



Efficiency of EMB Medium Combined with MALDI-TOF in Isolating and Identifying Coliform Lactose Fermenters and Non-Lactose Fermenters from Wastewater

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

In Côte d'Ivoire, the sewer network is largely underdeveloped, resulting in the absence or incompleteness of sanitation infrastructure in many urban and rural areas. This lack of infrastructure leads to untreated wastewater being frequently discharged into rivers and other sources of drinking water. The contamination of water sources by untreated wastewater spreads waterborne diseases, which particularly affect children and vulnerable populations. Coliforms, as key indicators of the microbiological quality of water, play a crucial role in assessing health risks. The presence and diversity of these coliforms can provide insights into the extent of water contamination and the potential health risks to the population. This study focuses on the diversity of lactose-fermenting and non-lactose-fermenting coliforms in the wastewater of Abidjan. The objective is to use MALDI-TOF mass spectrometry for the rapid and accurate identification of these coliforms, thereby providing a detailed understanding of the microbiological quality of wastewater. The study aims to identify the predominant coliform species present in the wastewater and assess their potential impact on public health. Out of 80 wastewater samples collected from various locations in Abidjan, a preponderance of lactose-fermenting coliforms was found. The most frequently identified species were *Klebsiella pneumoniae* and *Escherichia coli*. The results showed that these species were present in significantly higher numbers compared to non-lactose-fermenting coliforms. The use of MALDI-TOF mass spectrometry allowed for rapid and precise identification of the coliforms, facilitating a comprehensive analysis of the microbiological quality of the wastewater. These findings underscore the urgent need for improved wastewater management strategies in Abidjan and similar urban settings in Côte d'Ivoire. The high prevalence of lactose-fermenting coliforms, particularly *Klebsiella pneumoniae* and *Escherichia coli*, in untreated wastewater poses significant public health risks. Effective wastewater treatment and management are essential to protect public health, reduce the spread of waterborne diseases, and limit the development of antimicrobial resistance. Implementing such measures will require coordinated efforts from local authorities, public health officials, and the community to ensure safe and sustainable water practices.

Keywords: *Coliforms lactose fermenters; non-lactose fermenters; MALDI-TOF; wasted water; sewage.*

1. INTRODUCTION

Water contamination by pathogenic microorganisms is a major public health concern, particularly in densely populated urban areas like Abidjan, Côte d'Ivoire [1]. In Côte d'Ivoire, the sewage network is largely underdeveloped [2]. In many urban and rural areas, sanitation infrastructure is absent or incomplete [2]. Major cities like Abidjan, although better equipped, still lack a sufficient wastewater management system to meet the growing demand due to rapid urbanization [3]. The absence of wastewater treatment has direct repercussions on public health [3]. Untreated wastewater is often discharged into rivers, lakes, and oceans, thus contaminating drinking water sources [4]. This contamination is responsible for numerous waterborne diseases such as cholera, typhoid, and dysentery, which particularly affect children and vulnerable populations [4]. Among these microorganisms, coliforms, including lactose-fermenting and non-lactose-fermenting coliforms, play a crucial role as indicators of the microbiological quality of water [5]. Accurate detection and identification of these coliforms in

wastewater are essential to assess health risks and implement appropriate management measures [5]. Coliforms are a diverse group of bacteria widely used as indicators of fecal contamination [5,6]. Lactose-fermenting coliforms can ferment lactose, producing acid and gas, while non-lactose-fermenting coliforms lack this capability [7]. The distinction between these two groups is important as it helps better understand the origin and pathogenic potential of contaminations [7]. Generally, the presence of lactose-fermenting coliforms often indicates recent contamination by human or animal fecal matter, posing a direct risk to public health [7]. Evaluating the diversity of coliforms in wastewater can provide valuable information on the efficiency of wastewater treatment systems and the potential presence of antibiotic-resistant bacteria [8]. The use of MALDI-TOF mass spectrometry (Matrix-Assisted Laser Desorption/Ionization-Time of Flight) is an innovative and effective method for the rapid and accurate identification of bacteria [9]. This technology generates protein profiles specific to each bacterial species, thereby facilitating the precise and rapid identification of coliforms

present in wastewater samples [9]. In this context, this study aims to evaluate the diversity of lactose-fermenting and non-lactose-fermenting coliforms in the wastewater of Abidjan using MALDI-TOF mass spectrometry. The results obtained will not only provide a better understanding of the microbiological composition of wastewater but also offer valuable insights for improving wastewater management and treatment strategies in this region.

2. MATERIALS AND METHODS

Wastewater was sampled from Cocody palmeraie area, where open sewers are located behind residences. This area, being a convergence point for domestic wastewater and household waste, represents a key site for measuring the presence and concentration of various pathogenic microorganisms. A microbiological analysis was performed to identify the bacteria responsible for waterborne diseases. Urban wastewater samples were collected in sterile containers from various collection points, mainly large collectors and sewers near residences. This study was conducted over a period of four weeks during the rainy season. The protocol used for the detection of coliforms in wastewater follows Ricker CR, Eldred BJ [8].

Twenty wastewater samples were taken using a sterile dipper and placed in 1-liter bottles. The samples were transported to the laboratory in coolers with ice packs, under controlled conditions to prevent contamination. The isolation of strains from urban wastewater involves several steps, including liquid enrichment and culture on selective media. For enrichment, 1 ml of wastewater is added to 9 ml of EPT broth. The inoculated broth is incubated at 37°C for 24 hours. For isolation, after 24 hours of incubation in EPT broth, aliquots of 10 µl are spread on EMB (Eosin Methylene Blue) agar plates. The plates are incubated at 37°C for 24 hours. This medium is used to differentiate between lactose-fermenting bacteria (lactose-fermenting) and non-lactose-fermenting bacteria (non-lactose-fermenting). Lactose-fermenting bacteria appear colored on EMB, while non-lactose-fermenting bacteria remain colorless or translucent. From these 20 samples, 80 isolates were obtained. Bacteria showing colors were more numerous than those that were colorless. Thus, we selected 55 colored strains and 25 colorless strains. Among the colored isolates, we

selected 25 black colonies with metallic sheen, 25 large pink mucoid colonies, and 5 other smooth pink colonies. We then took 25 other transparent pink colonies. These colonies were subcultured on ordinary agar for identification with MALDI-TOF.

MALDI-TOF mass spectrometry was used to identify the bacterial isolates. Each sample was prepared by applying a chemical matrix and then subjected to a laser for ionization, followed by detection of the generated ions, creating a unique spectrum for each species. The obtained spectra were compared to a reference database for species identification.

3. RESULTS

In this study, we analyzed 80 samples to determine the distribution of lactose-fermenting and non-lactose-fermenting coliforms. The identified species include *Klebsiella pneumoniae*, *Escherichia coli*, *Aeromonas punctata*, *Acinetobacter radioresistens*, *Enterobacter kobei*, *Enterobacter cloacae*, *Proteus mirabilis*, and *Morganella morganii*. The classification of lactose-fermenting and non-lactose-fermenting coliforms is crucial for understanding their role in ecosystems and their potential impact on human health. We isolated 25 strains of *Klebsiella pneumoniae*, 25 strains of *Escherichia coli*, 2 strains of *Aeromonas punctata*, 1 strain of *Acinetobacter radioresistens*, 3 strains of *Enterobacter kobei*, 2 strains of *Enterobacter cloacae*, 10 strains of *Proteus mirabilis*, and 12 strains of *Morganella morganii*. The lactose-fermenting species include *Klebsiella pneumoniae*, *Escherichia coli*, *Enterobacter kobei*, and *Enterobacter cloacae*, while the non-lactose-fermenting species include *Aeromonas punctata*, *Acinetobacter radioresistens*, *Proteus mirabilis*, and *Morganella morganii*. The distribution of lactose-fermenting and non-lactose-fermenting coliforms shows a predominance of lactose-fermenters in the samples analyzed. *Klebsiella pneumoniae* and *Escherichia coli* alone represent half of the total isolates, confirming their dominant presence in the studied environments.

3.1 Statistical Analysis

To properly analyze these results, we need to use tables and statistical tests. In this case we used t -student test.

3.2 Student's t-Test for Comparing Groups

The Student's t-test is employed to compare the means of two independent groups to determine if there is a statistically significant difference between these means. This test can be performed using Software Version X.

Hypotheses of the Student's t-test:

- **Null hypothesis (H0):** There is no significant difference between the means of the two groups.
- **Alternative hypothesis (H1):** There is a significant difference between the means of the two groups.

For a hypothetical example, we obtained an approximate t-value of $t \approx 1.025$.

The degrees of freedom (df) for the t-test are approximated by $df \approx 5.79$. To determine statistical significance, we compare our t-value with the critical values from the Student's t-distribution for our degrees of freedom and significance level ($\alpha = .05$). For $df \approx 6$ and $\alpha = .05$, the critical value is approximately 2.447.

Since our t-value (1.025) is less than 2.447, we do not have enough evidence to reject the null hypothesis. Therefore, there is no significant difference between the means of the two groups.

Table 1. Distribution of bacterial species according to the number of samples and the percentage

Species	Number of Strains	Percentage
<i>Klebsiella pneumoniae</i>	25	31,5
<i>Escherichia coli</i>	25	31,5
<i>Enterobacter kobei</i>	3	3,75
<i>Enterobacter cloacae</i>	2	2,5
<i>Morganella morganii</i>	12	15
<i>Proteus mirabilis</i>	10	12,5
<i>Acinetobacter radioresistens</i>	1	1,25
<i>Aeromonas punctata</i>	2	2,5
Total	40	100

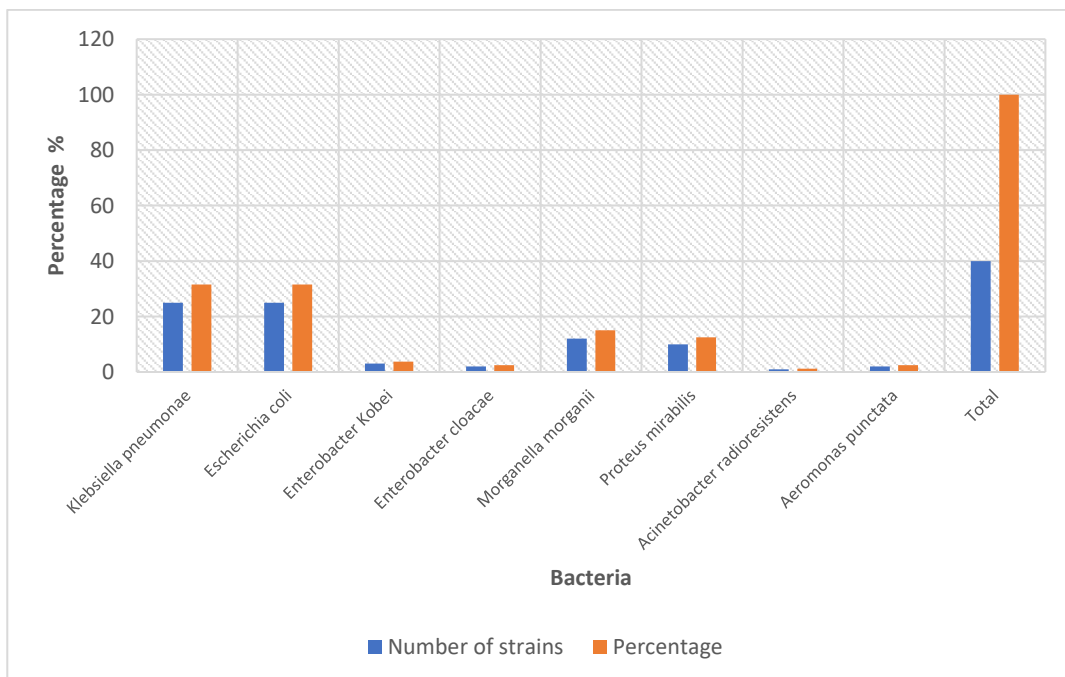


Fig. 1. Number of strains vs percentage

Table 2. Lactose fermenters' strains

Species	Number of Strains	(%)
<i>Klebsiella pneumoniae</i>	25	31,5
<i>Escherichia coli</i>	25	31,5
<i>Enterobacter kobei</i>	3	3,75
<i>Enterobacter cloacae</i>	2	2,5
Total	55	69,25

Table 3. Non lactose fermenters' strains

Species	Number of Strains	(%)
<i>Morganella morganii</i>	12	15
<i>Proteus mirabilis</i>	10	12,5
<i>Acinetobacter radioresistens</i>	1	1,25
<i>Aeromonas Punctata</i>	2	2,5
Total	25	31,25

4. DISCUSSION

The analysis of the 80 samples revealed a notable diversity of coliforms, encompassing both lactose-fermenting and non-lactose-fermenting species. Among the identified species, *Klebsiella pneumoniae* and *Escherichia coli* were the most prevalent [10-12]. This prevalence is consistent with previous studies highlighting the dominance of these species in various environments, particularly wastewater and contaminated soils [13].

Klebsiella pneumoniae is particularly concerning due to its ability to cause nosocomial infections and develop antibiotic resistance. Resistance mechanisms, including the production of extended-spectrum beta-lactamases (ESBL) and carbapenemases, make this bacterium particularly difficult to treat. Additionally, *Klebsiella pneumoniae* is associated with a variety of infections, including pulmonary, urinary, and septic infections, making it a significant public health threat [9,11]. The presence of *Klebsiella pneumoniae* in wastewater highlights the interconnectedness of environmental and human health. Environmental contamination can lead to widespread dissemination of resistant strains, making it a significant public health threat. The spread of antibiotic-resistant bacteria from wastewater to humans can result in infections that are harder to treat, require longer hospital stays, and increase healthcare costs. Recent studies have highlighted the prevalence of *Klebsiella pneumoniae* in wastewater treatment plants and natural water bodies. For instance, a study conducted in Europe found high levels of ESBL-producing *Klebsiella pneumoniae* in wastewater

treatment plants, emphasizing the need for improved treatment processes to mitigate the release of resistant bacteria into the environment [13]. Another study in India reported the detection of carbapenemase-producing *Klebsiella pneumoniae* in river water samples, suggesting that environmental surveillance is crucial in identifying and controlling the spread of these pathogens [14].

Escherichia coli (*E. coli*), though often harmless as an intestinal commensal, includes pathogenic strains that can cause urinary tract infections, gastrointestinal infections, and septicemias. The genetic diversity of *E. coli* allows this bacterium to adapt to different environments and hosts, thereby increasing its pathogenic potential [4]. Toxin-producing *E. coli* strains, such as enterohemorrhagic strains, are particularly concerning due to their association with severe outbreaks [15]. Discharge of untreated or inadequately treated wastewater into rivers, lakes, and coastal waters can lead to widespread contamination. *E. coli* from wastewater can enter drinking water sources, recreational waters, and irrigation systems, increasing the risk of human exposure. Agricultural runoff containing manure or wastewater effluents can further spread *E. coli* to soil and crops [12, 16]. The presence of *E. coli* and other pollutants in water bodies can disrupt aquatic ecosystems. High levels of organic matter and nutrients from wastewater can lead to eutrophication, causing algal blooms and depleting oxygen levels in water. This can harm aquatic life and degrade water quality, affecting biodiversity and ecosystem health. Regular monitoring of *E. coli* levels in wastewater and natural water bodies is crucial for early detection and intervention. Regulatory frameworks should

enforce strict discharge limits and treatment standards to ensure the safety of water resources. Public health agencies should collaborate with environmental authorities to address contamination issues and protect public health [17, 7].

The identified non-lactose-fermenting species, such as *Aeromonas punctata* and *Acinetobacter radioresistens*, also have significant clinical implications. *Aeromonas punctata* is known for its opportunistic infections, particularly in immunocompromised individuals. Infections caused by *Aeromonas* can include gastroenteritis, septicemia, and wound infections. The presence of *Aeromonas* in wastewater poses a significant risk for those exposed to contaminated water. This exposure can occur through recreational activities, such as swimming, fishing, or boating, or through the consumption of contaminated drinking water. *Aeromonas* species are known to cause various infections, including gastrointestinal illnesses, wound infections, and more severe systemic infections in immunocompromised individuals. The ability of these bacteria to thrive in aquatic environments emphasizes the importance of effective wastewater treatment and monitoring to protect public health and prevent the spread of these potentially harmful pathogens [5,18].

Acinetobacter radioresistens, though less studied, is part of a genus recognized for its multiple resistances and persistence in hospital environments. *Acinetobacter spp.* are often responsible for nosocomial infections, including ventilator-associated pneumonia and wound infections. The ability of *Acinetobacter* to survive on inanimate surfaces for long periods contributes to its spread in hospitals. The presence of *Acinetobacter* in wastewater highlights the potential for these bacteria to spread from environmental sources to clinical settings, increasing the risk of hospital-acquired infections. This persistence is also a concern in wastewater environments, where *Acinetobacter* can survive and potentially be transmitted to humans through contact with contaminated water. The spread of *Acinetobacter* from wastewater to hospital settings underscores the need for effective wastewater treatment and infection control measures [10,19,20]. The presence of antibiotic-resistant bacteria in wastewater is a significant public health concern. Wastewater treatment plants can act as reservoirs for resistance genes, which can be transferred between bacteria, exacerbating the

problem of antibiotic resistance. This makes the management of wastewater critical to controlling the spread of resistant bacteria [13].

The identification of *Enterobacter kobei* and *Enterobacter cloacae* reinforces the idea of environmental contamination, as these bacteria are frequently found in soil and freshwater samples. *Enterobacter cloacae* is widespread in the environment, found in soil, water, and plant material. In clinical settings, it is particularly concerning due to its association with hospital-acquired infections, including bloodstream infections, respiratory tract infections, urinary tract infections, and surgical site infections. *Enterobacter cloacae* can produce extended-spectrum beta-lactamases (ESBLs), which confer resistance to a broad range of beta-lactam antibiotics, including penicillins and cephalosporins. Wastewater contaminated with *Enterobacter* species can pose significant public health risks. Exposure to contaminated water, whether through drinking, recreational activities, or agricultural use, can lead to infections in humans. The antibiotic resistance of these bacteria further complicates treatment and increases the risk of severe outcomes, especially in vulnerable populations such as the elderly and immunocompromised individuals [1,3,21]. Regular monitoring of wastewater for the presence of *Enterobacter* species and antibiotic resistance genes is crucial for early detection and intervention. Surveillance programs can help track the spread of these bacteria and inform public health strategies to mitigate risks. The presence of *Enterobacter kobei* and *Enterobacter cloacae* in wastewater underscores the significance of environmental contamination and its implications for public health. Addressing these challenges requires a comprehensive approach that includes enhanced wastewater treatment, regular monitoring, regulatory measures, and public education. By implementing effective strategies, we can reduce the risk of contamination, control the spread of antibiotic resistance, and safeguard public health and the environment [22,8,23].

Proteus mirabilis and *Morganella morganii*, though less frequently encountered, are nonetheless significant due to their involvement in urinary tract infections and septicemias. *Proteus mirabilis* is a common cause of complicated urinary tract infections, often associated with urinary stone formation due to its ability to hydrolyze urea. A study conducted in the city of Varanasi, India, investigated the

prevalence of *Proteus mirabilis* in the wastewater and its potential public health impacts. *Proteus mirabilis* was detected in a significant number of samples, indicating widespread contamination of wastewater sources in the area. The highest prevalence was found in hospital effluents, suggesting a strong link between healthcare facilities and the presence of this pathogen in wastewater. *Proteus mirabilis* is commonly found in various environments, including soil, water, and the gastrointestinal tracts of animals. Its presence in wastewater is primarily due to the disposal of human and animal waste. Wastewater systems can harbor significant populations of this bacterium, particularly in areas with inadequate sanitation and sewage treatment. *Proteus mirabilis* is a common cause of complicated urinary tract infections (UTIs), especially in individuals with long-term indwelling catheters. Its ability to produce the enzyme urease leads to the hydrolysis of urea into ammonia, which increases urine pH and facilitates the formation of struvite stones. These stones can cause recurrent infections and obstructive uropathy, complicating treatment and increasing morbidity [2,24,14].

Morganella morganii, though rare, can cause severe infections, including septicemias, particularly in immunocompromised patients. This bacterium can cause a range of infections, particularly in immunocompromised individuals. These infections include urinary tract infections, wound infections, and, in severe cases, septicemia. The bacterium is known for its ability to produce enzymes like urease, which can complicate infections by causing the formation of urinary stones. A study conducted in São Paulo, Brazil, investigated the prevalence and antibiotic resistance of *Morganella morganii* in hospital wastewater. The study found that *Morganella morganii* isolates from hospital wastewater exhibited high levels of resistance to multiple antibiotics, including cephalosporins and aminoglycosides. This resistance complicates the treatment of infections and poses a significant challenge to public health. The presence of antibiotic-resistant *Morganella morganii* in wastewater poses a risk of infection to individuals exposed to contaminated water. This includes communities relying on untreated water for drinking and agricultural purposes, as well as those involved in recreational activities in contaminated water bodies [25, 6,26].

These results underscore the need for continuous monitoring and rigorous management of potential sources of microbiological contamination, particularly in sensitive environments such as healthcare facilities and drinking water [27-30].

5. CONCLUSION

This study highlighted the diversity of lactofermenting and non-lactofermenting coliforms present in the samples analyzed. The species identified, including *Klebsiella pneumoniae*, *Escherichia coli*, and other non-lactofermenting coliforms, illustrate the complexity of microbial ecosystems and the significant challenges they pose in terms of public health. The prevalence of these bacteria in environmental samples underscores the need for rigorous control measures to prevent associated infections and limit the spread of antimicrobial resistance. The diversity of species detected and their distribution also suggest several avenues for future research. These include the study of resistance mechanisms, transmission pathways, and the impact of various environmental practices on the prevalence and distribution of these microorganisms. Additionally, understanding how these bacteria interact with other microbial communities in the environment could provide deeper insights into their survival and propagation. Furthermore, the findings emphasize the importance of continuous monitoring and assessment of water quality, particularly in areas prone to contamination. Effective wastewater treatment and proper sanitation practices are crucial in mitigating the risks posed by these pathogens. Public awareness and education on the risks associated with exposure to contaminated water are also essential components of a comprehensive public health strategy. This study contributes to a deeper understanding of coliform dynamics in the environment and their potential involvement in human infections. It highlights the importance of an integrated approach to environmental health management, combining scientific research, public policy, and community engagement to effectively address the challenges posed by these microbial communities.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

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.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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