Matrix-assisted laser desorption/ionization time-of-flight mass spectrometric identification and antifungal susceptibility analysis of *Candida* species isolated from patients with invasive yeast infections in five university hospitals

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Abstract

In this multicenter study, we compared the performance of the Bruker Biotyper MS system and VITEK 2 YST systems for invasive yeast identification, investigated the distribution of isolated species, and evaluated the antifungal susceptibility profiles of *Candida albicans*, *Candida parapsilosis*, and *Candida tropicalis*. In cases of discrepant results lack of identification with either method, molecular identification techniques were employed. We tested 216 clinical isolates, and concordance between the two methods was observed for 192/216 isolates (88.9%). For five unidentified strains (2.3%), an internal transcribed spacer (ITS) sequencing approach was used. In brief, matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-ToF MS) provided short turnaround times and more reliable results than those of Vitek 2 YST. In Wuhan, *C. albicans*, *C. parapsilosis*, *Candida glabrata*, and *C. tropicalis* were the most common pathogens (93.0%) in patients with candidemia. *Cryptococcus neoformans* was mainly detected in cerebrospinal fluid samples (88.9%). *Trichosporon asahii* were all isolated from drainage fluids in the Surgery. *Candida albicans* was clearly susceptible to azoles, while *C. parapsilosis* and *C. tropicalis* displayed differences in susceptibility to azoles. Our findings provide a basis for the practical application of MALDI-ToF MS for identification and for the use of ATB FUNGUS 3 to characterize the susceptibility of *Candida* spp., thereby providing significant data for therapeutic decisions.

Keywords Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry · *Candida* · Yeast · Identification · Antifungal susceptibility test

Introduction

The frequency of invasive fungal infections has significantly increased in immunocompromised and critically ill patients,

and these infections have become a public health concern worldwide [1–4]. Infections due to pathogenic yeasts and yeast-like isolates in hospitalized patients are associated with high mortality rates and high costs [5–8].

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Candida species remain the most common pathogens among yeasts and yeasts-like isolates. Among the 15 reportedly infectious *Candida* species, *Candida albicans*, *Candida glabrata*, *Candida parapsilosis*, *Candida tropicalis*, and *Candida krusei* cause 95% of infections, and *C. albicans* is the predominant pathogen [8–10]. Early and accurate antifungal therapy would improve clinical outcomes [8–11]. Because antifungal susceptibility profiles differ substantially among species, rapid and accurate identification of yeasts and yeasts-like isolates enables determining effective antifungal therapeutic strategies.

Biochemical procedures are routinely used in clinical laboratories; however, they are time-consuming and yield inconclusive results. Molecular methods are expensive, tedious, technically demanding, and are not accessible in many diagnostic laboratories [12–16]. To address these limitations, many studies have reported that matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-ToF MS) is rapid, accurate, and sensitive, with a high resolution and high-quality detection range. It overcomes the limitations of traditional labor-intensive and time-consuming methods, greatly improving the fungal detection in clinical practice [17–35].

In this study, first, the efficiency of MALDI-ToF MS for routine identification of yeast was evaluated in real time. Second, we surveyed the local distribution of invasive yeast species. Third, we determined the antifungal susceptibility profiles of *Candida* species to assess the susceptibility rate in Wuhan city in a multicenter analysis.

Materials and methods

Yeast isolates

Yeast strains were collected between January and December 2015 from five university hospitals in Wuhan city. The strains were consecutively isolated from various sterile clinical samples obtained from non-AIDS inpatients and outpatients during the study duration, including the blood ($n = 74$), drainage fluids ($n = 39$), ascites fluid ($n = 38$), bronchoalveolar lavage fluid ($n = 19$), catheter ($n = 10$), cerebrospinal fluid ($n = 9$), pus, and other sterile samples ($n = 27$). Yeast isolates from the oral cavity, urine, stool, skin, gastrointestinal tract, sputum, and other sources were excluded. Strains of the same species from the same site for a particular patient, which were recovered at different time points, were considered duplicates and were also excluded. Finally, 216 strains were obtained and processed using standard microbiological procedures by inoculating yeasts onto Sabouraud dextrose agar (Detgerm Microbiology Technology, Guangzhou, China), followed by incubation at 35 °C for 24–48 h.

Microbial identification via VITEK 2

The VITEK 2 Compact (bioMérieux) with the YST Card was used with a routine laboratory system, and the results were interpreted in accordance with the manufacturer's instructions. *Candida parapsilosis* ATCC 22019 was used as a control.

Microbial identification via MALDI-ToF Bruker MS

Identification of all isolates was conducted using MALDI Biotyper in accordance with the protocol provided by Bruker Daltonics. The standard solution was prepared with 50% acetonitrile, 47.5% double-distilled water, and 2.5% trifluoroacetic acid and deposited on the prepared 48-well stainless steel target slides (Bruker Daltonics GmbH, Bremen, Germany) for peak correction. Thereafter, α -cyano-4-hydroxycinnamic acid (HCCA portioned, number 255344, Bruker Daltonik GmbH, Bremen, Germany) was vortexed thoroughly with 250 μ L of standard buffer prepared as the MALDI matrix for measurements. Pure yeast isolates (each from a single colony) were directly smeared onto the target slide, thereafter, 1 μ L of 70% formic acid (Sigma-Aldrich) directly onto the dried yeast spot that was dried once more prior to adding the 1 μ L HCCA matrix. For *Cryptococcus* species, besides the direct “on-slide testing” method, an additional protein extraction procedure was performed in accordance with the manufacturer's recommendation. For each spectrum, 240 shots in 40-shot steps from different positions of the target spot (automatic mode) were collected and analyzed. Internally, spectra were calibrated using *Escherichia coli* (ATCC 8739) ribosomal proteins every day. A threshold score of ≥ 2.000 was used for identification at the species identification, scores between 1.700 and 1.999 were used for identification at the genus level, and scores of < 1.700 indicated an unreliable identification.

Molecular identification method

For internal transcribed spacer (ITS) sequencing, the universal primers ITS1 (5'-TCCGTAGGTGAACCTGCGG-3') and ITS4 (5'-TCCTCCGCTTATTGATATGC-3') were used. The sequences were compared to the reference sequences available in GenBank by searching against the database using the nucleotide BLAST tool (blast.ncbi.nlm.nih.gov). The results were considered acceptable if the homology with other entries in the databases was $> 99.5\%$.

Criteria for the final species identification of isolates

When VITEK 2 YST and Bruker Biotyper yielded identical results for species-level identification, the result was considered final. In the case of discrepant results or unidentified status using either method, the sequencing results were used

Table 1 Comparison of results regarding yeast identifications via Bruker Biotyper and Vitek 2 Compact

Strains (n)	Correctly identified (%)	
	Bruker Biotyper	Vitek 2 Compact
<i>C. albicans</i> (100)	100.0	92.0
<i>C. parapsilosis</i> (35)	100.0	88.6
<i>C. tropicalis</i> (33)	100.0	87.9
<i>C. glabrata</i> (23)	100.0	100.0
<i>C. krusei</i> (3)	66.7	66.7
<i>C. haemulonii</i> (3)	100.0	100.0
<i>C. guilliermondii</i> (2)	50.0	50.0
<i>C. metapsilosis</i> (1)	0	0
<i>C. lusitaniae</i> (1)	0	0
<i>C. pelliculosa</i> (1)	0	0
<i>Cryptococcus neoformans</i> (12)	100.0	83.3
<i>Trichosporon asahii</i> (2)	100.0	50.0
Total (216)	97.7	88.9

Chi-square value = 13.34, *P* value = 0.000

for the final identification. For comparisons between conventional and MALDI-ToF MS identification methods, a chi-squared test was used.

Antifungal susceptibility analysis

The commercial antifungal susceptibility test strips ATB FUNGUS 3 (bioMérieux, La Balme-les Grottes, France), each comprising 16 pairs of cupules, including two growth control wells and the following five antifungal drugs at various concentrations: 5-flucytosine (4,16 µg/mL), amphotericin B (0.5–16 µg/mL), fluconazole (1–128 µg/mL), itraconazole (0.125–4 µg/mL), and voriconazole (0.06–8 µg/mL). The manufacturer's instructions, a suspension with a turbidity of 2 McFarland standards was prepared and 20 µL of this suspension was transferred to an ampule of ATB FUNGUS 3 Medium. Thereafter, 135 mL of the inoculated medium was transferred into each cupule. After incubation at 35 °C for 24 h for *Candida* species, the strips were read visually. In accordance with the manufacturer's instructions, the minimum inhibitory concentrations (MICs) were determined on the basis

of the growth scores for each of the cupules compared with those for the control cupules. For 5-flucytosine, fluconazole, itraconazole, and voriconazole, the MICs defined as sensitive to these antifungal agents were less than or equal to 4 µg/mL, 8 µg/mL, 0.125 µg/mL, and 1 µg/mL, respectively. Quality control was performed using the CLSI-recommended strain *C. parapsilosis* ATCC 22019.

Results

Final species identification of isolates

As shown in Table 1, the majority of invasive fungal infections were caused by *Candida* spp. (93.5%), including *C. albicans* (*n* = 100), *C. parapsilosis* (*n* = 35), *C. tropicalis* (*n* = 33), *C. glabrata* (*n* = 23), *C. krusei* (*n* = 3), *C. haemulonii* (*n* = 3), *Candida guilliermondii* (*n* = 2), *Candida metapsilosis* (*n* = 1), *Candida lusitaniae* (*n* = 1), and *Candida pelliculosa* (*n* = 1). After *Candida* spp., *Cryptococcus neoformans* (*n* = 12) was the most common, followed by *Trichosporon asahii*.

Performance of each identification method

The conventional VITEK 2 YST method accurately identified 192 of 216 (88.9%) isolates, and 211 of 216 (97.7%) isolates were accurately identified using Bruker Biotyper MS. For the remaining five (2.3%) isolates, the final identification was obtained via direct DNA sequencing. As shown in Table 2, five isolates were incorrectly identified by VITEK 2 YST, and one *C. pelliculosa* isolate was misidentified as *C. parapsilosis* on Bruker MS. One *C. metapsilosis*, one *C. guilliermondii*, one *C. lusitaniae*, and one *C. krusei* isolate were identified correctly; however, these results were unreliable, based on the low scores of < 1.7.

The origin and distribution of pathogenic yeasts

Of 216 patients with invasive fungal infections, 130 were male and 86 were female (male:female = 1.5:1), and the age range was 0.004 to 88 years (50.5 ± 22.6 years). Isolates were most frequently obtained from blood samples (74 strains, 34.3%), followed by drainage fluids (39 strains, 18.1%) and

Table 2 Details of the discordant results between Bruker Biotyper and Vitek 2 Compact

Species	Bruker Biotyper (score)	Vitek 2 Compact (ID lever %)
<i>C. metapsilosis</i>	<i>C. metapsilosis</i> (1.572)	<i>C. parapsilosis</i> (91%)
<i>Meyerozyma guilliermondii</i>	<i>C. guilliermondii</i> (1.602)	<i>C. famata</i> (59%)/ <i>C. guilliermondii</i> (41%)
<i>C. lusitaniae</i>	<i>C. lusitaniae</i> (1.515)	<i>C. famata</i> (50%)/ <i>C. lusitaniae</i> (50%)
<i>C. krusei</i>	<i>C. krusei</i> (1.637)	<i>C. lusitaniae</i> (50%)/ <i>C. krusei</i> (50%)
<i>C. pelliculosa</i>	<i>C. pelliculosa</i> (1.431)	<i>C. parapsilosis</i> (50%)/ <i>C. pelliculosa</i> (50%)

Fig. 1 The distribution of pathogenic yeasts in different samples. Others: pleural fluid, gastric fluid, dialysis fluids were included

ascites fluid (38 strains, 17.6%). The primary taxon isolated from cerebrospinal fluid was *Cryptococcus neoformans* (Fig. 1).

The distribution of invasive yeast infection sources was primarily surgery (75, 34.7%), followed by intensive care units (61, 28.2%) and medicine (54, 25.0%). *Cryptococcus neoformans* was primarily isolated from medicine (91.7%). *Trichosporon asahii* were all isolated from surgery. The three most frequent species isolated from surgical samples were *C. albicans*, *C. parapsilosis*, and *C. tropicalis*. The most frequent medicine isolates were *C. albicans*, *C. tropicalis*, and *Cryptococcus neoformans* (Fig. 2).

Susceptibility testing

In vitro susceptibility profiles of *Candida* spp. to four antifungal agents are listed in Table 3. According to the guidelines outlined in ATB FUNGUS 3 instruction manual, for the three main *Candida* spp. strains, high susceptibilities to 5-flucytosine and amphotericin B were detected; however, *C. albicans* displayed susceptibility to azoles, whereas *C. parapsilosis* and *C. tropicalis* exhibited significantly lower susceptibilities to these agents (Chi-square value = 501.0 and 486.0 respectively, *P* value = 0.000).

Fig. 2 The distribution of pathogenic yeasts in different units. Others: ENT, obstetrics, and gynecology were included

Table 3 In vitro susceptibilities of *C. albicans*, *C. parapsilosis*, and *C. tropicalis* to antifungal agents as determined by ATB FUNGUS 3 testing

Antibiotics	<i>C. albicans</i>			<i>C. parapsilosis</i>			<i>C. tropicalis</i>		
	S%	I%	R%	S%	I%	R%	S%	I%	R%
5-Fluorocytosine	100.0	0.0	0.0	100.0	0.0	0.0	100.0	0.0	0.0
Fluconazole	96.0	4.0	0.0	65.7	31.4	2.9	60.6	36.4	3.0
Itraconazole	98.0	2.0	0.0	71.4	28.6	0.0	66.7	30.3	3.0
Voriconazole	98.0	2.0	0.0	71.4	25.7	2.9	66.7	30.3	3.0

S% = percentage of susceptible strains; I% = percentage of intermediate strains; R% = percentage of resistant strains

Discussion

In this study, 216 yeasts isolated from sterile samples obtained at five Wuhan university hospitals were routinely identified via biochemical methods and the Bruker Biotyper MS system, which is considered a feasible alternative diagnostic tool for the rapid and accurate identification and differentiation of yeasts and yeast-like isolates [12, 13, 16–28]. The Vitek 2 YST system is one of the most widely used biochemical approaches in microbiology laboratories; it is advertised as being able to identify common and rare *Candida* species, and to perform better than the API ID32C and Auxacolor yeast identification systems for rare species identification [36, 37].

MALDI-ToF MS identification is based on the acquisition and comparison of the exclusive protein profiles of a strain to a reference library, using spectral pattern matching. This method is used to differentiate between closely related species or subspecies, which are typically difficult to differentiate using biochemical methods (e.g., *C. parapsilosis*/*C. metapsilosis*/*Candida orthopsilosis*) [23–25]. Our results are similar to those of previous reports, indicating that the five most common *Candida* species are *C. albicans* (46.3%), *C. parapsilosis* (16.2%), *C. tropicalis* (15.3%), *C. glabrata* (10.6%), and *C. krusei* (1.4%) [5–7, 38, 39]. For non-*C. albicans* *Candida* species, *C. parapsilosis* was more common than *C. tropicalis*, similar to the results obtained in studies from North America and in China [5, 39].

We identified more isolates using the Biotyper system than the Vitek 2 YST system for yeasts (211/216 [97.7%] versus 192/216 [88.9%]; $P = 0.000$). According to some studies [15, 30, 34, 40–43], it is difficult to definitively distinguish between *Candida famata* and *C. guilliermondii* by phenotypic methods. In this study, the Vitek 2 YST system erroneously identified *C. metapsilosis* as *C. parapsilosis*, similar to previous results [15, 30, 34, 42, 43]. The low identification scores for some strains might be explained on the basis of the use of Sabouraud dextrose agar, which is economical and widely used in routine microbiology laboratories [15, 29].

The present results are similar to those of previous studies [44–46] showing that *C. albicans*, *C. parapsilosis*, *C. glabrata*, and *C. tropicalis* are the most common pathogens

in patients with candidemia. *Cryptococcus neoformans* was primarily isolated from CSF samples (88.9%). *Trichosporon asahii* were all isolated from drainage fluids in the surgery.

In contrast to developed countries, multicenter and long-term antifungal surveillance data are lacking from China. Among the commercialized antifungal susceptibility test methods, ATB FUNGUS 3 is the most widely used method. The overall agreement between ATB and the broth microdilution method was 99.1% [47]. In the present study, *C. parapsilosis* and *C. tropicalis* showed significantly less sensitivities to azoles compared to *C. albicans*.

In summary, the present results provide clinically useful data related to the identification of yeast infections. The MALDI-ToF MS method provides short turnaround times and a standardized working protocol for yeast identification. An examination of the susceptibility of clinical isolates to azoles yielded high agreement, indicating that it is useful to confirm antifungal susceptibility profiles and is a practical approach to determine target drugs for therapy. The present study serves as an important basis for the practical application of MALDI-ToF MS for microbial identification and for routine use of ATB FUNGUS 3 for susceptibility surveillance of invasive yeast isolates.

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Compliance with ethical standards

Conflicts of interest The authors declare that they have no conflicts of interest.

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References

- Denning DW, Kibbler CC, Barnes RA (2003) British Society for Medical Mycology proposed standards of care for patients with invasive fungal infections. *Lancet Infect Dis* 3:230–240

2. Miceli MH, Diaz JA et al (2011) Emerging opportunistic yeast infections. *Lancet Infect Dis* 11:142–151
3. Schelenz S, Barnes RA, Barton RC, Cleverley JR, Lucas SB, Kibbler CC, Denning DW (2015) British Society for Medical Medical Mycology best practice recommendations for the diagnosis of serious fungal diseases. *Lancet Infect Dis* 15:461–474
4. Tuite NL, Lacey K (2013) Overview of invasive fungal infections. *Methods Mol Biol* 968:1–23
5. Wang H, Xiao M, Chen SC et al (2012) In vitro susceptibilities of yeast species to fluconazole and voriconazole as determined by the 2010 National China Hospital Invasive Fungal Surveillance Net (CHIF-NET) study. *J Clin Microbiol* 50:3952–3959
6. Zhang L, Xiao M, Wang H, Gao R, Fan X, Brown M, Gray TJ, Kong F, Xu YC, Warnock DW (2014) Yeast identification algorithm based on use of the Vitek MS system selectively supplemented with ribosomal DNA sequencing: proposal of a reference assay for invasive fungal surveillance programs in China. *J Clin Microbiol* 52:572–577
7. Wang H, Fan Y-Y, Kudinha T et al (2016) A comprehensive evaluation of the Bruker Biotyper MS and Vitek MS matrix-assisted laser desorption ionization–time of flight mass spectrometry systems for identification of yeasts, part of the National China Hospital Invasive Fungal Surveillance Net (CHIF-NET) study, 2012 to 2013. *J Clin Microbiol* 54:1376–1380
8. Pfaller MA, Diekema DJ (2007) Epidemiology of invasive candidiasis: a persistent problem. *Clin Microbiol Rev* 20:133–163
9. Lewis RE (2009) Overview of the changing epidemiology of candidemia. *Curr Med Res Opin* 25:1732–1740
10. Diekema D, Arbefeville S, Boyken L, Kroeger J, Pfaller M (2012) The changing epidemiology of healthcare-associated candidemia over three decades. *Diagn Microbiol Infect Dis* 73:45–48
11. Yapar N (2014) Epidemiology and risk factors for invasive candidiasis. *Ther Clin Risk Manag* 10:95–105
12. Marklein G, Josten M, Klanke U, Muller E, Horre R, Maier T, Wenzel T, Kostrzewa M, Bierbaum G, Hoerauf A, Sahl HG (2009) Matrix-assisted laser desorption ionization-time of flight mass spectrometry for fast and reliable identification of clinical yeast isolates. *J Clin Microbiol* 47:2912–2917
13. Chen JH, Yam WC, Ngan AH et al (2013) Advantages of using matrix-assisted laser desorption ionization–time of flight mass spectrometry as a rapid diagnostic tool for identification of yeasts and mycobacteria in the clinical microbiological laboratory. *J Clin Microbiol* 51:3981–3987
14. Eddouzi J, Lohberger A, Vogne C, Manai M, Sanglard D (2013) Identification and antifungal susceptibility of a large collection of yeast strains isolated in Tunisian hospitals. *Med Mycol* 51:737–746
15. Jamal WY, Ahmad S, Khan ZU et al (2014) Comparative evaluation of two matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-ToF MS) systems for the identification of clinically significant yeasts. *Int J Infect Dis* 26:167–170
16. Fraser M, Brown Z, Houldsworth M et al (2016) Rapid identification of 6328 isolates of pathogenic yeasts using MALDI-ToF MS and a simplified, rapid extraction procedure that is compatible with the Bruker Biotyper platform and database. *Med Mycol* 54:80–88
17. Marinach-Patrice C, Fekkar A, Atanasova R et al (2010) Rapid species diagnosis for invasive candidiasis using mass spectrometry. *PLoS One* 5:e8862
18. Bizzini A, Greub G (2010) Matrix-assisted laser desorption ionization time-of-flight mass spectrometry, a revolution in clinical microbial identification. *Clin Microbiol Infect* 16:1614–1619
19. Stevenson LG, Drake SK, Shea YR, Zelazny AM, Murray PR (2010) Evaluation of matrix-assisted laser desorption ionization-time of flight mass spectrometry for identification of clinically important yeast species. *J Clin Microbiol* 48:3482–3486
20. Bader O, Weig M, Taverne-Ghadwal L, Lugert R, Groß U, Kuhns M (2011) Improved clinical laboratory identification of human pathogenic yeasts by matrix-assisted laser desorption ionization time-of-flight mass spectrometry. *Clin Microbiol Infect* 17:1359–1365
21. Pinto A, Halliday C, Zahra M, van Hal S, Olma T, Maszewska K, Iredell JR, Meyer W, Chen SCA (2011) Matrix-assisted laser desorption ionization-time of flight mass spectrometry identification of yeasts is contingent on robust reference spectra. *PLoS One* 6:e25712
22. Theel ES, Schmitt BH, Hall L, Cunningham SA, Walchak RC, Patel R, Wengenack NL (2012) Formic acid-based direct, on-plate testing of yeast and *Corynebacterium* species by Bruker Biotyper matrix-assisted laser desorption ionization–time of flight mass spectrometry. *J Clin Microbiol* 50:3093–3095
23. Posteraro B, De Carolis E, Vella A et al (2013) MALDI-ToF mass spectrometry in the clinical mycology laboratory: identification of fungi and beyond. *Expert Rev Proteomics* 10:151–164
24. Sendid B, Ducoroy P, Francois N et al (2013) Evaluation of MALDI-ToF mass spectrometry for the identification of medically-important yeasts in the clinical laboratories of Dijon and Lille hospitals. *Med Mycol* 51:25–32
25. Buchan BW, Ledeboer NA (2013) Advances in identification of clinical yeast isolates by use of matrix-assisted laser desorption ionization–time of flight mass spectrometry. *J Clin Microbiol* 51:1359–1366
26. Bader O (2013) MALDI-ToF-MS-based species identification and typing approaches in medical mycology. *Proteomics* 13:788–799
27. Mancini N, De Carolis E, Infumari L et al (2013) Comparative evaluation of the Bruker Biotyper and Vitek MS matrix-assisted laser desorption ionization–time of flight (MALDI-ToF) mass spectrometry systems for identification of yeasts of medical importance. *J Clin Microbiol* 51:2453–2457
28. Lacroix C, Gicquel A, Sendid B, Meyer J, Accoceberry I, François N, Morio F, Desoubeaux G, Chandenier J, Kauffmann-Lacroix C, Hennequin C, Guitard J, Nassif X, Bougnoux ME (2014) Evaluation of two matrix-assisted laser desorption ionization-time of flight mass spectrometry (MALDI-ToF MS) systems for the identification of *Candida* species. *Clin Microbiol Infect* 20:153–158
29. Hamprecht A, Christ S, Oestreicher T, Plum G, Kempf VAJ, Götting S (2014) Performance of two MALDI-ToF MS systems for the identification of yeasts isolated from bloodstream infections and cerebrospinal fluids using a time-saving direct transfer protocol. *Med Microbiol Immunol* 203:93–99
30. Chao QT, Lee TF, Teng SH, Peng LY, Chen PH, Teng LJ, Hsueh PR (2014) Comparison of the accuracy of two conventional phenotypic methods and two MALDI-ToF MS systems with that of DNA sequencing analysis for correctly identifying clinically encountered yeasts. *PLoS One* 9:e109376
31. Panda A, Ghosh AK, Mirdha BR, Xess I, Paul S, Samantaray JC, Srinivasan A, Khalil S, Rastogi N, Dabas Y (2015) MALDI-ToF mass spectrometry for rapid identification of clinical fungal isolates based on ribosomal protein biomarkers. *J Microbiol Methods* 109:93–105
32. Singhal N, Kumar M, Kanaujia PK et al (2015) MALDI-ToF mass spectrometry: an emerging technology for microbial identification and diagnosis. *Front Microbiol* 6:791
33. Lévesque S, Dufresne PJ, Soualhine H, Domingo MC, Bekal S, Lefebvre B, Tremblay C (2015) A side by side comparison of Bruker Biotyper and VITEK MS: utility of MALDI-ToF MS technology for microorganism identification in a public health reference laboratory. *PLoS One* 10:e0144878
34. Stefaniuk E, Baraniak A, Fortuna M, Hryniewicz W (2016) Usefulness of CHROMagar *Candida* medium, biochemical methods–API ID32C and VITEK 2 compact and two MALDI-ToF MS systems for *Candida* spp. identification. *Pol J Microbiol* 65:111–114

35. Lee HS, Shin JH, Choi MJ, Won EJ, Kee SJ, Kim SH, Shin MG, Suh SP (2017) Comparison of the Bruker Biotyper and VITEK MS matrix-assisted laser desorption/ionization time-of-flight mass spectrometry systems using a formic acid extraction method to identify common and uncommon yeast isolates. *Ann Lab Med* 37:223–230
36. Meletiadiis J, Arabatzis M, Bompola M, Tsiveriotis K, Hini S, Petinaki E, Velegraki A, Zerva L (2011) Comparative evaluation of three commercial identification systems using common and rare bloodstream yeast isolates. *J Clin Microbiol* 49:2722–2727
37. Posteraro B, Efremov L, Leoncini E, Amore R, Posteraro P, Ricciardi W, Sanguinetti M (2015) Are the conventional commercial yeast identification methods still helpful in the era of new clinical microbiology diagnostics? A meta-analysis of their accuracy. *J Clin Microbiol* 53:2439–2450
38. Pfaller MA, Diekema DJ, Gibbs DL, Newell VA, Meis JF, Gould IM, Fu W, Colombo AL, Rodriguez-Noriega E, and the Global Antifungal Surveillance Group (2007) Results from the ARTEMIS DISK global antifungal surveillance study, 1997 to 2005: an 8.5-year analysis of susceptibilities of *Candida* species and other yeast species to fluconazole and voriconazole determined by CLSI standardized disk diffusion testing. *J Clin Microbiol* 45: 1735–1745
39. Pfaller MA, Diekema DJ, Gibbs DL, Newell VA, Ellis D, Tullio V, Rodloff A, Fu W, Ling TA, and the Global Antifungal Surveillance Group (2010) Results from the ARTEMIS DISK global antifungal surveillance study, 1997 to 2007: a 10.5-year analysis of susceptibilities of *Candida* species to fluconazole and voriconazole as determined by CLSI standardized disk diffusion. *J Clin Microbiol* 48: 1366–1377
40. Castanheira M, Wooslev LN, Diekema DJ et al (2013) *Candida guilliermondii* and other species of *Candida* misidentified as *Candida famata*: assessment by Vitek 2, DNA sequencing analysis, and matrix-assisted laser desorption ionization–time of flight mass spectrometry in two global antifungal surveillance programs. *J Clin Microbiol* 51:117–124
41. Jensen RH, Arendrup MC (2011) *Candida palmioleophila*: characterization of a previously overlooked pathogen and its unique susceptibility profile in comparison with five related species. *J Clin Microbiol* 49:549–556
42. Ninghui G, Bing W, Wei R et al (2015) Application of PCR and high-resolution melting for rapid identification of yeasts routinely isolated in a clinical microbiology laboratory. *Ann Clin Lab Sci* 45: 680–685
43. Pulcrano G, Iula DV, Vollaro A, Tucci A, Cerullo M, Esposito M, Rossano F, Catania MR (2013) Rapid and reliable MALDI-ToF mass spectrometry identification of *Candida non-albicans* isolates from bloodstream infections. *J Microbiol Methods* 94:262–266
44. Vasilyeva NV, Raush ER, Rudneva MV, Bogomolova TS, Taraskina AE, Fang Y, Zhang F, Klimko NN (2018) Etiology of invasive candidosis agents in Russia: a multicenter epidemiological survey. *Front Med* 12:84–91
45. Berrio I, Maidonado N, De Bedout C et al (2018) Comparative study for 147 *Candida* spp. identification and echinocandins susceptibility in isolates obtained from blood cultures in 15 hospitals in Medellín, Colombia. *J Glob Antimicrob Resist* 13:254–260
46. Li D, Xia R, Zhang Q, Bai C, Li Z, Zhang P (2017) Evaluation of candidemia in epidemiology and risk factors among cancer patients in a cancer center of China: an 8-year case-control study. *BMC Infect Dis* 17:536
47. Zhang L, Wang H, Xiao M, Kudinha T, Mao LL, Zhao HR, Kong F, Xu YC (2014) The widely used ATB FUNGUS 3 automated readings in China and its misleading high MICs of *Candida* spp. to azoles: challenges for developing countries' clinical microbiology labs. *PLoS One* 9:e114004