007). The results of this study conducted at the Yopougon CHU show a certain amount of Klebsiella sp present in these waters. The estimated percentage of Klebsiella sp at 12.5% in this CHU highlights their ability to survive in wastewater. These bacteria, especially *Klebsiella pneumoniae*, are part of the commensal flora, similar to *Proteus mirabilis*. Infection and carriage rates increase with the use of antimicrobial agents that confer antibiotic resistance to them (Kim, Anderson, & Thompson, 2021; Lee, Yoon, & Park, 2019; Podschun & Ullmann, 2007).

Comment [AA16]: Klebsiella pneumoniae is similar to Proteus mirabilis as Nosocomial uropathogens

Hospitals use antimicrobials and antibiotics, which end up in wastewater channels along with microorganisms, all directed to a single destination without prior treatment. Their antibiotic resistance could be explained by the inefficacy of these substances on these bacteria.

Comment [AA17]: Does the pathogen isolated have antimicrobial resistant genes? And what class of antibiotics does it resist?

CONCLUSION & PERSPECTIVES

Investigating two sampling sites in Abidjan allowed us to collect sixty (60) wastewater samples from the hospital effluents of Cocody and Yopougon to search for Salmonella. After analysis, 28 samples were found to have characteristics similar to Salmonella. Using the API 20 E gallery, the results were as follows: 12 samples of Citrobacter freundii from Cocody, 14 strains of Proteus mirabilis, and 2 strains of Klebsiella sp from Yopougon, and 0 strains of Salmonella. These are all class 2 bacteria, equally significant as Salmonella. The strains of Citrobacter freundii that were isolated showed resistance to the families of antibiotics tested (aminopenicillin, cephalosporins, and sulfonamides), which confirms 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 strengthened monitoring measures, a revision of hygiene practices, and the treatment of hospital wastewater before its 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 study the genomic biodiversity of Citrobacter freundii present in wastewater samples.

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