J R Coll Physicians Edinb 2018; 48: 114–9 | doi: 10.4997/JRCPE.2018.203 **PAPER**

Rapid species identification of *Candida* directly from blood culture broths by Sepsityper-MALDI-TOF mass spectrometry: impact on antifungal therapy

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Abstract

Rapid identification of *Candida* species facilitates pathogen-directed therapy with either fluconazole or an echinocandin.

Method We applied Sepsityper matrix-assisted laser desorption ionisation-time of flight mass spectrometry (MALDI-TOF-MS) technology on positive blood culture broths for rapid species identification.

Results Of the 74 patients with candidaemia, 25 had the species identified on the day of the positive blood culture directly from the broth (rapid identification group) while the remaining 49 had the species identified from culture (conventional identification group). Three (13.6%) out of 22 treated patients in the rapid identification group received echinocandin compared to 20/45 (44.4%) in the conventional identification group. The appropriateness of therapy was 90.9% in the rapid identification group and 62.2% in the conventional identification group (p = 0.01). Cost savings were more than £10,000 in the first three days of treatment.

Conclusion Sepsityper-MALDI-TOF-MS is a useful tool in supporting antifungal stewardship programmes.

Keywords: antifungal stewardship, MALDI-TOF, rapid identification, Sepsityper

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Introduction

Candidaemia is a significant cause of sepsis, with *C. albicans* and *C. glabrata* accounting for the majority of bloodstream infections caused by yeasts.[1] There has been a shift in the epidemiology of candidaemia in Scotland with a rise in the proportion of *C. glabrata* over the last decade.[1,2] An emergent multi-resistant species, *C. auris*, recently caused a major outbreak in England, which highlights the importance of invasive yeast infections.[3] Risk factors for candidaemia include broad-spectrum antibiotics, total parenteral nutrition, use of chemotherapeutic agents, admission to intensive care units, and the presence of central venous catheters.[4] Fluconazole and echinocandins (anidulafungin, caspofungin, and micafungin) are widely used for treating candidaemia. Echinocandins are very expensive compared to fluconazole but are also more reliable. However, widespread use of echinocandins may lead to emergence of resistance.[5] The antifungal susceptibility of *Candida* is generally predictable if the infecting species is known (Table 1).[6] However, conventional diagnostic methods to identify the infecting species may take 72 h.[7] In order to optimise the use of echinocandins, a clinical risk-factor based approach has been suggested by the Infectious Diseases Society of America (IDSA).[6] This approach relies on certain risk factors for *C. glabrata* bloodstream infection including diabetes, malignancy, history of recent azole use, and advanced age. In patients with these risk factors, echinocandin therapy is indicated with a subsequent switch to fluconazole if the identified species is likely to be susceptible. Echinocandins are also favoured in patients who are haemodynamically unstable or are neutropaenic as these patients are more likely to suffer adverse consequences if their initial treatment is suboptimal. Fluconazole is an alternative in patients without risk factors for *C. glabrata*. Guidelines from the European Society of Clinical Microbiology and Infectious Diseases (ESCMID) favour the use of echinocandin over fluconazole irrespective of risk factors.[8]

Rapid identification of microbial species provides opportunities for antimicrobial stewardship programmes by reducing turnaround time, thereby favouring targeted rather than empirical prescribing. In relation to candidaemia, identification

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Candida species	Fluconazole	Echinocandin	Recommended therapy	Basis for recommendation
C. albicans	S	S	Fluconazole	Reduced cost
C. glabrata	I or R	S	Echinocandin	Better efficacy
C. parapsilosis	S	S or I	Fluconazole	Reduced cost, better efficacy
C. krusei	R	S	Echinocandin	Efficacy
C. dubliniensis	S	S	Fluconazole	Reduced cost
C. kefyr	S	S	Fluconazole	Reduced cost
C. lusitaniae	S	S	Fluconazole	Reduced cost
C. tropicalis	S	S	Fluconazole	Reduced cost

S, sensitive; I, intermediate; R, resistant

Table 1 Showing recommended therapy based on the common susceptibility pattern of various *Candida* species isolated during the study

of species on the day of positive blood culture eliminates the need for risk stratification by targeting treatment directed towards the infecting species at the time of commencement of therapy. At University Hospital Crosshouse, we introduced matrix-assisted laser desorption ionisation-time of flight mass spectrometry (MALDI-TOF-MS) (Bruker Daltoniks, Germany) in January 2014 as part of laboratory modernisation following approval from the laboratory directorate. This enabled us to offer rapid identification of bacteria and yeasts from positive blood cultures, replacing the biochemical identification systems which are based on sugar fermentation reactions. In this report we describe our findings in relation to the usefulness of MALDI-TOF-MS technology in streamlining antifungal therapy.

Methods

Patients and setting

The microbiology laboratory based at University Hospital Crosshouse, Kilmarnock, provides diagnostic services to a population of 368,000 covered by NHS Ayrshire & Arran. Adult patients with candidaemia from January 2014 to September 2017 were identified with the help of Laboratory Information Management Systems. Patient demographics and information on risk factors including past medical history of diabetes or malignancy, use of azole antifungal agents in the 30 days prior to candidaemia, and presence of haemodynamic instability (systolic blood pressure ≤ 90 mmHg with requirement for vasoconstrictor agents),[9,10] were obtained from the electronic patient management system, Laboratory Information Management Systems, and case records.

Appropriateness of antifungal agents and costs

Fluconazole and micafungin are available on our hospital formulary for the treatment of candidaemia. Microbiologists offer advice on treatment based on patient factors and laboratory findings in consultation with the clinical teams and the patient. Details of treatment received by the patients were obtained from the electronic medicines management system. Defined daily dosages (DDD) for micafungin (standard dose 100 mg daily) and intravenous fluconazole (standard dose 800 mg loading followed by 400 mg daily) were 100 mg and 200 mg and the acquisition cost per DDD was £341 and £29.28, respectively.[11] Although fluconazole can also be administered orally for the treatment of candidaemia, we assumed that all dosages were administered by intravenous route for the purpose of analysis. To ascertain cost savings, we audited our findings against the treatment recommendation made by the IDSA guideline for each episode of candidaemia.[6] Cost saving was the difference between the cost of the DDDs that might have been consumed had therapy been based on the IDSA guideline and the cost of DDD actually consumed where results of rapid species identification were available at the time of prescribing. We assumed that for fluconazole-susceptible isolates, switching of therapy from micafungin to fluconazole would have normally occurred after three days.[11] For the purpose of data analysis, echinocandins were considered as appropriate/recommended treatment in patients with *C. glabrata* and *C. krusei* while fluconazole was appropriate treatment for all other species isolated during the study period (Table 1).

Microbiological investigations

Blood cultures were processed using the BacT/Alert 3D system (bioMerieux, Marcy-l'Etoile, France) with 5 days incubation period. Positive blood culture broths were inoculated on to Sabouraud's agar as part of conventional diagnostics. After 24–48 h incubation, subsequent growth from culture plates was identified by MALDI-TOF-MS, which is a new laser-based diagnostic technique. In addition, positive blood culture bottles were subjected to same day rapid identification by a special technique, Sepsityper MALDI-TOF-MS, which is directly applied to fluid specimens such as blood culture broths.[12] Rapid identification by Sepsityper MALDI-TOF-MS was routinely available only on weekdays (Monday–Friday, 9am to 5pm). Antifungal susceptibility testing was carried out using the YeastOne colourimetric microdilution test (ThermoScientific, Trek Diagnostic Systems, West Sussex, UK). Results were communicated to the clinical teams on the day they were generated.

Statistical analysis

The chi-square (or Fisher exact test where a cell value was 0) was used to compare the categorical variables and p values of ≤ 0.05 were considered to indicate statistical significance.

Table 2 Comparing demographic, microbiological, and treatment parameters in patients with and without rapid species identification based on Sepsityper MALDI-TOF-MS

Variables	Rapid identification	Conventional identification	Total	p value
Total number of patients	25	49	74	-
Mean age	58	66.8	63.8	-
Male/Female	11/14	26/23	37/37	0.46
Total number of *Candida*	25	50	75	-
C. albicans	13	25**	38	0.87
C. glabrata	5	12	17	0.69
C. parapsilosis	5	6	11	0.35
C. krusei	1	3	4	0.71
C. dubliniensis	0	2**	2	0.54
C. kefyr	0	1	1	1
C. lusitaniae	1	0	1	0.33
C. tropicalis	0	1	1	1
Total number of patients treated	22/25 (88%)	45/49 (91.8%)	67/74 (90.5%)	0.59
Patients with risk factors	17/22 (77.2%)	40/45 (88.8%)	57/67 (85%)	0.21
Treated with fluconazole	19/22 (86.3%)	25/45 (55.5%)	44/67 (65.6%)	0.01*
Treated with echinocandin	3/22 (13.6%)	20/45 (44.4%)	23/67 (34.3%)	0.01*
Fluconazole appropriate/recommended	17/19 (89.4%)	21/25 (84%)	38/44 (86.3%)	0.6
Echinocandin appropriate/ recommended	3/3 (100%)	7/20 (35%)	10/23 (43.4%)	0.06
Overall treatment appropriate/ recommended	20/22 (90.9%)	28/45 (62.2%)	48/67 (71.6%)	0.01*
30 day crude mortality – all patients	8/25 (32%)	10/49 (20.4%)	18/74 (24.3%)	0.27
30 day crude mortality – patients who received treatment	6/22 (27.2%)	8/45 (17.7%)	14/67 (20.8%)	0.36
30 day crude mortality – haemodynamically unstable patients	1/2 (50%)^	1/2 (50%)^^	2/4 (50%)	1

*Statistically significant; **One patient had mixed infection with *C. albicans* and *C. dubliniensis*; ^One patient died by the time of detection of candidaemia, one successfully treated with fluconazole; ^^Both patients treated with fluconazole

Results

Seventy-four patients (age range 26–93) had candidaemia during the study period yielding 75 *Candida* isolates (one patient had mixed infection). Most patients had at least one clinical risk factor for infection with *C. glabrata* including age ≥ 65 years (n = 40), diabetes (n = 30), malignancy (n = 30), and prior azole use (n = 3), with some having multiple risk factors. However, *C. glabrata* was recovered in only a minority of patients with risk factors. Four patients were haemodynamically unstable at the time of candidaemia and only one patient was neutropaenic. Table 2 gives the details of species that were isolated. Five isolates were resistant to fluconazole (four isolates of *C. krusei* and one *C. glabrata*), 16 were intermediate (*C. glabrata*), while the remaining were susceptible. Thirty-four out of 74 blood cultures flagged positive outside routine working hours and were subjected to conventional diagnostic methods only. The remaining 40 positive blood cultures were subjected to Sepsityper MALDI-TOF-MS. We were able to identify the species of *Candida* in 25 (62.5%) of these 40 samples. Thus, in 49 patients we did not have the benefit of rapid species identification while in 25 patients rapid identification was successful. Comparative data of the two patient groups are shown in Table 2.

Species identification based on conventional techniques

Forty-five of 49 patients whose *Candida* isolates were only identified following growth on solid media were treated with an antifungal agent: 20 (44.4%) received echinocandin therapy and 25 (55.5%) received fluconazole. Four were not treated: two had their treatment withdrawn by the time bloodstream infection was detected and in the remaining two, yeasts were dismissed as contaminants on clinical grounds. Although an overwhelming number (44/49) of patients had risk factors for *C. glabrata*, only 12 had *C. glabrata* recovered from their bloodstream. On matching the prescribed treatment with the isolates, 28 (62.2%) of 45 patients were found to have been treated with an antifungal agent appropriate for the species (Table 2).

Table 3 Showing the effect of identification by Sepsityper MALDI-TOF on antifungal prescribing categorized by various groups

No. of patients	Rapid identification	Risk factors for *C. glabrata*	Therapy suggested by IDSA	Therapy suggested by MALDI-TOF	Actual treatment
Group 1: No effect: Rapid identification did not alter risk-factor based prescribing					
2	*C. albicans*	None	FLC	FLC	FLC
1	*C. lusitaniae*	None	FLC	FLC	FLC
1	*C. parapsilosis*	None	FLC	FLC	FLC
1	*C. glabrata*	M	MCF	MCF	MCF
1	*C. krusei*	DM, E	MCF	MCF	MCF
Group 2: Cost saving by avoiding unnecessary use of micafungin					
1	*C. albicans*	E	MCF	FLC	FLC
1	*C. albicans*	H	MCF	FLC	FLC
4	*C. albicans*	DM	MCF	FLC	FLC
2	*C. albicans*	E, M	MCF	FLC	FLC
1	*C. albicans*	DM, E, M	MCF	FLC	FLC
Group 3: Targeted therapy with cost saving or greater expenditure					
1	*C. glabrata*	None	FLC	MCF	MCF
1	*C. parapsilosis*	DM	MCF	FLC	FLC
3	*C. parapsilosis*	E	MCF	FLC	FLC
Group 4: Therapy not compliant with either IDSA or rapid identification					
1	*C. glabrata*	E	MCF	MCF	FLC
1	*C. glabrata*	E, M	MCF	MCF	FLC

DM, diabetes; E, elderly; FLC, fluconazole; H, haemodynamically unstable; IDSA, Infectious Diseases Society of America; M, malignancy; MCF, micafungin

Rapid species identification

Of the 25 patients with candidaemia who had the species identified on the same day, 22 (88%) received antifungal therapy (two had died by the time candidaemia was detected while in one case, the yeast was dismissed as a contaminant). If the treatment had been based on the clinical practice guidelines of the IDSA, 17 patients would have received micafungin and the remaining 5 would have received fluconazole. However, only 3 (13.6%) of 22 patients received micafungin and the remaining 19 (86.3%) received fluconazole, which may have been influenced by the rapid identification of the infecting species. Rapid species identification thus had a demonstrable effect on the choice of therapy which helped reduce the cost of treatment by preferential use of fluconazole in patients infected with species that are usually susceptible to it. Rapid identification also led to targeted treatment: preferential use of fluconazole in patients with *C. parapsilosis* and micafungin in patients with *C. glabrata* or *C. krusei*. Prescribing was significantly more appropriate in the rapid identification group with 20 (90.9%) of 22 patients receiving appropriate therapy compared to only 62.2% for patients in whom rapid identification was not available ($p = 0.01$) (Table 2). Data from the groups of patients are summarised in Table 3.

There were no discrepancies between the Sepsityper MALDI-TOF-MS aided direct identification from broths and the subsequent MALDI-TOF-MS based identification from culture plates.

Cost savings

We estimated cost savings of £13,299 related to avoidance of 39 DDDs of micafungin in 13 patients each receiving 1 DDD of micafungin per day for 3 days (Table 3: Groups 2 and 3). We incurred an expenditure of £3045.12 on the 104 DDDs of fluconazole for 13 patients each receiving 8 DDDs of fluconazole over the first 3 days (Table 3: Groups 2 and 3) giving a net cost saving of £10,253.88 and average cost saving of £466.08 per successful species identification in patients who received therapy.

The overall 30-day crude mortality was 24.3% (21.7% for patients commenced on echinocandin, 20.4% for patients commenced on fluconazole).

Discussion

Individual species of *Candida* have predictable antifungal susceptibility patterns, although exceptions are encountered. Species identification is most relevant for *C. glabrata* and *C. krusei* as fluconazole is relatively or absolutely contraindicated in their treatment. There are also concerns about the activity of echinocandins against *C. parapsilosis*. Consequently, the IDSA guidelines recommend echinocandins in situations where the risk of *C. glabrata* is high.[6] However, experts agree that treatment of candidaemia needs to be individualised.[13] In this regard, rapid species identification is beneficial because early appropriate treatment of candidaemia

reduces mortality.[14] In addition, the pharmacokinetic and pharmacodynamic data also influence the choice of therapy, e.g. fluconazole may be the preferred agent for infections in the urinary tract or the central nervous system where echinocandins penetrate poorly. Azoles, on the other hand, have significant drug interactions compared to echinocandins. Source control, central venous catheter removal, funduscopic examination, echocardiography, and daily blood cultures until a documented clearance of bloodstream infection are also recommended in all cases of candidaemia.[8] Our data reveal a significantly greater degree of appropriate usage of antifungal agents when species identification was available.

We also show that risk factors are poorly discriminatory as we recovered *C. glabrata* from only a minority of patients. The evidence base for risk-stratification is weak.[15] Risk assessment for *C. glabrata* is rendered obsolete with the availability of rapid species identification by Sepsityper MALDI-TOF-MS, which merits consideration in future updates of guidelines. The use of Sepsityper MALDI-TOF-MS may have led to optimisation of antifungal therapy, which is supported by antimicrobial stewardship programmes.[16] We made significant savings which might be more than what we estimated because the proportion of patients who get step-down therapy in clinical practice is only 20–40%.[17] Also, our projected savings would be more if we audited against the ESCMID guidelines which advise commencing therapy with echinocandins for all patients prior to the knowledge of infecting species.[8] In our analysis, we assumed that fluconazole was given by the intravenous route which further moderated the cost savings as oral fluconazole is cheaper compared to the intravenous formulation. On the other hand, our data reveal that fluconazole was still the favoured therapy for patients who did not have the benefit of rapid identification despite the fact that an overwhelming majority of them had risk factors for *C. glabrata* (Table 2), perhaps because the association of risk factors with *C. glabrata* is not convincing.[15] In the rapid identification group, we would not necessarily have used micafungin solely on account of risk factors. The real benefit of rapid testing is facilitation of pathogen-directed therapy.

Our findings support the Scottish strategy on antimicrobial stewardship. The Scottish Antimicrobial Prescribing Group supports optimisation of antimicrobial prescribing through quality improvement initiatives.[18] An important barrier to antimicrobial optimisation is the delay in obtaining results. Previous studies based on cost-minimisation models have predicted a cost saving of $1800 per patient with the use of rapid diagnostic tools.[19] McMullan et al. demonstrated a statistically non-significant reduction in empirical antifungal usage in an intensive care unit setting following introduction of a serum polymerase chain reaction assay which discriminates between the fluconazole-susceptible and -resistant species.[20] We focused on definitive therapy in proven cases of candidaemia which enabled us to measure the appropriateness of treatment. Rapid testing has usefulness in invasive bacterial infections also, e.g. differentiating, on the day of the positive blood culture, *Staphylococcus aureus* from coagulase-negative staphylococci, the latter usually being contaminants.

There are drawbacks in our study. The dataset has limited numbers. We have reported a success rate of 62.5% with Sepsityper MALDI-TOF-MS for direct identification of *Candida* species from blood culture broths, which is within the range of 56–62.5% reported in the literature but it still needs improvement.[21,22] A greater success rate has also been reported.[23] Our basis for classifying the appropriateness of the antifungal agents was the predicted susceptibility pattern based on species identification. In practice, echinocandins are suitable treatment for many *Candida* species in addition to *C. glabrata* and *C. krusei*. Anidulafungin therapy was found to be superior compared to fluconazole in a randomised controlled trial on candidaemia in which a majority of patients had infection with *C. albicans*.[24] However, the fact that the IDSA recommends echinocandins for patients who are at risk of *C. glabrata* and fluconazole as an alternative in patients without risk factors implies that the expected susceptibility profile based on species identification should be considered when choosing antifungal treatment. Changes in epidemiology of *Candida*, local susceptibility profile, local guidelines on the choice of antifungal agents and the decline in the acquisition cost of echinocandins may affect the reproducibility of our results. Occasionally, the susceptibility pattern might not be on predictable lines. We have previously reported a case of echinocandin-resistant *C. albicans*.[25] On the other hand, pathogen-directed targeted treatment might still be beneficial by streamlining antifungal therapy with reduction in the risk of development of resistance. Our data suggest that Sepsityper MALDI-TOF-MS supports antifungal stewardship by optimising therapy in patients with candidaemia with a possible relationship between rapid species identification and antifungal usage.

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