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BLINDED COMPARISON IN IDENTIFICATION OF YEASTS: CONVENTIONAL METHODS VS BD PHOENIX™

MAYALARIN TANIMLAMASININ KÖRLEME KARŞILAŞTIRILMASI: GELENEKSEL YÖNTEMLER İLE BD PHOENİX™

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ABSTRACT

Objective: In recent years, the epidemiology of fungal infections has begun to change due to many reasons. The correct identification of fungal agents by laboratories is very critical in terms of directing the treatment. The aim of this study is to compare yeast identifications obtained by conventional methods evaluated by a mycologist versus BD Phoenix™ 100 System (Becton Dickinson, USA).

Materials and Methods: Various fungal cultures of years 2020-2023 in Balıkesir Atatürk City Hospital were included. Isolated yeasts were double-blindly evaluated in identification by conventional methods (colony morphology, germ tube formation, urease positivity, cycloheximide resistance, morphology on cornmeal tween 80 agar, thermotolerance, ascospore existance, production of metallic green sheen on eosin-methylene blue agar, capsule formation and pellicle formation on sabouraud dextrose broth) versus BD Phoenix™ 100 System (Becton Dickinson, USA).

Results: A total of 353 yeast isolates obtained from different specimens were included. *Candida albicans, Candida parapsilosis* complex, *Candida glabrata* complex, *Candida tropicalis, Candida dubliniensis, Candida kefyr, Saprochaete capitata, Candida krusei, Candida lipolytica, Candida inconspicua* and *Trichosporon asahii* were identified in species-level by both methods, while *Rhodotorula* spp. and *Saccharomyces* spp. were compatibly identified in genus-level by conventional methods, but in species-level by automated method. Six isolates identified as *Candida* spp. conventionally were identified as *Candida sake, Candida zeylanoides, Candida lambica, Candida guilliermondii* complex by automated system. Five isolates could not be identified by both methods, and two isolates were defined as *S.capitata* by only the automated system. Overall, 92.9%, 99.4% and 100% compatibility were observed in species-level, genus-level and in top five species, respectively

Conclusions: Species-level identification takes an important place in order to guide treatments and laboratories should optimize their diagnostic capacities. Although conventional methods are generally unsuccessful in identification of rare species, total compatibility was observed for common species. Laboratories should evaluate their patient profile and capacities to choose either one of them.

Keywords: fungal infections, fungi, yeasts, identification, *Candida*

ÖZET

Amaç: Son yıllarda fungal enfeksiyonların epidemiyolojisi son yıllarda birçok sebebe bağlı olarak değişmeye başlamıştır. Fungal etkenlerin laboratuvarlarca doğru tanımlanabilmesi tedavinin doğru yönlendirilmesi açısından çok kritiktir. Bu çalışmadaki amaç mikoloji uzmanı + konvansiyonel yöntemlerle elde edilen sonuçlar ile Phoenix™ 100 sistemi (Becton Dickinson, ABD) tanımlamalarının karşılaştırılmasıdır.

Gereç ve Yöntem: Balıkesir Atatürk Şehir Hastanesi'nde 2020-2023 yılları arasındaki hastaların çeşitli mantar kültürleri çalışmaya dahil edildi. Üreyen maya mantarlarına çift-kör olarak koloni morfolojisi, germ tüp, üreaz, siklohekzimid direnci, mısır unu tween 80 agar, ısı toleransı, aside dirençli boyama ile askospor gözlenmesi, eozin-metilen-mavisi besiyerinde metalik refle görünümü, kapsül incelemesi ve Sabouraud dekstroz buyyonda zar oluşumu şeklindeki geleneksel yöntemler ve BD Phoenix™ 100 sistemi (Becton Dickinson, ABD) ile tanımlama yapıldı.

Bulgular: Çeşitli kültürlerden üretilmiş 353 maya mantarı çalışmaya dahil edildi. *Candida albicans, Candida parapsilosis* kompleks, *Candida glabrata* kompleks, *Candida tropicalis, Candida dubliniensis, Candida kefyr, Saprochaete capitata, Candida krusei, Candida lipolytica, Candida inconspicua ve Trichosporon asahii* tür düzeyinde; *Rhodotorula* spp. ve *Saccharomyces* spp. cins düzeyinde uyumlu tanımlanmıştır. Konvansiyonel olarak *Candida* spp. olarak tanımlanan 6 izolat, sistem tarafından *Candida sake, Candida zeylanoides, Candida lambica ve Candida guilliermondii* kompleks şeklinde tanımlandı. Beş izolat iki yöntemle de tanımlanamazken, konvansiyonel olarak tanımlanamayan iki izolat da sistem tarafından *S. capitata* olarak tanımlandı. Tür düzeyinde %92,9; cins düzeyinde %99,4; en sık enfeksiyon etkeni olan 5 tür için %100 uyum görüldü.

Sonuçlar: Tür düzeyinde doğru tanımlama tedavinin yönlendirilmesi açısından önemlidir ve laboratuvarlar doğru sonuç vermek için tanısal kapasitelerini optimize etmelidirler. Her ne kadar en sık kullanılan konvansiyonel yöntemler nadir türlerde görece yetersiz görülse de, sık görülen türlerde otomatize sistem ile arasında tam bir uyum söz konusudur. Laboratuvarlar, kapasitelerine ve hasta profillerine göre maliyet etkinlik analizi ile iki yöntemi de tercih edebilirler.

Anahtar Kelimeler: fungal enfeksiyonlar, mantarlar, mayalar, identifikasyon, *Candida*

INTRODUCTION

A shift of epidemiology in fungal infections (FIs) has been observed for a while by microbiology societies, that is because of not only usage of differrent antifungals, but also increasing awareness and evolved diagnostic methods which are extremely useful in identification of fungi (1). Invasive fungal infections (IFIs) usually progress with serious mortality and morbidity, especially in the population with various predisposing factors (1,2). Although the ranking differs accoding to geographic location, clinical

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status and underlying diseases, in overall, the "Top 5" of infection-causative yeasts are *Candida albicans, Candida glabrata* complex, *Candida parapsilosis* complex, *Candida tropicalis* and *Candida krusei*, respectively (1-3).

Species-level identification has a crucial role in treatment of FIs (particularly IFIs), since their antifungal resistance (AFR) profile may differentiate (4). Several guides such as The European Society of Clinical Microbiology and Infectious Diseases (ESCMID) were published to lead both diagnosis and treatment in accordance with species-level identification (4-8). Conventional methods are widely used in identification, but particularly in the last decade, phenotypic automated devices [BD Phoenix™ 100 System (Becton Dickinson, MA, USA); VITEK-2 System (bioMérieux, Marcyl'Etoile, France)] are adapted to identification of yeasts and even antifungal susceptibility testing (AFST). Furthermore, new technologies like matrix-assisted laser desorption ionization time of flight mass spectrometry (MALDI TOF MS) are being used for identification of yeasts with high success, alongside with just crawling-phased studies on AFST (9).

Due to serious cost of automated devices, cost and effect analysis is essential in choosing the way of identification, particularly for local laboratories. On the other hand, rapid and accurate results can be very effective on prognosis of patients. Thus, optimization of identification by laboratories requires performance data of exprerienced staff and automated devices. The aim of this study is to observe the compatibility of conventional methods and Phoenix™ 100 System (Becton Dickinson, MA, USA) in identification of clinical yeasts from various cultures.

MATERIALS AND METHODS

Sample

Routine cultures obtained from patients in all age groups of Balıkesir Ataturk City Hospital for a 3-year period (2020 – 2023) were included. Stardard mycological cultivation methods were used according to international guidelines (10,11). Only the first fungal positive samples or the first isolates in different episodes of the same patients were investigated.

Methods

All isolated yeasts were routinely identified by Phoenix™ 100 System Yeast ID kit (Becton Dickinson, MA, USA) for a 3-year period (2020 – 2023). The storaged (-20°C; tryptic soy broth with 15% glycerol) isolates were blindly coded, subcultured and subjected to identification by a medical mycologist using conventional methods, including colony morphology on Sabouraud dextrose agar (SDA) (RTA Laboratories, Kocaeli, Türkiye), germ tube formation, conidial morphology, urease positivity (RTA Laboratories, Kocaeli, Türkiye), cycloheximide resistance, morphology on cornmeal tween 80 agar (GBL Laboratories, Istanbul, Türkiye), thermotolerance, ascospore existance with acid-fast staining, producing metallic green sheen on eosin-methylene blue (EMB) agar (RTA Laboratories, Kocaeli, Türkiye), indian ink staining for capsule formation and observation for pellicle formation in sabouraud dextrose broth (SDB) (RTA Laboratories, Kocaeli, Türkiye). The study had two armed-double blinded structure and all isolates were studied dublicately. *Candida parapsilosis* ATCC 22019, *Candida krusei* ATCC 6258, *Candida albicans* ATCC 90028 and *Candida dubliniensis* CD36 were used as quality control strains.

Statistical analysis

This study is a prospective descriptive analysis, compatibility ratios were shared.

Ethical Approval

Approved by Ethical Board of Istanbul Medipol University. Date: 26.01.2023, Number: 90.

RESULTS

A total of 353 yeast isolates were included to the study. *Candida albicans* was the most common species (n=142), followed by *Candida parapsilosis* complex (n=128), *Candida glabrata* complex (n=19), *Candida tropicalis* (n=12), *Candida dubliniensis* (n=8), *Candida kefyr* (n=7), *Saprochaete capitata* (n=2), *Candida krusei* (n=2), *Candida lipolytica* (n=1), *Candida inconspicua* (n=1) and *Trichosporon asahii* (n=1), which were identified in species-level by both methods. *Rhodotorula* spp. (n=1) and *Saccharomyces* spp. (n=5) were identified in genus-level by conventional method, and in species-level by automated method. Conventionally identified five *Candida dubliniensis* and six *Candida* spp. strains were resulted as *Candida albicans* (n=5), *Candida sake* (n=1), *Candida zeylanoides* (n=1), *Candida lambica* (n=3) and, *Candida guilliermondii* complex (n=1) by the automated system. Five isolates could not be identified by both methods and two isolates that could not be identified conventionally were defined as *S. capitata* by the automated system. In overall, 92.9%, 99.4% and 100% compatibility were observed in species-level, genus-level and in top 5 most common species, respectively. All results were presented in Table 1 and Table 2, and examples of cornmeal tween 80 agar pictures were given in Figure 1.

DISCUSSION

There is an increasing trend of frequency in IFIs as one of major public health problems, since they also showed several high-profile outbreaks, including ongoing ones such as *Candida auris*. Their significantly high mortality and morbidity varies depending on various factors such as immune status of the host, fungal species and its AFR profile. Although the most frequent species remain standing

Table 1. Special features in conventional identification.

Species	SDA Morphology	GT	CM Morphology	AC	Urease	CR	45°C Growth*	ASC	EMB	CAP	PF
Candida albicans	Cream colored, smooth colonies	Yes	blastoconidia, pseudohyphae, some true hyphae, terminal chlamydospore	None	None	Yes	Yes	None	NA	None	None
Candida parapsilosis complex	Cream colored, lacy colonies	None	clustered blastoconidia, giant cell, curved pseudohyphae, giant cell in some species	None	None	None	NA	None	NA	None	None
Candida glabrata complex	Small, cream colored, smooth colonies	None	ovoid cells, pseudohyphae absent	None	None	None	NA	None	NA	None	None
Candida tropicalis	Cream colored	None	teardrop blastoconidia, long pseudohyphae, true hyphae absent, relatively bigger cells	None	None	None	NA	None	NA	None	Yes
Candida dubliniensis	Cream colored, smooth colonies	None	blastoconidia, pseudohyphae, very few true hyphae (some absent), terminal multi/uni-chlamydospore	None	None	Yes	None	None	NA	None	None
Candida kefyr	Cream colored, smooth colonies	None	elongated blastoconidia	None	None	Yes	NA	None	Metallic Green	None	None
Saccharomyces spp.	Cream colored, smooth colonies	None	ovoid cells, budding cells, pseudohyphae absent	None	None	None	NA	Few	NA	None	None
Saprochaete capitata	Cream colored, wrinkled colonies	None	true hyphae, pseudohyphae?, arthroconidia, annelloconidia?, blastoconidia?	None	None	Yes	NA	None	NA	None	Yes
Trichosporon asahii	powdery, irregularly wrinkled, crumblike colonies	None	true hyphae with arthroconidia, blastoconidia, pseudohyphae	Yes	Yes	None	NA	None	NA	None	Yes
Candida krusei	Dry, dull colonies	None	elongated cells, pseudohyphae, elongated “crossmatchstick” blastoconidia	None	None	None	NA	None	NA	None	Yes
Candida inconspicua/ norvegensis/ krusei	Dry, dull colonies	None	elongated/oval cells, pseudohyphae	None	None	None	NA	None	NA	None	None
Rhodotorula spp.	Reddish pink, moist, mucoid colonies	None	pseudohyphae absent	None	Yes	None	NA	None	NA	Yes	None
Candida lipolytica	Cream colored, smooth colonies	None	elongated blastoconidia, pseudohyphae, septated true hyphae	None	Yes	Yes	NA	None	NA	None	None
Not identified	Cream colored, smooth colonies	None	blastoconidia, few short pseudohyphae (some species)	None	None	Yes (2 sp.)	NA	None	NA	None	1 sp. (weak)
Not identified (*Geotrichum* spp?)	White colored, moist colonies	None	true hyphae with arthroconidia or annelloconidia?, blastoconidia absent	Yes	None	None	NA	None	NA	None	Yes

SDA: sabouraud dextrose agar medium; GT: Germ Tube; CM: cornmeal tween 80 agar; AC: arthroconidia; CR: cycloheximide resistance; ASC: ascospore; EMB: eosin methylene blue agar medium; CAP: Capsule; PF: pellicle formation. *45°C growth analysis were performed with 48h incubation on SDA media.

as front-runners (*Candida* and *Aspergillus*), rare or previously-unreported/under-reported infections have been increasingly observed in the last decades, including major outbreaks wordwide (1,3,12,13). Furthermore, dramatic elevations in incidence of antifungal-resistant infections like multidrug-resistant fungi were reported (3).

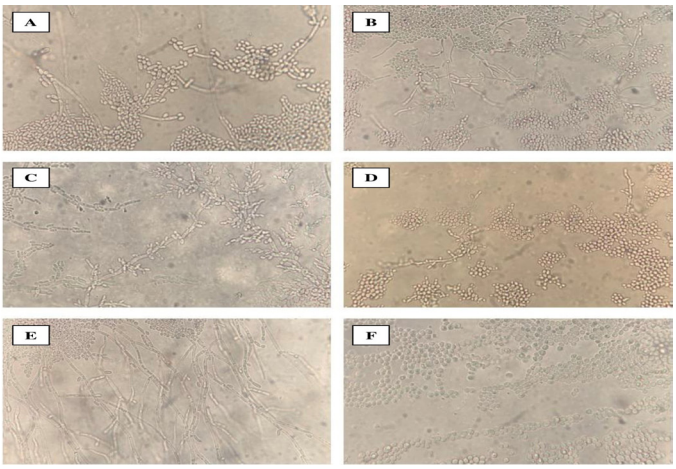

Figure 1: Examples of morphologies on cornmeal tween 80 agar after 72h of incubation at 25°C (40x).
(A: Candida inconspicua/norve-gensis/krusei; B: Candida parapsilosis complex; C: Candida lambica; D: Candida zeylanoides; E: Candida lipolytica; F: Saccharomyces cerevisiae)

Table 2. Comparison of conventional and Phoenix automated system identifications.

n	Conventional ID	BD Phoenix™
142	*Candida albicans*	*Candida albicans*
128	*Candida parapsilosis* complex	*Candida parapsilosis* complex
19	*Candida glabrata* complex	*Candida glabrata* complex
12	*Candida tropicalis*	*Candida tropicalis*
8	*Candida dubliniensis*	*Candida dubliniensis*
5	*Candida dubliniensis*	*Candida albicans*
7	*Candida kefyr*	*Candida kefyr*
5	*Saccharomyces* spp.	*Saccharomyces cerevisiae*
3	*Saprochaete capitata*	*Saprochaete capitata*
1	*Trichosporon asahii*	*Trichosporon asahii*
1	*Candida krusei*	*Candida krusei*
1	*Candida inconspicua/ norvegensis/krusei*	*Candida krusei*
1	*Rhodotorula* spp.	*Rhodotorula mucilaginosa*
1	*Candida lipolytica*	*Candida lipolytica*
1	*Candida lipolytica*	*Candida sake*
10	Not identified	Not identified
3	Not identified	*Candida lambica*
1	Not identified	*Candida sake*
1	Not identified	*Candida zeylanoides*
1	Not identified	*Candida inconspicua*
1	Not identified (*Geotrichum* spp?)	*Saprochaete capitata*
1	Not identified	*Candida guilliermondii* complex

Recent epidemiological changes are sourced by due to a variety of factors such as the widespread use of prophylactic/pre-emptive antifungals, increasing amounts and types of susceptible populations, changes in immunoeffective medications and chemotherapeutics, prolonged life expectations and increased elder populations in accordance with increased healthcare services (indwelling catheters, etc), and environmental changes (e.g.;global warming) (3). *Candida* spp. cause majority of FIs, most common species being *C.albicans*, *C.glabrata* complex, *C.tropicalis, C.parapsilosis* complex, and *C.krusei*. Although *C.albicans* has the first rank, increasing trend in incidence of non-albicans *Candida* infections is observed in most centers. In addition, non-candidal and non-cryptococcal yeasts/yeast-like organisms (e.g.; *S. capitata, T. asahii, Geotrichum* spp.) have been isolated more frequently (1,3,13). In a 16-year Italian survey of bloodstream infections, a significant trend was clearly towards non-*albicans Candida* infections, alongside with isolations of rare species such as *C. guilliermondii* complex, *C. lusitaniae, C. norvegensis, C. inconspicua, C. famata, C. intermedia, C. zeylanoides*, and *Candida pelliculosa* (14). In a 12-year data of various cultures from Turkey, a similar decrease was observed in the isolations of *C. albicans* was found, while there were siginificant rises in non-candidal yeasts, alongside with rare *Candida* spp. (1). These alterations have been mainly reported for the 15 years that can be observed even in wider international surveys (15). Especially in hospitalised patients, non-candidal and non-cryptococcal yeast infections are more frequently encountered, which created a question of therapeutic options along with their susceptibility patterns (13). This condition creates not only an epidemiologic change, but also significant alterations in AFR. Accurate diagnosis and rapid antifungal implementation were showed to have a crucial role in prognosis of patients, since several studies indicated that AFR had significant clinical reflection in respond to antifungal treatment (4). A wide meta-analysis on this topic stated “The 90-60 rule” that notified a huge difference in prognosis of infections in accordance with AFR (16). However, neither CLSI nor EUCAST could have defined clinical breakpoints for these so-called rare species, due to severe insufficiency of data (13). Thus, mycological societies published guidelines for “probable” efficient treatment in such cases, but it is clearly necessary and important to make species-level (at least genus level) idendification, since these species show wide variations in their susceptibility patterns. (e.g.; *Trichosporon* spp are intrinsically resistant to echinocandins) (8,13). Generally both ESCMID and Sanford guides recommend primarily echinocandin treatment in common yeast infections, on the contrary, echinocandin usage is recommended to be avoided against these rare species (5-7,13,17). So, accurate identification preferably to species level has direct

crucial role in patient treatment and this requirement seems to increase correlated with ongoing epidemiologic changes.

Acquired antifungal resistance is also a growing problem. Recently, fluconazole resistance in *C.glabrata* complex and *C.parapsilosis* complex, echinocandin resistance in *C.glabrata* complex and *C.krusei* and multidrug-resistance in *C.auris* are accepted as concerning issues. In addition, cryptic species (siblings) in the same species complex may also show different AFR profile (4). Generally, fluconazole resistance, with an ongoing increase, is <1% in *C. albicans* and approximately 11% in *C. glabrata* complex, <10% in *C. tropicalis* and varies as 2-5% in *C. parapsilosis* complex. The serious increasing pattern of AFR in *C. glabrata* complex and echinocandin resistance in *C. krusei* have been topics of severe concerns worldwide (4). In the first multicenter candidemia study from Turkey, 7.7% of *C. parapsilosis* complex isolates were fluconazole resistant, while unexpectedly low fluconazole resistance was observed in *C.glabrata* complex isolates and there was no fluconazole-resistant isolate of *C. tropicalis*, along with absence of any echinocandin-resistant strain of any species (18). However, in 2021, Sig et al (19) from Turkey stated the first unfortunate news of *C. glabrata* complex isolates that showed both phenotypic and molecular echinocandin resistance. In addition, the recent spreading threat of *C. auris* has changed the general perspective substantially, since 93%, 35% and 7% of *C. auris* isolates are resistant to fluconazole, amphotericin B and echinocandins, respectively (4). Thus, societies like ESCMID published different guidelines recommending different treatments according to the type of infection, antifungal susceptibilities and the infectious agent (5-8,13). In overall, type of species is strongly associated with outcome, not only because of their AFR, but also their individual virulence and features (20-22). A recent multinational European study reported that adherence to clinical guideline recommendations in treatments significantly improved the patient outcomes (23). Since all guidelines require at least genus-level idenfication, as a result, capacity of laboratories in identification to species-level has major importance for clinical outcome.

In our study, both traditional methods and automated device were used for identification, and 92.9% compatibility was found in species-level. Although this rate seems to be well, 10 isolates (2.8%) could not be identified by the device, while number of unidentified strains was fewer (n=7) among traditionally-identified ones. On the other hand, the device identified all species (except unidentified ones) to the species-level, but eighteen isolates could not be traditionally identified to such degree. Eight of these isolates were also noted as *Candida inconspicua/norvegensis/krusei* (Cannot be discriminated morphologically), and they were clinically reported with a warning expressing "probable fluconazole resistance" due to *C.krusei*. This condition, actually, conflicts with the expressions above, since species-level identification is the major goal. However, these isolates are rare species (like *Saccharomyces* spp. and rare *Candida* spp.) and according to guidelines, their treatment protocol does not still require such species-level identification (8,13). In addition, AFST is recommended in particularly IFIs for the rare non-*albicans Candida* spp. to lead the treatment (4). So, for now, it seems this is not a major issue that it does not signifcantly affect the antifungal treatment. On the other hand, conventionally-unidentified but device-identified eight species might have caused treatment differences. According to guidelines, in at least two cases among these species, *Saprochaete capitata* and *Candida inconspicua*, device identification result directly leaded the clinicans to different treatment protocols, since *Candida inconspicua* has the potential of fluconazole resistance and echinocandins are not accepted to be effective against *Saprochaete capitata* (8,13). The other six species are actually rare *Candida* spp. that require primarily echinocandin treatment, which is already the first choice of clinicians according to Sanford guide (17). It is notable here that genus-level identification had 99.4% compatibility.

C.albicans, C.glabrata complex*, C.tropicalis, C.parapsilosis* complex and *C.krusei* are the most frequent yeasts causing IFIs worldwide (Top 5) (1). In our study, there was a %100 agreement between two methods. These species generally cover nearly 90% of all infections (like our study) and they have relatively known antifungal susceptibility pattern (24). Acquired resistance are also most commonly observed in these species (as expected), so it has great importance to identify these species correctly. It was found, in overall, that with an experienced staff, traditional methods are enough to identify top 5, excluding laboratory workload and costs. Furthermore, five of *Candida dubliniensis* (n=13) isolates were identified as *C.albicans* by automated system, which we were not able to confirm by other methods. Nevertheless, *C.albicans* recently re-named by several reserchers as a complex, that includes siblings *C.dubliniensis* and *C.africana* (25). These species are actually hard to discriminate by phenotypic methods, except with successful performance of MALDI-TOF MS. Both siblings generally have same antifungal susceptibility pattern with a low/infrequent AFR, but it is even less common in *C.dubliniensis* (26). Thus, regarding both treatment and AFR, disagreement of our results seems to be a minor issue.

Novel identification methods like MALDI-TOF MS and molecular sequencing-based methods have significantly high costs and their fungal database are on updating process, although they provide good results in identification of yeasts. On the other hand, phenotyopic methods like BD Phoenix™ 100 (Becton Dickinson, MA, USA), VITEK-2 (bioMérieux, Marcyl'Etoile, France), API ID32C (bioMérieux, Marcyl'Etoile, France), AuxaColor (Bio-Rad, Marnes-la-Coquette, France) and RapID Yeast Plus (Thermo Fisher/

Remel, Lenexa, KS, USA) are easy-to-use, are relatively low cost and do not require higly experienced staff. Their performances were subjected to several studies that their results varied especially for uncommon yeasts (9,27). Generally, automated systems provide higher accuracy than manual ones, with slightly better performance of BD Phoenix™ 100 (Becton Dickinson, MA, USA), however MALDI TOF MS has become a game changer not only with its better performance, especially regarding very poor performances of automated systems for *C.auris*, but also its significantly much shorter turnaround time (28-31). It is crucial for a laboratory to be aware of the diagnostic capacity of conventional and automated methods [BD Phoenix™ 100 (Becton Dickinson, MA, USA) and VITEK-2 (bioMérieux, Marcyl'Etoile, France)]. It seems, joint application of different methods might be more beneficial in facilities without MALDI TOF MS device. We believe these new methods have also a role in expansion of infectious fungal species diversity, which might increase in the future concordant with improved spectrum database of these devices.

This study has a few limitations. First, we could not confirm the identifications by molecular methods. Molecular analysis is the gold standard in case of performance measurements, however our study focuses on correlation of commonly used identification methods in routine microbiology laborotories. Due to expensive nature, its requirement of experienced staff and advanced laboratory, molecular analysis cannot be performed by many local/ regional laboratories, thus, conventional and automated methods are in use in many facilities. Secondly, we did not have any collection of other uncommon species to test, such as *Geotrichum, Kodamaea, Malassezia, Pseudozyma, Sporobolomyces,* so we were not able to study. Finally, only a limited number of ATCC strains, which belonged to common species could be included. Unfortunately we were not able to provide reference strains of uncommon species.

CONCLUSION

Species spectrum of FI-causative fungi have been widening, with significant variations on AFR. Species-level identification takes an important place in order to guide treatments and laboratories should optimize their diagnostic capacities. Although MALDI-TOF MS has opened a new era, laboratories that are not able to provide this device, may still need conventional methods in addition to automated devices based on biochemical tests.

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