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Original Research Article

Assessment of Vitek 2 system and MALDI-TOF MS for identification of lactose-fermenting bacteria: Comparing with whole-genome sequencing - As a gold standard

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Abstract

Introduction: The Vitek 2 system is used widely for ID and AST of clinical bacterial isolates. MALDI-TOF-MS is often used to study accuracy of species identification. Recently, WGS is considered as a gold standard for species identification. The AST data is based on species of individual bacterial species; erroneous ID can lead to wrong AST interpretation and faulty antibiotic prescriptions.

Aim and objective: To study the percentage agreement in species identification of lactose fermenters isolated from clinical specimens by the Vitek 2 system and MALDI-TOF-MS with WGS as a gold standard.

Materials and Methods: A prospective study was conducted from May 2023 to December 2024 at a tertiary care hospital in Waghodia, Vadodara, Gujarat, India. This study included 117 clinical isolates collected from patients admitted to ICUs, specifically of Escherichia coli and *Klebsiella pneumoniae*. All isolates were identified by the Vitek 2 compact system, MALDI-ToF-MS, and whole-genome sequencing.

Result: out of 69 *K. pneumoniae*, 67 were correctly identified by the Vitek 2 compact system and 54 by the MALDI TOF MS system. In *E. coli*, the MALDI TOF MS system identified 45 (45/48) and 42 (42/48) correctly identified by the Vitek 2 compact System.

Conclusion: Identification of Clinical isolates to the species level is vital for the prediction of intrinsic resistance and antibiotic susceptibility as per CLSI guidelines. The VITEK 2 shows good concordance with WGS and can be used as a sole system for species identification of lactose fermenters like *Klebsiella pneumoniae* and *E. coli.*

Keywords: MDR lactose fermenters, WGS, MALDI-TOF MS, VITEK 2 system, Percentage of agreement.

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1. Introduction

Automation in diagnostic bacteriology has enhanced the quality of culture and antibiotic susceptibility reports.[1] Traditional culture methods of identification of bacteria up to the species level were based on phenotypic characteristics like colony morphology, staining properties, and biochemical reactions.[2] These methods are time-consuming and cumbersome.[2] Bacterial species identification is essential for effective patient care, particularly in critical care settings with common severe infections.[1] Prompt and accurate detection of the clinically relevant pathogens is vital for targeted treatment strategies, ultimately leading better patient outcomes, reduced morbidity, and lower mortality rates.[1,2]

Many clinical microbiology laboratories currently employ the Vitek 2 system[3] and Matrix-Assisted Laser Desorption Ionization Time-Of-Flight Mass Spectrometry (MALDI-TOF MS) to rapidly identify organisms.[2] The CLSI and EUCAST are two major standards for reporting AST

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based on the available data.[2,3] Since the reference data depends on the correct identification of species of bacteria, an inaccurate species identification could lead to an erroneous susceptibility report.[2,17]

Both conventional methods for identifying bacteria and pathogens are based on different principles.[3,4] The VITEK 2 system generates accurate results based on colorimetric analysis of the combination of biochemical tests and matching with the database algorithm.[3] MALDI-TOF MS involves the direct analysis of mass-to-charge ratios (m/z) of molecular ions, like protein fingerprinting from bacterial cells, and these fingerprints are compared with a reference database for identifying bacteria by various algorithms.[4,5] MALDI-TOF MS can detect microorganisms at concentrations as low as 10^2 -10^4 cells, depending on the species, making obtaining reliable identifications even with minimal sample amounts possible.[4]

In the present study, we aimed to assess the percentage agreement in identification between the two currently used methods (VITEK 2 and MALDI-TOF-MS) and next-generation sequencing (WGS).

2. Materials and Methods

A study was conducted on different specimens received in the laboratory for the period from May 2023 to December 2024 from ICUs in a tertiary care hospital.

2.1. Identification by vitek 2 compact system

Specimen preparation involved suspending a pure culture of the isolate in 0.45% saline to achieve a turbidity equivalent to a 0.5 McFarland standard. Clinical isolates were identified by inoculating a Gram-negative identification card (GN ID) and analyzing it using the Vitek 2 system.

2.2. Identification by MALDI-TOF MS

(Bruker MALDI biotype, version 4.1.100)

A small sample from a freshly grown bacterial colony was carefully applied to the MALDI target plate and immediately overlaid with 1 µL of CHCA (α-cyano-4-hydroxycinnamic acid) matrix solution.[4] After drying, the prepared target plate was inserted into the mass spectrometer for analysis.[4] Bacterial identification was subsequently performed using the Bruker MALDI Biotyper software (version 4.1.1).

2.3. Identification by molecular method

DNA was extracted from culture by the manual cetyltrimethylammonium bromide (CTEB) method, and a purity of the extracted bacterial DNA was checked by QIAxcel Advanced System.[6] Nextera XT DNA Library Prep Kit was used for library preparation, and for gene sequencing, an Illumina novaseq machine was used.

2.4. Data analysis

Quality of Raw data as checked by the Linux command line, criteria were <50 bp sequencing were removed, and trimming was done at 10-15 bp from the last portion. The Quast report would be checked for Contig length N50. N50 should be greater than 1 lack bp. Good fastaq file assembled and converted into a contig. fasta format using online software, BV-BRC version 3.35.5.[7]

2.5. Tool used for genomic data analysis

PubMLST (https://pubmlst.org/) was used for the Genomic identification of bacterial species.[8]

In this study, WGS was used as a reference method for comparing bacterial identification data analyzed by VITEK2 and MALDI-TOF MS.

3. Results

A total of 679 *K.pneumoniae*, 412 *E. coli*, and 43 *Enterobacter sp.* isolated from different clinical specimens. Out of these 1134 isolates, 117 multidrug-resistant lactose-fermenting bacteria (confirmed by WGS) were isolated from different clinical specimens included in the study.

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

Type of Specimen	Total Number of isolates
Blood	49
Respiratory specimens (Secretions and sputum)	31
Pus	12
Body fluids (Ascitic fluid, pleural fluid, peritoneal fluid, bile fluid)	12
Central venous catheter tip	2
CSF (cerebrospinal fluid)	1
Urine	10
Total	117

Out of these 117 isolates, *Klebsiella pneumoniae* were 69 (56%) and *Escherichia coli* were 48 (39%) identified by WGS, which work as a reference when compared with the Vitek 2 system and MALDI TOF MS separately.(**Table 1**)

A high percentage of agreement of Vitek 2 with WGS shows in *K. pneumoniae* (97.1%), whereas in *E. coli*, it is 87.5%.(**Table 2**)

Data shows a high percentage of agreement of MALDI TOF MS with WGS seen in *E. coli;* however, variation was noted in *Klebsiella pneumoniae* at the genus level (78.26%) and species level (66.66%).(**Table 3**)

Table 2: Comparative analysis of the Vitek 2 system with WGS.

Name of organisms	Genetic Identification by WGS (n)	Identification by the Vitek 2 system (Genus & Species levels) (n)	Misidentified by the Vitek 2 system (n)	Percentage of Agreement (%)
E. coli	48	42	6	87.5
K. pneumoniae	69	67	2	97.1

Table 3: Comparative analysis of WGS with MALDI TOF MS.

Name of organisms	Genetic Identification by WGS (n)	By MALDI TOF-MS				Percentage of Agreement (%)
		Identified at the Genus level (n)	Identified at the species level (n)	Misidentified (n)	Unidentified (n)	
E. coli	48	45	45	1	2	93.75
K. pneumoniae	69	54	46	1	14	78.26

4. Discussion

The lactose fermenter, like *E. coli*, is commonly found in community-acquired infections, while *Klebsiella pneumoniae* is an opportunistic pathogen, and most resistant forms are found in hospital-acquired infections.[10] *Klebsiella pneumoniae* has, over the last two decades, developed many mechanisms of antibiotic resistance and has become a threat globally.[11] The right species identification of lactose fermenters to the species level is of prime importance for targeted therapy.[19]

Several studies have compared the performance of Vitek 2 and MALDI-TOF MS for bacterial identification. Kassim et al. reported 87.3% correct identification at the genus and species levels using Vitek 2, compared to 100% with MALDI-TOF MS.[12] In the same study, both Vitek 2 and MALDI-TOF MS achieved 100% (33/33) correct identification of *K. pneumoniae*. [12] Elvira Garza-González et al. observed a 99.2% agreement between Vitek 2 and MALDI-TOF MS for *E. coli* (868/884) and a 99.3% agreement for *K. pneumoniae* (365/368) at both the genus and species levels.[13] Madhavan et al. found that Vitek 2 identified bacteria at the genus and species levels with 97% and 90.9% accuracy, respectively, while MALDI-TOF MS achieved 96% and 92.9% accuracy at the same levels.[14] They also used 16S rRNA gene sequencing to resolve discordant results.[14]

Two studies compared the accuracy of MALDI-TOF MS and VITEK 2 for microbial identification.[16,18] A study by Ibraheem et al.[18] evaluated 416 microbial isolates and found that MALDI-TOF MS achieved 100% accuracy at the genus level, compared to a 1.96% error rate for the VITEK 2 system. At the species level, MALDI-TOF MS had a 1.68% error rate, while VITEK 2 had a 5.04% error rate.[18] Conversely, Madhavan et al. reported that VITEK 2 had a slightly higher genus-level identification rate (97% vs. 96% for MALDI-TOF MS), but MALDI-TOF MS performed better at the species level (92.9% vs. 90.9% for VITEK 2).[16]

Rarely there is misidentification by the Vitek 2 system.[20] This can be, as explained by the manufacturers, could be multifactorial, like the age of the culture, the medium, saline diluent concentration, pH, cell suspension density, card lots, and the database and algorithm of the machine. (Vitek 2 user manual; bioMérieux, Inc., Durham, NC). [20] Popovic et al in the study noted that methods used for sample preparation, cultivation of bacteria, incubation time, and culture conditions of bacteria affect the identification of bacteria by MALDI TOF.[17] Encapsulated strains can hinder efficient lysis, leading to poor spectral quality, which may result in misidentification when using MALDI-TOF MS.[4,21]

In Clinical laboratories, most of the routine identification is done by the Automation systems, either Vitek 2 or MALDI TOF MS,[15] using a reference database to provide accurate identification with considerably low cost.[13,16] In contrast, whole-genome sequencing (WGS) is not routinely used due to its high cost and technically demanding complex procedures.[15] The VITEK 2 is the most widely used ID and AST instrument in clinical microbiological laboratories.[3] The present study will aid in the possible predictive value of ID and AST by Vitek 2 and its comparison with WGS.

5. Conclusion

Identification of Clinical isolates to the species level is vital for the prediction of intrinsic resistance and antibiotic susceptibility as per CLSI guidelines. VITEK 2 is widely used for identification and for testing antibiotic susceptibility in accredited microbiology laboratories in developing countries including India. Earlier studies compared VITEK 2 identification accuracy with MALDI TOF MS. Now that whole-genome sequencing is available, we attempted to compare both these methods with WGS as a gold standard. The study suggests that VITEK 2 has good concordance with WGS and can be used as sole identification system for species identification of lactose fermenters particularly for most frequently encountered MDR *Klebsiella pneumoniae* and *E. coli*.

6. Ethical Committee Approval

Approving body: Parul University Institutional Ethics Committee for Human Research [PU-IECHR]. Approval number: PUIECHR/PIMSR/00/081734/5813.

7. Conflict of Interest

None.

8. Source of Funding

None.

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