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Rapid characterisation of *Candida albicans* by pyrolysis mass spectrometry

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Summary. Clinical isolates (41) of *Candida* spp. from three possible outbreaks of nosocomially-acquired infection were compared by pyrolysis mass spectrometry (PMS) and by a combined morphotyping and resistotyping (M-R typing) method. Both systems characterised all the isolates and distinguished one isolate of *C. tropicalis* and another of *C. glabrata* from the 39 isolates of *C. albicans*. Results from both systems suggested that cross-infection with a single strain contributed to two of the outbreaks. In several instances, more than one strain of *C. albicans* was found amongst multiple isolates from the same patient. PMS is a simple, rapid and objective technique capable of characterising *C. albicans* isolates; discrimination was similar to M-R typing.

Introduction

The epidemiology of *Candida albicans* infections is poorly understood because of the lack of a reliable typing system. Early attempts at typing *Candida* spp. relied upon phenotypic variations that lacked sensitivity and reproducibility, were open to observer bias and often could not be confirmed by other methods. Combinations of methods such as morphotyping, resistance patterns and serological agglutination reactions, used by reference centres, have never been entirely satisfactory.^{1,2} Of these combined methods, morphotyping plus resistotyping (M-R typing) is the most suitable presently available.¹

New approaches based on DNA analysis have been introduced,^{3,4} but these require specialised equipment, expensive reagents and considerable technical expertise. Furthermore, they are slow, labour intensive, and gel-to-gel variation limits the number of isolates that can be conveniently examined. These approaches have thus been termed “fingerprinting” methods rather than typing methods.

Pyrolysis mass spectrometry (PMS) has been used successfully in rapid inter-strain comparisons for many bacterial species,⁵ and has been used to authenticate two reference strains of *C. albicans*.⁶ In this report the results of M-R typing and PMS characterisation of *C. albicans* isolates from three possible hospital outbreaks are compared.

Materials and methods

Source of isolates

Clinical isolates of *Candida* spp. from three geographically distinct hospitals were selected for the study. These comprised: nine isolates from seven patients on an intensive care unit (hospital A; table I); 25 isolates from four patients on one ward of hospital B, over a 4-month period (table II); and seven isolates from a neonatal intensive care unit (hospital C; table III).

Morphotyping and resistotyping

Morphotyping was performed by a modification of the method of Phongpaichit *et al.*⁷ Coding was simplified to include only six fringe states and two colonial surface types.⁸ Recognised fringe types were: no fringe (O); discontinuous (D); narrow fine (N-F); narrow coarse (N-C); wide fine (W-F) and wide coarse (W-C). The surface state characters were not used as they showed poor reproducibility.⁸

Resistotyping and code nomenclature were as described previously.⁸ Chemical concentrations and codes were: sodium selenite (13 g/L, A); boric acid (25 g/L, B); cetrимide (3 g/L, C); malachite green (0.01 g/L, D); CuSO₄ (25 g/L, E); benzalkonium chloride (1 g/L, F); chlorhexidine (2 g/L, G); mercurochrome (2.5 g/L, H); sodium arsenate (50 g/L, I); 5-fluorocytosine (0.75 g/L, J) and NaCl (93.75 g/L, K). Resistotype control (RC) strains were RC6, RC7, RC8, RC9 and RC10.^{8,9} When resistotyping gave unusual results, isolates were identified by the API 20C AUX system (API-bioMérieux UK Ltd).

Fig. 1. Ordination diagram of spectral data for duplicate subcultures of nine isolates of yeast from outbreak A. In this and subsequent figures, the axes represent the first two canonical discriminant functions (complex statistically derived axes reflecting the major spectrum differences) and the means of triplicate analysis of subcultures of the same isolate have been joined.

Table I. Details of nine isolates from hospital A

Isolate no.	Identity	Source*	M-type†	R-type		M-R group	PMS group
A1	<i>C. albicans</i>	Blood culture	N-C	AbCDEFG	K	AM1	AP1
A2	<i>C. albicans</i>	Blood culture	N-C	AbCDEFG	K	AM1	AP1
A3	<i>C. albicans</i>	Blood culture	D	a BC E G		AM2	AP2
A4	<i>C. albicans</i>	Blood culture	D	a BC E G		AM2	AP2
A5	<i>C. albicans</i>	Urine	D	ABCDEFG	ijk	AM1	AP1
A6	<i>C. tropicalis</i>	Blood culture	N-F	AB D	K	AM3	AP3
A7	<i>C. glabrata</i>	Faeces	O	a B D F H	Kl	AM4	AP4
A8	<i>C. albicans</i>	Sputum	N-C	A Cd EFG	k	AM1	AP1
A9	<i>C. albicans</i>	Sputum	N-C	AbCDEFG	K	AM1	AP1

* Isolates from the same patient are bracketed.

† See *Materials and methods* for codes.

Interpretation of M-R typing results

Isolates were considered to be distinguishable when there was a difference of two major characters⁸ (P. Hunter, personal communication). A single major difference was scored as a difference in fringe type, and good growth compared with no growth in resistotyping.

PMS

Cultures were blind-coded such that only their hospital source was known. The patient number was also given for isolates from hospital B. Isolates were grown on freshly prepared diagnostic sensitivity test (DST) agar plates incubated aerobically overnight at 37°C, then subcultured in duplicate on DST. Each

Fig. 2. Ordination diagram of spectral data on duplicate subcultures of six isolates of *C. albicans* from patient I, hospital B.

subculture was allocated a separate PMS number and pyrolysed in triplicate, at 530°C on a Horizon Instruments PYMS 200X pyrolysis mass spectrometer (Horizon Instruments Ltd, Heathfield, Sussex). Spectra for masses 51–200 were recorded on floppy disk, together with pyrolysis sequence number and total ion count.

PMS data analysis

After iterative normalisation to correct for variations in sample size, triplicate spectra sets for each subculture were labelled as distinct groups. The 150 mass ions were subjected to principal component (PC) and then canonical variate (CV) analyses, as described previously.^{10,11} Results of these analyses are represented as ordination diagrams of the first two principal component canonical variates (PCCV1 versus PCCV2).

Isolates were considered distinct if the line joining the means of the duplicate subculture replicates did not overlap or closely abut those of other strains. Clear outliers were removed from the dataset, and the data for the remaining isolates were then re-analysed in

repeated steps. This process was terminated when the means of the groups resulting from triplicate analyses of separate subcultures of the same isolate were at least as different as those of different isolates. Such isolates were considered indistinguishable by PMS.

PMS datasets for isolates from hospitals A, B and C were analysed separately. PMS datasets for isolates from individual patients were also analysed separately for hospital B.

Results

Reproducibility of M-R typing

Resistotyping was performed on all isolates on three separate occasions with two different batches of chemicals. The same result was required for each separate chemical on two occasions, with the third result being no more than one-half difference divergent. All chemicals satisfied these criteria, with the exception of malachite green (D). None of the duplicate cultures of an isolate differed to the degree that they would be resolved into different groups.

Table II. Details of 25 isolates of *C. albicans* from hospital B

Patient no.	Isolate no.	Source	M-type	R-type	M-R group	PMS group
I	B1	Tracheal aspirate	W-F	a BCd E G j	BM1	BP1
	B2	Mouth	N-C	a BC E G j	BM1	BP1
	B3	Mouth	W-F	a b CDEFG I K	BM2	BP2
	B4	Tracheal aspirate	N-C	a BC E G j	BM1	BP1
	B5	Tracheal aspirate	N-C	a BC E G j	BM1	BP1
	B6	Throat	W-F	a BCDEFG I K	BM2	BP2
II	B7	Throat	N-F	a BCDEFG I Kl	BM3	BP3
	B8	Tracheal aspirate	N-F	a BCDEFG I Kl	BM3	BP3
	B9	Gastric aspirate	N-F	a BCDEFG I Kl	BM3	BP3
	B10	Wound	N-F	a BCDEFG I Kl	BM3	BP3
	B11	Throat	N-F	a BCDEFG I Kl	BM3	BP3
	B12	Gastric aspirate	N-F	a BCDEFG I Kl	BM3	BP3
	B13	Wound	W-F	a BCDEFG I Kl	BM3	BP3
	B14	Wound drain	N-F	a BCDEFG I Kl	BM3	BP3
III	B15	Gastric aspirate	N-F	G	BM4	BP4
	B16	Throat	D	A CDEFG K	BM5	BP5
	B17	Perineum	W-F	A C EFG i K	BM5	BP6
	B18	Gastric aspirate	D	A Cd EFG K	BM5	BP6
	B19	Urine	W-F	A C EFG i K	BM5	BP6
	B20	Rectum	N-F	A Cd e FG K	BM5	BP6
IV	B21	Throat	N-C	A CDEFG i K	BM6	BP7
	B22	Gastric aspirate	N-C	A CDEFG i K	BM6	BP7
	B23	Mouth	N-C	A CDEFG i K	BM6	BP7
	B24	Tracheal aspirate	N-C	A CDEFG i K	BM6	BP7
	B25	Perineum	W-F	Ab CDe FG i j k	BM6	BP8

Fig. 3. Ordination diagram of spectral data on duplicate subcultures of six isolates of *C. albicans* from patient III, hospital B.

Fig. 4. Ordination diagram of spectral data on duplicate subcultures of five isolates of *C. albicans* from patient IV, hospital B.

Hospital A

PMS and M-R typing each resolved four groups of isolates from this hospital, and the isolate groupings were identical by the two methods (fig. 1, table I). Pyrolysis group AP1, and M-R typing group AM1, comprised isolates A1, A2, A5, A8 and A9. Pyrolysis group AP2, and M-R typing group AM2 comprised isolates A3 and A4. Two single member groups comprised isolates A6 (AP3, AM3) and A7 (AP4, AM4); in view of the unusual resistotypes of these isolates, they were identified in the API 20C AUX system, and found to be *C. tropicalis* and *C. glabrata*, respectively.

Hospital B: within-patient comparisons

PMS and M-R typing each resolved two groups of isolates from patient I, with identical isolate groupings (fig. 2, table II). PMS group BP1, and M-R typing group BM1, comprised isolates B1, B2, B4 and B5. BP2 and BM2 comprised isolates B3 and B6 respectively.

All isolates from patient II were indistinguishable by PMS (BP3; data not shown) and were homogeneous by M-R typing (BM3; table II).

PMS resolved three distinct groups (BP4–BP6) of isolates from patient III (table II, fig. 3). BP4 comprised isolate B15; this strain was also distinct in M-R typing (BM4). BP5 comprised isolate B16, and BP6 comprised isolates B17, B18, B19 and B20. In M-R typing the isolates of BP5 and BP6 formed a single group (BM5).

PMS resolved two distinct groups (BP7 and BP8) of isolates from patient IV (table II, fig. 4). BP7 comprised isolates B21, B22, B23 and B24, and BP8 comprised isolate B25. In M-R typing the isolates of BP7 and BP8 formed a single group (BM6).

Hospital B: PMS comparison of all isolates

PMS comparison of all 25 isolates from hospital B (fig. 5) suggested that some strains were common to, but not necessarily predominant in, all of the patients. Isolates of PMS groups BP2 (patient I) were indistinguishable from isolates of BP3 (patient II), BP6 (patient III) and BP7 (patient IV). All were part of a cluster of 18 isolates distinguished from the remaining seven by PMS. With M-R typing, this cluster of 18 isolates could be separated into three distinct groups comprising isolates of (i) BM2 plus BM3; (ii) BM5 and (iii) BM6.

Fig. 5. Ordination diagram of spectral data on duplicate subcultures of 24 isolates of *C. albicans* from hospital B.

Hospital C

With PMS, three distinct groups (CP1, CP2 and CP3) of isolates were found (table III, fig. 6). CP1 comprised isolates C1, C2, C3, C6 and C7; CP2 comprised isolate C4; and CP3 comprised isolate C5. With M-R typing all seven of these isolates formed a single group (CM1).

Discussion

PMS and M-R characterisation yielded identical divisions of the isolates from hospital A. The two non-*C. albicans* isolates from this outbreak were readily distinguished from the *C. albicans* isolates by PMS; similar large species differences were seen in the study of Magee *et al.*⁶ for *C. krusei*, *C. tropicalis* and *C. albicans*. PMS characterisation of isolates from hospital C distinguished three groups of isolates, whereas M-R typing failed to differentiate between these isolates.

PMS comparison of multiple isolates from individual patients from hospital B confirmed the M-R findings that all isolates from patient II were indistinguishable, as were the majority of isolates from patient IV, but patients I and III appeared to carry multiple strains. It is known that immunocom-

promised patients may be colonised with a succession of different strains of *C. albicans*,¹² and that healthy women may carry genetically distinct strains of *C. albicans* simultaneously in different anatomical locations.¹³ Therefore, it is essential, that epidemiological investigations of potential outbreaks include all isolates from each patient. More broadly, PMS indicated that all four patients involved in the potential outbreak at hospital B had yielded some closely similar isolates, suggesting nosocomial acquisition. However, for M-T typing, only patients I and II yielded indistinguishable isolates.

Overall, there was reasonable agreement between the M-R typing and PMS results. M-typing depends on detailed observation of colonial morphology, generating a bewildering array of codes that are difficult to compare within one laboratory and almost impossible to reproduce reliably in different centres.² However, the modified coding system described⁸ simplifies analysis.

In resistotyping there are problems in defining clear, usable criteria for the distinction between growth and no growth. It is also difficult to standardise inocula² and this can cause inter-assay variation. In this study a "definitive" set of resistotyping results was obtained by repeating the resistotyping at least three times and ensuring that the same pattern was obtained at least

Fig. 6. Ordination diagram of spectral data on duplicate subcultures of seven isolates of *C. albicans* from hospital C.

Table III. Details of seven isolates of *C. albicans* from the neonatal intensive care unit in hospital C

Isolate no.	Source	M-type	R-type	M-R group	PMS group
C1	Baby	D	Ab C d e F G	K CM1	CP1
C2	Baby	D	Ab c E F G i	K CM1	CP1
C3	Baby	D	Ab C e F G	K CM1	CP1
C4	Baby	D	Ab C E F G i	K CM1	CP2
C5	Baby	W-F	Ab C D E F G j	K CM1	CP3
C6	Incubator	D	Ab C d e F G	K CM1	CP1
C7	Baby	D	Ab C d e F G	K CM1	CP1

twice. Biotyping,¹⁴ serotyping¹⁵ and typing by sensitivity to killer toxins¹⁶ have been used to characterise *Candida* spp., but they are either insufficiently discriminatory between *C. albicans* strains or show poor intra- and inter-laboratory reproducibility.^{1,2}

Typing by iso-enzyme pattern differences detected by electrophoretic separation on polyacrylamide gels has been described¹⁷ but the procedures are time-consuming and require specialised equipment. Furthermore, inter-gel variations preclude comparisons of isolates run on separate gels, therefore this approach is unsuited to analysis of large isolate sets.²

Genotypic methods of strain delineation in *C. albicans* have concentrated on electrophoretic karyo-

typing by use of contour-clamped homogeneous electric fields (CHEF) and restriction endonuclease analysis (REA) of genomic DNA.^{3,4} Vazquez *et al.*⁴ examined 35 separate clinical isolates of *C. albicans* and found 17 distinct types by REA and 23 types by CHEF. However, both techniques are expensive, labour-intensive and unlikely to become widely available to routine laboratories.

In contrast, PMS is a simple and rapid technique, capable of comparing up to 50 isolates, each in duplicate, in a single run, with a minimum of sample preparation.

Most other typing schemes may be perturbed by phenotypic switching in *C. albicans*, which appears to be caused by spontaneous variations during growth resulting in different colonial morphology.¹ Switching is associated with concomitant alteration in cell physiology and biochemistry. Even karyotype differences have been reported to be associated with the colonial changes when switching occurs.^{18,19} Methods to induce switching all involve stressing the organism, such as growing at low temperature on nutrient-limited medium. Therefore, switching is perhaps less likely to be a problem in methods involving growth at 37°C on rich media, and there was certainly no obvious colonial variation observed in this study. However, as PMS allows samplings to avoid obvious colonial variants

and no subsequent cell division is possible, PMS results should not be affected by the switching phenomenon.

PMS typing of *C. albicans* is quick, simple and capable of comparing large numbers of isolates.

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