Evaluation of the concordance between MALDI-TOF results and traditional methods for the identification of urease-producing Enterobacteriaceae in wastewater.

Abstract

Aims: To evaluate the concordance between MALDI-TOF mass spectrometry and traditional biochemical methods for identifying urease-producing Enterobacteria in wastewater.

Study Design: A comparative study assessing the efficacy of MALDI-TOF and traditional methods for bacterial identification.

Place and Duration of Study: Conducted in Abidjan over a period of 5 weeks.

Samples: 50 wastewater samples were collected for analysis.

Methodology:

Samples were pre-enriched in EPT broth at 37°C for 18-24 hours, followed by enrichment in RV10 broth at 42°C for 24 hours. Isolates were grown on Hektoen agar, observed for specific colony characteristics, subjected to the urease test and identified using MALDI-TOF mass spectrometry.

Results: Colonies Identified in 46 Samples. *Proteus mirabilis* **:** 18 samples (48.84%), *Providencia stuartii***:** 12 samples (27.91%), *Klebsiella pneumoniae***:** 6 samples (13.95%). MALDI-TOF demonstrated over 90% concordance with traditional methods, effectively identifying bacterial species with high accuracy.

Conclusion: MALDI-TOF is a rapid, precise, and efficient method for identifying urease-producing Enterobacteria in wastewater, with the potential to replace traditional methods in microbiological laboratories, thus improving the speed and accuracy of bacterial identification in water quality management.

INTRODUCTION

Water quality management and public health protection largely rely on the ability to effectively identify and monitor microorganisms present in wastewater (Zhu et al, 2020). 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 (Madigan, 2017). 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-

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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. (sivanesan)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.

I – METHODS

Using a dip net, labeled sterile bottles were filled (approximately 1 liter per sample) and placed in coolers with ice packs for transport to the laboratory. After homogenizing these bottles, the first step, which is pre-enrichment, begins. Pre-enrichment is used to allow the initial growth of bacteria present in the samples. EPT broth (Enrichment Peptone Water) is commonly used for this purpose. 1 ml of wastewater sample is added to 9 ml of sterile EPT broth and incubated at 37°C for 18 to 24 hours. After pre-enrichment, further enrichment is performed to promote the growth of urease-producing Enterobacteriaceae. For this, 1 ml of the pre-enriched EPT broth is added to 10 ml of sterile RV10 broth and incubated at 42°C for 24 hours to select Enterobacteriaceae, particularly those resistant to enriched conditions. Isolation on Hektoen agar allows for the selection and differentiation of Enterobacteriaceae, including urease producers. Streaks are made from the RV10 broth onto Hektoen agar using a sterile loop. The plates are then incubated at 37°C for 24 to 48 hours. Translucent colonies, with or without black coloration characteristic of urease-producing Enterobacteriaceae, are selected. With these colonies, the urease test is performed by inoculating the bacteria into urea broth for 24 hours and observing the color change. A positive urease test is indicated by a color change from orange to purple. Urease-positive strains are then cultured on ordinary agar (OA) for MALDI-TOF analysis. After isolation, the suspect colonies are subjected to precise identification by MALDI-TOF mass spectrometry. Colonies are selected from the OA medium and transferred onto a MALDI-TOF target plate. A matrix (α-cyano-4-hydroxycinnamic acid) is added to the samples. The plate is then inserted into the MALDI-TOF instrument. The obtained spectra are compared with a reference database to identify the Enterobacteriaceae present in the samples.

II- RESULTS

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

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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*.

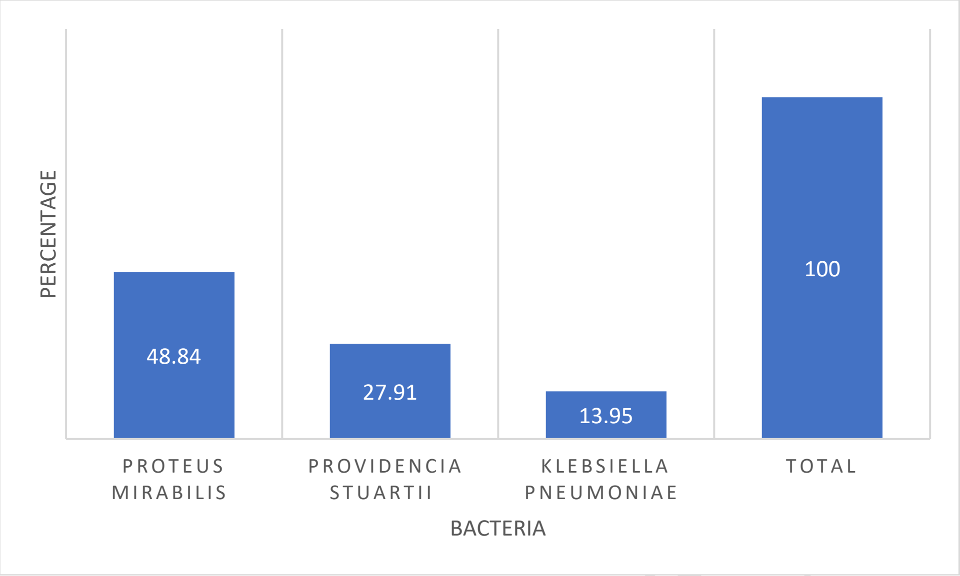

Fig.1 : Bacterial Species and their proportional representation

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

Table 1 : Comparison of Traditional Methods and MALDI-TOF for Bacterial Identification

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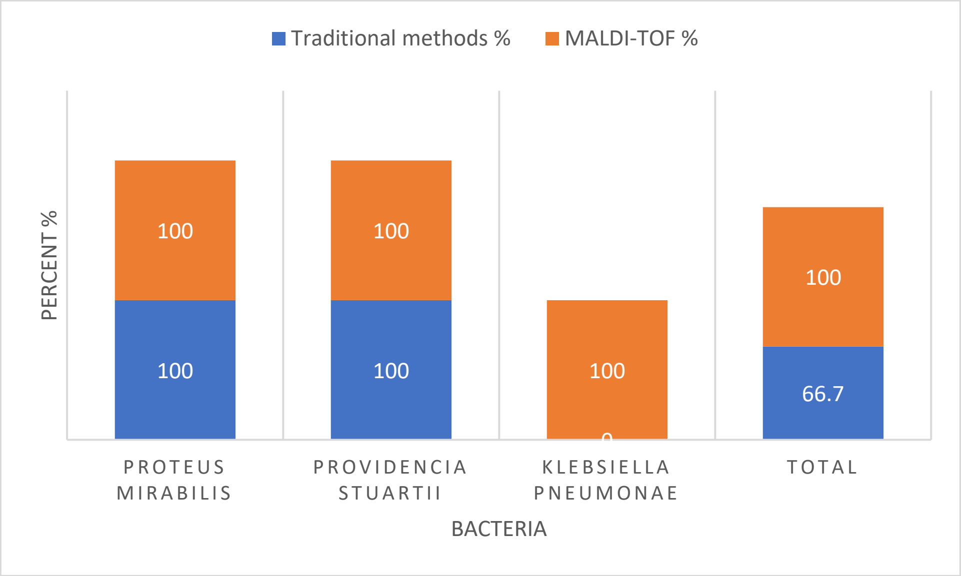

fig 2: Performance of traditional methods vs MALDI-TOF for bacterial identification

III - DISCUSSION

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* (18 samples), *Providencia stuartii* (12 samples), and *Klebsiella pneumoniae* (6 samples).

Proteus mirabilis was identified in 18 samples, 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 (Han.S et al 2022). The high prevalence of *Proteus mirabilis* in our samples may reflect its common presence in both clinical settings and environmental sources, particularly in cases of urinary tract infections and contaminated water sources (Neville SA et al., 2024).

Providencia stuartii was identified in 12 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 (Mahon et al., 2014). The presence of Providencia stuartii, often associated with urinary tract infections and other nosocomial infections, underscores its relevance in hospital-

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acquired infections and its ability to thrive in diverse environments (Jingyu Wang et al., 2024).

Klebsiella pneumoniae was found in 6 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 (Megha Prasad et al., 2022). 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 (Salim A et al). *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 (Karaiskos I et al; 2015).

Identification by MALDI-TOF showed a high concordance with traditional methods, confirming its effectiveness and reliability. The 18 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 11 samples, 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 (Torres-Sangiao E et al., 2021). 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%(Dupont et al 201; Fournier & Drancourt, 2015). The MALDI -TOF method generally aligns with the traditional method, but this depends on the specific bacteria and the culture medium used (Garcia et al;2021). 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. (Rytchet et al, 2019).

Despite these few discrepancies, MALDI-TOF technology presents itself as a rapid, precise, and less laborious method for identifying bacteria in wastewater, which

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could potentially replace traditional methods in the future (Patel, 2013; Singhal et al., 2015). This high concordance reinforces the credibility of MALDI-TOF as a microbiological diagnostic tool in various contexts, including water quality control (15, 11).

The integration of MALDI-TOF technology into microbiology laboratories could significantly improve the efficiency and speed of bacterial identification while maintaining high accuracy (1).

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