

Species identification of lactose non-fermenting isolates from ventilator-associated pneumonia using VITEK 2 and matrix-assisted laser desorption ionization time-of-flight mass spectrometry: A comparative study with next-generation sequencing

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ABSTRACT

Bacterial identification to species level is essential for appropriate and effective patient care, particularly in critical care units in patients with severe infections. In many microbiology laboratories where automation is available, the VITEK 2 system is widely utilized for the identification and antimicrobial susceptibility testing (AST) of clinical isolates. To assess the accuracy of bacterial identification, this study was undertaken to evaluate the concordance and percentage agreement, between the VITEK 2 system and matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOF-MS)-based biomarker identification, with next-generation sequencing (NGS) serving as a gold standard. A total of 97 multidrug-resistant isolates were recovered from blood and respiratory specimens in patients on ventilator. The initial identification of these isolates was performed using VITEK 2 followed by identification by MALDI-TOF-MS and NGS. Our study revealed a high degree of concordance between the identification methods, with a 94.8% agreement rate between VITEK 2 and NGS, and a 92.7% agreement rate between MALDI-TOF-MS and NGS at the genus level. The study suggests that the VITEK 2 system is a viable and sufficient option for bacterial identification and susceptibility testing for appropriate antibacterial treatment in intensive care units wherever VITEK 2 is used as the primary ID and AST system.

1. INTRODUCTION

Ventilator-associated pneumonia (VAP) is the most common hospital-acquired infection among intensive care unit (ICU) patients worldwide [1]. This condition poses significant clinical challenges, leading to prolonged mechanical ventilation, extended ICU stays, and notably higher morbidity and mortality risks [2]. The primary causes of VAP are multidrug-resistant (MDR) pathogens, particularly *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, and *Staphylococcus aureus* [2,3].

Bacterial identification is essential for effective patient care, particularly in critical care settings with common severe infections [4]. Prompt and accurate detection of the responsible pathogens is important for targeted treatment strategies, ultimately leading to enhanced patient outcomes, reduced morbidity, and lower mortality rates [4,5].

In clinical microbiology laboratories, bacterial identification primarily relies on phenotypic analysis, encompassing multiple parameters

such as growth patterns on culture media, colony morphology, Gram staining, and biochemical reaction profiles [4,5].

In most Indian laboratory settings, the VITEK 2 system, a fluorescence-based technology developed by BioMérieux, is widely utilized for identifying clinical isolates and determining their antimicrobial susceptibility in accordance with Clinical and Laboratory Standards Institute (CLSI) and European Committee on Antimicrobial Susceptibility Testing (EUCAST) guidelines [5,6]. However, conventional diagnostic methods have certain limitations, including inconclusive results due to prior antibiotic exposure, the fastidious nature of certain microorganisms, and the restricted sensitivity of culture-based diagnostic methods [7]. Moreover, traditional culture-based methods are often delayed in providing definitive identification and antimicrobial susceptibility testing (AST) results [5,7].

Matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOF-MS) is a cost-effective and rapid technique for the identification of clinically significant microorganisms, including bacteria and yeast. This method uses a reference database to provide accurate identifications in a remarkably short turn-around time of just minutes [8]. Notably, MALDI-TOF-MS can detect microorganisms at concentrations as low as 10^2 – 10^4 cells, depending on the species,

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making it possible to obtain reliable identifications even with minimal sample amounts [9-11].

The advent of molecular techniques has improved the diagnosis of infectious diseases, offering unparalleled accuracy and rapid turnaround time. This advancement is particularly crucial in ICU settings, where timely and precise diagnoses are essential for guiding effective treatment strategies and improving patient outcomes [7].

This study investigates the potential of cutting-edge diagnostic techniques to enhance the accurate identification of bacterial pathogens, ultimately leading to improved patient treatment outcomes. Specifically, we aim to evaluate the effectiveness of advanced diagnostics in managing VAP in critically ill patients.

To assess the accuracy of bacterial identification, a comparative study was undertaken to evaluate the concordance and correlation between the VITEK 2 system and MALDI-TOF-MS-based biomarker identification, with next-generation sequencing (NGS) serving as a confirmatory reference method.

2. MATERIALS AND METHODS

A 1-year study was conducted at a tertiary care hospital in Baroda, Gujarat, India. A total of 97 lactose non-fermenting (LNF) bacterial isolates, verified by NGS as a reference method, were incorporated into this study. The MDR status of the isolate was decided based on WHO criteria by feeding the AST data into WHONET software. The MDR isolates included 41 from blood specimens and 56 from respiratory specimens, specifically 43 from endotracheal secretions and 13 from tracheal secretions obtained from patients on mechanical ventilation.

2.1. Identification by VITEK 2 Compact System

Clinical isolates were identified using the gram-negative identification card and the AST N406 card for antibiotic susceptibility testing on VITEK 2. The expected identification is probably more than 90%.

All the isolates were plated on Nutrient agar before the identification by MALDI-TOF-MS and the same was used for DNA extraction for NGS.

Table 1: Total number of isolates obtained from different clinical specimens.

Specimens	Total no. of isolates
Respiratory specimens	56
Blood	41
Total	97

Table 2: Comparative analysis of bacterial identification by VITEK 2 and MALDI-TOF MS.

Isolates	No. of isolates	VITEK 2				MALDI-TOF-MS			
		No ID	MIS ID	Correct identification		No ID	MIS ID	Correct identification	
				Genus level	Species level			Genus level	Species level
<i>Acinetobacter baumannii</i>	59	0	3	56	54	2	2	55	55
<i>Pseudomonas aeruginosa</i>	33	0	2	31	29	2	1	30	29
<i>Burkholderia cepacia</i>	2	0	0	2	2	0	0	2	2
<i>Stenotrophomonas maltophilia</i>	3	0	0	3	3	0	0	3	3
Total no. of isolates (%)	97	0	5 (5.15)	92 (94.8)	88 (90.7)	4 (4.12)	3 (3.09)	90 (92.7)	89 (91.7)

*No ID: No identification, MIS ID: Misidentification, MALDI-TOF-MS: Matrix-assisted laser desorption ionization time-of-flight mass spectrometry

2.2. Identification by MALDI-TOF-MS: (Bruker MALDI Biotyper, Version 4.1.100)

A small sample from a freshly grown bacterial colony was applied to the MALDI target plate and immediately overlaid with 1 µL of α -cyano-4-hydroxycinnamic acid matrix solution [12]. Following drying, the prepared target plate was inserted into the mass spectrometer for subsequent analysis. Bacterial identification was performed utilizing the Bruker MALDI Biotyper software, version 4.1.100.

2.3. Molecular Method (NGS)

Bacterial DNA was manually extracted using the cetyltrimethylammonium bromide method [13]. DNA quality was verified through gel electrophoresis, and once validated, the DNA was used to prepare libraries according to the Illumina Nextera XT kit protocol. Library quality was subsequently evaluated using the QIAxcel instrument. Finally, the prepared libraries were sequenced using the Illumina NextSeq platform.

2.4. Genome Annotation and Assembly

Genomic annotation and assembly were performed using BVVRC version 3.35.5 [14]. Isolate identification was facilitated by PubMLST software [15].

In this study, NGS was used as a reference method for comparing bacterial identification data analyzed by VITEK 2 and MALDI-TOF-MS.

3. RESULTS

This study included 97 LNF multidrug resistance bacterial isolates comprising 41 from blood and 56 from respiratory specimens of mechanically ventilated patients [Table 1].

Table 3: % of error rate in VITEK 2 and MALDI-TOF in comparison of NGS.

Summarized data	Percentage (%)
% of agreement between MALDI TOF and VITEK 2	93
% agreement between VITEK 2 and NGS-correct ID at the genus level	94.80
% agreement between MALDI-TOF and NGS-correct ID at the genus level	92.70
% of genus level error rate-VITEK 2	5.20
% of species-level error rate-VITEK 2	9.30
% of genus level error rate-MALDI-TOF-MS	7.30
% of species-level error rate-MALDI-TOF-MS	8.30

MALDI-TOF-MS: Matrix-assisted laser desorption ionization time-of-flight mass spectrometry, NGS: Next-generation sequencing

Table 4: Comparison of MALDI TOF MS and VITEK 2 data.

References	No. of isolates	MALDI-TOF		VITEK 2	
		Genus level (%)	Species level (%)	Genus level (%)	Species level (%)
Van Veen <i>et al.</i> [23]	327	95.10	86	92.20	83.10
Guo <i>et al.</i> [5]	1025	100	93.37	-	-
Jamal <i>et al.</i> [27]	806.00	97.30	93.20	98.60	96.40
Kassim <i>et al.</i> [31]	383	97.60	97.40	95.70	88
Wang <i>et al.</i> [12]	1181	99.50	95.70	-	-
Madhavan <i>et al.</i> [32]	100	96	92.90	97.00	90.90
Ibraheem <i>et al.</i> [24]	416	100	98.32	99.04	94.96
Surányi <i>et al.</i> [33]	42	95.20	66.70	-	-
Rodriguez-Temporal <i>et al.</i> [34]	1330	99.70	99.10	-	-
Our study	97	92.70	91.70	94.80	90.70

MALDI-TOF-MS: Matrix-assisted laser desorption ionization time-of-flight mass spectrometry.

The 97 bacterial isolates consisted of 59 *A. baumannii*, 33 *P. aeruginosa*, 2 *Burkholderia cepacia*, and 3 *Stenotrophomonas maltophilia*.

Table 2 presents a comparative analysis of bacterial identification using VITEK 2 and MALDI-TOF MS. The results indicate that VITEK 2 accurately identified 56 isolates at the genus level and 54 at the species level for *A. baumannii*. In comparison, MALDI-TOF MS correctly identified 55 isolates at both the genus and species levels.

For *P. aeruginosa*, VITEK 2 correctly identified 31 out of 33 isolates at the genus level and 29 at the species level. Meanwhile, MALDI-TOF-MS identified 30 isolates at the genus level and 29 at the species level.

Both VITEK 2 and MALDI-TOF-MS successfully identified all isolates of *B. cepacia* and *S. maltophilia* at both the genus and species levels.

An examination of the bacterial identification data presented in Table 2 revealed discrepancies in the identification of certain isolates. In particular, MALDI-TOF failed to identify 2 *A. baumannii* isolates and 2 *P. aeruginosa* isolate. In addition, MALDI-TOF misidentified 2 *A. baumannii* isolates and 1 *P. aeruginosa* isolates.

Out of 97 isolates, VITEK 2 accurately identified 92 of the 97 LNF isolates at the genus level and 88 at the species level. This resulted in a 5.2% error rate at the genus level and a 9.3% error rate at the species level. In comparison, MALDI-TOF-MS correctly identified 90 out of 97 LNF isolates, with error rates of 7.3% at the genus level and 8.3% at the species level.

Table 3 represents the percentage agreement between the different identification methods. The results indicate that VITEK 2 and NGS had a 94.8% agreement, whereas MALDI-TOF-MS and NGS showed a 92.7% agreement. The percentage agreement between MALDI-TOF-MS and VITEK 2 was 93%.

4. DISCUSSION

VAP is a significant healthcare-associated infection, predominantly triggered by a group of LNF bacteria, particular *A. baumannii*, *P. aeruginosa*, *S. maltophilia*, and *B. cepacia*. Accurate detection and identification of these bacterial pathogens are essential for predicting intrinsic antibiotic resistance of the pathogen as well as guiding targeted antibiotic therapy and effective infection prevention strategies [2,4,5].

The VITEK 2 system’s ID-GN card is a 64-well card designed to identify gram-negative bacilli. It utilizes 41 fluorescent biochemical tests, including 18 specific enzymatic tests [16]. The identification process

relies on analyzing the collective reactions generated across all wells, which are then quantified using colorimetric detection methods [17].

The MALDI-TOF-MS technique has two key limitations. First, it requires isolated bacterial colonies from fresh cultures for rapid analysis [18]. Second, unable to distinguish between subspecies or strains with differing virulence factors or antimicrobial resistance profiles [18,19]. Consequently, these limitations compromise prompt clinical diagnosis of bacterial infections and cause delays in selecting effective antibiotic treatments [18,20].

Antibiotic susceptibility reporting aligns with CLSI/EUCAST standards [21,22]. Selection of antibiotics is based on species-specific intrinsic resistance patterns, species-based minimum inhibitory concentration and breakpoints of antibiotics [21,22]. Conversely, errors in bacterial identification can cause serious consequences, including misguided therapeutic strategies and inaccurate AST reporting.

Our study revealed a high concordance rate of 93% between MALDI-TOF MS and VITEK 2. This finding compares favorably to a similar study by Van Veen *et al.*, which reported a concordance rate of 82.3% [23].

Table 4 compares our findings with other research studies on the identification of bacteria using MALDI-TOF-MS and VITEK 2 at both the genus and species levels. These studies employed molecular methods such as polymerase chain reaction, 16S Sanger sequencing, or NGS as confirmatory methods.

A study conducted in 2014 by Guo *et al.*, evaluated 1,025 isolates using MALDI-TOF MS and VITEK 2. The findings revealed that MALDI-TOF MS achieved 100% accuracy at the genus level, while VITEK 2 had an error rate of 0.58% [5]. At the species level, the error rates were 5.56% for MALDI-TOF-MS and 6.24% for VITEK 2 [5]. In contrast, our study found that MALDI-TOF-MS had a genus-level error rate of 7.3% and a species-level error rate of 8.3%. Meanwhile, VITEK 2 had a genus-level error rate of 5.2% and a species-level error rate of 9.3%.

Our study showed that MALDI-TOF-MS effectively identified LNF Gram-negative bacteria (GNB) at the genus level, achieving a success rate of 92.7%. This result is relatively lower than the findings reported by Wang *et al.*, which indicated that MALDI-TOF MS identified GNB with an accuracy of 99.5% [12].

A study conducted by Ibraheem *et al.*, evaluated the accuracy of MALDI-TOF-MS and VITEK 2 in identifying 416 microbial isolates. The results showed that MALDI-TOF-MS achieved 100% accuracy

at the genus level, whereas VITEK 2 had a 0.96% error rate. At the species level, the error rates for MALDI-TOF-MS and VITEK 2 were 1.68% and 5.04%, respectively [24].

A study conducted by Guo *et al.*, on the comparative analysis of MALDI-TOF-MS and VITEK 2 revealed that the percentage agreement between MALDI-TOF-MS and VITEK 2 was 92.59% [5]. Garza-González *et al.*, found a 95% agreement [8], whereas Ibraheem *et al.* reported 93.26% [24]. Our findings were also close, showing a 93% agreement.

Alterations in the bacterial genome, including genetic mutations [25], deletions, or the acquisition of new genes through horizontal transfer [26] can modify the bacterial proteome. This, in turn, may impact the accuracy of MALDI-TOF-MS identification, potentially leading to delayed or incorrect identification of bacterial species.

MALDI-TOF-MS is a popular tool for identifying bacterial species. However, limitations can lead to misidentification or non-identification of certain species. These challenges arise from incomplete databases, particularly for variant antimicrobial-resistant (AMR) bacteria, closely related species, and suboptimal spectral quality [27,28].

Research conducted by Anderson *et al.*, revealed that the culture medium used can influence the confidence scores obtained from MALDI-TOF analysis, particularly when employing the direct method. This effect was notably pronounced in *Pseudomonas* species cultured on MacConkey or Cetrimide media [29].

Popovic *et al.*, in the study, noted that methods used for sample preparation, cultivation of bacteria, incubation time, and culture condition of bacteria affect the identification of bacteria by MALDI TOF [28].

Research conducted by Homem De Mello De Souza *et al.* examined the laboratory identification of rare glucose non-fermenting GNB associated with cystic fibrosis using MALDI-TOF-MS. Their findings indicated that MALDI-TOF-MS achieved a 75% accuracy rate for identification, which was validated through 16S rDNA gene sequencing [30]. In our study, we obtained higher accuracy rates, with 92.7% of isolates correctly identified by MALDI-TOF-MS and 94.8% by VITEK 2, both compared to the NGS method.

Most studies suggest that MALDI-TOF-MS outperforms VITEK 2 in identifying bacteria at both the genus and species levels. Our research supports this finding at the species level, with MALDI-TOF-MS demonstrating superior identification capabilities compared to VITEK 2. However, our results indicate that genus-level identification is less accurate with MALDI-TOF-MS, potentially due to the focus on AMR bacteria or limitations in the database [30]. In contrast to the VITEK 2 system, which is limited to identifying bacteria and *Candida* species, MALDI-TOF-MS offers broader identification capabilities, encompassing a wide range of bacteria, fungi, *Actinomyces* and nontuberculous mycobacteria.

5. CONCLUSION

Our study revealed that the accuracy of the VITEK 2 system in identifying bacteria is closely comparable to that of NGS, which is considered the gold standard method. This makes VITEK 2 a viable and adequate option for bacterial identification and susceptibility testing in most settings where automation is available. This is crucial for the effective management of patients. However, we found that the number of isolates such as *B. cepacia* and *S. maltophilia* was very limited. More isolates are needed to support our findings regarding these specific bacteria. As the study was conducted only on MDR organisms it remains to be seen whether or not similar identification

limitations occur in non-MDR organisms. Future studies should be conducted to evaluate the performance of VITEK 2 with a broader range of clinical bacterial isolates.

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7. AUTHORS' CONTRIBUTIONS STATEMENT

All authors made substantial contributions to conception and design, acquisition of data, or analysis and interpretation of data; took part in drafting the article or revising it critically for important intellectual content; agreed to submit to the current journal; gave final approval of the version to be published; and agree to be accountable for all aspects of the work. All the authors are eligible to be an author as per the International Committee of Medical Journal Editors (ICMJE) requirements/guidelines.

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The authors report no financial or any other conflicts of interest in this work.

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No direct experimentation was done on Humans or Animals.

11. DATA AVAILABILITY

All data generated and analysed are included within this research article.

12. PUBLISHER'S NOTE

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13. USE OF ARTIFICIAL INTELLIGENCE (AI)-ASSISTED TECHNOLOGY

The authors declares that they have not used artificial intelligence (AI)-tools for writing and editing of the manuscript, and no images were manipulated using AI.

14. INFORMED CONSENT STATEMENT

No direct experimentation was done on Humans or Animals.

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