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ORIGINAL ARTICLE

Comparison of the Autof MS1000 with the Vitek MS in the Identification of Bacterial and Yeast Isolates

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SUMMARY

Background: **Matrix-assisted laser desorption-ionization-time of flight mass spectrometry (MALDI-TOF) has been widely used in clinical microbiology laboratories as a rapid and reliable tool for pathogen identification. The aim of this study was to evaluate the diagnostic performance of the newly-developed Autof MS1000 in comparison with the Vitek MS.**
Methods: **A total of 578 clinical isolates consisting of 136 Enterobacterales, 76 non-fermenting Gram-negative bacilli, 17 other Gram-negative bacilli, 240 Gram-positive cocci, 30 Gram-positive bacilli, 52 anaerobic bacteria, and 27 yeasts were tested simultaneously by the two systems. The direct smear method was performed for bacteria and the formic acid extraction method for yeasts. Discrepant results were confirmed by 16S rRNA or ITS region sequencing.**
Results: **The Autof MS1000 and Vitek MS identified 93.3% and 95.5% of the strains at the species level, respectively. Three isolates (0.3%) yielded "no identification" results with Vitek MS, and no "unreliable" results (0%) were obtained with Autof MS1000. The Autof MS1000 and Vitek MS misidentified 1.5% and 1.4% of the isolates, respectively. Overall, there was significant agreement between the two systems ($p < 0.001$). In terms of identification times, the Autof MS1000 was approximately three times faster than the Vitek MS.**
Conclusions: **Our results demonstrate that the Autof MS1000 provides comparable results to the Vitek MS and can be used for the rapid identification of microorganisms. Furthermore, this study highlights the need for any MALDITOF MS system to implement regular database expansion for the identification of rarely encountered microorganisms.**
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KEYWORDS

MALDI-TOF mass spectrometry, Autof MS1000, Vitek MS, bacteria, yeast

INTRODUCTION

In the management of infectious diseases, rapid and accurate diagnosis of the causative microorganism is crucial for effective treatment. Culture-based diagnostic methods used in most clinical microbiology laboratories are time-consuming and labor-intensive and are increasingly being replaced by matrix-assisted laser desorp-

tion/ionization time-of-flight mass spectrometry (MALDI-TOF MS) [1]. This method is based on protein profiling of microorganisms by ionizing their protein structures and then passing them through an electric field. The profiles obtained are compared with the data in the system's library for identification. The microorganism proteins used as the basis for identification are mainly ribosomal proteins that are less affected by environmental conditions [2,3].

The great benefit of MALDI-TOF MS, which is very fast, reliable, and cost-effective compared to classical methods, in the diagnosis of clinical bacterial and yeast isolates has been proven in many studies [4,5,6]. Thanks to this method, the diagnosis of slow-growing or rarely encountered pathogens is no longer an obstacle, and both reference and clinical microbiology laboratories have started to incorporate MALDI-TOF MS into their routine procedures [7,8]. MALDI-TOF MS technology for pathogen identification, which started with Bruker Biotyper (Bruker Daltonik GmbH, Leipzig, Germany) and Vitek MS (bioMerieux, Marcy-I'Etoile, France), continues to evolve with new products manufactured in other countries, and the number of options available to users is increasing [1,9-12]. One of these alternatives, the Autof MS1000, was developed by Autobio Diagnostics (Zhengzhou, China) and has been used since 2020.

Despite the existence of several studies comparing this novel platform with the Bruker Biotyper, which is one of the most widely used MS systems, the studies that compare it with another well-established system, Vitek MS, are limited to only some anaerobic bacteria or fungi [13-15]. The aim of this study was to investigate the diagnostic performance of the Autof MS1000 in comparison with Vitek MS in the routine workflow of a clinical microbiology laboratory. To the best of our knowledge, the present study is the first in our country to evaluate the Autof MS1000, covering aerobic and anaerobic bacteria and yeast isolates.

MATERIALS AND METHODS

Study design, bacterial and yeast strains

This prospective study was conducted from February 2024 through April 2024 in the Microbiology Laboratory of Haydarpasa Numune Research and Training Hospital. All isolates were prospectively recovered from various clinical specimens (e.g., blood, urine, stool, cerebrospinal fluid, pus, biopsy, wound, respiratory tract, and screening swab samples) sent from different medical wards.

To better reflect the diversity of isolates that can be challenging to identify in a clinical microbiology laboratory, anaerobes, other rare bacteria, and yeast strains with known sequence analysis from our laboratory's culture collection were also included in the study.

MALDI-TOF MS analyses

Sample processing

Bacterial and yeast isolates were obtained after aerobic and/or anaerobic incubation of clinical samples on 5% sheep blood agar, chocolate agar, MacConkey agar, and Sabouraud dextrose agar media (bioMerieux, France) as part of the standard laboratory workflow. The frozen isolates were subcultured twice under the appropriate culture conditions to obtain pure cultures. Freshly grown overnight colonies were taken from the same medium at the same time and identified in parallel by the direct smear method for bacteria and by the formic acid extraction method for yeasts on Autof MS1000 and Vitek MS systems. In the first stage, only one spot per colony was processed. Further testing was conducted in cases where there was no spectrum observed, or where there was inconsistency between the two systems at the genus or species level of identification. To avoid any potential identification errors due to contamination or incorrect colony selection, the related isolate was subcultured for pure growth and then retested. This result was considered the final result.

Autof MS1000

All procedures were performed according to the manufacturer's instructions. The target plate of the Autof MS1000 is disposable and has 96 spots. In the direct transfer procedure, a single colony was smeared directly onto the target plate. After drying, 1 µL of matrix solution (α-cyano-4-hydroxycinnamic acid, CHCA) was applied to the target plate and dried at room temperature. In the formic acid extraction method, a single colony was smeared onto the target plate, 1 µL of 70% formic acid was applied and dried, and then the matrix solution was applied. The Autof MS1000 instrument was calibrated daily using the calibrator. The identification result was interpreted according to the manufacturer's recommendations as "reliable species identification" for range 9 - 10, "reliable genus identification" for range 6 - 8.9, and "no reliable identification" for range < 6. The Autof MS1000 database used in this study contains 5,053 species, out of which 4,302 are bacteria and 751 are fungi.

Vitek MS

All procedures were performed according to the manufacturer's instructions. Fresh colonies were picked from agar plates with a 1 µL plastic loop and were spotted onto the disposable target plates. One microliter of the matrix solution (α-cyano-4-hydroxycinnamic acid, Vitek MS CHCA) was added onto the smears. After air drying at room temperature, the target plates were then loaded into the device.

The calibration and quality control of each group of 16 samples was performed using *Escherichia coli* ATCC 8739. A confidence value, the percent probability, was calculated by the software to reflect the concordance of the observed spectrum with the Vitek MS database. A high-confidence result is obtained with a confidence

value above 99%, a low-confidence result is obtained with a confidence value between 60% and 99%, and no identification is obtained when the confidence value is below 60%. The Vitek MS database version 3.2 used in this study contains 1,316 species, out of which 1,095 are bacteria and 221 are fungi.

Identification of discrepant results

In the case of discrepant results or no identification result for one or both methods, isolates underwent sequencing analyses of the 16S rRNA gene for bacteria or the internal transcription spacer (ITS) region for yeasts. The DNA isolation process was conducted using the GeneMATRIX Bacterial & Yeast kit (EURx, Gdansk, Poland). Sequencing reactions were carried out on an ABI 3730XL sequencer (Applied Biosystems, Foster City, CA, USA). The primers used for 16S rRNA gene amplification were
5′-AGAGTTTGATCMTGGCTCAG-3′(27F) and
5′-TACGGYTACCTTGTTACGACTT-3′(1492R),
while the primers used for ITS region amplification were
5′-TCCGTAGGTGAACCTGCGG-3′(ITS1) and
5′-TCCTCCGCTTATTGATATGC-3′(ITS4).
The sequences obtained were submitted to the BLAST software (https://blast.ncbi.nlm.nih.gov/Blast.cgi) for comparison with the sequences in the GenBank database, and the identification percent > 98.7% was considered at the species level. For bacteria that could not be accurately distinguished by 16S rRNA sequencing (i.e., *Shigella* spp., *Streptococcus pneumoniae*), further phenotypic methods, biochemical tests, and serological tests were performed.

Interpretation of the identification results

The identification was considered correct if the analysis performed by the Autof MS1000 provided the same results as the analysis using the Vitek MS and met the acceptance criteria of both systems (≥ 99% confidence value for the Vitek MS and score of ≥ 9 for the Autof MS1000). To compare system performance, MALDI-TOF MS results were classified into the following categories: correct identification at the species level, correct identification at the genus level, no identification, and misidentification.

Comparison of the test time

In order to compare the identification times of the two MS systems under equal conditions, 48 isolates were smeared on Vitek MS and Autof MS1000 target plates and placed on the respective devices simultaneously. The time required from the placement of the target plate in the instruments to the analysis of the 48 spots was measured.

Statistical analysis

Statistical analysis was performed using SPSS 20.0 statistical analysis software (IBM Corporation, Armonk, NY, USA). Categorical variables were compared with Pearson´s chi-squared test. The agreement between the systems was evaluated by Kappa statistic. A two-tailed p-value < 0.05 was considered statistically significant.

RESULTS

A total of 578 isolates were identified from various clinical specimens during the study period. The 578 clinical isolates consisted of 136 Enterobacterales, 76 non-fermenting Gram-negative bacilli (GNB), 17 other Gram-negative bacilli, 240 Gram-positive cocci (93 *Staphylococcus* spp., 72 *Streptococcus* spp., 75 *Enterococcus* spp.), 30 Gram-positive bacilli, 52 anaerobic bacteria, and 27 yeasts.

Out of the 578 isolates, the Autof MS1000 and Vitek MS correctly identified 93.3% (n = 539) and 95.6% (n = 552) of the strains at the species level, respectively, while the identification at the genus level was 5.2% (n = 30) and 2.6% (n = 15), respectively. The correct results of the Autof MS1000 were Enterobacterales 89.7%, non-fermenting GNB 97.4%, other GNB 94.1%, Gram-positive cocci 96.6%, Gram-positive bacilli 90%, anaerobic bacteria 78.8%, and yeasts 100%. The correct results of the Vitek MS were Enterobacterales 85.3%, non-fermenting GNB 97.4%, other GNB 100%, Gram-positive cocci 99.6%, Gram-positive bacilli 96.7%, anaerobic bacteria 98.3%, and yeasts 96.3%. No significant differences were observed between the number of isolates identified to the species or genus level by the two MALDI-TOF MS systems, with the exception of a lower species-level identification rate for anaerobic bacteria in the Autof MS1000 system ($p < 0.001$). With regard to bacterial species identified at the genus level, *Salmonella* spp. strains (n = 7) were responsible for the significant decrease in the percentage of correct species-level identification for both systems. A small number of Gram-positive cocci (n = 7) (i.e., *Enterococcus faecalis*, *Enterococcus casseliflavus*, *Staphylococcus haemolyticus*, *Staphylococcus aureus*) were classified at the genus level due to the low scores (6 - 8.9), despite being identified at the species level by Autof MS1000.

While three isolates (0.3%) yielded “no identification” results with Vitek MS, no "unreliable" results (0%) were obtained with Autof MS1000. The Autof MS1000 and Vitek MS misidentified 1.5% (n = 9) and 1.4% (n = 8) of the isolates, respectively. The two systems were similar in terms of non-identification and misidentification rates (Table 1).

Supplemental Table S1 presents the detailed identification results by species for the Autof MS1000 and Vitek MS systems. The results demonstrated that 524 out of 584 isolates were identified to the species level by both systems, 8 isolates were identified to the genus level by both systems, and 7 isolates were misidentified by both systems. These findings indicate a significant agreement between the results of the two systems (Kappa value = 0.373, $p < 0.001$).

Table 2 shows the discrepant results obtained by the

Table 1. Comparison of identification results of Autof MS1000 and Vitek MS.

Group of microorganisms	Autof MS1000					Vitek MS			
	n	Species n (%)	Genus n (%)	No ID n (%)	Mis ID n (%)	Species n (%)	Genus n (%)	No ID n (%)	Mis ID n (%)
Enterobacterales	136	122 (89.7)	8 (5.9)	0 (0)	6 (4.4)	116 (85.3)	13 (9.6)	1 (0.7)	6 (4.4)
Non-fermenting GNB	76	74 (97.4)	1 (1.3)	0 (0)	1 (1.3)	74 (97.4)	1 (1.3)	0 (0)	1 (1.3)
Other GNB	17	16 (94.1)	0 (0)	0 (0)	1 (5.9)	17 (100)	0 (0)	0 (0)	0 (0)
Gram-positive cocci	240	232 (96.7)	7 (2.9)	0 (0)	1 (0.4)	239 (99.6)	0 (0)	0 (0)	1 (0.4)
Gram-positive bacilli	30	27 (90)	3 (10)	0 (0)	0 (0)	29 (96.7)	1 (3.3)	0 (0)	0 (0)
Anaerobic bacteria	52	41 (78.8)	11 (21.1)	0 (0)	0 (0)	51 (98.1)	0 (0)	1 (1.9)	0 (0)
Yeasts	27	27 (100)	0 (0)	0 (0)	0 (0)	26 (96.3)	0 (0)	1 (3.7)	0 (0)
Total	578	539 (93.3)	30 (5.2)	0 (0.0)	9 (1.5)	552 (95.6)	15 (2.6)	3 (0.5)	8 (1.4)

GNB Gram-negative bacilli, ID identification.

Table 2. Isolates that yielded misidentification, no identification, or discordant results by Autof MS1000 and Vitek MS.

Reference result	Identification by Autof MS100	Score	Identification by Vitek MS	Confidence level
Acinetobacter baylii **(n = 1)**	***Acinetobacter soli***	9.5	***Acinetobacter baumannii***	99.9%
Neisseria mucosa **(n = 1)**	***Neisseria macacea***	9.2	***Neisseria mucosa***	99.9%
Shigella sonnei **(n = 4)**	***Escherichia coli***	9.6	***Escherichia coli***	99.9%
Shigella flexneri **(n = 2)**	***Escherichia coli***	9.4	***Escherichia coli***	99.9%
Cronobacter sakazakii **(n = 1)**	***Cronobacter sakazakii***	9.5	no identification	0%
Streptococcus mitis **(n = 1)**	***Streptococcus pneumoniae***	9.4	***Streptococcus mitis***	99.9%
Streptococcus pneumoniae **(n = 1)**	***Streptococcus pneumoniae***	9.5	***Streptococcus mitis***	99.9%
Bacteroides nordii **(n = 1)**	***Bacteroides nordii***	9.6	no identification	0%
Cyberlindrea fabianni **(n = 1)**	***Cyberlindrea fabianni***	9.5	no identification	0%

two MALDI-TOF MS systems. One *Acinetobacter baylii* isolate was identified as *Acinetobacter soli* by Autof MS1000 and as *A. baumannii* by Vitek MS. One *Neisseria mucosa* isolate was misidentified as *Neisseria macacea* by Autof MS1000. Both MS systems misidentified six isolates of *Shigella* spp. (*Shigella sonnei*, *Shigella flexneri*) as *E. coli*. The Vitek MS failed to identify one *Cronobacter sakazakii* (external quality control isolate in our culture collection), whereas the Autof MS1000 was able to identify this isolate to the species level. One *S. pneumoniae* isolate was misidentified as *S. mitis* by Vitek MS, and one *S. mitis* isolate was misidentified as *S. pneumoniae* by Autof MS1000. For anaerobic bacteria, one *Bacteroides nordii* isolate was identified to species level by Autof MS1000, whereas Vitek MS failed to identify this isolate. Among yeast isolates, the emerging resistant yeast *Candida auris* was identified to species level by both MS systems. One *Cyberlindrea fabianni* (*Candida fabianni*) was identified to species level by Autof MS1000 but not by Vitek MS. It was noted that two of the three unidentified isolates on Vitek MS (*Bacteroides nordii* and *Cyberlindrea fabianni*) were not included in the current database.

In terms of the test time, the Autof MS 1000 performed the identification procedure faster than the Vitek MS. The average time from placement of the target plate into the instruments to analysis of 48 spots was 15 (12 - 18 minutes) and 45 (40 - 47 minutes) minutes for Autof MS1000 and Vitek MS, respectively ($p < 0.01$).

DISCUSSION

In the last decade, several MALDI-TOF MS systems have been introduced for the identification of bacteria and yeasts in clinical microbiology laboratories. In this study, we evaluated the performance of the recently de-

veloped Autof MS1000 system and compared it with the widely used Vitek MS.

Previous studies demonstrated that the Autof MS 1000 correctly identified the aerobic Gram-negative bacteria to the species level with a range of 99.8% - 92.86% [7, 9,12]. In our study, the vast majority of Enterobacterales, non-fermenters, and other fastidious Gram-negative bacteria were correctly identified to the species level by both instruments. For all Gram-negative bacteria tested, the species-level accuracy of the Autof MS1000 and Vitek MS was 92.6% and 90.3%, respectively. The genus-level identification of seven *Salmonella* spp. Isolates was the primary factor contributing to the observed decline in the percentage of correct species-level identifications for both systems. Consequently, biochemical and serological tests remain essential for precise identification of *Salmonella* spp. given the limitations of MALDI-TOF MS [12,16]. As reported in many other studies, *Shigella* spp. and *E. coli* species are very closely related and difficult to distinguish due to their similar protein spectra [1,2]. We also observed that both MS systems misidentified all of six *Shigella* spp. isolates as *E. coli*. However, since *Shigella* spp. infections belong to the group of diseases that must be reported to the health authorities, as in our country, and due to their different pathogeneses, correct identification is very important [17]. Therefore, laboratory methods based on serological and biochemical characteristics are still needed to distinguish these microorganisms. Taking all this into account, the slightly lower identification rate of both MS systems for Gram-negative bacteria in our study compared to previous studies may be explained by the fact that the number of *Salmonella* spp. and *Shigella* spp. isolates was very low or absent in other studies. Apart from this, other enteropathogens, such as *Vibrio* spp., *Aeromonas* spp., and *Plesiomonas* spp., were correctly identified by both systems.

Building on the discussion of Gram-negative bacteria identification, a condition related to *Cronobacter sakazakii*, not reported in previous studies of Autof MS1000, was observed in our study. *Cronobacter sakazakii* can cause high mortality (80%) in infants and is often isolated from cases of infant meningitis [8]. During the study, this isolate was obtained from the external quality control cerebrospinal fluid sample sent to our laboratory. It was identified at the species level by Autof MS1000 but not by Vitek MS. This also highlights the importance and necessity of testing the accuracy of MALDI-TOF MS systems in identifying not only common clinical isolates, but also isolates that cause rare but serious infections, as an indicator of laboratory diagnostic capacity.

Difficulties in the identification of *Acinetobacter* spp. isolates at species level are among the known limitations of MS systems [7,17]. In this study, an isolate identified as *Acinetobacter baumannii* by Vitek MS was identified as *Acinetobacter soli* by Autof MS1000, but sequence analysis revealed this isolate to be *Acinetobacter baylyi.*

Identification of coagulase negative staphylococci (CNS) at the species level is essential for the correct diagnosis of bloodstream infections, in order to differentiate between causative and contaminant bacteria. For the identification of CNSs, Autof MS1000 showed highly accurate identification results comparable with those of the Vitek MS, in line with previous studies [7, 9,12]. As reported in several studies, the thick peptideglycan layer of Gram-positive bacteria confers them increased resistance to lysis, resulting in poor protein spectra during analysis [4,17]. Probably for this reason, in our study, although some Gram-positive bacteria could be identified to species level by Autof MS1000, they were classified to genus level due to low scores (7.8 - 8.9). Similar findings were also reported by Xiong et al. [9] and Porte et al. [4]. Wang et al. [18] compared three different pre-treatment methods with Autof MS 1000 and Bruker MS and found that the formic acid extraction method was more suitable for both MS methods to overcome this problem observed in Gram-positive bacteria.

For enterococci, both systems could identify the vast majority of *E. faecalis* and *E. faecium* isolates, which is in line with previous studies [9,12]. For vancomycin-resistant enterococci surveillance screening, it is important to differentiate the species with intrinsic resistance to vancomycin. Park et al. [7] reported in their study comparing Autof MS1000 with Bruker Biotyper that an *E. gallinarum* isolate was misidentified as *E. casseliflavus* by both systems. In contrast to this, we observed that all *E. gallinarum* isolates were correctly identified to the species level by Autof MS1000 and Vitek MS.

Streptococcus pneumoniae is an important human pathogen often associated with community-acquired pneumonia, meningitis, sepsis, and otitis media. In addition, the *S. mitis* group, although mostly present in the human oral flora, can also cause various infections [5,19]. Because of their different clinical manifestations and importance, accurate identification of *S. pneumoniae* and *S. mitis/oralis* species is crucial for the appropriate therapy. However, limitations of MALDI-TOF MS systems in distinguishing *S. pneumoniae* from *S. mitis* group species have been reported in several studies [9,17,19]. The main cause is that *S. pneumoniae* is very closely related to *S. mitis* and *S. oralis*. In our study, one of the three *S. pneumoniae* isolates was identified as *S. mitis/oralis* by Vitek MS. Similar findings were also reported by other authors in previous studies [1,20]. In contrast to this, there are also studies showing that Vitek MS misidentified *S. mitis/oralis* isolates as *S. pneumoniae* [5,19]. Ma et al. [9] and Xiong et al. [12] correctly identified all *S. pneumoniae* isolates with Autof MS1000, and Park et al. [7] were also able to identify a small number of *S. mitis* and *S. pneumoniae* at species level with this system. In our study, the Autof MS1000 correctly identified all *S. pneumoniae* isolates, whereas it misidentified one *S. mitis* isolate as *S. pneumoniae*. Given the limitations of both systems, complementary tests such as optochin sensitivity and bile solubility should be

used for confirmation in the identification of these closely related strains. Therefore, MALDI-TOF MS analysis still cannot be used alone for the identification of these organisms in microbiology laboratories.

Anaerobic bacteria are often difficult to isolate using conventional methods due to their slow growth, and traditional phenotypic identification methods can be time-consuming and laborious [9,17]. By overcoming these technical difficulties, MALDI-TOF MS systems provide rapid and adequate identification of clinically relevant anaerobes [3,15]. When the studies investigating the performance of Autof MS1000 in the identification of anaerobic bacteria were examined, it was observed that the number and species of isolates were quite low. Xiong et al. [9] analyzed 8 *Bacteroides* spp. and 7 *Clostridium* spp. isolates and correctly identified all of them at species level. Ma et al. [12] similarly found the performance of Autof MS1000 to be quite good. Park et al. [7] reported that 12 anaerobic bacteria of different species were identified to the species level by Autof MS 1000, with the exception of one *Eggerthella lenta*, which was identified to the genus level. One of the strengths of our study is that it is more comprehensive than previous studies in terms of providing data on 52 clinically relevant anaerobes consisting of 10 genera and 20 different species. In contrast to other studies, the species level identification rate was significantly lower for anaerobic bacteria in Autof MS1000 than Vitek MS (78.8% versus 96.3%) in our study. Yet, all *Bacteroides fragilis*, one of the most isolated anaerobes in clinical specimens, were correctly identified by both systems, as reported by many other authors [9,15]. On the other hand, an isolate of *Bacteroides nordii* was identified at species level by Autof MS1000 but not by Vitek MS due to lack of database. Therefore, updating and expanding the databases are essential for improving the identification rate for every MS system. Although Autof MS1000 identified 12 strains at the species level, the results were classified at the genus level due to low identification scores. This situation may raise alternative suggestions for Autof MS1000, such as lowering the threshold value for anaerobes or applying pre-treatment methods as recommended in previous studies for other MS systems [18].

The accurate identification of yeasts at the species level is crucial for the initiation of targeted therapy, as each species has a distinct antifungal susceptibility profile. Furthermore, rapid identification of multidrug-resistant strains such as *C. auris* is essential for the implementation of infection control measures and the prevention of their spread in hospitals [2,6,13]. In a comprehensive study covering 1,228 yeast isolates, Yi et al. [13] observed that the Autof MS1000 and Vitek MS systems correctly identified 99.2% and 89.2% of isolates, respectively. The authors stated that the Autof MS1000 exhibited a greater capacity for yeast identification than the Vitek MS, mainly because the reference database of phylogenetically closely related yeast species of the Vitek MS was poor. In our study, Autof MS1000 and Vitek MS systems identified 100% and 96.3% of the isolates at species level, respectively, with no statistically significant difference between the two systems. Both systems correctly identified all *Candida* species, including *C. auris*. However, Vitek MS failed to identify a *Cyberlindrea fabianni* isolate, which reported to have been misidentified as *Candida utilis* by phenotypic methods [21,22]. Teke et al. [23] also reported that Vitek MS was unable to identify this species because it was not included in the database. So far, only a few reports of human infection cases caused by *Cyberlindrea fabianni* have been published [21,22]. It can be predicted that the number of case reports will increase worldwide as MS systems expand their databases to include *Cyberlindrea fabianni*.

When comparing the time for identification, the Autof MS1000 was approximately three times faster than the Vitek MS, which is in accordance with the findings of Park et al. [7]. This shorter identification time of the Autof MS1000 may prove beneficial in facilitating the routine operations of microbiology laboratories, particularly those with a high workload.

Some limitations of the study should be mentioned. Firstly, we could not perform 16S rRNA or ITS sequencing for all the 578 isolates, but only for those with inconsistent identification or “no identification” results from both instruments. Secondly, filamentous fungi and mycobacteria could not be included in the study.

Our results indicated that the Autof MS1000 showed comparable identification performance to the Vitek MS. Accordingly, it may be suitable for the use in clinical microbiology laboratories as a primary option for the identification of routine clinical bacterial and yeast isolates. However, improved sample extraction protocols should be considered for better identification of anaerobic bacteria. This study also underscores the importance for any MS system to implement regular database expansion and updates for the identification of rarely encountered microorganisms. We believe that our data will make a valuable contribution to the current literature on new MS systems, which are undergoing rapid evolution. Further studies with a greater diversity of microorganisms are needed to confirm our findings.

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Ethical Approval Statement:
The study was approved by the Research Ethics Committee of the Haydarpasa Numune Research and Training Hospital, University of Health Sciences (protocol no. HNEAH-KAEK 2024/KK/25).

Declaration of Interest:
There are no conflicts of interest in connection with this paper. The article has not been published previously in any language, in whole or in part, and is currently not under consideration for publication elsewhere.

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