ORIGINAL ARTICLE MYCOLOGY

Evaluation of two matrix-assisted laser desorption ionization-time of flight mass spectrometry (MALDI-TOF MS) systems for the identification of *Candida* species

C. Lacroix[1], A. Gicquel[2], B. Sendid[3], J. Meyer[4], I. Accoceberry[5], N. François[3], F. Morio[2], G. Desoubeaux[6], J. Chandenier[6], C. Kauffmann-Lacroix[7], C. Hennequin[8], J. Guitard[8], X. Nassif[4] and M.-E. Bougnoux[4]

1) *Service de Mycologie-Parasitologie, Hôpital Saint-Louis AP-HP, Université Paris Diderot Paris 7, Paris,* 2) *Laboratoire de Mycologie-Parasitologie CHU, Nantes,* 3) *Laboratoire de Mycologie-Parasitologie CHRU, Inserm U995, Lille,* 4) *Service de Microbiologie, Hôpital Necker-Enfants Malades AP-HP, Université Paris-Descartes, Paris,* 5) *Laboratoire de Mycologie-Parasitologie CHU, Bordeaux,* 6) *Laboratoire de Mycologie-Parasitologie CHU, Tours,* 7) *Laboratoire de Mycologie-Parasitologie CHU, Poitiers, and* 8) *Laboratoire de Mycologie-Parasitologie Hôpital Saint-Antoine AP-HP, Paris, France*

Abstract

Candida spp. are responsible for severe infections in immunocompromised patients and those undergoing invasive procedures. The accurate identification of *Candida* species is important because emerging species can be associated with various antifungal susceptibility spectra. Conventional methods have been developed to identify the most common pathogens, but have often failed to identify uncommon species. Several studies have reported the efficiency of matrix-assisted laser desorption ionization-time of flight mass spectrometry (MALDI-TOF MS) for the identification of clinically relevant *Candida* species. In this study, we evaluated two commercially available MALDI-TOF systems, Andromas™ and Bruker Biotyper™, for *Candida* identification in routine diagnosis. For this purpose, we investigated 1383 *Candida* isolates prospectively collected in eight hospital laboratories during routine practice. MALDI-TOF MS results were compared with those obtained using conventional phenotypic methods. Analysis of rDNA gene sequences with internal transcribed regions or D1-D2 regions is considered the reference standard for identification. Both MALDI-TOF MS systems could accurately identify 98.3% of the isolates at the species level (1359/1383 for Andromas™; 1360/1383 for Bruker Biotyper™) vs. 96.5% for conventional techniques. Furthermore, whereas conventional methods failed to identify rare or emerging species, these were correctly identified by MALDI-TOF MS. Both MALDI-TOF MS systems are accurate and cost-effective alternatives to conventional methods for mycological identification of clinically relevant *Candida* species and should improve the diagnosis of fungal infections as well as patient management.

Keywords: Andromas system, Bruker Biotyper system, *Candida* spp. identification, conventional methods, MALDI-TOF mass spectrometry, molecular identification
Original Submission: 17 December 2012; **Revised Submission:** 27 January 2013; **Accepted:** 24 February 2013
Editor: E. Roilides
Article published online: 7 March 2013
Clin Microbiol Infect 2014; **20:** 153–158
10.1111/1469-0691.12210

Corresponding author: M.-E. Bougnoux, Unité de Parasitologie-Mycologie, Service de Microbiologie, Hôpital Necker-Enfants Malades, 146 rue de Sèvres, Paris 75015, France
E-mail: marie-elisabeth.bougnoux@nck.aphp.fr

Introduction

Candida yeasts are responsible for frequent and severe infectious complications in immunocompromised patients and those undergoing invasive procedures [1]. While six species of *Candida* (i.e. *Candida albicans*, *Candida tropicalis*, *Candida glabrata*, *Candida parapsilosis*, *Candida kefyr* and *Candida krusei*) are currently considered the leading aetiological agents of candidiasis, several other *Candida* species have been reported as significant pathogens [2]. At present, up to 40 distinct *Candida* species have been shown to infect humans [3]. Identification of these new clinically relevant species has been made possible due to the development of molecular identification [4–6]. An accurate identification of *Candida* species is of major importance, as several of these emerging species have distinct antifungal susceptibility profiles and/or could be

associated with specific clinical settings [7]. Conventional methods routinely used in a mycology laboratory for identification of yeasts traditionally included a combination of phenotypic methods, including morphological and biochemical features. However, these methods have been developed to identify only the most common pathogens and often failed to identify emerging/cryptic species.

Indeed, molecular taxonomic studies have revealed several new *Candida* species among phenotypically distinct species [8–10]. For instance, recent studies have revealed that some clinical isolates previously identified as *C. glabrata* using conventional methods actually belong to the closely related species *Candida nivariensis* and *Candida bracarensis* [5]. Similarly, some isolates formerly misidentified as *C. parapsilosis* actually represent isolates of *Candida metapsilosis*, *Candida orthopsilosis* or *Lodderomyces elongisporus* [9,10]. Molecular identification, by sequencing genomic regions of the rDNA genes, is considered the 'reference standard' for accurate identification of *Candida* at the species level [4–6]. However, sequencing of clinical isolates is still time-consuming and not standardized; it cannot therefore be used as a routine method for yeast identification.

In recent years, several studies have reported the performance of matrix-assisted laser desorption ionization-time of flight mass spectrometry (MALDI-TOF MS) for the identification of clinically relevant *Candida* species [11–14]. This method could represent a valid and rapid alternative to both conventional and molecular methods for yeast identification in routine practice. Currently, there are four commercial systems available: the MALDI Biotyper (Bruker Daltonics, Bremen, Germany), the AXIMA@SARAMIS database (AnagnosTec, Potsdam, Germany and Shimadzu, Duisburg, Germany), and lately the Andromas (Andromas, Paris, France) and VITEK MS systems (bioMérieux, Marcy l'Etoile, France)[15]. However, this method has not yet been comparatively evaluated in a large number of clinical isolates concomitantly with accepted standards.

In this study, we compared the identification efficiency of two MALDI-TOF MS systems, Andromas™ and Bruker Maldi Biotyper™, with that of conventional methods on a large panel of 1383 clinical isolates of *Candida* obtained from eight French university hospital laboratories. Our results demonstrate that both techniques are reliable, the Andromas™ strategy being easier to perform in clinical microbiology laboratories as it does not require protein extraction.

Materials and Methods

Candida isolates

The *Candida* isolates included in this study have been prospectively isolated over a 2-month period from patients hospitalized in haematology, intensive care and renal transplant units of eight French university hospitals. All isolates, from both superficial and deep sites, were collected by the laboratories in sequential order without any selection criteria. Isolates were cultured on chromogenic media (CHROMagar Candida™ medium (Becton-Dickinson, Le Pont-de-Claix, France)) for 48 h at 35°C. Growing colonies were checked for mixed cultures and transferred to a new plate. The isolates growing in pure culture were identified routinely in each of the eight laboratories by conventional methods using the same protocol of identification described below. The isolates were cryopreserved at −80°C until use. In total, 1383 *Candida* isolates have been included.

Conventional methods

Isolates identified as *C. albicans* on CHROMagar Candida™ media were screened with the Bichro-Dubli™ test (Sofibel, Levallois-Perret, France) to identify *C. dubliniensis* [10–12]. Isolates suspected to be *C. glabrata* or *C. krusei* were tested using the RTT Glabrata™ (Sofibel) and Krusei-color™ (Sofibel) methods, respectively [10,13]. All other isolates were identified using ID32C AUX™ identification strips (bioMérieux), according to the manufacturer's instructions. We defined an identification score of $\geq$98% by the ID32C AUX™ method as the threshold for accurate identification at the species level.

MALDI-TOF MS

The 1383 isolates were identified blindly by the two MALDI-TOF systems in two independent laboratories.

Analysis by the MALDI-TOF Andromas™ system

Part of a pure colony of each pure culture was transferred to an Andromas™ target well using a disposable loop, overlaid with 1 μL of formic acid 70% (v/v) and dried. One microlitre of a saturated solution of α-cyano-4-hydroxycinnamic acid, 50% acetonitrile and 2.5% trifluoroacetic acid (HCCA; matrix solution) was then added and allowed to co-crystallize with the sample. Samples were processed in a MALDI-TOF spectrometer (Andromas™, Paris, France) with the control software [12,16–18]. Positive ions were extracted with an accelerating voltage of 20 kV in linear mode. The spectra were analysed and compared in an *m/z* range of 3000–20 000 Da with Andromas software (Andromas™). The Andromas software identified the number of common peaks between the spectra of the tested isolate and the species-specific spectral fingerprints of the reference strains in the database. Briefly, for each isolate, all peaks with an intensity >0.1 were retained and were compared with the peaks for the species-specific spectral fingerprints of each reference strain, taking into account possible variations in the *m/z* value of $\pm$10. Subsequently, the

percentage of common peaks was obtained using the formula: 100 × (number of common peaks between the tested isolate and the species-specific spectral fingerprint/total number of peaks specific to the species-specific spectral fingerprint). An acceptable identification was given if the percentage of common peak was ≥70% of those of a species-specific profile in the database. A 10% difference between the first two species diagnostics having the best match in the database is also required to give species identification.

Analysis by the MALDI-TOF Biotyper™ system

Samples were prepared according to the manufacturer's instructions. Briefly, two to three yeast colonies were transferred using a 10-μL inoculating loop into 300 μL of distilled water and 900 μL of ethanol (Sigma-Aldrich, St Louis, MO, USA). The suspension was pelleted after centrifugation at 6000 *g* for 3 min, dried, and reconstituted in 50 μL of 70% formic acid (Sigma-Aldrich). After incubation for 30 min, 50 μL of acetonitrile (Sigma-Aldrich) was added. The suspension was then centrifuged at 6000 *g* for 3 min. A volume of 1.5 μL of the supernatant was applied to a 96-spot Anchorchip™ target (Bruker Daltonics, Inc., Bremen, Germany) plate and dried. A saturated solution of 1.8 μL of MALDI matrix (HCCA; Bruker Daltonics, Inc., Bremen, Germany) was applied to the fungal smear and dried. Measurements were performed with a Microflex™ mass spectrometer (Bruker Daltonics, Wissembourg, France) using the FlexControl™ software (version 3.3.108.0). Mass spectra ranging from 2000 to 20 000 Da were acquired in a linear, extraction mode with positive polarity. The spectrum was imported into the Biotyper software (version 3.0; Bruker, Karlsruhe, Germany). The generated spectrum of biomarkers for each sample was then compared with reference spectra in the Bruker library. Identifications from MALDI-TOF were classified using modified score values proposed by the manufacturer: a score ≥2 indicated identification to the species level; a score between 1.7 and 1.99 indicated identification to the genus level; and a score of <1.7 indicated no identification.

Molecular identification

Molecular identification of isolates was performed by amplification and sequencing of the ITS rDNA regions without a prior DNA extraction step (colony-PCR), as previously described [19]. Amplification of the ITS rDNA was achieved using the universal primers ITS1 (TCCGTAGGTGAACC TGCGG) and ITS4 (TCCTCCGCTTATTGATATGC) [20]. Nucleotide sequences were assembled using Seqscape software (Applied Biosystems, Foster City, CA, USA.). Molecular identification was achieved by comparing sequence data with the GenBank database using the BLAST algorithm (http://blast.ncbi.nlm.nih.gov/Blast.cgi) or with the MycoBank database (http://www.cbs.knaw.nl/collections/BioloMICSSequences.aspx). For some isolates, identification was also confirmed by amplification of the D1-D2 region of the 28S rDNA using NL1 (GCATATCAATAAGCGGAGGAAAAG) and NL4 (GGTCCGTGTTTCAAGACGG) primers [20]. A per cent similarity of ≥98% between the unknown sequence and the closest matching sequence from the reference database was used as the criterion to classify an isolate to the species level.

Criteria for final species identification of isolates

All isolates were subjected to conventional identification methods, and MALDI-TOF Andromas™ and Biotyper™ systems.

The final species identification of isolates was performed as follows: an accurate identification of the species *C. albicans*, *C. dubliniensis*, *C. krusei* and *C. tropicalis* was confirmed if the three methods (conventional methods, and Andromas™ and Biotyper™ MALDI-TOF systems) yielded the same species identification. When discrepant results were observed for the three methods or when only two out of three methods yielded identification, ITS rDNA and/or D1-D2 sequencing was performed and was considered as the final species identification. For the remaining *Candida* species, the final identification of isolates was by molecular identification, regardless of the results obtained by the three methods.

Results

Final species identification of isolates

We analysed 1383 isolates obtained from the eight diagnostic laboratories during routine practice. For 950 isolates (68.7%), the use of conventional methods and the two MALDI-TOF systems resulted in the accurate identification of 836 isolates of *C. albicans*, 31 of *C. dubliniensis*, 44 of *C. krusei* and 39 of *C. tropicalis*. For the 433 (31.3%) remaining isolates, final identification was obtained by rDNA sequencing. In all, final identification at the species level was obtained for all the 1383 isolates that encompassed 20 *Candida* species (Table 1).

Performance of each identification method

Biochemical methods could accurately identify 1336 of the isolates (96.5%). Forty-four isolates (3.5%) were misidentified and three isolates were not identified (two *C. albicans* and one *C. bracarensis*). Isolates belonging to the species complexes were misidentified or not identified by conventional biochemical methods. Similarly, none of the uncommon species, *C. fabianii* (*n* = 17), *C. sphaerica* (*n* = 1), *C. fermentati* (*n* = 3)

TABLE 1. Discrepancies and errors in conventional methods and MALDI TOF MS systems (Andromas™ and Bruker Biotyper™) for identification of *Candida* species

	No. of isolates					
			MALDI-TOF identification			
	Conventional identification		Andromas™		Biotyper™	
Final identification	No identification	Misidentification	No identification	Misidentification	No identification	Misidentification
C. albicans (n = 838)	2	0	0	0	0	0
C. dubliniensis (n = 32)	0	0	1	0	0	0
C. glabrata (n = 176)	0	0	0	1	0	0
C. bracarensis (n = 1)	1	0	0	0	1	0
C. tropicalis (n = 81)	0	1	0	0	0	0
C. parapsilosis (n = 78)	0	3	0	0	0	0
C. orthopsilosis (n = 3)	0	3	0	0	0	0
C. krusei (n = 44)	0	0	0	0	0	0
C. kefyr (n = 60)	0	1	O	0	O	0
C. lusitaniae (n = 21)	0	4	0	0	0	0
C. inconspicua (n = 12)	0	8	0	0	0	0
C. guilliermondii (n = 9)	0	0	0	0	0	0
C. fabianii (n = 17)	0	17	17	0	13	4
C. intermedia (n = 3)	0	2	0	0	0	0
C. magnoliae (n = 1)	0	0	1	0	0	0
C. sphaerica (n = 1)	0	1	0	0	1	0
C. fermentati (n = 3)	0	3	2	1	2	1
C. ethanolica (n = 1)	0	1	0	1	1	0
C. utilis (n = 1)	0	0	0	0	0	0
C. lipolytica (n = 1)	0	0	0	0	O	0
Total (n = 1383)	3	44	21	3	18	5

and *C. ethanolica* (n = 1), was identified correctly by biochemical methods.

The MALDI-TOF MS Andromas™ system was able to accurately identify 1359 isolates (98.2%). Three isolates (0.24%) were misidentified: one *C. parapsilosis* instead of a *C. glabrata*, one *C. albicans* instead of a *C. fermentati*, and one *C. guilliermondii* instead of a *C. ethanolica*. Twenty-one isolates belonging to four species were not identified because these species were not included in the database (*C. fabianii* and *C. magnoliae*) or because of no spectral acquisition (one *C. dubliniensis* and two *C. fermentati*).

The MALDI-TOF MS BioTyper™ system could accurately identify 1360 isolates (98.2%). Five isolates (0.36%) were misidentified: two *C. parapsilosis* instead of *C. fabianii*, one *C. albicans* instead of *C. fabianii*, one *C. kefyr* instead of *C. fabianii*, and one *C. guilliermondii* instead of *C. fermentati*. Eighteen isolates belonging to five species could not be identified because these species were not included in the database (*C. bracarensis, C. sphaerica*, *C. ethanolica, C. fermentati* and *C. fabianii*).

Discrepancies and errors in the three systems of identification (biochemical methods, and Andromas™ and Biotyper MALDI-TOF MS systems™) are given in Table 1.

Discussion

This study shows that the MALDI-TOF MS Andromas™ and Biotyper™ systems appear to be fast and powerful techniques for the accurate identification of *Candida* species routinely isolated in medical laboratories, both providing a high rate of correct identification at the *Candida* species level (98.2% each). Both MALDI-TOF systems appear more reliable than the conventional phenotypic methods that are used routinely (96.5% of correct identification). These findings are in accordance with recent studies showing comparable results, with yeast identification rates of 97.6% with Biotyper 2.0 and 96.1% with Saramis MALDI-TOF systems [11,21].

Importantly, we demonstrated an excellent agreement between identifications by MALDI-TOF MS and those provided by rDNA sequencing. Indeed, only three (0.7%) and five (1.1%) isolates of the 433 requiring molecular identification were misidentified, respectively, by MALDI-TOF MS Andromas™ and Biotyper™ systems, while 44 (10.2%) misidentifications were obtained using conventional methods. Unlike previous studies, in which molecular identification was performed only when discrepant results were noted between the compared methods [11,21], all identifications at the species level (except for *C. albicans, C. dubliniensis, C. krusei* and *C. tropicalis* with an ID32C score of $\geq$98%) have been confirmed by molecular methods in the present study. Therefore, our results strongly highlight the accuracy of two MALDI-TOF systems for *Candida* species identification. Additionally, the two MALDI-TOF MS systems were able to discriminate isolates belonging to species complexes at the species level. However, most of the rare *Candida* species were not identified using MALDI-TOF MS. Indeed, specific spectra were not included in the databases at the time of our study.

Once the spectra were included (on obtaining firm identification of these isolates by sequence analysis), subsequent identification of these species resulted in a perfectly matching profile.

The MALDI-TOF database can be updated when a species is absent from the database; however, the high level of expertise required for molecular identification is sometimes a pitfall. In our study, *C. fabianii* identification was difficult because the genomic databases did not contain the complete ITS region of *C. fabianii* as a single nucleotide sequence and misidentification with other yeast species (e.g. *Lindnera* (*Pichia*) *mississippiensis*) could have occurred. Partial sequencing of the *EF1*α gene facilitated correct identification of this species [22]. One limitation of mycological identification from clinical samples is in the analysis of mixed cultures; in particular, distinct species show similar colours when using chromogenic media. We encountered this situation in our study in the analysis of a mixed culture containing both *C. albicans* and *C. dubliniensis*. This mixture could only be detected by molecular analysis; biochemical and MALDI-TOF MS analysis could only identify one of the two species. One improvement that can be easily incorporated into MALDI-TOF MS would be to screen several colonies presenting the same colour from the same culture plate.

Notably, the two MALDI-TOF MS systems use distinct sample preparation protocols. An initial extraction step of the yeast is necessary for the Biotyper protocol, which is time-consuming; however, when using the Andromas™ system, cell lysis is performed directly on the steel plate. This difference in the extraction steps of the two systems could clearly have an impact on identification speed in routine laboratory practice. However, some studies have recently reported that spectra obtained by on-target lysis with the Bruker system can be sufficient for identification of yeasts [23,24].

In conclusion, the present study indicates that the MALDI-TOF MS systems Andromas and Biotyper are reliable and cost-effective techniques for the identification of clinically relevant *Candida* species. Identification would be faster and more precise than when using conventional non-automated methods. Furthermore, spectral databases should be regularly updated by suppliers to improve identification rates. The availability of MALDI-TOF MS in the clinical laboratory will improve the diagnosis of fungal infections and patient management, allowing appropriate antifungal therapy.

Acknowledgement

This study was supported by a grant from Gilead Pharmaceuticals.

Transparency Declaration

XN and MEB are shareholders of Andromas SAS.

References

1. Pfaller MA, Diekema DJ. Epidemiology of invasive candidiasis: a persistent public health problem. *Clin Microbiol Rev* 2007; 20: 133–163.
2. Hazen KC. New and emerging yeast pathogens. *Clin Microbiol Rev* 1995; 8: 462–478.
3. Miceli MH, Diaz JA, Lee SA. Emerging opportunistic yeast infections. *Lancet Infect Dis* 2011; 11: 142–151.
4. Chen YC, Eisner JD, Kattar MM *et al.* Identification of medically important yeasts using PCR-based detection of DNA sequence polymorphisms in the internal transcribed spacer 2 region of the rRNA genes. *J Clin Microbiol* 2000; 38: 2302–2310.
5. Leaw SN, Chang HC, Sun HF, Barton R, Bouchara JP, Chang TC. Identification of medically important yeast species by sequence analysis of the internal transcribed spacer regions. *J Clin Microbiol* 2006; 44: 693–699.
6. Cornet M, Sendid B, Fradin C, Gaillardin C, Poulain D, Nguyen HV. Molecular identification of closely related Candida species using two ribosomal intergenic spacer fingerprinting methods. *J Mol Diagn* 2011; 13: 12–22.
7. Borman AM, Szekely A, Palmer MD, Johnson EM. Assessment of accuracy of identification of pathogenic yeasts in microbiology laboratories in the United kingdom. *J Clin Microbiol* 2012; 50: 2639–2644.
8. Lockhart SR, Messer SA, Gherna M *et al.* Identification of Candida nivariensis and Candida bracarensis in a large global collection of Candida glabrata isolates: comparison to the literature. *J Clin Microbiol* 2009; 47: 1216–1217.
9. Lockhart SR, Messer SA, Pfaller MA, Diekema DJ. Lodderomyces elongisporus masquerading as Candida parapsilosis as a cause of bloodstream infections. *J Clin Microbiol* 2008; 46: 374–376.
10. Tavanti A, Davidson AD, Gow NA, Maiden MC, Odds FC. Candida orthopsilosis and Candida metapsilosis spp. nov. to replace Candida parapsilosis groups II and III. *J Clin Microbiol* 2005; 43: 284–292.
11. Bader O, Weig M, Taverne-Ghadwal L, Lugert R, Gross U, Kuhns M. Improved clinical laboratory identification of human pathogenic yeasts by matrix-assisted laser desorption ionization time-of-flight mass spectrometry. *Clin Microbiol Infect* 2011; 17: 1359–1365.
12. Bille E, Dauphin B, Leto J *et al.* MALDI-TOF MS Andromas strategy for the routine identification of bacteria, mycobacteria, yeasts, Aspergillus spp. and positive blood cultures. *Clin Microbiol Infect* 2012; 18: 1117–1125.
13. Haigh J, Degun A, Eydmann M, Millar M, Wilks M. Improved performance of bacterium and yeast identification by a commercial matrix-assisted laser desorption ionization-time of flight mass spectrometry system in the clinical microbiology laboratory. *J Clin Microbiol* 2011; 49: 3441.
14. Tan KE, Ellis BC, Lee R, Stamper PD, Zhang SX, Carroll KC. Prospective Evaluation of a Matrix-Assisted Laser Desorption Ionization-Time of Flight Mass Spectrometry System in a Hospital Clinical Microbiology Laboratory for Identification of Bacteria and Yeasts: a Bench-by-Bench Study for Assessing the Impact on Time to Identification and Cost-Effectiveness. *J Clin Microbiol* 2012; 50: 3301–3308.
15. Bader O. MALDI-TOF-MS-based species identification and typing approaches in medical mycology. *Proteomics* 2013; 13: 788–799.
16. Alshawa K, Beretti JL, Lacroix C *et al.* Successful identification of clinical dermatophyte and Neoscytalidium species by matrix-assisted

laser desorption ionization-time of flight mass spectrometry. *J Clin Microbiol* 2012; 50: 2277–2281.
17. Farfour E, Leto J, Barritault M *et al.* Evaluation of the Andromas matrix-assisted laser desorption ionization-time of flight mass spectrometry system for identification of aerobically growing Gram-positive bacilli. *J Clin Microbiol* 2012; 50: 2702–2707.
18. Degand N, Carbonnelle E, Dauphin B *et al.* Matrix-assisted laser desorption ionization-time of flight mass spectrometry for identification of nonfermenting gram-negative bacilli isolated from cystic fibrosis patients. *J Clin Microbiol* 2008; 46: 3361–3367.
19. Luo G, Mitchell TG. Rapid identification of pathogenic fungi directly from cultures by using multiplex PCR. *J Clin Microbiol* 2002; 40: 2860–2865.
20. White T, Burns T, Lee S, Taylor T. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: Innis MA, Gelfand DH, Sninsky JJ, White TJ, eds, *PCR protocols: a guide to methods and applications*. San Diego: Academic Press, 1990; 315–322.
21. Sendid B, Ducoroy P, Francois N *et al.* Evaluation of MALDI-TOF mass spectrometry for the identification of medically-important yeasts in the clinical laboratories of Dijon and Lille hospitals. *Med Mycol* 2013; 51: 25–32.
22. Gabriel F, Noel T, Accoceberry I. Lindnera (Pichia) fabianii blood infection after mesenteric ischemia. *Med Mycol* 2012; 50: 310–314.
23. Goyer M, Lucchi G, Ducoroy P, Vagner O, Bonnin A, Dalle F. Optimization of the preanalytical steps of matrix-assisted laser desorption ionization-time of flight mass spectrometry identification provides a flexible and efficient tool for identification of clinical yeast isolates in medical laboratories. *J Clin Microbiol* 2012; 50: 3066–3068.
24. Steensels D, Verhaegen J, Lagrou K. Matrix-assisted laser desorption ionization-time of flight mass spectrometry for the identification of bacteria and yeasts in a clinical microbiological laboratory: a review. *Acta Clin Belg* 2012; 66: 267–273.