EJMI

DOI: 10.14744/ejmi.2021.56223
EJMI 2021;5(3):354–359

Research Article

Identification of Dermatophytosis Agents by Matrix-Assisted Laser Desorption Ionization Time of Flight Mass Spectrometry

Ayse Baris

Department of Medical Microbiology, University of Health Sciences, Sisli Hamidiye Etfal Training and Research Hospital, Istanbul, Turkey
Department of Medical Microbiology, Istanbul Medical Faculty, Istanbul University

Abstract

Objectives: We aimed to evaluate the identification of dermatophytes with Matrix-assisted laser desorption ionization time-of-flight mass spectrometry(MALDI-TOF MS).

Methods: 138 dermatophytes were identified by applying ethanol-formic acid-acetonitrile extraction protocol to mold subcultures on solid and broth media, using fungal library of MALDI Biotyper(Bruker Daltonics,Germany) system. Scores of ≥1.7 and ≥2.0 were used as identification threshold scores. Morphology-based techniques were used as the diagnostic standard methods.

Results: With the threshold score of ≥2.0; 17.6% of 136 isolates extracted from solid media and 59% of 61 isolates extracted from liquid media could be identified correctly. When the threshold is reduced to 1.7; 61.8% of 136 isolates extreacted from solid media and all of 61 isolates extracted from liquid media could be identified correctly. Regardless of the extraction method, 38.4% and 82.6% of the isolates could be identified at the species level when the threshold score was accepted as ≥2.0 and ≥1.7, respectively($p<0.001$).

Conclusion: The MALDI-TOF-MS system is a quite useful complement to conventional methods for dermatophyte diagnosis, particularly when the threshold score was accepted as ≥1.7. Primarily extraction from solid media, and if identification cannot be made, subsequent subculture to liquid medium will provide benefits in terms of workload, time, and cost.

Keywords: Identification of dermatophyte, MALDI-TOF-MS, MALDI Biotyper, protein extraction

Cite This Article: *Baris A. Identification of Dermatophytosis Agents by Matrix-Assisted Laser Desorption Ionization Time of Flight Mass Spectrometry. EJMI 2021;5(3):354–359.*

Dermatophytosis is a globally important disease caused by pathogenic keratinolytic fungi in humans and animals, affecting approximately 20-25% of the world population and 30-70% of adults are asymptomatic carriers.[1,2] In the last two decades, there has been a dramatic increase in the incidence of dermatophytosis in humans as a result of increased socioeconomic problems, international travel and migration, and contact with animals (especially pets).[3] Epidemics and self-limiting outbreaks with dermatophytes have occasionally been reported in the literature.[4] The source of the infection can be soil (geophilic species), animals (zoophilic species) or human (anthropophilic species).[5] While pathogenic dermatophytes used to include *Microsporum, Trichophyton and Epidermophyton* genera; with the recent taxonomic studies, new genera such as Nannizzia, Lophophyton, Arthroderma and Paraphyton have been added to the group.[6]

Address for correspondence: Ayse Baris, MD. Saglik Bilimleri Universitesi, Sisli Hamidiye Etfal Egitim ve Arastirma Hastanesi, Tibbi Mikrobiyoloji Anabilim Dali, Istanbul, Turkey
Phone: +90 212 373 50 00 **E-mail:** aysebarisacb@gmail.com

Submitted Date: August 24, 2021 **Accepted Date:** September 20, 2021 **Available Online Date:** September 22, 2021

The treatment process can be uncertain in dermatophytosis infections due to the appearance of clinical features such as circular lesions, scaling and multifocal alopecia, which are similar to some other skin diseases. Unless infections are detected and treated in time, they can cause irreversible damage to skin, hair and nails. Isolation and determination of the agent are also effective on the regulation of the species-specific treatment regimen. Rapid identification of etiologic agents and source of infection has also a great importance to prevent further spread to human and/or animals.[2]

Identification of dermatophytes is traditionally based on the examination of microbiological and biochemical features.[1] Conventional methods require special protocols, expertise, experience, and are time-consuming.[2] On the other hand, DNA -sequence -based identification, the recommended gold standard, is expensive and time -consuming for a routine microbiology laboratory.[7] Another promising technique for the identification of microorganisms is the fingerprint analysis of protein extract using the matrix -assisted laser desorption/ionization time -of-flight mass spectrometry (MALDI -TOF MS) method.[1] In last decade, MALDI -TOF MS has been widely used in clinical diagnostic laboratories for the rapid, simple and reliable identification of pathogen microorganisms, such as bacteria, yeasts and filamentous fungi.[7,8] The aim of this study is to evaluate the identification of dermatophytes with the MALDI TOF MS system in a routine microbiology laboratory.

Methods

In our study, dermatophytosis agents isolated from superficial body surfaces (scalp, nails, skin scrapings) that are sent to microbiology laboratory between 01 January 2015- 31 December 2020 with suspicion of dermatophytosis were included. Only the first isolate of the patients was included in the study, and repetitive samples were excluded. The samples were inoculated into two BBL™ Sabouraud Dextrose Agars with Chloramphenicol (Becton Dickinson, USA) and a BD Mycosel Agar with cycloheximide and chloramphenicol (Becton Dickinson, USA) plates. Direct microscopic examination was performed with 10-30% KOH for each sample. Species identification was made with conventional methods and accepted as reference method by examining colony morphology, microscopic features, characteristic features such as the ability to grow at different temperatures (28°C, 37°C) on potato dextrose agar (Becton Dickinson, USA), corn meal agar (Oxoid,), Sabouraud dextroz agar (Becton Dickinson, USA) and urease agar (Becton Dickinson, USA) for some isolates.

Species Identification by MALDI-TOF MS

The analysis of mass spectra were performed by a MALDI Biotyper Microflex LT (Bruker Daltonics GmbH, Bremen, Germany) mass spectrometer using the MALDI Biotyper FlexControl software package (version 3.1, Bruker Daltonik, GmbH), and the Filamentous Fungi Library 1.0 (Bruker Daltonik, GmbH) was used as the analysis database. In the MALDI-TOF system, protein extraction as a preliminary step was recommended for identification of microorganisms such as fungi, mycobacteria whose cell wall structures are different from bacteria. Ethanol formic acid extraction protocol were prepared according to the standart operating procedure (SOP) of the manufacturer. Mold colony in solid medium, was applied in our laboratory between 2015-2018. After 2019, the method of extraction from liquid medium has also been used in routine identification. Firstly, the solid medium was extracted and the results with score values above 2.0 were reported. For the isolates with an identification score below 2.0, the liquid medium was subcultured and the extraction method was applied two days later. Protein extraction was performed on 136 of the isolates from solid medium, and on 61 isolates by subculture to liquid medium. In 59 of these isolates, both extraction methods were applied simultaneously. For identification, the highest score obtained by spotted to the target plate at least four times was accepted for each isolate.[9] According to the manufacturer's recommendation, the results obtained with scores of ≥2.0 were accepted as 'reliable species identification', while the results obtained with scores between 1.7-2.0 were admitted as 'reliable identification at the genus level'. In our study, besides the manufacturer's recommendation, a threshold score of ≥1.7 was also evaluated. In the performance evaluation of the MALDI-TOF-MS system in all isolates, regardless of the protein extraction method, the highest score was accepted as the identification score.

Six dermatophyte isolates sent to the laboratory for mycological evaluation from the College of American Pathologist (CAP), an external quality control program, were used for quality control.

Protein Extraction from Solid Medium:

İsolates were inoculated on SDA medium at 28 °C for 2–5 days until the colonies reached a size of about 2 cm diameter. The mycelia were gently collected with a sterile inoculating loop, and the fungal material (approximately 2–3 mm in diameter) was suspended in a microtube containing 900 µL of anhydrous ethyl alcohol and 300 µL of HPLC sterile water. After a 10-min centrifugation step at 13,000 rpm, the pellet was resuspended in 50 µL of 70% formic acid.

After shortly vortexed, 50 µL of acetonitrile was added. The suspension was then centrifuged at 13,000 rpm for 2 min, and four spots of 1 µL of supernatant per isolate were deposited on the steel target plate. Last, the samples were covered with 1 µL of HCCA (α-cyano-4-hydroxycinnamic acid) matrix solution. The target was subsequently loaded into the MALDI Biotyper instrument and measurements were carried out in linear positive-ion mode within a mass range of 2-20 kDa.

Protein Extraction from Liqiud Medium

A small amount was inoculated from the mould colony on the agar surface into the tube containing 10 ml of Sabouraud dextrose broth (SDB). The liquid medium was rotated at room temperature at a constant speed of 20 rpm for two days in a rotator device (Rotator SB2 (Carl Roth GmbH & Co KG, Germany) until sufficient colonies were grown. At the end of the period, the tubes taken from the rotator were allowed to settle for 10 minutes. 1.5 mL was taken from the precipitate and transferred to the eppendorf tube. It was centrifuged at 13,000 rpm for 2 minutes. 1.2 ml of sterile distilled water was added to the pellet and centrifuged at 13,000 rpm for 2 minutes. The supernatant was removed and the same procedure was repeated. The standard ethanol formic acid extraction method mentioned above was applied by adding 300 µL of HPLC distilled water and 900 µL of EtOH on the pellet.

Statistical Analysis

SPSS 15.0 for Windows program was used for statistical analysis. Descriptive statistics were given as numbers and percentages for categorical variables, and mean, standard deviation, minimum and maximum for numerical variables. Rates in dependent groups were compared with the Mc Nemar Test. Statistical alpha significance level was accepted as $p<0.05$.

Results

Of the 138 isolates included in the study, 75 (54.3%) were obtained from female patients and 63 (45.7%) from male patients. The mean age of the patients was 43.0±21.9 (4-94). In the distribution of patients according to dermatophyte isolated body regions; 51 tinea unguium (nail), 26 tinea corporis (trunk), 21 tinea pedis (foot), 16 tinea capitis (scalp), 14 tinea cruris (inguinal), six tinea manum (hand), and three tinea facei (face) infections were determined. The species distribution of 138 isolates in the identification made with the conventional method is shown in Table 1.

When the threshold score of MALDI Biotyper system identification rates at the species level is ≥2.0; 17.6% were identified correctly by extraction from solid media and 59% by extraction from liquid media. When the threshold score value is reduced to 1.7; 61.8% of the isolates could be identified correctly by extraction from solid media, and all 61 isolates could be identified correctly by extraction from liquid media. When the performance of the system in all isolates was evaluated regardless of the extraction method, 38.4% of the isolates could be identified at the species level when the threshold score was accepted as ≥2.0, and 82.6% when the threshold score was reduced to ≥1.7 ($p<0.001$) (Tables 2, 3).

In the evaluation of extraction methods based on the reference method, the rate of correct identification by extraction from broth was found to be statistically significantly higher at both threshold scores than identification by extraction from solid medium. ($p<0.001$) (Tables 3-5).

In both extraction methods and in the collective evaluation of all isolates, when a score of ≥1.7 was accepted as the threshold value for identification at the species level, the correct identification rates were found to be significantly higher than the correct identification rates at the threshold score of ≥2.0 (Tables 3, 5).

Table 1. Species distribution of the isolates identified by conventional method

Species	n (%)
Trichophyton rubrum	100 (72.5)
Microsporum canis	27 (19.6)
Trichophyton tonsurans	5 (3.6)
Trichophyton spp.	3 (2.2)
Nannizzia gypsea (formerly Microsporum gypseum)	2 (1.4)
Trichophyton mentagrophytes	1 (0.7)
Total	138 (100)

Table 2. Distribution of isolates according to applied protein extraction method and MALDI-TOF MS threshold scores

Protein Extraction Method	The Number of Isolates Described to MALDI-TOF-MS Score		
	<1.7	1.7-2.0	≥2.0
Extraction from solid medium (n=136)	54	58	24
Extraction from broth (n=61)	0	25	36
All MALDI TOF MS identification (n=138)*	24	61	53

*The evaluation when the highest score is accepted, regardless of the extraction method.

Table 3. The performance evaluation of isolates according to applied protein extraction method and threshold score values

Method	n	Score ≥1.7		Score ≥2.0		p
		no identification	correct identification	no identification	correct identification	
Extraction from solid medium	136	52 (38.2)	84 (61.8)	112 (82.4)	24 (17.6)	<0.001
Extraction from broth medium	61	-	61 (100)	25 (41.0)	36 (59.0)	<0.001
All MALDI TOF MS *	138	24 (17.4)	114 (82.6)	85 (61.6)	53 (38.4)	<0.001

*The evaluation when the highest score is accepted, regardless of extration method.

Table 4. Score distributions of isolates in which two protein extraction methods were applied simultaneously

Protein Extraction Method	Distribution according to MALDI-TOF-MS Score (n)		
	<1.7	1.7-2.0	≥2.0
Extraction from solid medium (n=59)	28	22	9
Extraction from broth medium (n=59)	0	24	35

Discussion

In this study, by MALDI-TOF MS system, 82.6% of the isolates could be identified at the species level when the threshold score was accepted as ≥1.7. the rate of correct identification by extraction from broth was higher than identification by extraction from solid medium.

Clinical course in the dermatophyte infections can differ according to ecological source of agents. Moderate and chronic infection develops with anthropophilic species, whereas severe inflammation occurs in zoophilic and geophilic species.[5] Rapid and accurate identification of etiologic agents and route of infection is of great importance for providing specific antifungal therapy and preventing further spread to humans and/or other animals.[2] Dermatophytes are conventionally identified by examining morphological and microscopic features.[1] In this technique, colony formation, sporulation and additional physiological tests last approximately six weeks. Evaluation requires experienced personnel. This technic requires sufficient incubation time to ensure the development of fruiting bodies.[10] Some limitations due to lack of sporulation, intraspecies morphological variability, and interspecies morphological similarity makes the identification of the dermatophytes difficult by coventional methods.[1,11]

Over the past few years, matrix -assisted laser desorption/ionization time -of-flight mass spectrometry (MALDI -TOF MS) has been widely used in clinical diagnostic laboratories for the rapid, simple and reliable identification of microbial pathogens.[7,8] The success rate of the MALDI-TOF MS system for species identification of dermatophytes ranges from 13.5% to 100% in the literature.[12] The factors that cause this wide range of distribution are cultural characteristics (medium, incubation time), sample preparation (extraction method) and the system used (device, spectrum library).[9,12-15] Commercially, two systems (Bruker Biotyper (Bruker Daltonics, Bremen, Germany), and Vitek MS (bioMérieux, Marcy l'Etoile, France) systems) have been approved by the US Food and Drug Administration for the identification of bacteria and yeasts.[13]

Several identification techniques have been described in the MALDI TOF MS system, including simple direct transfer, with or without formic acid extraction, complate protein extraction with ethanol-formic acid and acetonitrile.[7,12,13] Methods used for the identification of bacteria and yeasts as simple direct transfer method to the target plate are unsuccessful in dermatophyte identification.[7] Fungi require additional processing steps to break the cell wall, extract proteins and inactivate the isolate.[1]

Species identification in MALDI-TOF MS systems is based

Tablo 5. Evaluation of the performance when two extraction methods were applied simultaneously

Method	Score ≥1.7		Score ≥2.0		p*
	no identification n (%)	correct identification n (%)	no identification n (%)	correct identification n (%)	
Extraction from solid medium (n=59)	28 (47.46)	31 (52.54)	50 (84.75)	9 (15.25)	<0.001
Extraction from broth medium (n=59)	-	59 (100)	24 (40.68)	35 (59.32)	<0.001

on the analysis of an isolate-specific peptide spectrum comparable to a microorganisms protein fingerprint. An unknown microorganism is identified by comparing its spectrum with reference spectra in a library.[12] Spectra in the filamentous fungi database of the MALDI Biotyper system are prepared by protein extraction from subcultures in broth.[13,16] In a study comparing solid and liquid media for the identification of non-Aspergillus mold in the same system, 15.4% of the isolates were identified at the species level, 57% at the genus level by extraction from solid media, while 57% and 34% were identified by extraction from broth, respectively.[15] In parallel, in our study, 17% of the 136 isolates extracted from solid media were identified at the species level and 42% at the genus level, while 59% of the 61 isolates subcultured to the broth were identified at the species level and 41% at the genus level. The difference in the two culture methods may be due to the change in the density of spore and hyphae structures due to colony growth in solid media, while mycelia develops with short-term incubation in liquid media.[15,16]

Since the species identification is strictly dependent on the number and quality of the spectra present in the library, the number of species in the databases can be increased by adding the spectra of standard strains to the data library in system. It has been reported in the studies that the identification rates made with in house expanded libraries are quite high compared to the identification made with commercial libraries.[12,14,17,18] Our analyses were performed without any library supplementation since in clinical management species identification.

Biotyper software generates a log score value range of 0-3 and recommends the scores of ^{3} 2.0 and ≥1.7 for species-level or genus-level identification, respectively.[13] Use of manufacturer-recommended spectral threshold scores results in relatively poor identification of fungi at the genus and species levels.[19] Hedayati et al.[19] reported that, of the 94 clinical isolates containing three genera and five species identified by the molecular method, 47% were able to be identified, assuming a threshold score of ≥2.0 in the MALDI Biotyper system. Recently, some researchers reported that lowering the threshold score value from 2.0 to 1.7 significantly increased the identification rates at the species level.[9,15-17,20] Schulthess et al.,[21] using the liquid broth and manufacturer-recommended interpretation criteria, genus and species identification rates were 78.3% and 54.2%, respectively. Reducing the species cutoff from 2.0 to 1.7 significantly increased species identification to 71.1%. Theel et al.[20] correctly identified 36.8 and 59.6 % of 171 isolates using the manufacturer's log score threshold 2.0 and a reduced log score threshold of 1.7, respectively. Karabicak et al.[17] improved the species level of identification from 31 to 89.7 % by lowering the log score threshold from 2.0 to 1.7. In our study, 38.4% of 138 dermatophte isolates were identified at the species level at a threshold score of 2.0 using the commercial library in the MALDI Biotyper system, while 82.6% were identified when the threshold score was reduced to 1.7. Our result supports the findings of the authors.[16,17,18,20,21] suggesting that lowering the identification threshold score will increase the efficiency of MALDI-TOF MS-based fungal identification.

There are some limitations in the species identification of dermatophytes with MALDI TOF MS systems. The most important of these is the inadequacy of existing commercial reference spectrum libraries. Another problem is that due to the similarity of molecular components it may be difficult to distinguish some species (such as *T mentagrophytes, T interdigitale,T tonsurans and T. rubrum, T. violaceum*).[1,17,22] In our study, due to similar morphology in three isolates, species identification could not be made with the conventional method, but identification could be made at the genus level. These three isolates; It could not be identified in a reliable score with the MALDI Biotyper system by extracting it from solid media.(one was as *T. rubrum* with a score of 1.72, and the other two were defined as *T. interdigitale* with the highest log scores of 1.3 and 1.6) Considering these throughputs, it seems that manufacturers should regularly update their data libraries to include more species and intraspecies variations for routine diagnosis of dermatophytes with the MALDI TOF MS system.[10]

The strengths of our study are the evaluation of high number of isolates compared to other publications in the literatüre and comparison of both solid and liquid media extractions. The fact that ITS sequencing was not available as the gold standard method could be considered as a limitation of our study. Besides, investigation of dermatophyte isolates with wider species diversity can produce more significant data.

Conclusion

In conclusion, we think that in the routine laboratory identification of dermatophytes, the application of a flow chart that includes protein extraction from solid media as the first step and performing protein extraction by subculture to liquid medium when the identification is not successful will be beneficial in terms of time and cost. In summary, the MALDI-TOF MS system is a fast, easy and inexpensive method and is an quite useful complement to the conventional methods.

Disclosures

Ethics Committee Approval: Non-Invasive Research Ethics Committee approval was obtained from Prof.Dr. Cemil Taşcıoğlu City Hospital Clinical Research Ethics Committee (16.08.2021,284).

Peer-review: Externally peer-reviewed.

Conflict of Interest: None declared.

References

1. Petrucelli MF, Abreu MH, Cantelli BAM, Segura GG, Nishimura FG, Bitencourt TA, et al. Epidemiology and diagnostic perspectives of dermatophytoses. J Fungi (Basel) 2020;6:310.
2. Begum J, Mir NA, Lingaraju MC, Buyamayum B, Dev K. Recent advances in the diagnosis of dermatophytosis. J Basic Microbiol. 2020;60:293–303. [CrossRef]
3. Gnat S, Łagowski D, Nowakiewicz A. Genetic predisposition and its heredity in the context of ıncreased prevalence of dermatophytoses. Mycopathologia 2021;186:163–76. [CrossRef]
4. Thakur R, Kalsi AS. Outbreaks and epidemics of superficial dermatophytosis due to trichophyton mentagrophytes complex and microsporum canis: global and ındian scenario. Clin Cosmet Investig Dermatol 2019;12:887–93. [CrossRef]
5. Gnat S, Łagowski D, Nowakiewicz A, Dyląg M. A global view on fungal infections in humans and animals: infections caused by dimorphic fungi and dermatophytoses. J Appl Microbiol. 2021 Mar 23. doi: 10.1111/jam.15084. [Epub ahead of print].
6. de Hoog GS, Dukik K, Monod M9 Packeu A, Stubbe D, Hendrickx M, Kupsch C, et al. Toward a novel multilocus phylogenetic taxonomy for the dermatophytes. Mycopathologia 2017;182:5–31. [CrossRef]
7. Intra J, Sarto C, Tiberti N, Besana S, Savarino C, Brambilla P. Genus-level identification of dermatophytes by MALDI-TOF MS after 2 days of colony growth. Lett Appl Microbiol 2018;67:136–43. [CrossRef]
8. Cassagne C, Normand AC, L'Ollivier C, Ranque S, Piarroux R. Performance of MALDI-TOF MS platforms for fungal identification. Mycoses 2016;59:678–90. [CrossRef]
9. Normand AC, Cassagne C, Gautier M, Becker P, Ranque S, Hendrickx M, et al. Decision criteria for MALDI-TOF MS-based identification of filamentous fungi using commercial and in-house reference databases. BMC Microbiol 2017;17:25. [CrossRef]
10. Robert MG, Cornet M, Hennebique A, Rasamoelina T, Caspar Y, Pondérand L, et al. MALDI-TOF MS in a medical mycology laboratory: on stage and backstage. Microorganisms 2021;9:1283.
11. Rudramurthy SM, Shaw D. Overview and update on the la boratory diagnosis of dermatophytosis. Clin Dermatol Rev 2017;1:3-11. [CrossRef]
12. L'Ollivier C, Ranque S. MALDI-TOF-based dermatophyte ıdentification. Mycopathologia 2017;182:183–92. [CrossRef]
13. Sanguinetti M, Posteraro B. Identification of Molds by Matrix-Assisted Laser Desorption Ionization-Time of Flight Mass Spectrometry. J Clin Microbiol 2017;55:369–79. [CrossRef]
14. Packeu A, De Bel A, l'Ollivier C, Ranque S, Detandt M, Hendrickx M. Fast and accurate identification of dermatophytes by matrix-assisted laser desorption ionization-time of flight mass spectrometry: validation in the clinical laboratory. J Clin Microbiol 2014;52:3440–3. [CrossRef]
15. Li Y, Wang H, Hou X, Huang JJ, Wang PC, Xu YC. Identification by matrix-assisted laser desorption ıonization-time of flight mass spectrometry and antifungal susceptibility testing of non-aspergillus molds. Front Microbiol 2020;11:922. [CrossRef]
16. Lau AF. Matrix-assisted laser desorption ıonization time-of-flight for fungal ıdentification. Clin Lab Med 2021;41:267–83.
17. Karabıçak N, Karatuna O, İlkit M, Akyar I. Evaluation of the bruker matrix-assisted laser desorption-ıonization time-of-flight mass spectrometry (MALDI-TOF MS) system for the ıdentification of clinically ımportant dermatophyte species. Mycopathologia 2015;180:165–71. [CrossRef]
18. da Cunha KC, Riat A, Normand AC, Bosshard PP, de Almeida MTG, Piarroux R, et al. Fast identification of dermatophytes by MALDI-TOF/MS using direct transfer of fungal cells on ground steel target plates. Mycoses 2018;61:691–7. [CrossRef]
19. Hedayati MT, Ansari S, Ahmadi B, Taghizadeh Armaki M, Shokohi T, Abastabar M, et al. Identification of clinical dermatophyte isolates obtained from Iran by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry. Curr Med Mycol 2019;5:22–6. [CrossRef]
20. Theel ES, Hall L, Mandrekar J, Wengenack NL. Dermatophyte identification using matrix-assisted laser desorption ionization-time of flight mass spectrometry. J Clin Microbiol 2011;49:4067–71. [CrossRef]
21. Schulthess B, Ledermann R, Mouttet F, Zbinden A, Bloemberg GV, Böttger EC, et al. Use of the Bruker MALDI Biotyper for identification of molds in the clinical mycology laboratory. J Clin Microbiol 2014;52:2797–803. 12. [CrossRef]
22. Shao J, Wan Z, Li R, Yu J. Species identification of dermatophytes isolated in China by matrix-assisted laser desorption ionisation-time-of-flight mass spectrometry. Mycoses 2020;63:1352–61. [CrossRef]