Carriage of *Candida* species and *C albicans* biotypes in patients undergoing chemotherapy or bone marrow transplantation for haematological disease

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SUMMARY Six hundred and seventy four yeast isolates obtained from routine microbiological screening of 153 patients with haematological disease were identified and *Candida albicans* isolates biotyped over nine months to determine longitudinal and cross sectional patterns of yeast colonisation. A yeast microflora persisted in many patients despite the routine prophylactic use of oral antifungal agents. Analysis of the yeast species isolated on a cross sectional basis showed that *C albicans* accounted for 65% of yeasts isolated from the oral cavity but only 45% of the faecal yeast flora. Longitudinal changes in yeast flora occurred significantly more often in faecal samples than in oral samples and significantly less often in sites colonised with *C albicans* than in sites colonised with other species. No associations were found between the yeasts isolated and the nature of antifungal prophylaxis used, or the extent of a patient's stay in hospital.

Patients with leukaemia and other forms of haematological malignancy comprise a group at high risk for systemic forms of candidosis.¹ While many surveys have examined the prevalence and prevention of clinically important *Candida* infection in such patients, very few have studied their yeast flora in the absence of overt fungal infection. Most studies of this type have involved patient populations with a wide spectrum of malignant diseases. Only Tomoda *et al* specifically surveyed patients receiving anti-leukaemia chemotherapy²: they found higher faecal *Candida* concentrations among 56 such patients compared with healthy controls and identified the *Candida* species in 29 isolates.

This study was designed to examine the *Candida* species and *C albicans* biotypes isolated in routine samples from patients undergoing chemotherapy or bone marrow transplantation for haematological disease. By identifying and biotyping all yeast isolates received from such patients, it was hoped to determine longitudinal as well as cross sectional patterns of yeast colonisation in this particular group of patients.

Patients and methods

The study group comprised 153 patients nursed in either a general ward, a 28 bed mixed haematology/

chest diseases ward, or a general paediatrics ward. About 40% of treatment courses were undertaken in single rooms using protective isolation techniques. The period of the study spanned October 1985 to July 1986. The patients had various haematological malignancies, predominantly acute myeloblastic leukaemia, and were receiving induction/remission chemotherapy according to several different protocols. Nineteen patients underwent allogeneic bone marrow transplantation for acute leukaemia, chronic granulocytic leukaemia, lymphoma or aplastic anaemia, and four patients had autografts for acute lymphoblastic leukaemia.

Almost all patients received antifungal prophylaxis during their hospital stay with oral amphotericin B 500 mg four times a day and ketoconazole 200 mg twice a day. Ten patients received ketoconazole alone and seven were given itraconazole 100 mg daily. Nine patients received no antifungal prophylaxis. All patients were given colistin (1.5×10^6 units 12 hourly by mouth), neomycin (500 mg 12 hourly by mouth), and 1% povidone iodine mouth washes (5 ml six hourly).

Routine microbiological screening entailed regular culture of oral or throat swabs and faeces on Sabouraud's glucose agar as well as on bacteriological culture media.

All cultures positive for yeast were stored at 4°C for two to eight weeks before they were processed for yeast

identification and biotyping. The protocol for identification and biotyping of yeast isolates was designed to maximise the probability of detection of instances where multiple yeast types occurred in a single sample. Three colonies were selected from each culture plate. When different colonial forms (determined by their size, hue, texture and morphological detail) were seen on the plate, examples of each form were included among the three selected. Where the colony characteristics seemed to be identical, three colonies were selected at random.

The triplicate isolates from each clinical sample were processed for presumptive species identification and for differentiation of *C. albicans* biotypes in physiological tests.³ Isolates were tested for growth at pH 1.55, resistance to cetrimide, and lack of growth on Difco MacConkey's agar to establish their presumptive identity as *C. albicans*. *C. albicans* isolates were further typed according to their ability to grow at pH 1.40, production of clear zones in protein agar, resistance to flucytosine, boric acid, and safranin, salt tolerance, and utilisation of urea, sorbose, and citrate. All isolates giving negative or equivocal results in the presumptive tests for identification of *C. albicans* were identified on the basis of their microscopic morphology and their profiles in API 20C tests, direct pour-plate auxanograms,⁵ or both. Individual *C. albicans* clones from a single clinical specimen were recorded as different biotypes when their biotype patterns were disparate in two or more tests.

For convenience, individual biotype patterns of *C. albicans* isolates were referred to by means of three digit codes. Each digit uniquely encodes the results of three of the nine tests where a positive result is scored 1, 2, or 4, and the digit represents the total score for these tests. For example, a result of + + - - + + - - + would be encoded as 364.

The biotyping tests were conducted entirely without any knowledge of the identity of the patients from whom the samples came. When the blind testing was completed, the results were matched to their sources. Where sequential samples from the same patient had been typed, the biotype pattern was similar throughout but with small variations. For example, in one

patient from whom 13 sequential samples yielded *C. albicans*, the biotypes found were 153 (n = 8), 157 (n = 3), and 053 (n = 2). As the biotyping system has been shown to possess a batch-to-batch reproducibility of only one or two test differences³ and as, in most cases, the results were obtained from many separate test batches, it was decided that interpretation of the above results as temporal variations in biotype was invalid in the longitudinal studies. For these studies alone, a computerised iterative procedure was used for each set of multiple sample results from a single patient to determine a "consensus" biotype pattern and to detect outlying (truly different) types. This procedure involved determining the most common pattern of positive and negative test results from the biotype data, then determining which individual isolates gave patterns that differed from this "majority" type by more than two test differences. The latter types were then resubjected to the pattern determination procedure. In the example given above, the "consensus" biotype for all the isolates was 153.

Results

A total of 674 cultures positive for yeast was received from 153 patients with leukaemia during the study period. Most isolates were from the oral cavity and from faeces. The remainder were from miscellaneous sources, particularly sputum, the vagina, the nose and perineal swabs. Seven isolates were from the tips of central venous catheters, three came from cerebrospinal fluid, and one each came from a blood culture and a biopsy specimen. The number of isolates from putatively deep seated sites was therefore considered to be too small to permit statistically valid interpretation. At least three isolates were obtained from 45 of the patients.

Isolates from both the mouth and the faeces of an individual patient were obtained in 57 instances on the same date. In 35 of these instances the *Candida* species or *C. albicans* biotype was the same in both specimens; in 18 the *Candida* species isolated were different, and in four the *C. albicans* biotypes isolated differed between the oral and faecal isolates.

Table 1 No (%) of yeast species and *C. albicans* biotypes in different types of sample from patients with haematological disease

Sample source (No of isolates)	<i>C. albicans</i> biotype			<i>C. glabrata</i>	<i>C. tropicalis</i>	Other spp
	1 - -	3 - -	Other			
Oral cavity (n = 84)	17 (20)	23 (27)	14 (17)	8 (10)	5 (6)	17 (20)
Stools (n = 82)	9 (11)	10 (12)	18 (22)	17 (21)	5 (6)	23 (28)
Other sources (n = 91)	16 (18)	20 (22)	19 (21)	14 (15)	5 (5)	17 (19)

Biotype group 1 - - includes isolates that were proteinase negative but pH 1.4 positive; biotype group 3 - - includes isolates that were positive in both these tests but sensitive to flucytosine.

Table 2 Incidence of *C. albicans* biotypes in different types of sample from patients with haematological disease

No of occurrences	Biotypes in oral cavity samples	Biotypes in stool samples	Biotypes in other samples
1	001, 004, 011, 015, 026, 033, 035, 053, 113, 115, 146, 171, 177, 251, 311, 315, 317, 337, 340, 345, 357, 371, 401, 557, 713	001, 003, 013, 016, 031, 033, 044, 071, 077, 113, 171, 210, 211, 213, 251, 253, 313, 317, 345, 347, 350, 351, 557	003, 013, 023, 031, 033, 055, 057, 077, 113, 131, 133, 201, 213, 255, 301, 313, 350, 355, 400, 451, 777
2	151, 153, 215, 355	117, 153	177, 317, 350, 351, 357, 377, 557
3	117	157	153, 214
4	313	055, 353	
5	157		
8			157, 353
9	353		

CROSS SECTIONAL SURVEY OF *CANDIDA* ISOLATES FROM PATIENTS WITH HAEMATOLOGICAL DISEASE

Tables 1 and 2 summarise the numbers of *Candida* species and *C. albicans* biotypes isolated in a cross sectional survey of the study group. For these compilations, only the first sample isolated from a particular source in any patient was included, so that no patient/sample combination is represented more than once. *C. albicans* was the most common species found from all sites (table 1), but other species, notably *C. glabrata* and *Saccharomyces cerevisiae*, were isolated reasonably often from faecal samples. The proportion of species other than *C. albicans* isolated from faeces was significantly greater than that from oral samples and other sources (χ^2 test, $p < 0.05$). The *C. albicans* biotypes isolated were mainly those with codes 1 – – or 3 – –; respectively, types that were pH 1.4 positive, proteinase negative, and pH 1.4 positive, proteinase positive (tables 1 and 2). Types 157 and 353 were the most common.

Mixtures of *Candida* species or *C. albicans* biotypes were obtained in 25 of the samples. Six of the oral samples contained *C. albicans* plus another *Candida* sp; one contained *C. glabrata* plus *C. parapsilosis*; and one contained *C. albicans* plus *S. cerevisiae*. Two oral samples contained two *C. albicans* biotypes. Among the faecal samples, *C. albicans* plus another *Candida* sp was found on six occasions, *C. albicans* plus *S. cerevisiae* once, and two *C. albicans* biotypes once. The remaining instances of mixed populations occurred in two samples of sputum, a vaginal swab, a nasal swab, a perineal swab and an eye sample.

YEAST FLORA IN INDIVIDUAL PATIENTS

Table 3 gives details of isolates obtained in 12 patients from whom at least 12 yeast positive samples were obtained. Except where stated below, all 12 patients received prophylactic cover with amphotericin B plus ketoconazole. The results are presented in detail for these patients because they convey more clearly than any analysis the often complex nature of the findings of this survey. They show that for most patients a

consistent yeast microflora was harboured at a given site. Case 1 who received no antifungal prophylaxis, was consistently colonised with *C. tropicalis*, except for a single occasion when *C. glabrata* was isolated from a stool sample. Case 2 was colonised orally and faecally mainly with *C. albicans* biotypes 015 and 215 (consensus biotype 215) on three separate admissions to the ward. *C. tropicalis* was also isolated frequently from oral samples during the first admission, and *C. glabrata* was present in several faecal samples during admissions 1 and 3.

Case 3 was colonised with *Candida* mainly in faecal samples on three separate admissions. *C. glabrata* was the species isolated most often, but *C. krusei* was encountered in some oral samples during the first two admissions, and *C. albicans* was isolated from single oral and faecal samples during the first admission. This patient received ketoconazole alone for antifungal prophylaxis but amphotericin B was added in subsequent admissions. Case 4 was also colonised with *Candida* mainly in faecal samples, but the flora was complex. A flora of *C. albicans* (consensus biotype 055) was present during the first three weeks of this patient's first admission, but this species was replaced first with a mixed flora of *C. krusei* and *S. cerevisiae*, then with *C. glabrata* and *S. cerevisiae*, a combination that persisted through the patient's second and third hospital admissions.

Case 5 was positive for *Candida* primarily in the oral cavity, and his *C. albicans* flora persisted through five hospital admissions. The *C. albicans* biotype patterns varied from sample to sample in this patient, but all isolates were within two test differences of the consensus type 353. Case 6 was colonised with *C. albicans* consensus biotype 345 in oral and faecal samples during three admissions. Her prophylactic antifungal treatment varied between admissions: itraconazole was used alone during the first admission; amphotericin B plus ketoconazole in subsequent admissions. Case 7 similarly retained a *C. albicans* flora (consensus type 353) throughout four hospital admissions, although in this patient occasional faecal samples yielded *C. glabrata* during the first two admissions.

Table 3 Details of yeasts isolated from patients for each week of their hospital stay

Case No	Admission	Week	Oral isolate	Stool isolate	Other isolate
1	1	1		<i>C tropicalis</i> (1)	
		4		<i>C tropicalis</i> (2)	<i>C tropicalis</i> (1)
		5	<i>C tropicalis</i> (2)	<i>C tropicalis</i> (1)	<i>C tropicalis</i> (1)
		6	<i>C tropicalis</i> (1)		<i>C tropicalis</i> (2)
		7	<i>C tropicalis</i> (1)	<i>C glabrata</i> (1)	<i>C tropicalis</i> (4)
2	1	8		<i>C tropicalis</i> (1)	<i>C tropicalis</i> (2)
		1	<i>C albicans</i> 015 (1), 215 (2)	<i>C glabrata</i> (1)	
		2	<i>C tropicalis</i> (2)		
		3	<i>C tropicalis</i> (1)	<i>C albicans</i> 215 (1)	
		4	<i>C albicans</i> 215 (1); <i>C tropicalis</i> (1)		
	2	5	<i>C albicans</i> 015 (1); 215 (2)	<i>C albicans</i> 215 (1); <i>C glabrata</i> (3)	
		6	<i>C tropicalis</i> (2)		
		7		<i>C albicans</i> 215 (1)	
		8	<i>C albicans</i> 215 (1)	<i>C albicans</i> 215 (2)	
		9	<i>C albicans</i> 215, 351 (1)	<i>C albicans</i> 215 (1), 217 (2)	
3	1	10	<i>C albicans</i> 015, 257 (1)	<i>C glabrata</i> (1)	
		2		<i>C albicans</i> 071 (1); <i>C glabrata</i> (1);	
		3		<i>C krusei</i> (2)	
		4	<i>C albicans</i> 011 (1)	<i>C glabrata</i> (2); <i>C krusei</i> (1)	
		5	<i>C glabrata</i> (1); <i>C krusei</i> (2)	<i>C glabrata</i> (2); <i>C krusei</i> (2)	
	2	6	<i>C krusei</i> (1)		
		7		<i>C glabrata</i> (1)	
		8		<i>C glabrata</i> (1)	
		9	<i>C krusei</i> (1)	<i>C glabrata</i> (2); <i>C krusei</i> (1)	
		10		<i>C glabrata</i> (1)	
4	1	1		<i>C glabrata</i> (2)	
		2		<i>C glabrata</i> (2)	
		3		<i>C glabrata</i> (2)	
		4		<i>C glabrata</i> (2)	
		5		<i>C albicans</i> 055 (1)	
	2	6		<i>C albicans</i> 055 (1)	
		7		<i>C albicans</i> 051 (1); <i>C krusei</i> (1);	
		8	<i>C glabrata</i> (1)	<i>S cerevisiae</i> (1)	
		9		<i>C krusei</i> (1); <i>S cerevisiae</i> (1)	
		10		<i>S cerevisiae</i> (1)	
5	1	1		<i>C glabrata</i> (1)	
		2		<i>C glabrata</i> (1)	
		3		<i>C glabrata</i> (1); <i>S cerevisiae</i> (1)	
		4		<i>C glabrata</i> (1)	
		5		<i>S cerevisiae</i> (1)	
	2	6		<i>C glabrata</i> (1); <i>S cerevisiae</i> (1)	
		7		<i>C glabrata</i> (1)	
		8		<i>S cerevisiae</i> (1)	
		9		<i>C glabrata</i> (1); <i>S cerevisiae</i> (1)	
		10		<i>C albicans</i> 253 (1)	
6	1	1	<i>C albicans</i> 353 (1)		
		2	<i>C albicans</i> 313 (1)		
		3	<i>C albicans</i> 353 (1)		
		4	<i>C albicans</i> 353 (1)		
		5	<i>C albicans</i> 313 (1), 353 (2)		
	2	6	<i>C albicans</i> 253 (1), 313 (1)		
		7	<i>C albicans</i> 313 (1)		
		8	<i>C albicans</i> 313 (1)		
		9	<i>C albicans</i> 317, 377 (1)		
		10	<i>C albicans</i> 353, 373 (1)		
7	1	1	<i>C albicans</i> 313, 353 (1)		
		2	<i>C albicans</i> 313, 353 (1)		
		3	<i>C albicans</i> 305, 345 (1)		
		4	<i>C albicans</i> 305, 345 (1)		
		5			
	2	6	<i>C albicans</i> 305 (1)		
		7	<i>C albicans</i> 345 (1)		
		8	<i>C albicans</i> 341 (2), 345 (1)		
		9			
		10			
8	1	1		<i>C albicans</i> 343 (1)	
		2		<i>C albicans</i> 353 (2)	
		3		<i>C albicans</i> 353; <i>C glabrata</i> (2)	
		4		<i>C glabrata</i> (2)	
		5		<i>C glabrata</i> (1)	
	2	6		<i>C albicans</i> 353 (2)	
		7	<i>C albicans</i> 313 (1)		
		8	<i>C albicans</i> 313 (1)		
		9	<i>C albicans</i> 153, 353 (1)		
		10			
9	1	1		<i>C albicans</i> 353 (1); <i>C glabrata</i> (1)	
		2		<i>C albicans</i> 313 (1)	
		3	<i>C albicans</i> 353 (3)		
		4	<i>C albicans</i> 353, 357 (1)		
		5		<i>C albicans</i> 353 (1)	
	2	6		<i>C albicans</i> 317 (1)	
		7	<i>C albicans</i> 317 (1)		
		8			
		9			
		10			

Table 3 continued

Case No	Admission	Week	Oral isolate	Stool isolate	Other isolate
8	1	8	<i>C albicans</i> 353, 357 (1)	<i>C albicans</i> 353, 313 (1)	
		9	<i>C albicans</i> 353 (1)		
		10	<i>C albicans</i> 353 (1)		
		11	<i>C albicans</i> 353 (1)		
		12	<i>C albicans</i> 353 (1)	<i>C albicans</i> 153, 353 (1)	
		1	<i>C albicans</i> 053 (1)		<i>C albicans</i> 053 (1)
		2	<i>S cerevisiae</i> (1)		
		4			<i>S cerevisiae</i> (1)
		5	<i>C albicans</i> 021, 345 (1)		<i>C albicans</i> 053, 345 (1)
		7	<i>C albicans</i> 031 (1)	<i>C albicans</i> 001, 031, 033 (1)	<i>C albicans</i> 021 (1), 053 (2)
		8	<i>C albicans</i> 000, 053 (1)	<i>C albicans</i> 063 (1)	
		9	<i>C albicans</i> 001 (1), 053 (2)		
9	1	10	<i>C albicans</i> 021, 345 (1)		<i>C albicans</i> 021 (1)
		11			<i>C albicans</i> 053 (1)
		12	<i>C albicans</i> 053 (1)		<i>C albicans</i> 053 (1)
		1		<i>S cerevisiae</i> (2)	
		5			<i>C albicans</i> 557 (1)
		6		<i>C krusei</i> (1)	
		11		<i>C glabrata</i> (3)	<i>C glabrata</i> (1)
		12			<i>C glabrata</i> (1); <i>C albicans</i> 077 (1)
	2	1		<i>C albicans</i> 146, 156 (1)	
	3	3	<i>C albicans</i> 146 (1)		
	3	1	<i>C albicans</i> 146 (1)		
10	1	2	<i>C albicans</i> 176 (1)		
		1	<i>C albicans</i> 340, 350 (1)	<i>C albicans</i> 350, 354 (1)	
		2		<i>C albicans</i> 250 (1)	
		3	<i>C albicans</i> 210, 351 (1)	<i>C albicans</i> 350 (1)	
		4	<i>C albicans</i> 350, 351 (1)		
		1	<i>C albicans</i> 310 (1); <i>C glabrata</i> (2)	<i>C albicans</i> 777 (1); <i>C glabrata</i> (1)	
		4	<i>C albicans</i> 314 (1)	<i>C albicans</i> 214 (1); <i>C glabrata</i> (1)	<i>C albicans</i> 350 (1)
		3		<i>C albicans</i> 353 (1)	
		4	<i>C albicans</i> 111, 153 (1)		<i>C albicans</i> 013 (1)
		5	<i>C albicans</i> 151, 350 (1)	<i>C albicans</i> 311 (1)	
		6	<i>C albicans</i> 053 (1)		
		7	<i>C albicans</i> 053, 213 (1)	<i>C albicans</i> 153 (1)	
11	1	8	<i>C albicans</i> 053, 211 (1)	<i>C tropicalis</i> (1)	
		10	<i>C albicans</i> 153 (1)		
		11		<i>C albicans</i> 111 (1)	<i>C albicans</i> 153 (1)
		14	<i>C albicans</i> 153 (2), 353 (1)	<i>C albicans</i> 051 (2), 353 (2)	
		15	<i>C albicans</i> 311 (1)	<i>C albicans</i> 211 (1)	
		16	<i>C albicans</i> 113 (1)	<i>C glabrata</i> (2)	
		17	<i>C albicans</i> 257 (1)		<i>C albicans</i> 113 (1)
		1	<i>C albicans</i> 213 (1)		
	2	1		<i>C albicans</i> 253, 353 (1)	
	3	1		<i>C albicans</i> 153 (1)	<i>C albicans</i> 153 (1)
	1	2	<i>C albicans</i> 153 (1)		<i>C krusei</i> (1)
		3			<i>C krusei</i> (1)
		4	<i>C famata</i> (1)		<i>C krusei</i> (1)
		5		<i>C albicans</i> 013 (1); <i>S cerevisiae</i> (1);	<i>C krusei</i> (1)
		2	<i>C krusei</i> (1)	<i>C krusei</i> (1); <i>S cerevisiae</i> (1)	
		5			<i>C krusei</i> (2)
		6		<i>C albicans</i> 051, 317 (1)	
		7	<i>C albicans</i> 117 (1)		<i>C albicans</i> 177, 214 (1)
		9	<i>C krusei</i> (1)	<i>C krusei</i> (1)	
		10	<i>C krusei</i> (1)		
	3	2	<i>C albicans</i> 257 (1); <i>C krusei</i> (1)		

The 12 patients included in the table were those for whom 12 or more yeast isolations were recorded in the study. Numbers of occasions on which each species or *C albicans* biotype were isolated are given in parentheses.

Case 8 is an example of a case in which the *C albicans* biotypes isolated seemed to vary considerably (table 3). Computer analysis suggested there were, in fact, two main biotypes and one less common biotype in this patient. Types 000, 001, 021 and 031 all fell within two test differences of consensus type 021; types 033, 053, 063, 353 all fell within two test differences of consensus type 053. The less common type was type 345, which was the consensus biotype for types 345 and 357. In addition to these three *C albicans* types,

S cerevisiae was recovered from one oral and one sputum specimen. Case 9 seems to have acquired a *C albicans* flora between his first and second hospital admissions. The three different yeast species recovered from his faeces during the first admission were not isolated at all during admissions 2 and 3. *C albicans* types 557 and 077, which were isolated from this patient's nose and perineum, respectively, during the first admission, bore no resemblance to the *C albicans* consensus type 146 that was isolated repeatedly during

admissions 2 and 3.

During her two admissions, Case 10 repeatedly yielded oral and faecal samples positive for *C. albicans* with a consensus biotype of 350. During the second admission the new biotypes 214 and 777 were recovered from individual faecal samples and *C. glabrata* was isolated from faeces on two occasions. Case 11 was colonised with two repeatedly isolated *C. albicans* biotypes: 033, 051, 053, 111, 113, 151, 153, 253 and 353 suggest a consensus biotype of 153; types 211, 213, and 311 correspond to the consensus biotype 211. Types 350 and 257 were also isolated once each from oral swabs, *C. glabrata* was isolated twice from faeces, and *C. tropicalis* was isolated once.

Finally, case 12 was colonised throughout three hospital admissions mainly by *C. albicans* and *C. krusei*, but the *C. albicans* biotypes conformed to no particular pattern or majority consensus, and two other yeast species, *C. famata* and *S. cerevisiae*, were isolated on rare occasions.

LONGITUDINAL CHANGES IN YEAST FLORA DURING AND BETWEEN HOSPITAL ADMISSIONS

The results in table 3 showed that even if the complication of changes in *C. albicans* biotypes were disregarded, the yeast microflora showed substantial variations from time to time in some patients while it remained very consistent in others. The full body of isolation data was therefore analysed to determine the incidence and nature of changes in yeast flora within a single hospital admission and between admissions.

For analysis of changes in commensal yeast flora among the study population within one continuous period in the ward, data were analysed for a single hospital admission only for each patient, and the analysis was restricted entirely to oral and faecal yeast isolates. For inclusion in the analysis a patient had to have a minimum of two yeast positive cultures from either oral swabs or faeces during the hospital admission. Data that met these criteria were available for 38 patients with yeast positive oral swabs and for 44 patients with yeast positive faeces (table 4). The consensus biotype results for *C. albicans* isolates were used to avoid overinterpretation of changes in biotype that might have resulted from technical considerations. The period of observation for each patient was calculated on the basis of the period between the first and last yeast positive cultures obtained within the admission, as the analysis was concerned only with species and biotype changes in a yeast positive flora: the data available were insufficient to permit analysis of acquisition and loss of yeast flora of any type.

The results for individual patients in table 3 show how rarely a complete exchange of one yeast species for another occurred within the study population. A change in flora was therefore defined as the

Odds, Kibbler, Walker, Bhamra, Prentice, Noone

Table 4 Appearance of new *Candida* species or *C. albicans* biotypes in oral or faecal flora of patients within one hospital admission

Sample source	Duration of observation*	No of patients	No (%) with new species	No (%) with new biotype
Oral cavity	1-14 days	18	3 (17)	4 (22)
	> 14 days	20	2 (10)	2 (10)
Stools	1-14 days	20	5 (25)	0
	> 14 days	24	19 (79)	1 (4)

*Time between first and last yeast positive cultures obtained.

appearance of any yeast species or *C. albicans* biotype different from that or those encountered in the earliest yeast positive sample. On this basis it was evident that changes in the yeast flora occurred significantly more often in faecal samples (57% showed some change) than in oral samples (29% showed some change); χ^2 test, $p < 0.02$ (table 4). In faecal samples the changes in flora occurred significantly more often after 14 days of yeast colonisation than within a 14 day period (table 4; χ^2 test, $p < 0.01$).

Of 49 patient admissions in which the positive oral or faecal sample contained *C. albicans*, a new yeast species was subsequently found in only 11 (22%); in 33 patient admissions where the first yeast isolated was a species other than *C. albicans*, a new species subsequently appeared in 18 (55%) (χ^2 test, $p < 0.02$). *C. albicans* and *C. glabrata* were the two species which seemed to be acquired most commonly as replacement or additional yeast flora among the patients studied (table 5). There were no statistical associations between changes in the yeast flora and the nature of antifungal prophylaxis used or the time of species change subsequent to the time of hospital admission.

Twenty five of the patients were admitted to the ward on at least two separate occasions during the study. For 15 of these patients there were at least two yeast positive samples from a single anatomical source within each admission, so that changes in flora between admissions could be assessed. Only three of the 15 patients showed any change in their yeast flora that could be interpreted as a change between admissions; the other 12 retained essentially the same yeast flora, sometimes throughout three or more admissions (table 3). The three flora changes were all of a different nature. One patient whose faecal isolates were *C. glabrata* and *S. cerevisiae* on the first admission consistently carried *C. albicans* type 146 on the second admission (case 9, table 3). One patient from whom only *C. albicans* was isolated from faeces during the first admission had *C. glabrata* present as well as *C. albicans* in faeces during the second admission (case 10). Finally, case 12, with a single oral swab positive for *C. famata* during the first admission, was colonised

Table 5 Occurrence of yeast species as additional or replacement flora in one hospital admission

Species	Incidence in oral samples (49 patients)	Incidence in faecal samples (33 patients)
<i>C albicans</i>	2	11
<i>C glabrata</i>	1	10
<i>C krusei</i>	1	3
<i>C parapsilosis</i>	1	1
<i>G tropicalis</i>	0	1
<i>S cerevisiae</i>	2	4

In patients from whom more than one yeast species was isolated subsequent to the first sample, each new species is counted separately.

orally with *C krusei* and *C albicans* during the second admission.

Discussion

As far as we know this study is the first broadly based study of commensal yeast colonisation among patients with haematological disease. Yeast species found among such patients occur in a complex manner and faecal samples are more likely than oral samples to show changes in the yeast species present during a patient's hospital stay.

The spectrum of yeast species isolated overall from these patients can be compared with that found among cancer patients of all types. Kiehn *et al* found that *C albicans* accounted for 73% of oral yeast isolates and 72% of faecal yeast isolates from their cancer patients.⁶ Tomoda *et al* found this species in 69% of faecal isolates from their cancer patients.² The figures for oral and faecal yeast species in our survey showed prevalences of *C albicans* of 64% and 45%, respectively, which indicates a lower preponderance of *C albicans* among the patients with leukaemia than among other cancer patients. Whether this difference is specifically related to haematological disease or to other factors such as the selective pressures of antifungal prophylaxis as used in our patients is not known.

A yeast flora persisted among many patients despite the use of prophylactic, orally administered antifungal drugs. No antifungal agent has yet been shown to be capable of eliminating the commensal yeast flora in patients with leukaemia, although agents such as amphotericin B and ketoconazole seem to reduce the incidence of life threatening systemic *Candida* infections among this population.⁷

Both the value and the inadequacies of the biotyping method used to differentiate *C albicans* strains in the present study have been shown very plainly. Indeed, this is the most substantial study in which *C albicans* biotyping has been applied blind to a very large number of yeast isolates over a long period of

time. The data in table 3 confirm the inherent reliability of the method, as in four of the patients (case 2, 4, 5 and 9) a highly acceptable consistency in biotypes was found over a period of time. Similarly consistent biotyping results were found in other patients who harboured *C albicans* over long periods but for whom the total number of yeast isolates in the study was fewer than for those included in table 3. On the other hand, results showing wider variations in biotype data were obtained longitudinally in cases 6, 7, 8, 9, and 10 although even among most of these patients there was a tendency towards a consistent underlying biotype pattern. Results from the *Candida* biotyping tests used in this study are known to be highly vulnerable to technical performance variables, particularly to variance in inoculum size,^{3,8} so the use of "consensus" biotype results determined by computer for each patient avoids overinterpretation of apparent changes in *C albicans*. On this basis it seems that patients with leukaemia change their *C albicans* biotype less often than they change the species of yeast with which they are colonised. Biotype results cannot be reliably compared between studies,⁸ so no attempt is made here to relate the *C albicans* biotypes found in this patient group with those in other surveys.

We can only speculate as to why the faecal yeast flora seems to change more often than the oral yeast flora among the patients studied. Because it seems that a site colonised with *C albicans* is significantly less likely to undergo a change in commensal yeast species than a site harbouring another species, the reduced initial prevalence of *C albicans* in the faeces of our patients seems to render the lower bowel more prone to colonisation by other yeasts. Certainly, *C albicans* adheres better than other yeast species to epithelial surfaces,¹ which may explain the comparative resilience of intestinal sites occupied by *C albicans* to recolonisation.

The relative consistency of our patients' commensal yeast flora, both within and between admissions to the haematology wards, provides little evidence for cross contamination of the patients with yeasts from the environment, staff, or other patients. Apparent outbreaks of systemic *Candida* infection have been documented in some intensive care units^{9,10} and a neonatal special care unit,¹¹ though not yet in specialist leukaemia wards. The results of our extensive survey suggest such outbreaks of candidosis result from the chance introduction into these closed units of strains with an unusually high propensity to colonise the patients, and that they do not represent an ever present hazard from an inherent tendency of "ward" strains of *Candida* to cross infect patients readily.

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References

- 1 Odds FC. *Candida and candidosis* 2nd ed. London: Baillière-Tindall, 1988.
- 2 Tomoda T, Nakano Y, Kageyama T. Variations in intestinal *Candida* populations in patients receiving antileukaemic therapy. *Bull Osaka Med School* 1984;30:14-8.
- 3 Odds FC, Abbott AB. A simple system for the presumptive identification of *Candida albicans* and differentiation of strains within the species. *Sabouraudia* 1980;18:301-18.
- 4 Odds FC, Abbott AB. Modification and extension of tests for differentiation of *Candida* species and strains. *Sabouraudia* 1983;21:79-81.
- 5 Land GA, Vinton AC, Adcock GB, Hopkins JM. Improved auxanographic method for yeast assimilations: a comparison with other approaches. *J Clin Microbiol* 1975;2:206-17.
- 6 Kiehn TE, Edwards FF, Armstrong D. The prevalence of yeasts in

Odds, Kibbler, Walker, Bhamra, Prentice, Noone

- clinical specimens from cancer patients. *Am J Clin Pathol* 1980;73:518-21.
- 7 Hann IM, Prentice HG, Corringham R, *et al.* Ketoconazole versus nystatin and amphotericin B for fungal prophylaxis in severely immunocompromised patients. *Lancet* 1982;i:826-9.
- 8 Odds FC, Auger P, Krogh P, Neely AN, Segal E. Biotyping of *Candida albicans*—results of an international collaborative survey. *J Clin Microbiol* 1989;27:1506-9.
- 9 Burnie JP, Odds FC, Lee W, Webster C, Williams JD. Outbreak of systemic *Candida albicans* in intensive care unit caused by cross-infection. *Br Med J* 1985;290:746-8.
- 10 Matthews R, Burnie JP. Assessment of DNA fingerprinting for rapid identification of outbreaks of systemic candidiasis. *Br Med J* 1989;298:354-7.
- 11 Phelps M, Ayliffe GAJ, Babb JR. An outbreak of candidiasis in a special care baby unit: the use of a resistogram typing method. *J Hosp Infect* 1986;7:13-20.

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