Assessment of MALDI-TOF Analysis in Contrast with Typical Microbiological Techniques for Identifying Microorganisms: The Case of a Tertiary Setting

Fouad M. Sait[1], Mohammed A. Alghamdi[2], Walleed H. Nasraldeen[3], Hesham A. Almohammadi[4], Wala F. Andejani[5]

King Abdulaziz University Hospital

Abstract

Background: A quick assessment of pathogens is key in patient management and care. Although traditional microbiological methods still stand out as dependable, they score low when it comes to efficiency and the rate of work done. Pathogen research, which was regarded as complicated in the past, is now easier and quicker thanks to MALDI-TOF MS (Matrix-Assisted Laser Desorption Ionization-Time of Flight Mass Spectrometry).

Objective: In this paper, we examine the use of MALDI-TOF MS in pathogen identification and its effectiveness against routine microbial culture at a level three hospital.

Methods: It spanned six months and the subjects that were examined included clinical specimens of blood, urine, and pneumonic sputum, from a total of 500 patients. After a using routine culture methods on clinical samples we employed MALDI/TOF MS techniques to detect pathogens using a mass spectrometer. Turnaround time, accuracy, diagnostic yield, cost per sample and error rates formed the set of metrics for comparison.

Results: MALDI-TOF MS achieved lower diagnostic turn-around times (6.2 hours vs. 36.5 hrs, $p < 0.001$), and higher identification accuracy (96.8% vs. 86.3% $p<0.001$). When compared with other technologies, MALDI-TOF also had higher levels of cost and efficiency diagnostic yield while achieving lower error rates (1.8% vs. 5.4%). Its high dependence on well maintained spectral databases and trained technologists is one of its key drawbacks.

Conclusion: The MALDI-TOF MS method is able to provide rapid, accurate, and economic identification of pathogens, thus distinguishing itself from conventional approaches thus serving as a great asset in the medical field. Improving and overcoming these drawbacks will subsequently increase its effectiveness and efficiency.

Keywords: MALDI-TOF MS; Pathogen Identification; Clinical Microbiology; Diagnostic Efficiency; Turnaround Time; Cost Analysis; Tertiary Hospital.

Introduction

Microbial pathogen detection forms an integral part of clinical microbiology especially in formulating treatment and infection control measures. As highlighted by Guo et al. (2014), most microbiological diagnosis relies on culture based techniques that`s accurate but, lengthy and strenuous to implement. This, in return makes biochemical tests saves for pathogen detection to waste time that should have been used for diagnosing and starting treatment.

Mass spectrometry aids in microbial diagnostics in conjunction with matrix assisted laser desorption ionization time of flight (MALDI TOF), this recently developed technology makes it possible to identify bacteria, fungi and other pathogens in seconds by examining their unique protein spectral fingerprints. This is claimed to enhance effectiveness by drastically improving turnaround time relative to traditional methods (Singhal et al, 2015).

Numerous comparative studies have compared the work of MALDI-TOF MS with traditional microbiological methods. These studies have examined the efficiency of these techniques on the basis of time, precision and their affordability. For example, The system has been able to identify a wide range of pathogens directly from clinical specimens without the need for more complex biochemical and molecular methods (Risch et al., 2010; Bazzi et al., 2017). Nevertheless, some weaknesses do remain such as the reliance on detailed spectral databases and problems with differentiating closely related organisms (Chen et al., 2021).

The objective of this study is evaluating the current role of MALDI-TOF MS combination with basic microbiological methods in pathogen detection in a tertiary care setup such as ours. Focusing specifically on the diagnostic accuracy, efficiency, and cost this work intends to balance the advantages and challenges involved in adopting the MALDI-TOF MS technique for day to day diagnostics.

Literature Review

Conventional Microbiological Methods in Pathogen Identification

Pathogen identification methods have relied on traditional microbiological techniques for a long time. These methods include culturing microorganisms on selective or differential media and then biochemically testing them to classify species based on their metabolic properties. While these methods are accepted as reliable, they are not the fastest being rather time-consuming (Guo et al., 2014). In some instances, it can take as long as 48 - 72 hrs for the pathogen growth to become identifiable. For some, especially slow pathogens, they can take even longer to grow which results in disturbed treatment plans. More so, older methods are also unable to deal with atypical or non drugable organisms which further limits their diagnostic versatility within complex clinical environments (Risch et al., 2010).

Introduction of MALDI-TOF Mass Spectrometry into Practice of Clinical Microbiology

The matrix-assisted laser desorption ionization time-of-flight mass spectrometry, or MALDI-TOF MS, is quickly being brought into the clinical arena as a new, effective technique in microbiology. By using this strategy, it constitutes an easy way of identifying microbes and significantly enhancing the accuracy using unique protein molecular fingerprints which are mass spectra. This is done by having all the fingerprints stored in a database for later comparison and species identification, this is unlike other conventional

methods. The expected time frame of results becomes much shorter than expected since the MALDI-TOF MS method can provide results within a few minutes (Singhal et al., 2015). This opportunity is complemented by the ability to deal with various sample types (bacteria, fungi, and mycobacteria) which are used in laboratories of today (Rychert, 2019).

Comparative Studies on MALDI-TOF and Traditional Methods

A considerable amount of work has been carried out to evaluate whether there is a significant difference between the testing capabilities of MALDI-TOF mass spectrometry and the typical microbiological practices. Traditionally, bacterial identification required the full complement of questions required by a clinical microbiologist, including microscopy, isolation, and growth in culture. However, Risch et al. (2010) and other researchers highlighted that MALDI-TOF MS was quicker and much more accurate than older methodologies, claiming success rates of over 95 for the majority of pathogens relevant to the clinical setting. Moreover, Bazzi et al. (2017) show that it Takes 30-50% shorter time for a diagnosis when MALDI-TOF MS is used in comparison to the conventional methods this time decrease is more useful when used in ICUs.

However, it must be mentioned that the performance of this method is dependent on the application of MALDI-TOF MS, particularly certain Maldi-TOF MS genomics which are more efficient with reference databases that are more detailed than average. For example, there are studies that report complications in differentiating between closely related species of Shigella spp. and Eschereichia coli or those that go on to MOPA (renin overproduction assays) indentification of rare pathogens (Chen et al., 2021). These weaknesses point out the requirement to expand and perfect the current spectral databases.

Operational and Cost Efficiency

Besides the benefits outlined above, MALDI-TOF MS has earned high appreciation in terms of cost efficiency. The pricing per sample however is lower than biochemical and molecular traditional methods, while the initial investment is steep. According to Singhal et al. (2015), MLDI-TOF MS complemented tests being conducted and the tests would negate 30 percent of tests being conducted by conventional techniques in high through put.

Challenges and Limitations

While noting its strengths, it should be mentioned that the introduction of the MALDI-TOF MS system has not been without the problems already mentioned. First, a competent personnel is required for the operation of the system and interpretation of the data generated and this sometimes proves to be a bottleneck in resource poor settings (Rychert, 2019). Second, being incapable of molecular-based work, this approach has been limited in its appeal as it involves protein-based identification only and cannot be used to detect genes which code for antimicrobial resistance or virulence factors, otherwise, molecular approaches such as polymerase chain reaction (PCR) or sequencing would usually be employed (Florio et al., 2018). With these limitations, it is clearly seen that while MALDI-TOF MS is a great tool, it is best applied as an adjunct in a multi modality approach for diagnosis.

Emerging Trends and Future Directions

While the previous paragraph elaborates on the limitations of MALDI-TOF systems, it also emphasizes the areas that are being explored for enhancement of this proton system. For instance, automated sample preparation systems and enhanced spectral database linking are being considered for implementation (Chen et al., 2021). This has been adjoined with extensions of its use outside clinical diagnostics such as environmental microbiology, food safety, and antimicrobial resistance profiling (Bazzi et al., 2017).

Conclusion

The body of research highlights the impact that the MALDI-TOF MS has in the field of clinical microbiology. Even traditional methods have their respective place, however the speed, the precision and the lower cost of MALDI emphasize the need for its application. Nonetheless, as this technology is to be used in everyday diagnosis some molecular methods are also required in order to solve the shortcomings of the technique. with such progress being made MALDI-TOF is set to become a staple technology in the fight against infectious diseases.

Methodology

Methodology

Study Design

The research adopted a prospective observational approach with the aim of assessing translational practices of MALDI-TOF MS and traditional microbiological approaches with regards to pathogen identification. The study was hosted in the microbiology department of a tertiary hospital for six months. The research was quoted valid by a review board in the hospital, and all data were thoroughly de-identified to preserve participant anonymity.

Sample Collection and Processing

In an effort to diagnose patients for microbial infection, a combination of clinical specimens was used, such as blood, urine, wound swabs, respiratory specimens, and fluids from the body. The samples were handled according to protocols and guidelines to guarantee sterilization and prevention of sample cross contamination.

1. *Blood cultures: These were handled manually and processed with BacT/ALERT or BACTEC automated blood culture systems.*

2. *Urine Samples: Cultures were carried on cystine-lactose electrolyte deficient (CLED) agar and blood agar for bacterial replication.*

3. *Wound and Respiratory: These were culturd on blood, MacConkey and chocolate agar for isolation of pathogens.*

4. *Body Fluids: All these were directly inoculated on enriched and selective media for bacterial growth.*

Microbial Identification

1. Traditional Microbiology Techniques

• Cultured plates were held under the proper temperature of 37 celsius for a period range of 18 to 48 hours under desired atmospheric conditions (these included aerobic, anaerobic or micro-aerob).

• The expansion of bacteria was detailed for observation and morphogenetic characteristics were classified based on their color and shape including the size of the colony.

• Analysis of catalase, coagulase, and oxidase tests were carried out for reinterpretation of the results to be the initial finding.

• The species level on the other hand, was performed through manual or automated biochemical systems such as Vitek 2 and many others.

2. MALDI-TOF MS

• The colonies of bacteria were selected from the culture plates, which now are determined as Bruker Biotyper or VITEK MS equipment.

• Samples were prepared by combining bacterial suspension on to the target plate then a matrix solution such as α-cyano-4-hydroxycinnamic acid.

• The obtained mass spectra are subjected to a reference database and species identification is undertaken.

• Results were categorized as high-confidence (score ≥ 2.0), low-confidence (1.7–1.99), or unreliable (< 1.7) based on manufacturer criteria.

Evaluation Metrics

The comparison of performance of MALDI TOF MS and ASR or other methods were assessed on the basis of:

1. Turnaround Time (TAT) TAT:

o It is calculated as the measurement duration from the time the sample is received to the time the last pathogen within the sample is identified.

2. Identification Accuracy:

o The isolated selected sample is checked against a gold-standard method which can be 16S rRNA sequencing.

3. Diagnostic Yield:

o Well, in real life, when a sample is taken, sometimes it happens that a final identification is not obtained because there are numerous other variants, the DIAGNOSTIC YIELD gives the percentage of such samples.

4. Cost Analysis:

o Obtained through an identification and a method analysis over a sample mean cost comprising labor and consumables.

5. Error Rates:

o Misidentification and failure to identify rates have been documented and compared with each other.

Data Analysis

- *For statistical analysis SPSS software (25.0 version) was utilized.*
- *Student t-test was utilized for comparison of continuous variables such as turnaround time.*
- *Chi-square test was used for comparison of categorical variables such as identification accuracy.*
- *P-values of < 0.05 were deemed as statistically significant.*
- *For every method of MPM PPM NPV and sensitivity were calculated.*

Workflow Integration and Quality Control

To equalise the results for a fair comparison:

1. Laboratory personnel performing traditional identification were kept in the darkness of the results of MALDI -TOF MS.

2. Both methods had been performed in the exact same test setting allowing parallel testing.

3. Control organisms were used every day to check the function of both identification systems.

Study Limitations

- *The reference database for MALDI-TOF MS may not contain some rare or fastidious pathogens.*
- *The results may not be applicable in other settings as the study was done in one teaching hospital which could make generalizability of the findings problematic.*

Study Limitations

- Certain rare or fastidious pathogens may not be adequately represented in the reference database of the MALDI-TOF MS system.

- The study was conducted in a single tertiary care hospital, which may limit generalizability to other settings.

Findings

Demographic and Sample Distribution

While conducting the research, a cumulative total of 500 clinical samples were examined with respect to their types and yield as portrayed in Table 1.

Table 1. Sample Distribution and Yield

Sample Type	Number of Samples	Positive Cultures	Yield (%)
Blood	150	85	56.7
Urine	120	95	79.2
Wound Swabs	100	60	60.0
Respiratory Samples	80	45	56.3
Body Fluids	50	30	60.0
Total	500	315	63.0

Turnaround time comparison

As indicated in Table 2, the average TAT of pathogen identification with MALDI-TOF MS was considerably reduced in comparison to other conventional techniques.

Table 2. Comparison of Turnaround Time

Method	Average TAT (hours)	Standard Deviation	p-value
Traditional Methods	36.5	± 8.4	
MALDI-TOF MS	6.2	± 2.3	<0.001

Identification Accuracy

As the gold standard (16S rRNA sequencing), 16S rRNA was also compared to Traditional Methods and MALDI-Remaining MS results. Comparing both of the results, it was revealed that the accuracy for MALDI-TOF MS is considerably higher.

Table 3. Identification Accuracy

Pathogen Type	Gold-Standard Confirmed Cases	Correct Identification (%)	p-value
		Traditional Methods	MALDI-TOF MS
Gram-Positive Bacteria	160	88.1	97.5
Gram-Negative Bacteria	120	85.0	96.7

Pathogen Type	Gold-Standard Confirmed Cases	Correct Identification (%)	p-value
Fungi	35	80.0	94.3
Overall	315	86.3	96.8

Cost Assessment

The cost analysis indicates that though the MALDI-TOF MS charge was considerably higher for the equipment, the cost per sample was greatly minimized attributing to the low consumed reagents and labor (Table 4).

Table 4. Cost Analysis

Method	Cost per Sample (USD)	Consumable Costs (USD)	Labor Costs (USD)
Traditional Methods	25.5	18.0	7.5
MALDI-TOF MS	8.5	5.0	3.5

Error Statistics and Shortcomings

Both methods report error rates which are presented in Table 5. MALDI—TOF MS exhibits less misidentification of identification than traditional methods which do poorly for fastidious organisms.

Table 5. Error Rates

Method	Misidentifications (%)	Unidentified Cases (%)
Traditional Methods	5.4	7.2
MALDI-TOF MS	1.8	2.5

Diagnostic Yield

MALDI-TOF MS also demonstrated a greater yield in diagnosis because it was able to determine the presence of unusual pathogens as well as those that need certain special conditions for culture.

Key Findings

1. Duration of Turnaround Time: As compared to the old methods, MALDI-TOF MS shortened the TAT by 83%, reaching an average of TAT 6.2 hours whilst traditional methods case around 36.5 hours.

2. MALDI-TOF MS Comparison with Other Thermophiles in a Bioreactor: From all the pathogens types, MALDI TOF MS yielded the most identification accuracy of 96.8% as compared to other thermophiles which exhibited an 86.3% identification accuracy.

3. Cost Efficiency: The cost incurred per sample in the case of MALDI-TOF MS was 66% lesser than vis-à-vis the traditional methods.

4. Error Rates: In relation to traditional methods, the use of MALDI-TOF MS showed the use of identification error of 1.8% while traditional methods showed an identification error of 5.4%.

Taking into consideration these findings, it can be concluded that MALDI-TOF MS is a useful diagnostic test because it enables faster, more accurate and cost effective pathogen identification as compared to the previously used microbiological methods.

Discussion

The results of this research corroborate the hypothesis that MALDI-TOF MS has a higher efficiency than traditional microbiological methods in the identification of pathogens for several criteria, including the speed of response to the request and accuracy of make identification, cost of identification, and the rate of errors made during this process. Such results can be explained within the context of the increase in literature supporting the implementation of MALDI-TOF MS in standard clinical microbiology practice.

Turnaround Time

TAT stands for Turnaround Time which refers to the duration of time taken to provide a laboratory result and is especially critical during times of clinical emergencies. In case of extending TAT, this simultaneously increases the likelihood of a negative patient outcome. Unconventional methods take the longest, averaging around 36.5 hours, their TATs are expected to remain high for a long time due to the need for biochemical and phenotypic testing followed by additional confirmatory tests. On the other hand, a significant short period of TAT was recorded during the use of MALDI-TOF MS as it averaged around 6.2 hours and this early detection ultimately led to timely administration of appropriate medications. In cases such as blood stream infection, the survival chances decrease rapidly with every minute wasted further validating the aforementioned point (Bazzi et al., 2017).

Precision and Diagnostic Yield

MALDI-TOF MS has achieved a better accuracy rate of specimen identification of 96.8% as against 86.3% that traditional methods achieved. Its specialty is interspersed with the current capacity to differentiate self-isolating and difficult pathogens. This enhanced accuracy has been especially beneficial for the fungi and Gram-negative bacteria, which are difficult for normal techniques to deal with (Singhal et al., 2015). The restriction of distinguishing between two closely related species such as E. coli and Shigella spp. which have a protein spectrum that grows over one another did the research expose in MALDI-TOF MS. These findings stress the importance of having relevant databases to mitigate such spectral shortcomings (Chen et al., 2021).

Cost Effectiveness

Given the price of the MALDI-TOF MS instruments, which at the current exchange rate is around $85,000, the usable cost per specimen, which comes to around $8.50 as opposed to $25.50 for the normal methods, would imply such an assortment of technologies would only be viable in high throughput laboratories. The decreased use of reagents, consumables and shorter times of labor save a lot. The normal method’s high upfront costs will in the coming years be recouped by the new system’s efficiency especially in tertiary hospitals that deal with many samples (Rychert, 2019).

Error Rates and Limitations

The rates of error were considerably lesser with MALDI-TOF MS, which misidentified 1.8% of gross samples in contrast to 5.4% of traditional methods. The more considerable gross error which most traditional tools have been caused by the trauma of either biochemically inert organisms or slow growing bacteria. But still, one of the cons of the MALDI-TOF MS is reliance on comprehensiveness of the database. This is an explicit reason why either rare or novel pathogens are poorly identified and thus some additional molecular methods are needed in such cases (Risch et al., 2010).Operational Challenges However, regardless of its clear strengths, there are areas of concern in respect of the introduction of MALDI-TOF MS technology. The personnel operating this technology has to be skilled because the operation and data interpretation required is very sophisticated, coupled with the fact that the initial installation of the device could be quite expensive for smaller laboratories. In addition, direct evaluation of antimicrobial resistance is out of the scope of MALDI-TOF MS technology which means that complementary techniques like PCR or molecular susceptibility assay test must be embraced with MALDI-TOF MS to enable complete diagnostic assessment (Florio et al., 2018).

Clinical Implications

In our opinion, the results of this study provide the best evidence for the integration of MALDI-TOF MS into the routine testing arsenal of tertiary care hospitals. Since it is rapid, precise and cost effective, it is likely to improve the quality of patient care in settings where rapid determination of pathogens is crucial. For example, an earlier diagnosis in septic patients could assist in the earlier withdrawal of broad-spectrum antibiotics, reducing the risk of antibiotic resistance development, and leading to better morbidity and mortality of such patients.

Study Limitations

This study has its shortcomings, even though it makes some contributions to the existing literature. It has been reported that this study was based in one tertiary hospital hence the generalizability of the analysis may be limited. Some rare pathogens were also not present in the study sample, and this could have an effect on how well diagnostic of MALDI-TOF MS is perceived. To verify these findings, more multicenter studies with larger, diverse samples are suggested.

Conclusion

It is evident from the above discussion of the findings of the research that MALDI-TOF MS has great potential for transformation in the field of clinical microbiology. The scan time is shorter, more accurate and less expensive than traditional methods which makes it a viable option. However, enhancing the spectral databases and merging with molecular diagnostics will be the area crucial for the technology to resolve its limitations and promote its integration into primary health care. With the advancements in the technology, there are good predictions of MALDI-TOF MS becoming in the centre of microbial diagnosis in the future.

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