Iranian Journal of Microbiology
Volume 18 Number 4 (August 2026) 473-481
DOI: http://doi.org/10.18502/ijm.v18i4.22190

ORIGINAL ARTICLE

Matrix assisted laser desorption/ ionization time of flight mass spectrometry (MALDI-TOF MS) for identification of anaerobic and microaerophilic bacteria from clinical specimens, in comparison with conventional techniques

Beena Antony[1*], Sherin Justin[1], Shruthi Bagambila[2], Jane Grace D'Souza[2], Rhea Nicola Pinto[2]

[1]Department of Microbiology, Fr. Muller Medical College, Karnataka, India
[2]Fr. Muller Research Center, Mangalore, Karnataka, India

Received: November 2025**, Accepted:** June 2026

ABSTRACT

Background and Objectives: MALDI-TOF MS offers a unique combination of flexibility, accuracy, automation and has found benefit in many fields of biological research. Here we investigated MALDI-TOF analysis for the identification of anaerobic and microaerophilic bacteria isolated from clinical specimen and also compared the results with conventional biochemical characterization.

Materials and Methods: The present study was conducted for a duration of 2 years and had obtained Ethical clearance. Bacteria obtained from 1514 specimens were subjected to MALDI-TOF in comparison with conventional techniques. MALDI-TOF was done by Extended Direct Transfer technique after a preparatory protein extraction.

Results: Of the 957 isolates of anaerobes and 214 microaerophilic organisms, 552 anaerobes and 154 microaerophilic isolates were identified up to species level giving concordant results by conventional techniques. Rest of the strains could be identified only up to genus level by biochemical characterization. MALDI-TOF MS was able to identify more isolates to the species level compared with conventional methods, including several new genera and species including *Clostridia*, *Peptostreptococcus* and *Lactobacilli*.

Conclusion: Newly recognized and taxonomically rearranged genera, frequently isolated anaerobic and microaerophilic species as well as closely related species could be identified accurately by employing MALDI-TOF MS. Implementation of this technique for identification will shorten the Turn Around Time, reduce the cost in the Microbiology laboratory and improve the patient care with better clinical outcome.

Keywords: Matrix-assisted laser desorption/ionization (MALDI) time-of-flight (TOF) mass spectrometry (MS); Anaerobes; Microaerophilic bacteria; Biochemical characterization

INTRODUCTION

Matrix-assisted laser desorption/ionization (MALDI) time-of-flight (TOF) mass spectrometry (MS) has found application in various fields of research and diagnosis in the last decade. As the name indicates, a matrix (α- cyano 4 hydroxy cinnamic acid) assists in the desorption and ionization of microbial analytes through the energy of a laser. Franz Hillenkamp & Michael Karas reported soft desorption

*Corresponding author: Beena Antony, Ph.D, Department of Microbiology, Fr. Muller Medical College, Karnataka, India. Tel: +9448024684 Email: beenafmmc@gmail.com; beenafmmc@fathermuller.in

ionization using an organic compound matrix and coined the term MALDI in 1980s (1). The unique combination of flexibility, accuracy, automated analysis is of benefit in many fields of biological research. To enumerate, the applications of MALDI-TOF include rapid and accurate identification of all aerobic and anaerobic bacteria, yeasts, moulds and *Mycobacteria* upto species level. Recent developments in the MALDI-TOF MS, made it possible for the rapid identification of bacteria directly from positive body fluid cultures as well as the selective detection of β-lactamases like carbapenemase and cephalosporinase. In terms of identification of anaerobic bacteria, MALDI-TOF MS has replaced 16SrRNA gene sequencing and gas liquid chromatography to become the method of choice (2).

However, this technique has not gained widespread use in our country due to very high expenses incurred as the initial cost of the instrument or the reluctance to leave the conventional techniques. A variety of models of MALDI-TOF machines are available in the market such as Bruker Daltonics (Germany), Vitek MS (BioMerieux), Axima Assurance MALDI-TOF MS, Shimadzu(LCMS-9030Q (Japan), Sigma- Aldrich- (Millipore,Sigma) including the latest Autof MS-1000, MALDI-TOF (HI media Pvt.Ltd.) Among these, the most widely accepted equipments in our country are Bruker Daltonics, Germany (Desk top Model) & Vitek MS (BioMerieux -Large Floor Model).

The Principle of MALDI TOF-MS is based on the detection of highly abundant ribosomal proteins in a mass range between 2000- 20,000 daltons. The matrix isolates the analyzed molecules and protects them from fragmentation.The laser being shot by the multiple 'laser shots', the microbial molecules are desorbed. Matrix, absorbs the laser light and vaporizes along with the sample, converts them to an ionized state. This ionized state gets charged and travel through a tube under vacuum (Time of Flight mass analyzer), based on the weight. A mass spectrum is generated with peaks specific to genera, species and strains. The organisms are identified by comparing the typical score value generated for each one, in comparison with the standard reference spectra. The spectra of each organism is the proteomic signature to identify them and it will be analyzed by the Biotyper software.

Several studies from the western countries had highlighted the efficacy of this technique for the diagnosis of anaerobic bacteria such as speciation of *Bacteroides fragilis* group (3, 4), identification of phylogenetically heterogeneous group of anaerobic gram positive cocci (5) identification of clostridia species (6) as well as detection of oral anaerobic bacteria from subgingival biofilm (7). Another study employed MALDI-TOF MS for the identification of HACEK group of bacteria including *Aggregatibacter actinomycetemcomitans*, the causative agent of periodontitis (8).

However, there are inadequate reports from the Indian Subcontinent regarding MALDI-TOF MS analysis on anaerobes and microaerophilic bacteria. Hence the present study aims to investigate the identification of anaerobic and microaerophilic bacterial isolates that recovered from clinical specimen, employing MALDI-TOF MS analysis and to compare the results with conventional biochemical characterization.

MATERIALS AND METHODS

The present study was carried out for a duration of 2 years, starting from January 2023 to December 2024. Ethical clearance was obtained. 957 isolates of clinically relevant non-duplicate anaerobes and 214 microaerophilic bacteria were analysed by MALDI-TOF MS and the results were compared with conventional identification.

The details of the system what we used are as follows:- Bruker Daltonics GmbH& Co, KG, Germany. Instrument model is Microflex LT /SH MALDI TOF system with MBT compass Software- MBT Compass Database which covers 4320 species of Bacteria & yeasts as well as 222 filamentous fungi.

The isolates obtained from a variety of specimens inoculated in Robertsons Cooked Meat medium or direct specimen transported without delay, that were sent routinely for anaerobic culture or for research projects were included in the study. Culture media employed for the anaerobes were Laked Blood Agar, Supplemented Brucella BA and Neomycin BA. Selective media such as Bacteroides Bile Esculin Agar for *Bacteroides fragilis* group, Cycloserin Cefoxitin Fructose agar for *Clostridioides difficile*, Rogosa SL agar for *Lactobacilli*, Bifidobacterium chrome agar for *Bifidobacteria* were included depending on the specimen. Anaerobic incubation was carried out using BD Gaspak jar or Don Whitley Anaerobic work station for 48-72 hours. If the growth was negative,

incubation was extended for up to 7 days. For the isolation of microaerophilic bacteria, Blood agar and Chocolate agar were employed whereas for the isolation of *Aggregatibacter actinomycetemcomitans* from periodontal specimen, Tryptic Soy Bacitracin Vancomycin (TSBV) agar was included. All these media were incubated in Carbon dioxide incubator. Associated aerobic bacteria and fungi were also detected from these specimens by inoculating in respective media such as Blood agar, MacConkeys agar (aerobes) and Sabouraud's Dextrose agar (fungi).

For the identification of the isolates, biochemical tests including oxidase, catalase, carbohydrate fermentation, arginine hydrolysis and differentiation by special potency discs were done according to the standard protocols (9-11).

The procedure for MALDI-TOF MS was performed by Extended Direct Transfer technique after a preparatory protein extraction. In brief, a pure and fresh colony of microbe was spot inoculated on target plate and applied with 1 µl formic acid. 1 µl commercially prepared matrix solution (α- cyano 4 hydroxy cinnamic acid) solution was added on to this spot. After drying, samples were submitted to multiple laser shots. The matrix absorbs laser energy, facilitating desorption and ionization of microbial proteins. The spectra generated from bacterial suspension was analyzed by the Biotyper software. A score of ≥ 2.0 represented species level, 1.700- 1.999 genus level and ≤ 1.7, suggested that No identification was possible. The characteristic mass spectra produced by various anaerobic isolates are shown in Fig. 1.

RESULTS

957 isolates of anaerobes and 214 microaerophilic bacteria were identified by MALDI-TOF in comparison with conventional techniques, which were obtained from 1514 specimens. Details of the various specimens are shown in Table 1.

Microaerophilic bacteria. 154 microaerophilic isolates (71.3%) were identified up to species level by conventional techniques as well as by MALDI-TOF MS, while 199 (92.13%) microaerophilic bacteria could be identified up to species level by MALDI-TOF MS. Details of microaerophilic organisms identified from these specimen by MALDI-TOF MS is summarized in Table 2.

Anaerobes. 552 (57.9%) of anaerobes were identified up to species level giving concordant results by conventional techniques using biochemical characterization and rest of the strains could be identified only up to genus level according to the standard protocols (7, 8). Totally, 909 (94.98%) anaerobes could be identified up to species level by MALDI-TOF in this study. Employing this technique, many new strains were identified up to species level, especially species of *Clostridia*, new genera proposed from *Peptostreptococcus*, *Lactobacillus* as well as numerous unusual isolates. Details of anaerobes identified in this study is given in Table 3. However, Porphyromonas- Prevotella species, new genera of *Lactobacillus* & Peptostreptococci, recently discovered genera including *Terrisporobacter*, *Moryella*, *Megasphera*, *Megamonas*, *Mitsuokella Catenibacterium*, *Collinsella*, *Solobacterium*, *Mobiluncus* could not be identified by biochemical characterization.

Score value analysis. Of the 957 isolates of anaerobes, 796 (83.18%) represented with high-confidence score value of ≥ 2.0 and 161 with low-confidence values (1.777-1.999). Those showing score below 2.0 were sub cultured and the procedure was repeated with fresh colonies and was assigned to species level except 48. A total of 43 isolates showing a score below 1.7 -(No identification Possible) were not included here, thus making it a total of 909 anaerobes (94.98%). A comparative evaluation of identification of the isolates by biochemical characterization and MALDI TOF-MS analysis is given in the Table 4.

To find out other associated pathogens in the specimens, they were subjected to aerobic and fungal culture according to the standard protocols. A total of 725 aerobic bacteria were obtained in the study which included –Gram Positive cocci 294 (*Staphylococcus aureus* -112, coagulase negative *Staphylococci* -46, Group A *Streptococci* – 36, Group B *Streptococci*-53, *Enterococci*- 47) Enterobacteriaceae 269 (*E. coli*-108, *Klebsiella* species 63, *Citrobacter* species-27, *Proteus* species- 18 and Non Typhoidal *Salmonellae*-53), non-fermenters -115 (*Pseudomonas aeruginosa*-48, *Burkholderia pseudomallei*-4, *Acinetobacter baumannii*-51 and *Stenotrophomonas maltophilia* 12) and *Corynebacterium* species-45. Two unusual isolates included one strain each of *Cupriavidus necator* and *Tsukumurella tyrosinosolvens*. A total of 95 Fungi were also isolated from these specimens consisting of *Candida* species-78, *Rhizopus*-11 and *Aspergillus* spe-

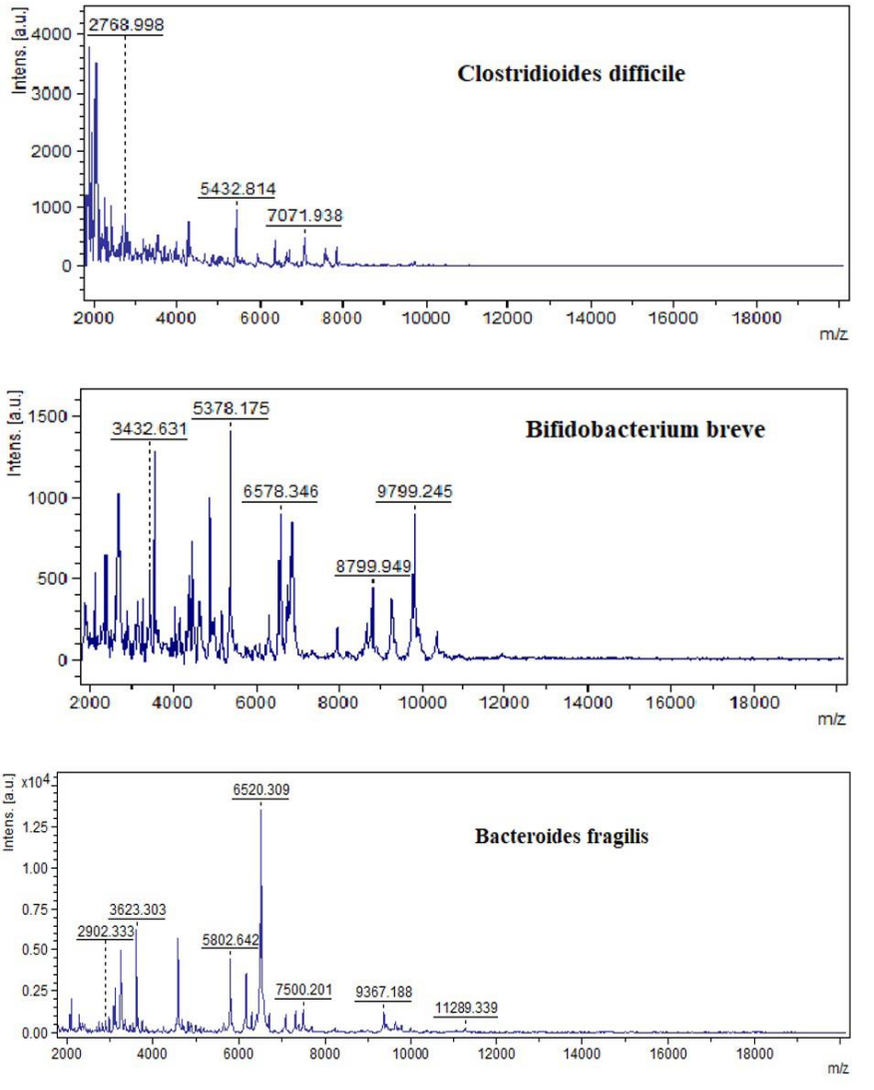

Fig. 1. Mass spectra of various anaerobes obtained in MALDI Biotyper software program (Bruker Daltonics)

Table 1. Details of specimen included in the study

No.	Specimen	No	%
1.	Pus from deep abscess	492	32.5
2	High vaginal Swabs	279	18.43
3.	Blood	73	4.82
4	Body fluids	187	12.35
5.	Faeces (For *C. difficile* & *Bifidobacteria*)	171	11.29
6.	Sub gingival plaques, Paper points, pus from dental abscess from Orodental infections	185	12.22
7.	Respiratory secretions	47	3.10
8.	Broncho alveolar lavage	80	5.28
	Total	1514	99.99

cies-6. These results indicates the nature of polymicrobial infections caused by anaerobes. As reported by other investigators, a combination of *B. fragilis* and *E. coli* was commonly found in this study as well. From vaginal secretions and pus from intra abdominal abscesses, 112 cases of such combinations were observed.

DISCUSSION

As the isolation and identification of anaerobes by phenotypic and DNA based molecular methods at a species level is time-consuming and laborious, many investigators recommended MALDI-TOF MS for the rapid identification of anaerobic bacte-

Table 2. Microaerophilic bacteria identified by MALDI TOF

Sl No	Genus	No	Species
1.	*Aggregatibacter*	35	*Aggregatibacter actinomycetemcomitans*
2.	*Gardnerella*	18	*Gardnerella vaginalis*
3.	Microaerophilic *Streptococci*	142	*S. salivarius* (31), *S. anginosus* (17) *S. mitis* (34), *S. oralis* (25) *S. parasanguinus* (16), *S. vestibularis* (5), *S. mutans* (7), *S. constellatus* (4), *S. downie* (1) *S. gordonii* (2)
4.	*Brucella*	3	*B. melitensis*
5.	*Granulicatella*	6	*Granulicatella adiacens*
6.	*Helicobacter*	4	*Helicobacter pylori*
7.	*Pedicoccus*	3	*Pedicoccus acidolactici*
8.	*Eikinella*	3	*Eikinella corrodens*
	Total	214	

Table 3. Details of anaerobic bacteria identified by MALDI TOF

Sl.No	Genus	No.	Species
	GNB (Non sporing)		
1.	*B. fragilis* group	186	*B. fragilis*- 94, *B. thetaiotaomicron* -47, *B. ovatus* – 22, *B. pyogenes* 11, *B. uniformis* – 10, *B. caccae*- 1, *B. xylanisolvens*-1
2.	*Phocaeicola*	5	*Phocaeicola vulgatus*
3.	*Parabacteroides*	11	*P. johnsonii* -5, *P. distasonis* -6
4.	*Porphyromonas* species	43	*P. gingivalis* -10, *P. asaccarolytica* -18 *Porphyromonas* species -15
5.	*Prevotella* sps.	84	*P. buccae* –25, *P. buccalis* -1, *P. oralis* -3 *P. melaninogenica* -12, *P. denticola*-7 *P. bivia* -22, *P. disiens*-3, *P. salivae* 1, *P. copri* -5, *P. histicola*-1, *P. jejuni* -1 *P. baroniae*-2, *P. pallens* 1
6.	*Fusobacterium*	44	*F. varium* 15, *F. nucleatum* -21 *F. canifelinum* -1, *Fusobacterium* species -7
7.	*Megamonas*	2	*Megamonas hypermegale*
8.	*Mitsuokella*	2	*Mitsuokella jalaludini*
	GPB Non-sporing		
9.	*Lactobacillus*	88	*L. acidophilus*-33, *L. gasseri* -13, *L. paracasei* -4, *L. delbruki* -10, *L. crispatus* -9, *L. johnsonii* -4 *L. gasseri* -10, *L. vaginalis* 2, *L. lactis* -1, *L. oris* -1, *L. sharpeae* 1
10.	*Limosilactobacillus*	11	*L. mucosae*-1, *L fermentum*-9 , *L. reuteri*-1
11	*Lactiplantibacillus*	12	*L. plantarum* 11, *L. pentosus*, -1
12	*Ligilactobacillus*	14	*L. ruminus* -8, *L. salivarius* 6
13	*Lacticaseibacillus*	5	*L. rhamnosus* -5
14.	*Levilactobacillus*	1	*L. brevis*
15.	*Cutibacterium*	40	*C. acnes* 36, *C. lymphophilum*-2, *C. avidum* 2
16.	*Eggerthella*	3	*Eggerthella catenaformis*
17.	*Bifidobacterium*	64	*B. bifidum*-10, *B. dentium*-1, *B. adolescentis*-16, *B. breve* 8, *B. longum* 17, *B. catenultum* 10 *B. pseudocatenulatum* -1, *B. ruminantium* 1

Table 3. Continuing...

18.	*Actinomyces*	14	*A. odontolyticus*-4, *A. naeslundii*-3 *A. europaeus* -2, *A. oris* – 1 *A. urogenitalis*-4
19.	*Moryella*	2	*Moryella indolegenes*
20 .	*Catenibacterium*	5	*Catenibacterium mitsuokai*
21.	*Solobacterium*	3	*Solobacterium moorei*
22.	*Alloscardovia*	1	*Alloscardovia omnicolens*
23.	*Mobiluncus*	9	*Mobiluncus* species
	GPC		
24.	*Peptostreptococcus*	72	*P. anaerobius* -32, *P. stomatis*-3 *P. asaccharolyticus* 20 *Peptostreptococcus* species -17
25.	*Peptoniphilus*	16	*P. harei* -4, *P. anaerobius* -6 *P. gorbachii* -2, *P. indolicus* 3, *P. grossensis*-1
26.	*Anaerococcus*	9	*A. hydrogenalis* -4, *A. vaginalis* -1 *A. octavius* -3, *A. murdochii* -1
27.	*Finegoldia*	50	*Finegoldia magna*
28.	*Collinsella*	2	*Collinsella aerofaciens*
	GNC		
29.	*Veillonella*	62	*V. parvula* 45, *V. atypica* 14, *V. dispar* 3
30.	*Acidaminococcus*	2	*Acidaminococcus intestini*
31.	*Megasphera*	1	*Megasphera elsdeni*
	Spore forming GPB		
32.	*Clostridium* species	66	*C. butyricum* -2, *C. perfringens* -35, *C. tertium* 7 *C. paraputrificum*-2, *C. sporogenes*-13, *C. sardiniense*-1, *C. ramosum* 1, *C. sordelli* -3 *C. innocum*1, *C. barati* 1
33.	*Clostridioides*	15	*Clostridioides difficile*-
34.	*Paraclostridioides*	11	*P. bifermentans*
35.	*Terrisporobacter*	2	*Terrisporobacter glycolicus*
	Total	957	

Table 4. Comparison of identification by MALDI TOF analysis and conventional techniques

Organisms	**Maldi ToF**	**Conventional**
Anaerobes (957)	909 (94.98%)	552 (57.9%)
Microaerophilic (214)	197 (92.06 %)	154 (71.96)
Time required for ident. of anaerobes after colonies appear	30 minutes	3-5 days
No. of sps. & genera in (214 microaeroph.)	8 genera and 17 species	5 genera and 8 species
No. of sps. & genera in (957 anaerobes)	35 genera and 103 species	13 genera and 47 species

ria. According to Nagy, newly recognized and taxonomically rearranged genera, frequently isolated anaerobic species as well as phylogenetically related species could be identified accurately by employing this rapid technique (3, 4). Keeping up with frequent taxonomic revisions and identifying isolates using conventional methods is challenging. For example, in the present study, *Moryella, Megasphera, Megamonas, Mitsuokella, Catonibacterium, Collinsella, Alloscardovia* were isolated as new genera whereas Terrisporobacter, *Phocaeicola, Parabacteroides, Clostridioides* as the revised nomenclature of anaerobes and *Solobacterium moorie, Acidaminococcus* as frequently isolated anaerobes. Among the aerobic

& microaerophilic bacteria *Granulicatella, Cupriavidus, Tsukumurella* – which are recently recognized genera, also could be identified accurately in our study, employing Bruker Maldi Biotyper.

A few specimens included in the study may be controversial such as Broncho Alveolar Lavage, respiratory secretions, faeces which are usually included in the rejection criteria for anaerobes. These specimens are likely to yield many anaerobes, but may be contaminated with normal flora. They can be ideal to understand current distribution and nomenclature of the isolates by MALDI-TOF MS. Faeces is the appropriate specimen for *Clostridioides difficile*, probiotic strains such as Bifidobacteria , Lactobacilli. These two genera are not well established pathogens, however there are reports regarding their pathogenicity in rare conditions such as septic prosthetic joint arthritis, Bacteremia, Meningitis, endocarditis and other systemic infections (12-16). We isolated these two genera from faeces to demonstrate the antagonistic action of probiotics against gastrointestinal & vaginal pathogens in another study. Inclusion of these specimens as well as the bacterial isolates was done to highlight the application of MALDI-TOF MS for the identification of these strains to species level.

The introduction of MALDI-TOF MS at our institute in 2022 greatly enhanced the identification of diverse anaerobic and microaerophilic bacteria. In the present study of 2 years duration, among the 214 microaerophilic bacteria, 8 genera and 17 species could be identified by MALDI-TOF MS, while only 5 genera and 8 species were identified by biochemical characterization. In the same way, of the 957 anaerobes, 35 genera and 103 species could be identified by MALDI-TOF whereas only 13 genera and 47 species by conventional techniques and biochemical characterization as shown in Table 4. Prior to the implementation of MALDI TOF-MS in our institute, isolates were identified based on gram stain, colony morphology, biochemical reactions including carbohydrate fermentation, aesculin hydrolysis, Indole, nitrate reduction, H2S production and sensitivity to special potency discs (9, 10). This required tedious efforts such as preparation of various culture media, prolonged incubation, expensive culture techniques as well as expertise in the field. Biochemical characterization of anaerobes required not less than 5days and microaerophilic bacteria not less than 3 days after the colonies appear on culture media. MALDI-TOF MS analysis could identify the isolates within 30 minutes once the pure colonies appear on the culture plates. MALDI-TOF showed superior performance in identifying all genera compared to the conventional biochemical methods, only disadvantage being initial high expenses of the instrument.

Alcala et.al recommended MALDI-TOF MS as a reliable technique for the identification of anaerobic isolates, allowing clinicians to streamline the most appropriate antibiotic therapy and manage patients accordingly (17). In a 4 year study they could identify overall 95.7% of the isolates to the species level. 68.5% of the total were reliably identified with high-confidence score values (≥2.0) and 95.0% with low-confidence values (score value ≥1.7). Only 2.1% of the isolates could not be reliably identified and required molecular methods for a final identification. In the present study, out of 957 isolates of Anaerobes, 796 (83.18%) represented with high-confidence score value of ≥ 2.0, 161 with low-confidence values (1.777-1.999). Forty-three anaerobic isolates could not be identified, possibly due to suboptimal culture conditions or mixed bacterial populations. In a multi-center study, the performance of the VITEK MS system in relation to 16S rRNA gene sequencing for the identification of clinical *Prevotella* isolates was assessed by Toprak et.al. Of the 508 isolates included in the study, 83.1% were identified to the species level, 90.4% to the genus level and 9.6% of the isolates were not identified correctly (18).

MALDI-TOF MS is an excellent tool for the identification of phylogenetically heterogeneous groups of micro-organisms such as anaerobic gram positive cocci as suggested by Veloo et. al (5). Major taxonomic revision happened in the genera of Peptostreptococcus and *Lactobacillus.* In the recent taxonomical revision, genus, *Peptostreptococcus* was reclassified including genera such as *Peptoniphilus, Anaerococcus, Finegoldia, Micromonas, Shleiferella*. In the same way, genus Lactobacillus was assigned recently to new genera such as *Limosilactobacillus, Lactiplantibacillus, Ligilactobacillus, Lacticaseibacillus, Levilactobacillus* based on the source of isolation. Phenotypic identification by conventional technique is a time consuming and cumbersome procedure in these cases. However, 130 strains of Lactobacilli and 147 strains of gram positive cocci could be assigned till species level according to the revised taxonomy by MALDI-TOF MS in our study as depicted in Table 3.

Li et.al evaluated the effectiveness of the ANC card

and MALDI-TOF MS techniques for identification of clinical anaerobic isolates employing 16S rRNA gene sequencing as reference method (19). Based on the results, they recommended Vitek MS is easier, rapid and more economical for each test, providing the updation of the databases currently available (19). In another study, Coltella et.al stated the superior performance of MALDI-TOF MS by analyzing 484 anaerobic bacteria (20). In their findings, the most prevalent genus was Clostridium (76.85 %), with 70% *C. difficile* isolates. The concordance of sensitivity and specificity for *C. difficile* was 94.08% and 100% respectively. For the other anaerobes, the sensitivity and specificity were 94.07% and 81.82% with a concordance of 93.15%. They observed a low performance for *Propionibacterium* (*Cutibacterium*) acnes and *Fusobacterium nucleatum* (20). In our findings, 66 isolates of *Clostridia* were obtained, predominated by *C. perfringens* followed by 15 isolates of *Clostridioides difficile.*

In a retrospective study by Watanabe et al., employing MALDI-TOF MS, the current trends of anaerobic Blood Stream Infections (BSI) was analysed which reflected the predominance of *Bacteroides* species (21). Regarding the clinical severity, *Clostridium* species affected the mortality rate of patients with anaerobic BSIs. They also observed that both Bacteroides and Clostridium often caused trans-abdominal and hepatobiliary focal infection. According to them, these findings may assist physicians and microbiologists in the diagnosis and treatment of anaerobic BSIs. (21)

According to a recent development, rapid identification of isolates from positive blood cultures and detection of emerging Carbapenem resistance in *Bacteroides fragilis* associated with cfiA-encoded class B metallo-beta-lactamases are possible by MALDI TOF as demonstrated by Johansson et al. (22) In the near future, typing of anaerobic bacteria on a subspecies level, determination of antibiotic resistance and direct identification of blood culture isolates will definitely revolutionize the amazing world of anaerobes (3).

In short, results of the present study and the experience of other investigators have revealed that by employing MALDI TOF, many newly recognized and taxonomically rearranged organisms could be identified accurately up to species level. Employing this technique, it will be possible to avoid automated and time- consuming microbial identification by biochemical characterization. If feasible implementation of MALDI TOF MS for identification will certainly shorten the Turn Around Time, reduce the cost in the Microbiology laboratory and improve the patient care, resulting in better clinical outcome.

CONCLUSION

MALDI-TOF MS analysis was employed for the identification of anaerobic and microaerophilic bacteria from clinical specimens in comparison with conventional techniques. Most of the strains could be identified accurately by employing MALDI TOF MS within half an hour, after the colonies appeared on culture media. Species identification of anaerobes and unusual microaerophilic bacteria is a tedious task for the Microbiologists which will be taken care by this instrument. Highly specific and cost effective MALDI-TOF MS represents a robust and efficient platform for the rapid identification of these bacteria in clinical laboratories. Adoption of this technique will also improve patient care with better clinical outcomes.

REFERENCES

1. Li D, Yi J, Han G, Qiao L. MALDI-TOF Mass Spectrometry in Clinical Analysis and Research. *ACS Meas Sci Au* 2022; 2: 385-404.
2. Patel R. MALDI-TOF MS for the diagnosis of Infectious Diseases. *Clin Chem* 2015; 61: 100-111.
3. Nagy E. Matrix-Assisted Laser Desorption/Ionization Time-Of-Flight Mass Spectrometry: a New Possibility for the Identification and Typing of Anaerobic Bacteria. *Future Microbiol* 2014; 9: 217-233.
4. Nagy E, Maier T, Urban E, Terhes G, Kostrzewa M. Species identification of clinical isolates of Bacteroides by matrix-assisted laser-desorption/ionization time-of-flight mass spectrometry. *Clin Microbiol Infect* 2009; 15: 796-802.
5. Veloo ACM, Erhardb M, Welkerb IM, Wellinga 1GW, Degenera JE. Identification of Gram-positive anaerobic cocci by MALDI-TOF mass spectrometry. *Syst Appl Microbiol* 2011; 34: 58-62.
6. Grosse-Herrenthey A, Maier T, Gessler F, Schaumann R, Böhnel H, Kostrzewa M, et al. Challenging the problem of clostridial identification with matrix-assisted laser desorption and ionization-time-of-flight mass spectrometry (MALDI-TOF MS). *Anaerobe* 2008; 14:

242-249.
7. Stingu CS, Rodloff AC, Jentsch H, Schaumann R, Eschrich K. Rapid identification of oral anaerobic bacteria cultivated from subgingival biofilm by MALDI-TOF-MS. *Oral Microbiol Immunol* 2008; 23: 372-376.
8. Couturier MR, Mehinovic E, Croft AC, Fisher MA. Identification of HACEK Clinical Isolates by Matrix-Assisted LaserDesorption Ionization–Time of Flight Mass Spectrometry. *J Clin Microbiol* 2011; 49: 1104-1106.
9. Willis AT (2014). Anaerobic Bacteriology: Clinical and Laboratory Practice. 3rd ed. Butterworth-Heinemann. Oxford, UK.
10. Jousimies-Somer HR, Summanen P, Citron DM, Baron EJ, Wexler HM, Finegold SM (2002). Wadsworth Anaerobic Bacteriology Manual. 6th ed. Star Publishing. Belmont, CA.
11. Procop GW, Church DL, Hall DS, Janda WM, Koneman EW, Schreckenberger PC, Woods GL (2017). Koneman's Color Atlas & Textbook of Diagnostic Microbiology. 7th ed. Wolters Kluwer Health. Philadelphia, PA.
12. Bush LM, De Almeida KN, Martin G, Perez MT. Probiotic associated Bifidobacterium septic prosthetic joint arthritis. *Infect Dis Clin Pract* 2014; 22: e39-e41.
13. Zhang W, Jia Q, Han M, Zhang X, Guo L, Sun S, et al. Bifidobacteria in disease: from head to toe. *Folia Microbiol (Praha)* 2024; 69: 1-15.
14. Esaiassen E, Hjerde E, Cavanagh JP, Simonsen GS, Klingenberg C; Norwegian Study Group on Invasive Bifidobacterial Infections. Bifidobacterium Bacteremia: Clinical Characteristics and a Genomic Approach To Assess Pathogenicity. *J Clin Microbiol* 2017; 55: 2234-2248.
15. Rossi F, Amadoro C, Colavita G. Members of the *Lactobacillus* Genus Complex (LGC) as Opportunistic Pathogens: A Review. *Microorganisms* 2019; 7: 126.
16. Cannon JP, Lee TA, Bolanos JT, Danziger LH. Pathogenic relevance of *Lactobacillus*: A retrospective review of over 200 cases. *Eur J Clin Microbiol Infect Dis* 2005; 24: 31-40.
17. Alcala L, Marín M, Ruiz A, Quiroga L, Zamora-Cintas M, Fernández-Chico MA, et al. Identifying Anaerobic Bacteria Using MALDI-TOF Mass Spectrometry: A Four-Year Experience. *Front Cell Infect Microbiol* 2021; 11: 521014.
18. Ulger Toprak N, Alida C M V, Urban E, Wybo I, Justesen US, Jean-Pierre H, et al. Performance of mass spectrometric identification of clinical Prevotella species using the VITEK MS system: A prospective multi-center study. *Anaerobe* 2018; 54: 205-209.
19. Li Y, Bing Gu, Liu G, Xia W, Fan K, Mei Y, et al. MALDI-TOF MS versus VITEK 2 ANC card for identification of anaerobic bacteria. *J Thorac Dis* 2014; 6: 517-523.
20. Coltella L, Mancinelli L, Onori M, Lucignano B, Menichella D, Sorge R, et al. Advancement in the routine identification of anaerobic bacteria by MALDI-TOF mass spectrometry. *Eur J Clin Microbiol Infect Dis* 2013; 32: 1183-1192.
21. Watanabe T, Hara Y, Yoshimi Y, Yokoyama kokuryo W, Fujita Y, Yokoe M, et al. Application of MALDI-TOF MS to assess clinical characteristics, risk factors, and outcomes associated with anaerobic bloodstream infection: a retrospective observational study. *Ann Clin Microbiol Antimicrob* 2021; 20: 42.
22. Johansson A, Nagy E, Sóki J. Instant screening and verification of carbapenemase activity in Bacteroides fragilis in positive blood culture, using matrix-assisted laser desorption ionization--time of flight mass spectrometry. *J Med Microbiol* 2014; 63: 1105-1110.