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Full Length Research Paper

Direct identification of yeasts from blood culture by MALDI-TOF mass spectrometry

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This study aimed to identify yeasts directly from positive blood cultures by MALDI-TOF mass spectrometry. This was a 5-month prospective study (February to June, 2015) carried out on bottles of blood cultures from in-patients at the Hôpital de l'ARCHET at the CHU, Nice. Positive blood culture broth was analysed by MALDI-TOF mass spectrometry using Sodium Dodecyl Sulphate (SDS) as the lysis buffer. Out of 64 samples, 51 (79.6%) were identified by MALDI-TOF mass spectrometry at the species level and 12 strains (18.7%) at the genus level. The isolated yeasts fell into six species: *Candida albicans* 37.2%, *Candida glabrata* 31.4%, *Candida parapsilosis* 15.7%, *Candida tropicalis* 5.9%, *Candida krusei* 5.9%, and *Candida lusitaniae* 3.9%. In comparison, 95.3% of species were identified from cultured colonies, the main ones being *C. albicans* (37.1%), *C. glabrata* (30.7%) and *C. parapsilosis* (16.1%). Identification of yeasts from blood culture bottles by MALDI-TOF mass spectrometry is a fast, reliable technique. However, analysis of colonies remains the best technique for identification and for antifungal imaging in order to refer the patient to the most appropriate therapy.

Key words: Yeasts, blood cultures, identification, MALDI-TOF, mass spectrometry.

INTRODUCTION

Invasive fungal infections incur high rates of morbidity and mortality (Seifert, 2009), but prompt initiation of appropriate therapy is associated with a favourable prognosis. Since there is often no clear clinical picture, biological tools, particularly blood cultures, play a major role in the diagnostic strategy (Sepharin, 2015).

Blood cultures are the standard diagnostic tools for bacteraemia and fungaemia (Ferroni et al., 2011). However, identifying species that have grown in a blood culture broth requires culture on a solid medium prior to antifungal imaging, which delays identification until 24 to 48 h after detection of positivity, delaying in turn prescription of a targeted therapy (Ferroni el al., 2010, 2011; Lavergne et al., 2013). For these reasons, attention was focussed on blood cultures and rapid identification of the yeasts responsible for fungaemia by MALDI-TOF mass spectrometry.

Microorganisms can be rapidly identified by MALDI-TOF mass spectrometry analysis of the macromolecules of the different bacteria, notably proteins, from colonies

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isolated on solid medium. By adapting this technology to identify microorganisms cultured in liquid medium, it is possible to identify species immediately, thereby speeding up diagnosis (Bougnoux, 2013).

The aim of this study is to avoid the culture stage and identify yeasts directly from positive blood cultures using the Bruker Microflex LT MALDI-TOF Biotyper mass spectrometry system. A significant amount of time would be gained in implementing the most effective therapy, compared with identification by mass spectrometry from colonies isolated after spotting on Sabouraud chromogenic medium.

MATERIALS AND METHODS

Clinical samples

The isolates analysed in this prospective study were recovered from clinical positive blood cultures in the Mycology Department of L'Archet Hospital in Nice, France, during a 5 month period. The arm of the study aimed to compare the performance of yeast colonies identification by MALDI-TOF mass spectrometry (Bruker Daltonik GmbH, Bremen, Germany) with an SDS-based (Sodium Dodécyle Sulfate SDS, Gen-Apex, Prolabo 500 g) lysis direct identification.

The majority of blood samples were collected in BACTECTM Mycosis-IC/F culture vials (BD Diagnostics, Le Pont de Claix, France). For pediatric blood specimens of less than 3 ml, samples were collected in BACTEC Ped PlusTM/F culture vials (BD Diagnostics). These culture vials were loaded in a BACTECTM FX (BD Diagnostics) automat, which alerts when significant microbial growth is detected. When a vial was flagged positive, a small sample was examined microscopically, and if yeasts or mycelium were observed, the original samples will be submitted to the two identification procedures performed in parallel: identification of colonies obtained after solid medium subculture and SDS lysis-based direct identification.

Identification by MALDI-TOF MS

Identification by MALDI-TOF mass spectrometry was carried out using yeast colonies obtained after 48 h incubation on Sabouraud gentamicin, chloramphenicol and CHROMagar media (Becton Dickinson, Le Pont de Claix, France) at 30°C. Protein extraction was carried out according to the manufacturer's instructions, with one to three colonies being removed using a 1 µl loop and suspended in 300 µl distilled water (Water HPLC, Prolabo BDH, Fontenay-sous-Bois, France) and 900 µl absolute ethanol (70%; CarloErba SDS, Val de Reuil, France).

To perform the SDS lysis-based technique, 1.8 ml of positive blood culture was transferred into a 2-ml microtube. After a centrifugation step of 13 000 *g* for 2 min, the supernatant was removed, the pellet was resuspended in 1.8 ml of sterile distilled water and the microtube was centrifuged at 13000 *g* for 2 min. Next, the supernatant was removed and the pellet was resuspended in 1.8 ml of 0.1% SDS in sterile distilled water and incubated for 10 min. After another wash step, the pellet was resuspended in a mixture of 900 µl ethanol and 300 µl sterile distilled water.

Protein extraction was performed identically for the two protocols. After a centrifugation step of 13000 rpm for 2 min, the supernatant was removed and the pellet was air-dried at room temperature for 10 min. Next, the pellet was resuspended in 50 µl of 70% formic acid (Sigma-Aldrich, Lyon, France) and incubated for 5 min. The same volume of 100% acetonitrile (Prolabo BDH) was then added to the sample. The mixture was incubated for 5 min and then centrifuged for 2 min at 13000 rpm. Subsequently, 1.2 µl of the supernatant was placed into duplicate on a target spot on a metallic plate (Anchorchip 96-spot; Bruker Daltonics, Inc.) and dried. Matrix solution (alpha-cyano-4-hydroxycinnamic acid; Bruker Daltonics, Inc; 1.2 µ l of a concentration of 10 mg/ml) was then added to the fungal protein extract and redried. The Microflex mass spectrometer is used routinely for yeast and bacterial isolate identification. As recommended by the company, *Escherichia coli* isolate is generally employed as a positive control but no yeast control was recommended. For standardization purposes a negative control (all reagents without a yeast isolate) and positive controls prepared from reference isolates (*Candida krusei* CBS 5314) was included in each set of analyses.

The samples were analysed using a Microflex LT Instrument (Bruker Daltonics GmbH, Bremen, Germany). The resulting spectra were analyzed using MALDI Biotyper version 3.0 software (Bruker Daltonics GmbH), which contains the 4111-entry reference version of the Bruker Daltonics database. The spectra generated for each yeast were compared to the reference spectra of all yeasts in this database. The identification scores were used as recommended by the manufacturer: ≥2 indicating species identification, while 1.7-1.9 = identification of a genus. Two successive scores between 1.7 and 1.9 with the same species allowed identification of the species, whereas a score <1.7 corresponding to different species did not allow any species identification. In the case of non-identification of the species, a second analysis was carried out after a new extraction, as described previously.

Statistical analysis

The statistical analysis of data was made from EPI INFO 6.04 software. The tests used were the Chi^2 test (χ^2) and Fisher's exact test at the risk of 5%. P-value<0.05 was considered to be statistically significant.

RESULTS

A total of 64 isolates belonging to six species and a single genus as *Candida* were identified using both techniques. There was, however, a discrepancy in the results: 51 (79.6%) strains were identified directly from the blood culture broth by MALDI-TOF mass spectrometry at the species levels, while 12 (18.7%) strains were identified at the genus level, whereas 95.3% of strains (62) were identified from cultured colonies at the species level and 4.6% (3 strains) at the genus level. Only one strain (1.6%) could not be identified from the broth. The yeast species were mainly *Candida albicans* 37.2% (19/51), *Candida glabrata* 31.4% (16/51), *Candida parapsilosis* 15.7% (8/51), *Candida tropicalis* 5.9% (3/51), *C. krusei* 5.9% (3/51) and *Candida lusitaniae* 3.9%. Two strains of a Gram-positive bacterium, *Staphylococcus epidermidis* were found associated with two strains of *C. lusitaniae*, all of which were identified at the genus level. The species isolated from the colonies were identical to those identified from the broth (Tables 1 and 2). Also, a mixed culture was found and was able to identify two yeasts from its colony: *C. glabrata* and *C. parapsilosis*,

Table 1. Identification of yeast grown in blood culture bottles.

Species	Good identification at the species level [N=51]	Identification at genus level [N=12]	No identification [N=1]
	n (%)		
C. albicans	19 (37.2)	3 (25)	0
C. glabrata	16 (31.4)	3 (25)	0
C. parapsilosis	8 (15.7)	3 (25)	0
C. tropicalis	3 (5.9)	1 (8.3)	0
C. krusei	3 (5.9)	0	0
C. lusitaniae	2 (3.9)	0	0
C. lusitaniae + *S. epidermidis*	0	2 (16.7)	0
No identification	0	0	1 (100)

N means total number of identification; n represents the number front of the percentage. *C: Candida, S: Staphylococcus.*

Table 2. Identification of yeast grown on solid media.

Species	Good identification at the species level [N=62]	Identification at genus level [N=3]	No identification [N=1]
	N (%)		
C. albicans	23 (37.1)	1 (33.3)	0
C. glabrata	19 (30.7)	0	0
C. parapsilosis	20 (16.1)	1 (33.3)	0
C. tropicalis	4 (6.5)	0	0
C. krusei	3 (4.8)	0	0
C. lusitaniae	3 (4.8)	1 (33.3)	0
C. lusitaniae +*S. epidermidis*	0	0	0
No identification	0	0	0

which were identified with a score greater than 2. Subjecting the unidentified yeast to a second extraction, a species of *C. albicans* was able to be isolated. There was a discrepancy in the result of one sample: one species of *C. grabata* isolated in the blood culture broth was identified as *C. albicans* by mass spectrometry of the colony, and this would need to be resolved by sequencing. MALDI-TOF spectrometry from cultures is a much more sensitive procedure than identification from broth, as 95.4% of strains were identified at the species level with the former, compared with 79.6% with the latter. The difference, however, is not statistically significant (χ^2=0.0041, P<0.05).

DISCUSSION

Several studies have been carried out on direct identification of yeasts from positive blood culture bottles using various protocols with varying results (Buchan et al., 2012; Drancourt, 2010; Ferreira et al., 2011; Idelevich et al., 2014; Lavergne et al., 2013; Marinach-Patrice et al., 2010; Yan et al., 2011).

While some authors have had very low success rates with fungi (Buchan et al., 2012; Idelevich et al., 2014), others have reported excellent levels of identification with different sample processing methods (Ferroni et al., 2010, 2011; Idelevich et al., 2014; Lavergne et al., 2013; Marinach-Patrice et al., 2010; Yan et al., 2011).

In our previous study, where we used 0.1% sodium dodecyl sulphate (SDS) as the lysis buffer, a level of identification was obtained at the species level of 79.6%. Idelevich et al. (2014) identified 62.5% of species, Jamal et al. (2013) identified 50%, both using the Bruker Sepsityper kit. Ferroni (2010), on the other hand, used saponin as the lysis buffer for 20 *Candida* strains and identified all species (100%), while Yan et al. (2011) identified 100% of species using the Sepsityper kit preceded by two additional washing steps. In their study, Lavergne (2013) and Marinach-Patrice (2010) used the same protocol as ours and were able to rapidly and precisely identify the main *Candida* species, thus avoiding the need for culture on a specific medium. In a second study carried out by Pulcrano et al. (2013), on *Candida* non-*albicans* with 0.5% SDS, all 82 strains were identified at the species level with a score greater than 2.

In contrast to these results, Buchan (2012) and Ferreira (2011) failed to identify any positive yeasts at the species level using the Sepsityper kit. In our series, only one genus of yeast, *Candida*, was isolated unlike Yan et al. (2011), who found yeasts belonging to two genera: *Candida* and *Cryptococcus.* Pulcrano et al. (2013) identified yeasts of the genera *Saccharomyces* and *Lodderomyces* in addition to the genus *Candida*.

C. albicans is the *Candida* spp. most frequently found in human pathology and was the most common species (37.2%) in our series, as in several other studies (Bassetti et al., 2006; Ferroni et al., 2010 ; Seraphin, 2015 ; Yan et al., 2011). In his thesis, Sepharin (2015) identified 96.6% of *C. albicans*, while Yan et al. (2011) identified 66.7%. The other species were *C. parapsilosis* (19%), *C. tropicalis* (11.9%) and *Cryptococcus neoformans* (2.4%). Ferroni et al. (2010) identified 10 species of *C. albicans* out of 20 *Candida* spp*.* Numerous other species of non-*Candida* yeasts and *Candida* non-*albicans* were also identified by Pulcrano et al. (2013), these being 53 species of *C. parapsilosis*, 11 species of *C. glabrata*, 8 species of *C. tropicalis*, 6 species of *Candida guillermondii*, 1 species of *Lodderomyces elongipsorus* and 1 species of *Saccharomyces cerevisiae*. *C. albicans* is the species most frequently responsible for invasive candidiasis, although according to Bille et al. (2012), other non-*albicans* species, including *C. tropicalis*, *C. parapsilosis*, *C. glabrata* and *C. krusei*, are becoming increasingly common. According to Arnold et al. (2010), fungal infections affect the length of hospital stay and increase treatment costs, so it is very important to correctly identify the fungi causing infection in order to promptly administrate targeted treatment. The results of the present study indicate that with sample processing and MALDI-TOF Biotyper analysis, microorganisms can be rapidly and reliably identified directly from bottles of positive blood cultures in 45 min. Jamal et al. (2013) reported an average time of 35 min, Feronni et al. (2010), 20 min.

The main advantage is the 1 to 2 days gained in putting in place the most appropriate therapy for patients with potentially life-threatening diseases (Yan et al., 2011). It was not possible to identify several yeast species in the same broth in the case of yeast associations, as many other studies have (Ferroni et al., 2010, 2011, Marinach-Patrice et al. 2010). On the other hand, it was possible to identify at the genus level two cases of co-infection with *Staphylococcus epidermidis*. In all cases, the yeast associations were identified from the colonies. With this technique, it was possible to carry out antifungal imaging with the aim of testing the susceptibility of the different species.

Conclusion

Identification of yeasts from blood culture bottles by MALDI-TOF mass spectrometry is a fast and reliable technique, with which it was possible to identify 79.6% of yeast species. However, identification from colonies remains the best technique for identification and for carrying out antifungal imaging in order to refer the patient to the most appropriate therapy.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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