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Article

Clinical Evaluation of VITEK MS PRIME with PICKME Pen for Bacteria and Yeasts, and RUO database for Filamentous Fungi

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Abstract: VITEK MS PRIME (bioMerieux), a newly developed Matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOF MS) system, alongside VITEK PICKME Pen (PICKME), offers easy sample preparation for bacteria and yeast. VITEK MS PRIME also offers two software platforms for filamentous fungi: the IVD database and the RUO database. Our study evaluated its identification agreement on 320 clinical isolates of bacteria and yeasts, comparing PICKME and traditional wooden toothpick sampling techniques against ASTA MicroIDSys Elite results. Additionally, we assessed the IVD (v3.2) and SARAMIS (v4.16) RUO databases on 289 filamentous fungi against molecular sequencing. The concordant rates for species-level identification of bacteria and yeasts were about 89.4% (286/320) between the PICKME and wooden toothpick, about 83.4-85.3% between VITEK MS PRIME and ASTA MicroIDSys Elite. Retesting with PICKME improved concordance to 91.9%. For filamentous fungi, species-level identification reached 71.3% with the IVD database and 85.8% with RUO, which significantly enhanced basidiomycetes' identification from 35.3% to 100%. Some strains in the IVD database, like *Aspergillus vesicolor*, *Exophiala xenobiotica*, and *Nannizzia gypsea* failed to be identified. VITEK MS PRIME with PICKME offers reliable and efficient microorganism identification. For filamentous fungi, combining the use of the RUO database can be beneficial, especially for basidiomycetes.

Keywords: MALDI-TOF mass spectrometry; VITEK MS PRIME; PICKME; filamentous fungi; yeast; bacteria

1. Introduction

Identifying microorganisms typically demands significant effort, time, and resources using various methods like physiological, serological, biochemical, and chemotaxonomic approaches. While genomic methods offer high reliability, they are slow and require extensive expertise [1]. In contrast, Matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOF MS) offers a swift, cost-efficient alternative capable of processing large volumes of samples simultaneously [2]. This technique has revolutionized conventional identification methods in laboratories by enabling rapid pathogen identification [3]. Although it is widely accepted for identifying bacteria and yeasts, there are still challenges when it comes to filamentous fungi [4]. The main challenge is the lack of a sufficient database and of an effective and rapid protein extraction method [5].

The VITEK MS PRIME (bioMérieux, Marcy l'Etoile, France) is a new version of the VITEK MALDI-TOF MS instrument developed by bioMérieux. This upgraded instrument features a continuous load-and-go sample loading system, prioritization for critical patient samples on urgent

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slides, and new internal components, all of which are designed to enhance sample processing speed and reduce handling time [6]. The VITEK MS PRIME system utilizes the VITEK MS IVD Database 3.2., but it also allows for additional analysis using the SARAMIS® Knowledge Base v4.16 (for research use only).

The VITEK-PICKME pen (hereafter referred to as PICKME, bioMérieux) is a device with a disposable nip that facilitates the user's ability to pick and smear colonies on the VITEK MS target slide, improving smear quality. Compared to conventional wooden toothpicks, PICKME provides better uniformity and thinner deposits [7]. A study highlighted by the American Society for Microbiology (ASM) Microbe 2019 showed its ability to decrease variability among users, reduce sample preparation time by 26-42%, and improve uniformity across different user experience levels [8].

In this study, bacterial and fungal isolates detected in clinical specimens were identified using the VITEK MS PRIME to confirm their identification capabilities. Both bacteria and yeast were tested using the PICKME and a conventional wooden toothpick, respectively. The results were also compared with the MicroIDSys Elite (ASTA, ASTA Corp., Suwon, Korea) MALDI-TOF MS currently used in our laboratory. For filamentous fungi, clinical isolates previously identified by molecular sequencing were analyzed using the VITEK MS IVD Database 3.2 and the SARAMIS® Knowledge Base V4.16 (RUO database), respectively to find the clinical utility of the RUO database.

2. Materials and Methods

2.1. Clinical Isolates

A total of 320 bacteria and yeast isolates were obtained from the International St.Mary`s hospital, consisting of 268 bacteria (124 gram-negative bacilli, 91 gram-positive cocci, 22 gram-positive bacilli, 16 anaerobes, 15 gram-negative cocci) and 52 yeasts (37 *Candida* species, 15 others). All isolates were confirmed by ASTA MicroIDSys Elite (ASTA), VITEK2 (bioMérieux), and/or molecular sequencing by Clinical and Laboratory Standards Institute (CLSI) guideline (MM18) [9].

For the evaluation of filamentous fungi, 289 fungal isolates deposited in the Korean nationwide fungal collection network by the National Culture Collection for Pathogens, were used. Fungal isolates compromising 31 genera and 79 species, were obtained from various clinical specimens from 10 hospitals in the Republic of Korea (Seoul, Incheon, Gyeonggi-do, Busan, Daejeon, Ganwon-do, and Jeju Island) between March 2017 and December 2020. They were recovered from various clinical specimens such as respiratory specimens, blood, urine, CSF, body fluids, tissue, eye, nose, ear, nail, skin, and hair. Preservation was done using liquid nitrogen in a deep freezer to minimize loss. The identification of the isolates involved macroscopic phenotypic identification/microscopic examination and molecular sequencing, as described in previous studies [2].

Approval for this study was obtained from the International St. Mary's Hospital, Catholic Kwandong University College of Medicine in Korea (IS22ESSE0006).

2.2. MALDI-TOF MS Analysis

For bacterial and yeast isolates, VITEK MS PRIME [6] and ASTA MicroIDSys Elite [2] systems were used according to the manufacturer`s instructions. Sample preparation was performed by direct smearing method, as per the manufacturer's instructions. VITEK MS PRIME was conducted using both the PICKME and a wooden toothpick. The spectra generated by VITEK MS PRIME were analyzed by the VITEK MS Software (version 1.1.0 – 203571250) and the VITEK MS IVD Database 3.2. ASTA MicroIDSys Elite experiments were conducted using the applying wooden toothpick. The MS spectra obtained by ASTA MicroIDsys Elite were analyzed with the reference library (CoreDB version 1.27.04). Duplicate spreading on a single-use target slide was performed for all samples.

Identification of filamentous fungi was performed using the VITEK MS Mold Kit, following the manufacturer's instructions. After experiments, filamentous fungi were analyzed in the IVD database first in VITEK MS PRIME. Strains that were not identified at the species level in the IVD database were analyzed once more with the RUO database version (SARAMIS® Knowledge Base V4.16).

2.3. Interpretation and Analysis of the Results

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For bacterial and yeast isolates, concordance was determined when both VITEK MS PRIME and ASTA MicroIDSys Elite provided the same species or genus identification at the species or genus level. In cases of no identification or discrepant results, 16S rRNA gene sequencing was used as a reference method. For filamentous fungi, consistency was determined if one or more of the duplicate results coincided with the sequencing result. Identification failure was recorded when two results were inconsistent or identification was not possible. Statistical analysis was performed using MedCalc Statistical Software version 19.2.1 (MedCalc Software Ltd, Ostend, Belgium), using Chi-square and Fisher's exact tests with a two-tailed p-value.

3. Results

3.1. Bacterial and Yeast Identification Using PICKME and Wooden Toothpicks

Using the wooden toothpicks, VITEK MS PRIME identified bacteria and yeast at the species level in 91.9% (294/320) of cases, while PICKME achieved species-level identification in 90.3% (289/320) (Table 1). The concordance rate of wooden toothpicks and PICKME in VITEK MS PRIME was 89.4% (286/320).

Among the 34 bacterial isolates with discrepancies between the wooden toothpick and PICKME (Table 2), eight isolates (2.5%) were correctly identified only by the wooden toothpick, three (0.9%) were correctly identified only by PICKME, and 23 isolates (5.9%) showed no identification (n=19) or incomplete identification (n=4).

Table 1. Bacterial and yeast identification by VITEK MS PRIME and ASTA MicroIDSys Elite.

	No. of isolates	**VITEK MS PRIME - wooden toothpick, n (%)**			**VITEK MS PRIME - PICKME, n (%)**			**ASTA MicroIDSys Elite, n (%)**			**Concordance rate, n (%)**		
		Correct ID (species level)	Incomplete ID (genus level)	No ID	Correct ID (species level)	Incomplete ID (genus level)	No ID	Correct ID (species level)	Incomplete ID (genus level)	No ID	Wooden toothpick and PICKME	Wooden toothpick and ASTA	PICKME and ASTA
Gram positive cocci	91	90 (98.9)		1 (1.1)	89 (97.8)		1 (1.1)	87 (95.6)	1 (1.1)	3 (including 2 misID) * (3.3)	89 (97.8)	86 (94.5)	85 (93.4)
Gram positive bacilli	22	18 (81.8)	1 (4.5)	3 (13.6)	16 (72.7)	1 (4.5)	5 (22.7)	21 (95.5)		1 (4.5)	16 (72.7)	18 (81.8)	16 (72.7)
Gram negative cocci	15	15 (100)			15 (100)			13 (86.7)	1 (6.7)	1 (6.7)	15 (100)	13 (86.7)	13 (86.7)
Gram negati	124	111 (89.5)	2 (1.6)	11 (8.9)	111 (89.5)	2 (1.6)	11 (8.9)	103 (83.1)	11 (8.9)	10 (8.1)	110 (88.7)	101 (81.5)	100 (80.6)

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ve bacilli												
Anaerobes	16	14 (87.5)	2 (12.5)	14 (87.5)	2 (12.5)	14 (87.5)		2 (12.5)	14 (87.5)	14 (87.5)	14 (87.5)
Yeast	52	46 (88.5)	6 (11.5)	44 (84.6)	8 (15.4)	44 (84.6)		8 (15.4)	42 (80.8)	41 (78.8)	39 (75.0)
Total	320	294 (91.9)	3 (0.9) 23 (7.2)	289 (90.3)	27(8.4 %)	282 (88.1)	13 (4.1)	25 (7.8)	286 (89.4)	273 (85.3)	267 (83.4)

* ASTA MicroIDSys Elite misidentified two Staphylococcus cohnii as Micrococcus lylae; Abbreviation: ID, identification; ASTA, ASTA MicroIDSys Elite, n; number.

Table 2. Discordant isolates between wooden toothpick and PICKME in VITEK MS PRIME (n=34).

Discordant isolates	**VITEK MS PRIME – wooden toothpick**		**VITEK MS PRIME– PICKME**		**ASTA MicroIDSys Elite**		**Re-tests**
	result	score	result	score	result	score	
Identified only in wooden toothpick (n=8)							
Aeromonas caviae	*Aeromonas caviae*	99.9	No identification		*Aeromonas caviae*	233	
Corynebacterium jeikeium	*Corynebacterium jeikeium*	99.9	No identification		*Corynebacterium jeikeium*	178	
Corynebacterium jeikeium	*Corynebacterium jeikeium*	99.9	No identification		*Corynebacterium jeikeium*	178	
Streptococcus anginosus	*Streptococcus anginosus*	99.9	No identification		*Streptococcus anginosus*	211	
Candida albicans	*Candida albicans*	95.3	No identification		*Candida albicans*	139	
Candida albicans	*Candida albicans*	99.9	No identification		*Candida albicans*	224	Correct ID in PICKME
Candida ciferrii	*Candida ciferrii*	99.9	No identification		No identification		Correct ID in PICKME
Candida parapsilosis	*Candida parapsilosis*	99.9	No identification		*Candida parapsilosis*	141	Correct ID in PICKME
Identified only in PICKME (n=3)							
Acinetobacter nosocomialis	No identification		*Acinetobacter nosocomialis*	90.7	No identification		Correct ID in wooden toothpick in VITEK

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Candida ciferrii	No identification		*Candida ciferrii*	99.9	No identification		Correct ID in wooden toothpick in VITEK
Candida tropicalis	No identification		*Candida tropicalis*	99.9	*Candida tropicalis*	214	Correct ID in wooden toothpick in VITEK
Identified only in genus level (n=4)							
Acinetobacter proteolyticus	No identification		*Acinetobacter gyllenbergii*	99.6	No identification		
Aeromonas dhakensis	*Aeromonas veronii/sobria*	50/50	*Aeromonas veronii/sobria*	50/50	No identification		
Paenibacillus polymyxa	*Paenibacillus peoriae*	99.9	*Paenibacillus peoriae*	99.9	No identification		
Pseudomonas granadensis	*Pseudomonas fluorescens*	99.7	No identification		No identification		
Misidentification (n=19)							
Acinetobacter seifertii	No identification		No identification		*Acinetobacter calcoaceticus*	137	
Aerococcus viridans	No identification		No identification		*Aerococcus viridans*	160	
Aeromonas dhakensis	No identification		No identification		*Aeromonas* sp	195	
Aeromonas dhakensis	No identification		No identification		*Aeromonas* sp	205	
Bacteroides thetaiotaomicron	No identification		No identification		No identification		Correct ID in ASTA
Comamonas acidovorans	No identification		No identification		*Comamonas acidovorans*	154	
Corynebacterium afermentans	No identification		No identification		*Corynebacterium afermentans*	169	
Corynebacterium afermentans	No identification		No identification		*Corynebacterium afermentans*	167	
Eikenella corrodens	No identification		No identification		Invalid Identification		
Fusobacterium nucleatum	No identification		No identification		No identification		

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Gardnerella vaginalis	No identification	No identification	Invalid Identification		
Pasteurella multocida	No identification	No identification	*Pasteurella multocida*	141	
Shewanella algae	No identification	No identification	No identification		Correct ID in wooden toothpick & PICKME in VITEK
Spingomonas paucimobilis	No identification	No identification	Invalid Identification		
Stenotrophomonas maltophilia	No identification	No identification	Invalid Identification		Correct ID in ASTA
Trichrosporon faecale	No identification	No identification	Invalid Identification		
Yarrowia galli	No identification	No identification	Invalid Identification		
Candida dubliniensis	No identification	No identification	*Candida dubliniensis*	153	Correct ID in wooden toothpick & PICKME in VITEK
Candida tropicalis	No identification	No identification	*Candida tropicalis*	153	

Abbreviation: ID, identification; VITEK, VITEK MS PRIME; ASTA, ASTA MicroIDSys Elite.

After retesting, eight isolates showed species-level agreement between the wooden toothpick and PICKME. Ultimately, 294 isolates (91.9%) showed species-level agreement between the wooden toothpick and PICKME. Additionally, two isolates showed genus-level agreement between PICKME and the wooden toothpick.

There are a total of 15 strains of Corynebacterium included (5 Corynebacterium striatum, 3 Corynebacterium diphtheriae, 2 Corynebacterium amycolatum, 2 Corynebacterium jeikeium, 2 Corynebacterium afermentans, and 1 Corynebacterium simulans). Among these strains, 11 were successfully identified at the species level using both the PICKME and wooden toothpick methods. However, for the 2 strains of Corynebacterium jeikeium, only the wooden toothpick method achieved species-level identification, while the PICKME method failed. Additionally, for the 2 strains of Corynebacterium afermentans, both the PICKME and wooden toothpick methods resulted in failed identification.

3.2. Bacterial and Yeast Identification Using VITEK MS PRIME and ASTA MicroIDSys Elite

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ASTA MicroIDSys Elite achieved a species-level identification rate of 88.1% (282/320). The concordant rates for species-level identification 85.3% (273/320) between the wooden toothpick and ASTA MicroIDSys Elite, and 83.4% (267/320) between PICKME and ASTA MicroIDSys Elite. Two isolates (Bacteroides thetaiotaomicron, Stenotrophomonas maltophilia) initially showed no identification in ASTA MicroIDSys Elite but were correctly identified upon retesting. As both isolates showed identification failure in VITEK MS PRIME, the agreement rate between ASTA MicroIDSys Elite and VITEK MS PRIME did not change after retesting. Overall, gram-positive bacilli including Corynebacterium exhibited higher accuracy in ASTA MicroIDSys Elite (95.5%) compared to VITEK MS PRIME (72.7-81.8%). The strains of Corynebacterium jeikeium and Corynebacterium afermentans that failed to be identified in VITEK MS PRIME were both successfully identified at the species level in ASTA MicroIDSys Elite.

3.3. Identification of Filamentous Fungi Using the IVD and RUO Databases

Among the 289 filamentous fungi, species-level identification using the IVD database was 71.3% (206/289), while genus or complex level identification was 75.4% (218/289) (Figure 1).

Additionally, when analyzed using the RUO database, species-level identification improved significantly to 85.8% (248/289), and genus or complex level identification increased to 88.2% (255/289). There was no significant difference in identification performance between the IVD and RUO databases for *Aspergillus* strains. However, for basidiomycetes, there was a significant improvement in identification performance when using the RUO database, with an increase from 35.3% to 100% (P<0.0001). Detailed results for each strain are summarized in Table 3.

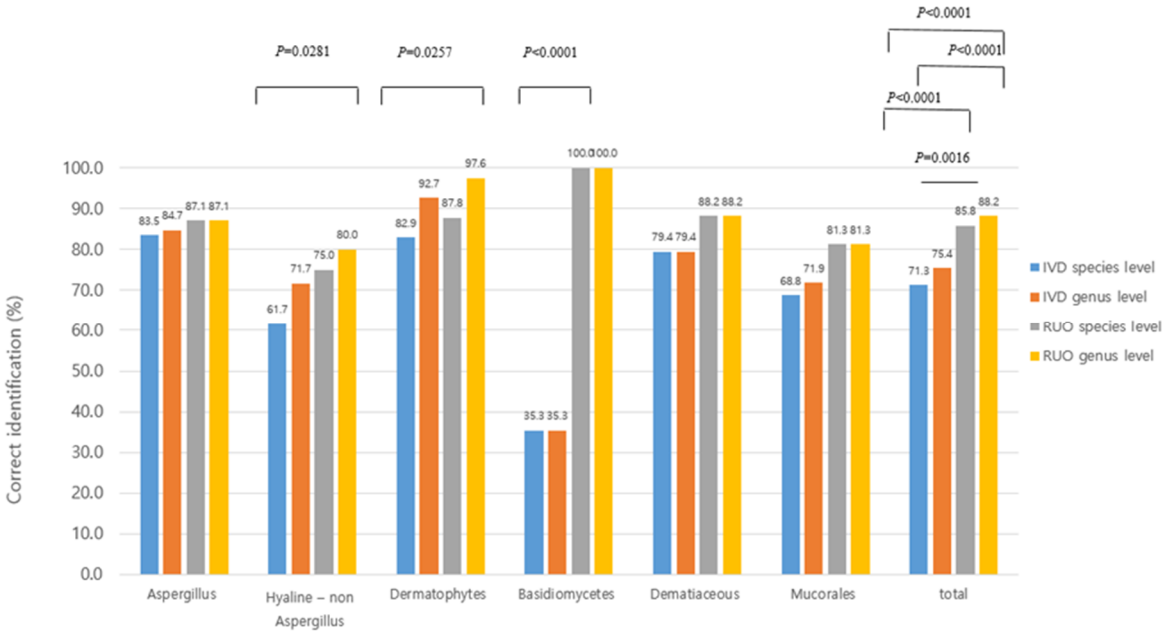

Figure 1. Correct identification percentages for molds by VITEK MS PRIME IVD database and RUO database (n=289).

Table 3. Bacterial and yeast identification by VITEK MS PRIME and ASTA MicroIDSys Elite.

		IVD DB			RUO DB		
Identification by DNA sequencing	No. of isolates	Species level ID (%)	Genus or complex level ID (%)	no ID (%)	Species level ID (%)	Genus or complex level ID (%)	no ID (%)
Hyaline – *Aspergillus*							
Aspergillus aculeatus	2			2			2
Aspergillus calidoustus	2	2					

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Aspergillus chevalieri	5			5			5
Aspergillus flavus	7	7					
Aspergillus fumigatus	7	7					
Aspergillus japonicus	1			1			1
Aspergillus lentulus	12	12					
Aspergillus nidulans	7	6		1	1		
Aspergillus niger	8	8					
Aspergillus ochraceus	1	1					
Aspergillus sydowii	14	13		1	1		
Aspergillus tamarii	2	1	1		1		
Aspergillus terrei	8	8					
Aspergillus tubingensis	6	6					
Aspergillus versicolor	3			3			3*
Total	**85**	**71**	**1**	**13**	**3**		**11**
Hyaline – non *Aspergillus*							
Acremonium sclerotigenum	1	1					
Beauveria bassiana	1	1					
Fusarium dimerum	1	1					
Fusarium equiseti	2		1	1	1		1
Fusarium oxysporum	2	2					
Fusarium solani	4	4					
Fusarium verticillioides	4	1	3		2	1	
Paecilomyces formosus	4			4			4
Paecilomyces variotii	2	2					
Penicillium chrysogenum	3	3					
Penicillium citrinum	9	8		1	1		
Penicillium crustosum	1			1	1		
Penicillium expansum	1			1		1	
Penicillium glabrum	2	2					
Penicillium janthinellum	2			2		1	1
Penicillium oxalicum	4			4	3		1
Penicillium toxicarium	1			1			1
Pseudallescheria boydii	1	1					
Purpureocillium lilacinum	8	8					
Rasamsonia argillacea	3	3					
Talaromyces marneffei	1			1			1
Talaromyces pinophilus	2		2				2
Talaromyces purpureogenus	1			1			1
Total	**60**	**37**	**6**	**17**	**8**	**3**	**12**
Dermatophytes							
Epidermophyton floccosum	1	1					
Microsporum canis	10	9		1	1		
Nannizzia gypsea	1			1			1*
Trichoderma	2	2					
Trichophyton erinacei	1	1					
Trichophyton interdigitale	3	2	1			1	
Trichophyton	3		3			3	
Trichophyton rubrum	17	16		1	1		
Trichophyton tonsurans	3	3					
Total	**41**	**34**	**4**	**3**	**2**	**4**	**1**

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Basidiomycetes							
Bjerkandera adusta	1	1					
Coprinellus radians	4			4	4		
Irpex lacteus	11	11					
Schizophyllum commune	18			18	18		
Total	**34**	**12**		**22**	**22**		
Dematiaceous							
Alternaria alternata	11	11					
Cladosporium	6	6					
Cladosporium halotolerans	2			2	2		
Colletotrichum	1			1			1
Exophiala xenobiotica	2			2			2*
Neoscytalidium dimidiatum	1			1			1
Scedosporium apiospermum	10	10					
Scopulariopsis brevicaulis	1			1	1		
Total	**34**	**27**		**7**	**3**		**4**
Mucorales							
Cunninghamella	3			3			3
Lichtheimia corymbifera	3	3					
Mucor circinelloides	5	5					
Mucor fragilis	1		1				1
Mucor irregularis	1			1			1
Mucor velutinosus	1	1					
Rhizomucor miehei	1			1			1
Rhizomucor pusillus	4			4	4		
Rhizopus microsporus	3	3					
Rhizopus oryzae	10	10					
Total	**32**	**22**	**1**	**9**	**4**		**6**
Others							
Eutypella scoparia	3	3					
Total molds	**289**	**206**	**12**	**71**	**42**	**7**	**34**

3.4. Identification Failure of Filamentous Fungi

A total of 34 isolates failed to be identified in VITEK MS PRIME using both the IVD and RUO databases (Table 4). Among them, six isolates were strains included in the IVD database, including *Aspergillus versicolor* (3), *Exophiala xenobiotica* (2), and *Nannizzia gypsea* (1). The remaining 28 isolates were strains included only in the RUO database, with *Penicillium* and *Talaromyces* strains accounting for 11 cases.

Table 4. Clinical filamentous fungi isolates failed to identify in VITEK PRIME MS (n=34).

Isolates included in IVD database		No. of isolates
Aspergillus species	*Aspergillus versicolor*	3
Dematiaceous	*Exophiala xenobiotica*	2
Dermatophytes	*Nannizzia gypsea*	1
Isolates included in only RUO database		
Aspergillus species	*Aspergillus aculeatus*	2
	Aspergillus chevalieri	5
	Aspergillus japonicus	1
Hyaline – non *Aspergillus*	*Fusarium equiseti*	1

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	Paecilomyces formosus	4
	Penicillium janthinellum	1
	Penicillium oxalicum	1
	Penicillium toxicarium	1
	Talaromyces marneffei	1
	Talaromyces pinophilus	2
	Talaromyces purpureogenus	1
Dematiaceous	*Colletotrichum gloeosporioides*	1
	Neoscytalidium dimidiatum	1
Mucorales	*Cunninghamella bertholletiae*	3
	Mucor fragilis	1
	Mucor irregularis	1
	Rhizomucor miehei	1

4. Discussion

Based on the species included in the VITEK MS IVD database, the performance of VITEK MS PRIME for the identification of bacteria and yeasts using the PICKME was remarkable. It provided quick and reliable identifications for 89.4% of the isolates, using the same laboratory workflow as the conventional method in various bacterial and yeast species. A previous study highlighted the significant role of the PICKME in selecting rough *Nocardia* colonies and creating thin deposits, essential for accurate MALDI-TOF identification [7]. The study involved adding formic acid directly to the deposit along with the cyano-4-hydroxycinnamic acid (CHCA) matrix solution to facilitate bacterial wall destruction and protein extraction. Despite being an extra step, this direct deposit analysis method proved much faster than the sample preparation recommended by the manufacturer and was compatible with standard bacterial identification workflows.

The quality of the deposit on the target can influence the spectra's quality. Proper training is needed to ensure the appropriate amount of colony transfer onto the plate [10]. Previous research reported significant differences in score distribution among testing institutions, even when using the same extraction method [11]. In this study, all unidentified isolates with wooden toothpicks and 37.5% of unidentified isolates with PICKME were correctly identified after retesting. Therefore, using the PICKME may lead to more consistent testing, unaffected by the examiner's capabilities.

Comparing VITEK MS PRIME to ASTA MicroIDSys Elite, the platform showed similar performance. Notably, the VITEK MS PRIME exhibited relatively higher rates of inaccurate identification in the Coryneform and aerobic/anaerobic gram-positive bacilli group, which aligns with a previous study [12]. This difference may be attributed to the thick peptidoglycan layer, potentially interfering with laser ionization. Interestingly, among the 19 strains not detected by VITEK MS PRIME, ASTA MicroIDSys Elite detected 7 strains at the species level and 3 strains at the genus level. Among the unidentified isolates in VITEK MS PRIME, *Aeromonas dhakensis*, *Comamonas acidovorans*, *Spingomonas paucimobilis*, and *Yarrowia galli* have been reported as unidentified isolates in previous systems and databases [13,14]. These species do not exist in the VITEK MS ver. 3.2 database, but all of them were reported as human pathogens [15–18]. Therefore, updating the database is needed to improve the distinguishing ability from other species.

For filamentous fungi, the VITEK MS PRIME using the updated IVD database successfully species-level identification in 71.3% (206/289). The use of the RUO database significantly improved the identification ability by up to 85.8% and especially, basidiomycetes showed a remarkable improvement.

The manufacturers' libraries for microbial identification are regularly updated to include more species and variations within a single species. However, they still face the challenge of incompleteness due to the vast diversity encountered in human pathology [10]. The VITEK MS v3.2

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database only covers 221 fungal species. While common molds are generally well covered, identifying rarer or cryptic species becomes more challenging. To improve the identification capability of rare filamentous fungi, developing a homemade library or utilizing open public databases for analysis is possible, but it may not be straightforward to implement in actual laboratories [10]. The VITEK MS PRIME provides a practical solution, allowing a seamless transition to the RUO database for strains that failed in the existing IVD database without additional experiments. This is expected to significantly aid in the identification of rare filamentous fungi.

The VITEK MS PRIME successfully identified *T. rubrum* and *T. Tonsurans* to the species level in IVD mode. However, it failed to achieve species-level identification for *T. interdigitale* and *T.mentagrophytes* even in RUO mode. This lack of accurate identification was also observed in a previous study using VITEK MS v3.0 [19]. Indeed, some strains (*Aspergillus vesicolor, Exophiala xenobiotica*, and *Nannizzia gypsea*) included in the IVD database, could not be identified using VITEK MS v3.2. These findings were also reported in the previous study, where Zvezdánova et al. reported correct identification rates of 86.4% for *Aspergillus* species and 65.7% for Mucorales [20]. It will be necessary to enhance the database for *Aspergillus* species, Mucorales, and Trichophyton strains to improve their identification in the VITEK MS PRIME.

In this study, VITEK MS PRIME with PICKME provides reliable results with high efficiency and allows standardized sample preparation. For filamentous fungi, VITEK MS PRIME provides reliable results for diverse filamentous fungi that are commonly isolated in clinical laboratories. For further identification, combining the use of the RUO database can be beneficial, especially for basidiomycetes.

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