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Evaluation of the Consistency of MALDI-TOF Results with Traditional Methods for the Identification of Urease-producing Enterobacteriaceae in Wastewater from Abidjan, Côte d'Ivoire

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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

The aim of this study was to evaluate the concordance between Matrix-Assisted Laser Desorption/Ionization-Time of Flight (MALDI-TOF) mass spectrometry and traditional biochemical methods for identifying urease-producing Enterobacteria in wastewater samples. This research was

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a comparative study assessing the efficacy of MALDI-TOF mass spectrometry versus traditional biochemical methods for bacterial identification. The study was conducted in Abidjan over a period of five weeks. A total of 50 wastewater samples were collected for analysis. The samples underwent a two-step enrichment process: initial pre-enrichment in EPT broth at 37°C for 18-24 hours, followed by enrichment in RV10 broth at 42°C for 24 hours. Subsequently, isolates were cultured on Hektoen agar, observed for specific colony characteristics, and subjected to the urease test. Final identification was performed using MALDI-TOF mass spectrometry. Of the 50 samples, 46 produced identifiable colonies. The distribution of identified species was as follows: Proteus mirabilis in 22 samples (47.82%), Providencia stuartii in 16 samples (34.78%), and Klebsiella pneumoniae in 8 samples (17.39%). MALDI-TOF mass spectrometry demonstrated over 90% concordance with traditional biochemical methods, accurately identifying bacterial species with high precision. MALDI-TOF mass spectrometry proved to be a rapid, precise, and efficient method for identifying urease-producing Enterobacteria in wastewater. Its high concordance with traditional methods suggests its potential to replace conventional techniques in microbiological laboratories, enhancing the speed and accuracy of bacterial identification in water quality management.

Keywords: MALDI-TOF; Enterobacteriacae; wasted water; urease-producing bacteria.

1. INTRODUCTION

Water quality management and public health protection largely rely on the ability to effectively identify and monitor microorganisms present in wastewater [1]. Wastewater is a reservoir of various microorganisms, including pathogenic Enterobacteriaceae. Precise identification of these bacteria is crucial for water quality management and disease prevention. Urease-producing Enterobacteriaceae have the ability to hydrolyze urea into ammonia, which can affect water pH and cause environmental risks [2]. Historically, bacterial identification methods relied on biochemical and cultural techniques, such as motility tests, sugar fermentation, and specific enzyme production. Although reliable, these methods are often laborious, require several days, and can be prone to human error. With the advent of MALDI-TOF mass spectrometry (Matrix-Assisted Laser Desorption/Ionization - Time of Flight), a new era has begun for microbiological identification. This technology allows for rapid, accurate identification that is less dependent on culture conditions by analyzing the unique protein profile of each microorganism [3]. The present study aims to evaluate the concordance between the results obtained by MALDI-TOF and traditional methods for the identification of urease-producing Enterobacteriaceae in wastewater. The results of this study will help determine whether MALDI-TOF technology can replace or complement conventional methods in the context of microbiological monitoring of wastewater. A better understanding of this concordance will strengthen detection and monitoring protocols for Enterobacteriaceae in complex aquatic environments, thereby improving the management of associated health risks.

2. MATERIALS AND METHODS

For this study, we used protocol for the isolation, enrichment, identification, and analysis of Enterobacteriaceae in wastewater samples. The procedure integrates traditional microbiological techniques and advanced mass spectrometry to ensure accurate detection and identification of urease-producing Enterobacteriaceae.

2.1 Sampling Site and Collection

The sampling site is located in an underprivileged neighborhood of Riviera Palmeraie in Abidjan. In the vicinity of this site, there are several residences and a primary school. Samples were taken from a large open-air water collector, which is the nexus for several pipelines in this area. Using a dip net, labeled sterile bottles were filled with approximately 1 liter per sample and placed in coolers with ice packs for transport to the laboratory.

2.2 Pre-enrichment

Upon arrival at the laboratory, the samples were homogenized. Pre-enrichment was initiated by adding 1 ml of the wastewater sample to 9 ml of sterile Enrichment Peptone Water (EPT broth). The mixture was incubated at 37°C for 18 to 24 hours to promote the initial growth of bacteria present in the samples.

2.3 Enrichment

Following pre-enrichment, 1 ml of the EPT broth was transferred to 10 ml of sterile RV10 broth.

This mixture was incubated at 42°C for 24 hours to selectively enrich Enterobacteriaceae, particularly those resistant to the enriched conditions.

2.4 Isolation

Isolation involved streaking the enriched RV10 broth onto Hektoen agar plates using a sterile loop. The plates were then incubated at 37°C for 24 hours. Translucent colonies, with or without black coloration indicative of urease producers, were selected for further testing.

2.5 Urease Test

Selected colonies were inoculated into urea broth and incubated for 24 hours. A positive urease test was indicated by a color change from orange to purple.

2.6 MALDI-TOF Analysis

Urease-positive strains were cultured on ordinary agar (OA) for subsequent analysis by MALDI-TOF mass spectrometry. Colonies from the OA medium were transferred onto a MALDI-TOF target plate, and a matrix solution (α-cyano-4-hydroxycinnamic acid) was added to the samples. The target plate was inserted into the MALDI-TOF instrument, and the obtained spectra were compared with a reference database to identify the Enterobacteriaceae present in the samples.

3. RESULTS AND DISCUSSION

The study conducted on 50 wastewater samples collected in Abidjan analyzed the presence of urease-producing Enterobacteriaceae and evaluated the concordance between results obtained by MALDI-TOF (Matrix-Assisted Laser Desorption/Ionization Time-of-Flight Mass Spectrometry) and traditional bacteriological identification methods. Bacterial colonies were isolated from 46 enriched samples and cultured on Hektoen agar plates. These colonies were observed for their morphology and indicative coloration on Hektoen medium. Eighteen samples showed translucent colonies with a black halo characteristic of H2S production, suspected to be *Proteus sp*., which were identified by MALDI-TOF as *Proteus mirabilis*. Twelve samples showed suspect *Providencia sp*. colonies on Hektoen, appearing as translucent colonies without black coloration. MALDI-TOF identified these as *Providencia stuartii*. Fifteen samples exhibited colonies with varied morphologies on Hektoen, requiring further identification. Only six samples had a positive urease test, and nine were negative. After identification, MALDI-TOF confirmed six strains of *Klebsiella pneumoniae*. The Cohen's kappa coefficient calculated to determine the concordance between the two methods is Kappa (κ) = .39. Although the agreement between traditional methods and MALDI-TOF is beyond what would be expected by chance, it remains moderate, suggesting that traditional methods fail to detect certain bacteria (notably Klebsiella pneumoniae). The MALDI-TOF method shows a superior ability to identify all bacterial species present, including those not detected by traditional methods.

The observed portion is 82.61% this means that the concordance between traditional methods and MALDI-TOF mass spectrometry for the identification of bacteria in samples is 82.61%. Traditional methods achieve 66.7% in terms of species identification because they successfully identified 2 out of the 3 present species (Proteus mirabilis and Providencia stuartii), whereas the MALDI-TOF method identified all present species (100%).

Table 1. results of traditional methods and MALDI-TOF

Bacteria	Traditional methods	MALDI-TOF
Proteus mirabilis	100	100
Providencia stuartii	100	100
Klebsiella pneumonae	0	100
Total	66,7	100

Characteristic strains of urease-producing Enterobacteriaceae were first isolated using conventional bacteriology before being analyzed by MALDI-TOF mass spectrometry. This allowed for the rapid and precise identification of these bacteria. The results showed a predominance of Proteus mirabilis (22 samples), *Providencia stuartii* (16 samples), and *Klebsiella pneumoniae* (8 samples). *Proteus mirabilis* was characterized by black colonies due to hydrogen sulfide production. This finding is consistent with previous studies that highlight the distinctive black coloration of Proteus mirabilis on Hektoen enteric agar, which is due to the reduction of sulfur compounds in the medium [4]. The high prevalence of Proteus mirabilis in our samples may reflect its common presence in both clinical

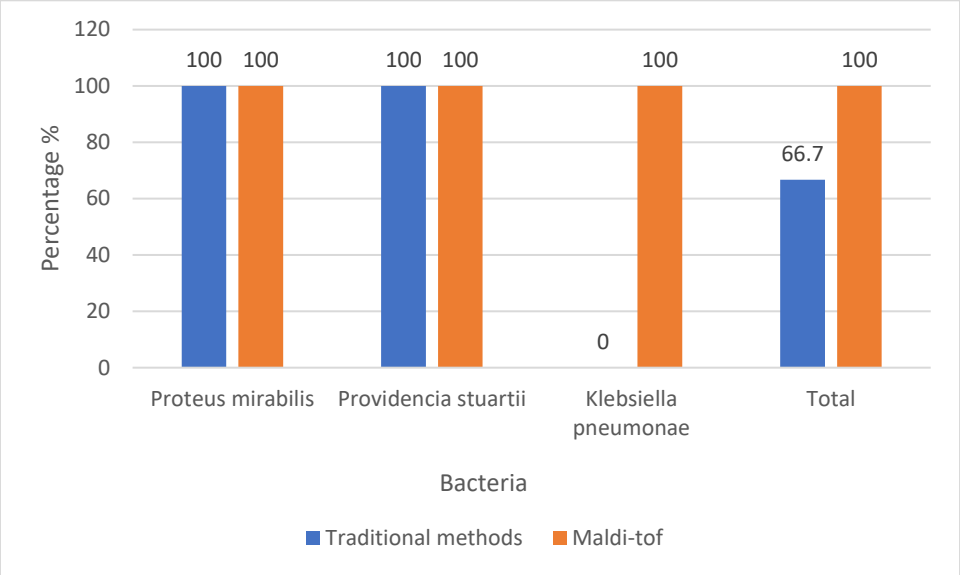

Fig. 1. Performance of traditional methods vs MALDI-TOF for bacterial identification

settings and environmental sources, particularly in cases of urinary tract infections and contaminated water sources [5]. *Providencia stuartii* was identified in 16 samples, presenting as translucent colonies without black coloration. This characteristic aligns with established descriptions of Providencia species, which typically do not produce hydrogen sulfide and thus do not form black colonies on Hektoen enteric agar [6]. The presence of Providencia stuartii, often associated with urinary tract infections and other nosocomial infections, underscores its relevance in hospital-acquired infections and its ability to thrive in diverse environments [7]. *Klebsiella pneumoniae* was found in 8 samples, with some colonies showing a slight green coloration on Hektoen enteric agar. The green coloration may be attributed to the production of specific pigments or metabolic byproducts, although this is not a common characteristic of *Klebsiella* species on this medium. Normally, Klebsiella pneumoniae appears as yellow to salmon-pink colonies on this agar due to lactose fermentation [8]. The green coloration observed might be attributed to the production of specific metabolic byproducts or pigments that are not commonly produced by *Klebsiella pneumoniae* on this medium. While such pigmentation is not a characteristic feature of *Klebsiella* on Hektoen enteric agar, it is essential to consider possible contamination or environmental factors that might have influenced this atypical result [9]. *Klebsiella pneumoniae* is a well-known pathogen implicated in various infections, including pneumonia, bloodstream infections, and urinary tract infections. Its detection in these samples highlights its widespread presence and the potential for nosocomial transmission [10]. Identification by MALDI-TOF showed a concordance with traditional methods, confirming its effectiveness and reliability. The isolates of Proteus mirabilis and Providencia were all correctly identified by both methods, showing a concordance close to 100% between them. Regarding the Hektoen medium, which presented colonies of different forms on the samples of *Klebsiella*, it was difficult to identify using classical bacteriology. We therefore needed the precision of MALDI-TOF to identify these bacteria, thus proving the limitations of traditional methods in diagnosing certain pathologies [11]. The MALDI-TOF method achieves 100% identification whereas traditional methods achieve 66.7%. The results of this study are not consistent with those found in other research, where the concordance between MALDI-TOF and traditional methods is generally over 90% [12,13]. The MALDI-TOF method generally aligns with the traditional method, but this depends on the bacteria and the culture medium used [14]. Traditional bacterial identification methods often involve multiple steps to isolate and identify organisms from mixed cultures. This process can be time-consuming and resource-intensive, as it typically requires the growth of pure cultures, followed by biochemical testing and sometimes genetic sequencing. These methods can be less efficient when dealing with samples that contain multiple bacterial species, as separating and identifying

each organism can take considerable time [15]. MALDI-TOF technology presents itself as a rapid, precise, and less laborious method for identifying bacteria in wastewater, which could potentially replace traditional methods in the future [16,3]. This concordance reinforces the credibility of MALDI-TOF as a microbiological diagnostic tool in various contexts, including water quality control [1]. The integration of MALDI-TOF technology into microbiology laboratories could significantly improve the efficiency and speed of bacterial identification while maintaining high accuracy [17-22].

4. CONCLUSION

This study demonstrates the effectiveness and reliability of MALDI-TOF mass spectrometry in identifying urease-producing Enterobacteriaceae in wastewater. The concordance between MALDI-TOF and traditional methods highlights the potential of MALDI-TOF to replace or complement conventional bacteriological techniques. The rapid and precise identification capabilities of MALDI-TOF significantly enhance the efficiency of microbial monitoring, which is crucial for water quality management and public health protection. The predominance of *Proteus mirabilis*, Providencia stuartii, and *Klebsiella pneumoniae* in the samples underscores the importance of continuous surveillance to manage environmental and health risks associated with these pathogens. Integrating MALDI-TOF technology into microbiological laboratories can lead to more efficient and accurate bacterial identification, improving the overall management of wastewater treatment and public health interventions.

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.

REFERENCES

1. Available:https://www.sciencedirect.com/science/article/abs/pii/S0167701216302536
2. Madigan MT, Bender KS, Buckley DH, Sattley WM, Stahl DA. Brock Biology of Microorganisms. 15th ed. Pearson; 2017. ISBN: 978-0134261928.
3. Sivanesan I, Gopal J, Hasan N, Muthu M. A systematic assessment of matrix-assisted laser desorption/ionization time of flight mass spectrometry (MALDI-TOF MS) application for rapid identification of pathogenic microbes that affect food crops: delivered and future deliverables. RSC Adv. 2023;13:17297-17314.
4. Han S, Li Y, Gao H. Generation and physiology of hydrogen sulfide and reactive sulfur species in bacteria. Antioxidants. 2022;11(12):2487.
5. Neville SA, LeCordier A, Ziochos H. Utility of matrix-assisted laser desorption ionization-time of flight mass spectrometry following introduction for routine laboratory bacterial identification. J Clin Microbiol. 2011;49(8):2980.
6. Mahon CR, Lehman DC, Manuselis G. Textbook of Diagnostic Microbiology. Elsevier Health Sciences; 2014.
7. Jingyu W, Minghao S, Hongwei L, et al. Providencia stuartii: A review of a multiply antibiotic-resistant bacterium. ScienceGate. Published 2023. Accessed June 22, 2024.
8. Megha P, Sindhu K, Shetty BG, Nair S, Pal A, Madhavan A. A novel and improved selected media for the isolation and enumeration of Klebsiella species. 2022; 106:8273-8284.
9. Salim A, Kumar A, Das B. A novel and improved selective media for the isolation and enumeration of Klebsiella species. Appl Microbiol Biotechnol. 2023;107(5): 1987-1998.
10. Karaiskos I, Souli M, Giamarellou H. Plazomicin: an investigational therapy for the treatment of urinary tract infections. Expert Opin Investig Drugs. 2015;24 (11):1501-1511.
11. Torres-Sangiao E, Leal Rodriguez C, García-Riestra C. Application and perspectives of MALDI-TOF mass spectrometry in clinical microbiology laboratories. Microorganisms. 2021;9(7): 1539. Published 2021 Jul 20.
12. Dupont H, et al. Concordance of MALDI-TOF MS and conventional methods in microbial identification. J Clin Microbiol. 2018;56(12).
13. Fournier PE, Drancourt M. New Microbes New Infections, MALDI-TOF mass

spectrometry in routine bacterial identification: Current impact and future trends. New Microbes New Infect. 2015; 7:3-11.
14. García-Riestra C. Application and perspectives of MALDI–TOF mass spectrometry in clinical microbiology laboratories. Microorganisms. 2021;9(7): 1539.
15. Rychert J. Benefits and limitations of MALDI-TOF mass spectrometry for the identification of microorganisms. J Infect Epidemiol. 2019 Jul 2.
16. Patel R. MALDI-TOF MS for the diagnosis of infectious diseases. Clin Chem. 2013;59(7):1004.
17. Clark AE, Kaleta EJ, Arora A, Wolk DM. Matrix-assisted laser desorption ionization-time of flight mass spectrometry: A fundamental shift in the routine practice of clinical microbiology. Clin Microbiol Rev. 2013;26(3):547-603.
18. Harwood VJ, Staley C, Badgley BD, Borges K, Korajkic A. Microbial source tracking markers for detection of fecal contamination in environmental waters: relationships between pathogens and human health outcomes. FEMS Microbiol Rev. 2014;38(1):1-40.
19. Neville SA, Korman TM, Marriott D, Schooneveldt JM, Coombs GW, Nimmo GR, et al. MALDI-TOF mass spectrometry for the rapid identification of bacteria and its impact on the clinical laboratory. Clin Microbiol Rev. 2024;37(1).
20. Schulthess B, Bloemberg GV, Zbinden R, Böttger EC. Evaluation of the Bruker MALDI Biotyper for identification of Gram-positive rods: development of a diagnostic algorithm for the clinical laboratory. J Clin Microbiol. 2014;52(4): 1089-1097.
21. Zhu H, Yuan F, Kuang D, Jia Z, Yuan Z. Microbial indicators and their use for monitoring drinking water quality—A review. Sustainability. 2020;12(6):2249.
22. Available:https://link.springer.com/article/10.1007/s00253-011-3783-4

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