Evaluation of Matrix-assisted laser desorption/ionization Time-of flight Mass spectrometry (MALDI TOF MS) and VITEK 2 in routine microbial identification

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SUMMARY
Background: Microbial Identification was done by phenotypic methods. VITEK-2 and Matrix-assisted laser desorption ionization-time of flight mass spectrometry (MALDI-TOF MS) are now being increasingly used in laboratories. Objectives: To compare and evaluate the usefulness of MALDI-TOF MS and VITEK-2 in routine microbial identification. Methods: The performances of MALDI-TOF MS and VITEK 2 were compared for identifying microorganisms. Results: MALDI-TOF MS and VITEK-2 correctly identified 96 % (96/100) and 97% (97/100) of the isolates upto the genus level. Conclusion: MALDI TOF MS and VITEK -2 gave comparable identification and error rates. The rapid reduction in turnaround time with MALDI TOF is a significant game-changer in the field of clinical microbiology.

Keywords: MALDI TOF MS, VITEK -2, Sequencing
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INTRODUCTION
Microbial identification in laboratories is achieved mainly by observing colony morphology, gram stain and biochemical reactions.¹ A laboratory usually takes 48-72 hrs after receiving the sample to identify the organism.² Vitek 2 System (Biomerieux-Vitek) is one of the automated systems which can identify gram-negative bacteria (GNB) within three hours.³ VITEK 2 can accurately identify 84.7% of the gram-negative bacteria within three hrs as shown by Funke et al.⁴ MALDI-TOF MS technology has a simple, high throughput, and low-cost technique plus a larger database and a more rapid turnaround time (few minutes).⁵ The prime objective was to use two systems to identify 100 isolates.

METHODS
This was a study conducted in Government Medical College, Alapppey from September 2018 to February 2019. Isolates(n=100) such as those belonging to Enterobacteriaceae, non-fermenting GNB, yeasts were used. This study was approved by the Institutional Ethics Committee (Reg.No. ECR/122/ Inst/KL/2013/RR-16) of Government Medical College, Alapppey (Reference Number-EC 38/2018).

VITEK used Gram-negative (GN), Gram-positive (GP) and Yeast cards for identification. An identification rate greater than 90% was considered acceptable. For MALDI-TOF MS, Isolate was smeared on the target slide with a wooden stick and was then smeared with 1 μL VITEK MS-CHCA (α-Cyano-4-hydroxycinnamic acid) and air-dried until the matrix and sample co-crystallized. In the case of yeast, 1.5 μL of 70% formic acid was added to each isolate and dried. Then, 1.5 μL of an α-cyano-4-hydroxycinnamic acid (CHCA) matrix solution was added and dried again. The mass spectra acquired for each sample were compared to the reference spectra. 16S rRNA gene sequencing was used to resolve all genus level discrepancies.

Errors and Identification standards
Concordant - If the isolates were correctly identified at the genus or species level by both systems.

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Major Errors – Discordant results at the genus level or if the results from one method were not definite.

Minor Errors- Discordant results at the species level but not at the genus level.

RESULTS
MALDI-TOF MS and VITEK-2 correctly identified 96% (96/100) and 97% (97/100) of the isolates up to the genus level. The identification rates for MALDI-TOF MS and VITEK-2 up to species level were 92.9% (92/99) and 90.9% (90/99) respectively (One Minor error was not taken for calculation up to species level as Sequencing was not done for this isolate). Among the 100 isolates, results for 93 isolates were in agreement up to genus level by both methods. Results for 87 isolates were in agreement up to species level by both methods (Table 1). So among the 100 isolates, 13 (13%) isolates produced discordant results between the two methods.

Table 1 Matching results by both MALDI TOF and VITEK

| Identification            | Number of Strains n (%) |
|---------------------------|-------------------------|
| Acinetobacter baumannii   | 16 (18.4)               |
| Acinetobacter nosocomialis| 1 (1.1)                 |
| Burkholderia cepacia      | 16 (18.4)               |
| Citrobacter freundii      | 2 (2.3)                 |
| Candida albicans          | 3 (3.4)                 |
| Candida tropicalis        | 7 (8)                   |
| Candida parapsilosis      | 2 (2.3)                 |
| Enterobacter cloacae      | 4 (4.6)                 |
| Escherichia coli          | 14 (16.1)               |
| Klebsiella pneumonia      | 2 (2.3)                 |
| Kodamaea ohmeri           | 1 (1.1)                 |
| Pseudomonas aeruginosa    | 2 (2.3)                 |
| Pseudomonas putida        | 2 (2.3)                 |
| Pseudomonas stutzeri      | 1 (1.1)                 |
| Salmonella group          | 4 (4.6)                 |
| Serratia marcescens       | 1 (1.1)                 |
| Shewanella putrefaciens   | 1 (1.1)                 |
| Staphylococcus haemolyticus| 1 (1.1)                  |
| Staphylococcus saprophyticus| 2 (2.3)                 |
| Stenotrophomonas maltophilia | 3 (3.4)            |
| Trichosporon asahii       | 2 (2.3)                 |

Major Errors
Among the seven isolates which were not in agreement at the genus level, two organisms were identified as Shigella sonnei and Escherichia coli by VITEK and MALDI-TOF MS respectively. Isolates were confirmed as Shigella sonnei by serotyping. These isolates were not processed as 16S rRNA gene sequencing should not be used to differentiate between E. coli and shigella.

So, by excluding these two organisms, there were five major errors (Table 2). These were resolved by sequencing. VITEK and MALDI TOF MS showed 3% and 2% errors respectively.

Table 2 Major Errors

| VITEK                               | MALDI TOF     | Sequencing          |
|-------------------------------------|---------------|---------------------|
| Sphingomonas paucimobilis           | Brevibacillus | Brevibacillus agri   |
| Sphingomonas paucimobilis           | Delfia acidovorans | Delfia taurulatensis |
| Cupriavidus pauculus                | Acinetobacter baumannii | Acinetobacter baumannii |
| Enterobacter cloacae                | Leclercia adecarboxylan | Enterobacter cloacae |
| Candida ciferii                     | Not identified | C.allociferii       |

Minor Errors
There were six isolates in our study for which there was agreement up to the genus level but there was discordance at species level. Among the three Candida species, two were identified as C. parapsilosis and C. orthopsilosis by VITEK and MALDI-TOF MS respectively. This is due to the fact that Candida orthopsilosis is not present in the database of VITEK. Among the three bacterial isolates, two were identified as Elizabethkingia anopheles and Citrobacter werkmanii by MALDI TOF MS and the corresponding VITEK identifications were E. meningoseptica and C. freundii respectively. This is due to the fact that E. anopheles and C. werkmanii are not present in the database of VITEK. So by excluding these organisms there were two minor errors, one of which included wrong identification of C. albicans as C. lusitaniae by VITEK-2 which was resolved by sequencing. The other minor error was the identification of an organism as Acinetobacter junii and Acinetobacter baumanii by VITEK-2 and MALDI-TOF respectively. 16S rRNA gene sequencing was used to resolve only one of the minor errors due to financial limitations.

DISCUSSION
VITEK-2 showed identification rates of 97% and 90.9% at genus and species level whereas MALDI TOF MS showed identification rates of 96% and 92.9% respectively at genus and species level. Guo et al reported identification rates of 99.60% and 93.37% up to genus and species level by MALDI TOF. 7 Our findings were comparable to study by Van Veen et al., who achieved identification rates of 97.1% and 92% up to genus and species level.8 This difference is due to the different choices of strains.
One major issue which was seen during the usage of MALDI TOF was the inability to differentiate between E. coli and shigella which has been previously documented. Another method for differentiating E. coli from Shigella was by using MALDI-TOF MS-based assay using ClinProTools software.

CONCLUSION
The results of this study showed that MALDI TOF MS and VITEK -2 gave comparable identification and error rates. However, the rapid reduction in turnaround time with MALDI TOF is a significant game changer in the field of clinical microbiology.

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REFERENCES
1. Carroll KC, Weinstein MP. Manual and automated systems for detection and identification of microorganisms. In: Murray PR, Baron EJ, Jorgensen JH, et al. eds. Manual of clinical microbiology. 9th ed. Washington, DC: ASM Press, 2007:192-217
2. Dellinger, R.P., Levy, M.M., Carlet, J.M. et al. Surviving Sepsis Campaign: International guidelines for management of severe sepsis and septic shock: 2008. Intensive Care Med 34, 17–60 (2008).
3. Joyanes P, del Carmen Conejo M, Martinez-Martinez L, Perea EJ. Evaluation of the VITEK 2 system for the identification and susceptibility testing of three species of nonfermenting gram-negative rods frequently isolated from clinical samples. J Clin Microbiol. 2001 Sep;39(9):3247-53.
4. Funke G, Monnet D, deBernardis C, von Graevenitz A, Freney J. Evaluation of the VITEK 2 system for rapid identification of medically relevant gram-negative rods. J Clin Microbiol. 1998 Jul;36(7):1948-52.
5. Martiny D, Busson L, Wybo I, El Haj RA, Dediste A, Vandenberg O. Comparison of the Microflex LT and Vitek MS systems for routine identification of bacteria by matrix-assisted laser desorption ionization-time of flight mass spectrometry. J Clin Microbiol. 2012 Apr;50(4):1313-25.
6. Christensen H, Nordentoft S, Olsen JE. Phylogenetic relationships of Salmonella based on rRNA sequences. Int J Syst Bacteriol. 1998 Apr;48 Pt 2:605-10.
7. Guo L, Ye L, Zhao Q, Ma Y, Yang J, Luo Y. Comparative study of MALDI-TOF MS and VITEK 2 in bacteria identification. J Thorac Dis. 2014 May;6(5):534-8.
8. van Veen SQ, Claas EC, Kuijper EJ. High-throughput identification of bacteria and yeast by matrix-assisted laser desorption ionization-time of flight mass spectrometry in conventional medical microbiology laboratories. J Clin Microbiol. 2010 Mar;48(3):900-7.
9. Deng J, Fu L, Wang R, et al. Comparison of MALDI-TOF MS, gene sequencing and the Vitek 2 for identification of seventy-three clinical isolates of enteropathogens. J Thorac Dis. 2014;6(5):539-544.
10. Fukushima M, Kakinuma K, Kawaguchi R. Phylogenetic analysis of Salmonella, Shigella, and Escherichia coli strains on the basis of the gyrB gene sequence. J Clin Microbiol. 2002 Aug;40(8):2779-85.
11. Khot PD, Fisher MA. Novel approach for differentiating Shigella species and Escherichia coli by matrix-assisted laser desorption ionization-time of flight mass spectrometry. J Clin Microbiol. 2013 Nov;51(11):3711-6.