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Original Research Article

Concordance of *Enterococcus* species identification by two different identification systems VITEK2 and MALDI-TOF, compared with WGS (gold standard)

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Abstract

Introduction: Bacterial species identification is vital for taking decision of antimicrobial therapy. Bacteria can be quickly identified through a system database, which is particularly significant for critically ill patients. This becomes particularly important in cases where antimicrobial susceptibility varies significantly between *Enterococcus faecium* and *Enterococcus faecalis*. According to the literature, *Enterococcus faecium* is intrinsically resistant to ampicillin and frequently exhibits a high rate of vancomycin resistance, which is quite rare in *Enterococcus faecalis.*

Aims & Objective: This study aims to compare the performance of two commercial systems, VITEK2 and MALDI-TOF (Matrix-assisted laser desorption-ionization time-of-flight mass spectrometry), in identifying *Enterococcus* species when compared against Whole Genome Sequencing, which is considered the gold standard.

Study Type: A cross-sectional comparative Diagnostic accuracy study.

Materials and Methods: Blood cultures were processed using the BacT/Alert system. Aerobic-positive cultures showing only one bacterial type were subjected to direct identification. 44 *Enterococcus spp.* isolated and identified by a biochemical-based system, VITEK2 and a proteomics-based system, MALDI-TOF. All the isolates were subjected to Whole Genome Sequencing for species identification, using the Illumina NovaSeq instrument.

Results: Among 44 *Enterococcus spp.* identified by VITEK2, 31 were identified as *Enterococcus faecium* and 13 were identified as *Enterococcus faecalis*. All *Enterococcus* were showing concordance report when it comes to identification at the Genus level, by both systems including VITEK2 and MALDI-TOF. And at the species level 84% of *Enterococcus faecium* were showing concordance with MALDI-TOF. And 92.3% *Enterococcus faecalis* were showing concordance with MALDI-TOF.

Conclusion: Quick and precise identification methods in the laboratory are required to enable the identification of bacterial species. In the current study, we associated the relative precision of VITEK2 and MALDI-TOF, using *Enterococci* isolates from clinical specimens, and compared the results, considering WGS as a gold standard method. Our results evidently exhibited that VITEK2 is a dependable tool for identifying *Enterococcus spp.* with superior inequivalent power with acceptable discrepancies.

Keywords: Bloodstream infections, *Enterococcus*, MALDI-TOF, VITEK2, Whole Genome Sequencing.

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1. Introduction

Enterococcus spp. are major culprits behind bloodstream infections in debilitated patients in the ICUs, especially among those with weakened immune systems, including organ transplant recipients, individuals undergoing chemotherapy or dialysis, the elderly, and those suffering from neutropenia.

Over the years, Enterococci have evolved from gut commensal flora to the most difficult to treat pathogen. Treatment of BSI due to *Enterococci* is a challenge to

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clinicians. *Enterococcus faecium* and *Enterococcus faecalis* are the furthermost frequent pathogens isolated from critically ill patients in ICUs.

Treatment of *Enterococci* is difficult due to the intrinsic resistance mechanism & tolerance to antibiotics. The clinical studies regarding the treatment of *Enterococci* are scarce and indicate that the treatment of *Enterococcal* infections is species-specific.[1,2]

VITEK2 is a popular tool in clinical microbiology laboratories, helping to identify bacteria by examining their phenotypic traits like culture characteristics, morphology of colonies, gram staining results, and several biochemical characteristics.[3]

While this method tends to be pretty accurate, it often takes about one to two days to get results. Traditional techniques can still slow down the diagnosis process, since they usually need several subcultures to pinpoint the species accurately. Additional understanding of phenotypic features is often subjective, necessitating substantial training & experience for precise identification. MALDI-TOF MS accomplished identification of bacteria rapidly, inexpensively, and with additional specificity.[4-8]

Sequencing methods may be executed for this, but they frequently result in extended turnaround times and add substantial expense. THE MALDI-TOF MS platform uses an investigative method, in which particles are ionized, divided as per their mass-to-charge ratio, & this measurement is defined by the period it takes for the particles to travel to a sensor that detects it at the terminal part of a time-of-flight tube. That subsequent spectrum, per mass-to-charge values plotted on axis X & intensity on axis Y, is compared to a database of spectra from already identified bacteria.[9-11]

Excluding species that are not included in the database & innately identical to each other.[9]

Owing to the minute amount of biomass desired, identification tests can generally be executed from the main culture plate, as long as a well-isolated colony is available. Sample preparation is quite simple, and analysis of more than 40 samples is possible in about an hour.[12]

Additionally, prior information about an organism being tested is not essential, so less experienced microbiologists can also perform the testing. This contributes to a lessening of time in the identification of bacteria.[13]

Every commercially accessible platform has an exclusive customary bacterial profile in its databases. The VITEK2 is FDA approved for the detection & identification of 332 bacteria.[14]

The MALDI-TOF is FDA approved for the detection & identification of 294 bacteria.[15,16]

In count of having diverse databases, VITEK2 & MALDI-TOF platforms correspondingly vary in the techniques & they match the spectrum of unknown bacteria to known bacteria.[17,18]

In this study, we compared the identification result of *Enterococcus spp.* by the VITEK2 with the biomarker-based system MALDI-TOF, with reference to the Whole genome sequencing method, considering it as a gold standard.

2. Materials and Methods

This cross-sectional comparative Diagnostic accuracy study was conducted at the Department of Microbiology, PIMSR, Gujarat from April 2023 to December 2024. All the blood culture bottles collected from patients suspected of bacteraemia are loaded into BacT/Alert (bioMe´rieux). This system continuously monitors bacterial growth and incubates blood culture bottles.

2.1. Inclusion and exclusion criteria

Aerobic positive blood culture bottles with microscopy showing a single type bacterium were included for identification by VITEK 2. Only first isolate per blood sample is included. Samples other than blood culture or blood culture exhibiting bacteria other than *Enterococci* were excluded. Repeated blood samples from one patient were excluded.

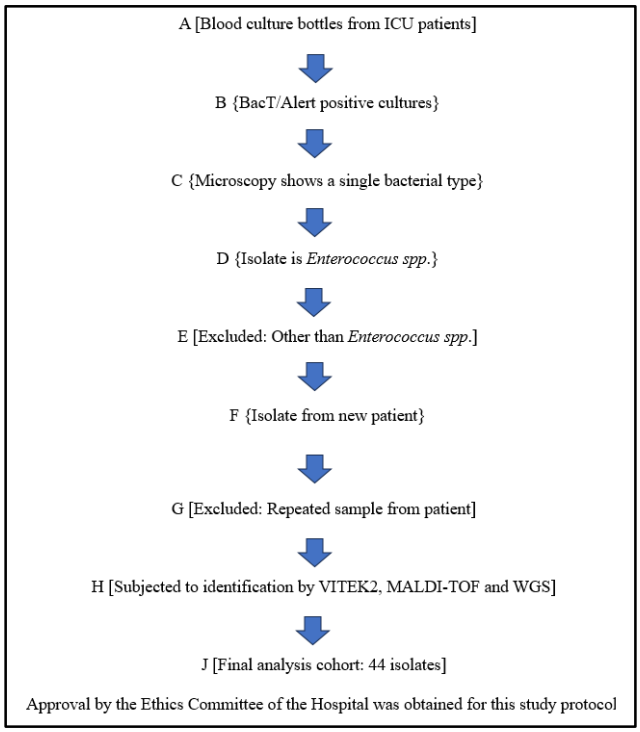

Figure 1: Flow diagram of sample collection

2.2. Methodology

The identification of blood isolates *Enterococcus spp.* was executed by the VITEK2 system (Biomérieux, Marcy l' Etoile, France) & MALDI-TOF MS (Bruker Daltonics,

Bremen, Germany). The processing of the blood specimens was done following standard operating procedures. The sample size was calculated to be 44 by prevalence of 3%, margin of error 5% and confidence interval 95%, by using statistical software open-EPI.

2.3. Identification protocol

2.3.1. VITEK2 analysis

Enterococcus spp. was identified using the Gram-positive ID card and the AST card for antibiotic susceptibility testing on VITEK 2. The anticipated identification is possibly more than 90%. All the isolates were plated on Nutrient agar before the identification by MALDI TOF MS, and the same remained useful for DNA extraction for WGS.

2.3.2. MALDI-TOF MS analysis

Freshly isolated *Enterococci* were tapped on target plates of MALDI-TOF & then covered with 1 µl matrix solution of α-cyano-4-hydroxycinnamic acid (10 mg/mL).[19]

This target plate was evaluated by the Bruker Autoflex Max system. Bacterial ID was performed using the Bruker MALDI Biotyper software of version number 4.1.100.

2.3.3. Whole genome sequencing analysis

DNA extraction: the manual method was performed using the cetyltrimethylammonium bromide (CTAB).[20]

Extracted quality of DNA was tested by gel electrophoresis method, and after validation, that DNA was utilized to make libraries as per the Illumina Nextera XT kit protocol.

The prepared libraries are then sequenced on the Illumina NovaSeq machine.

2.4. Data analysis

The resulting data from the machine was incorporated into different Bioinformatics software. *Enterococcal* identification was done using PubMLST software.[21]

In this study, WGS was utilized as a reference (gold standard) method for comparing bacterial ID data analysed via VITEK2 and MALDI-TOF MS.

3. Results

This cross-sectional comparative Diagnostic accuracy study included 44 *Enterococcus spp.* isolated from the blood of patients admitted to ICUs during April 2023 to December 2024.

Table 1 represents a comparison analysis of *Enterococcal* species identification using VITEK2 and MALDI-TOF.

From which 44 *Enterococcus* were further identified by WGS.

The results indicate that VITEK 2 & MALDI-TOF, both systems, accurately identified all 44 isolates at the genus level.

Table 2 and **3** represent a comparison analysis of *Enterococcal* species identification using VITEK2 against WGS and MALDI-TOF against WGS, respectively.

Table 1: Concordance rate in Species identification of *Enterococcus* between VITEK2 against MALDI-TOF.

ID by VITEK2 (44)	ID by MALDI-TOF (44)	Concordance rate N/N (%)
Enterococcus faecium (31)	*Enterococcus faecium (26)*	26/31 (84%)
Enterococcus faecalis (13)	*Enterococcus faecalis (12)*	12/13 (92.3%)

Note: N= number

Table 2: Concordance rate in Species identification of *Enterococcus* between VITEK2 against WGS.

ID by VITEK2 (44)	ID by WGS (44)	Concordance rate N/N (%)
Enterococcus faecium (31)	*Enterococcus faecium (30)*	30/31 (96.7%)
Enterococcus faecalis (13)	*Enterococcus faecalis (12)*	12/13 (92.3%)

Note: N= number

Table 3: Concordance rate in Species identification of Enterococcus between MALDI-TOF against WGS.

ID by MALDI-TOF (44)	ID by WGS (44)	Concordance rate N/N (%)
Enterococcus faecium (31)	*Enterococcus faecium (31)*	31/31 (100%)
Enterococcus faecalis (13)	*Enterococcus faecalis (08)*	08/13 (61.5%)

Note: N= number

4. Discussion

Enterococcal Bloodstream Infection is a significant cause of mortality and morbidity in hospitalized patients.

Correct identification up to species level of infective agent is of vital importance as the CLSI guidelines and breakpoint of antimicrobial agents is based on species of bacteria. Patients' outcomes can be improved by fast identification of causative bacteria, as instigation of antimicrobial therapy on time is vital for patients' prognosis, and it is related to higher survival rates and decreased healthcare costs.[22,23]

VITEK2 is the most widely used automated system for conducting ID and AST, in microbiology and diagnostic laboratories. MALDI-TOF is available only at certain laboratories in our country. So, that was the reason, we wanted to see, is VITEK2 system sufficient enough for

Identification system and AST for diagnosis up to species level, so we conducted this study.

In this study, we aimed to compare the ID results of biomarker-based MALDI-TOF and VITEK2 in the identification of *Enterococcus spp.*

Then we find out that, VITEK2 matches result more with WGS, indicating that it is robust identification system up to species level then MALDI-TOF.

MALDI-TOF is more efficient and faster method for direct identification of isolates from the specimen. It also useful for many non-culturable bacteria, *Mycobacterium tuberculosis*, *Nontuberculous mycobacteria (NTM)*, and fungi.

Efficient turnaround time in the identification of this bacterium delivers a timely response to the clinician, intended for focused treatment.[24,25]

A decrease in TAT for *Enterococci* identification benefits clinicians to treat patients quicker, and this leads to a decrease in the overall mortality ratio, the span of patient stays in the hospital, & the healthcare expenses related to patient care.[26]

Automation in commercial platforms, identifying bacteria with clinical significance, is at present useful in microbiology laboratories.[27]

VITEK2 is an automated platform regularly utilized in clinical microbiology laboratories, with the competence of carrying out antimicrobial susceptibility tests.[28]

The VITEK2 is a biochemical reaction-based identification system. ID-GPC cards were 64-well card intended to recognize gram-positive cocci. It operates by 43 tests, fluorescent & inhibitory tests, including 17 enzymatic tests. The identification method depends on inspecting the united reactions produced across every well & further quantified by colorimetric recognition procedures.[29]

The rapidity of MALDI-TOF MS in identifying bacteria supports quickly guiding treatment decisions, which is particularly grave when the infecting bacteria are unanticipated. For example, one case pronounced by von Rotz et al. in which a father and son duo were admitted to an ICU with sepsis and gastroenteritis-like symptoms, later a camping trip & unexpected bacteria were identified quickly by MALDI-TOF & based on that, treatment was started and their life was saved.[30]

Though there are exceptions, like failure to discriminate among related species, which can be because of inherent resemblance.[31]

In inherently alike bacteria, it is customary to report to the genus level. In cases where differentiation to the species level is clinically compulsory, supplementary tests must be executed. In the future, proteomic-based methods like MALDI-TOF may improve the discriminatory power & make it feasible to recognize bacteria at the strain or serotype level.[32]

Modifications in the bacterial genomes, including genetic mutation deletion, and the gaining of novel genes via horizontal transfer,[33] can alter the bacterial proteome. This may affect the accuracy of MALDI-TOF MS identification, potentially leading to late or incorrect identification of bacterial species.

One more reason of similar species may be incorrectly identified is a dearth of an adequate spectrum of bacteria in the database. In such a scenario, it is likely to acquire mis-identification at the species-level or no identification at all. For example, one study found that a misidentification was common among fungi named *Trichophyton* species.[34]

Mistakes in bacterial identification are reason of severe consequences & become responsible for erroneous therapy due to imprecise antimicrobial susceptibility reports.

A former study done by Moon et al. in which MALDI-TOF correctly identified 100% of the *Enterococci* at the genus level.[35] Alike to these studies, our results presented that VITEK2 & MALDI-TOF MS correctly identified all *Enterococcus sp*p. at the Genus level, whereas at the species level, VITEK2 identified 95.45% correctly, while MALDI-TOF MS identified 91%, when subjected to identification by the gold standard method, WGS.

Both systems identified *Enterococci* with a good discrimination between *E. faecalis* and *E. faecium*, but VITEK2 is more precise in correctly differentiating *Enterococcus* at the species level.[36]

In one previous study, Fang et al. showed that MALDI-TOF MS is more efficient than VITEK2 in identifying *Enterococcus species*.[37] While our study results indicate that VITEK2 is a better platform for the identification of genus *Enterococcus*, as well as species differentiation.

Another comparison study done by F. Febbraro et al. concluded that bacteria responsible for bacteremia, correctly identified at the 91.4% genus and species level, by MALDI-TOF when compared with VITEK2.[38] Our study showed agreement with results, in which VITEK2 has good identification rate for *Enterococcus spp*.

A similar comparative study report published by Garza-González E et al. concluded that when different methods are used for identification, like MALDI-TOF, VITEK2, and WGS, misidentification is seen among species of *Enterococci*.[39]

Our study shows agreement with this result as VITEK2 shows a concordance rate of 96.7% in the identification of *Enterococcus faecium*, while 92.3% in the identification of

Enterococcus faecalis, when compared against WGS. And MALDI-TOF shows agreement with this result as well. It shows a concordance rate of 100% in identification of *Enterococcus faecium*, while 61.5% in identification of *Enterococcus faecalis*, when compared against WGS.

There is scarcity of literature on *Enterococcus spp*. identification by VITEK2, MALDI-TOF and WGS. And this study is from very few of the literatures that gives data on *Enterococcus spp.* identification, done by all these methods.

With the use of different identification methods, *Enterococcus spp*. identification is inconclusive, and for confirmation, when the WGS method is used, which is the gold standard, but consumes time, and can delay therapeutics. WGS is considered as gold standard for identification of bacteria, therefore, ID results of MALDI-TOF and VITEK2 are compared with % differences in species ID are considered.

WGS data was considered conclusive for species identification, as sequence assembly and bioinformatic analysis, performed using PubMLST software, yielded a unique species match with a confidence score and coverage depth above a predefined threshold. Any isolates that produced ambiguous results were excluded from the final analysis. For the purpose of this study, all 44 isolates subjected to WGS analysis provided a single, definitive species identification, hence there was no need to handle discordant WGS results.

While some cases where misidentifications do not pose a clinical risk, in other instances, like *Enterococci*, there can be a significant clinical impact. It is problematic given that they have different levels of resistance among different species to different antibiotics.[40]

Our study also in an agreement with one such comparison study between VITEK2 and MALDI-TOF with WGS is done by Marathe and Shaikh et al.[41] where VITEK2 in correctly identifying bacteria at species level.

Alternatively, using backup methods such as sequencing can be as effective as long as the issue is known. This is one of the scarce studies done using WGS to compare the identification of clinical bacterial isolates of *Enterococci spp.* from ICU patients. CLSI and EUCAST guidelines are used to decide the MICs and breakpoints. The estimate of intrinsic resistance and break-points is grounded on precise identification of bacteria.

While MALDI-TOF offers the advantage of a rapid turnaround time, our findings demonstrate that VITEK2 is a more precise and reliable platform for species-level identification of *Enterococcus faecium* and *Enterococcus faecalis* when compared to the gold standard WGS method. These results are particularly relevant given the significant clinical implications of accurate species identification for appropriate antimicrobial treatment. Future studies with larger, more diverse isolate collections and formal statistical analyses are needed to confirm these findings.

5. Conclusion

Our results indicate that VITEK2 system is robust & sufficient for identification of *Enterococcus spp. (faecalis & faecium),* when compared with the gold standard method of Whole Genome Sequencing. *Enterococcus faecium* and *faecalis* were major *Enterococcus* species isolated from clinical specimens of BSI.

Identification of other spp. of *Enterococci* needed to be studied. Though MALDI-TOF is proven method of species identification, especially from direct specimens and other organisms like NTM, TB and Fungi etc.

6. Ethical Approval

Parul University Institutional Ethics Committee for Human Research (PU-IECHR). Approval number: PUIECHR/PIMSR/00/081734/5913.

No direct experimentation was done on Humans or Animals.

7. Funding of Source

None.

8. Conflict of Interest

None.

9. Acknowledgement

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