Candida species and *C. albicans* biotypes in women attending clinics in genitourinary medicine

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Summary. Yeasts were isolated from two or more anatomical sites in 198 women attending genitourinary clinics on at least two occasions. The yeast biotypes isolated concurrently from the vagina and urethra were the same in 138 (99%) of 140 instances, and 94% of 124 concurrent genital and anal isolates were of matching types, whereas only 75% of concurrent genital and oral isolates were of the same type. Mixtures of *Candida* spp. or *C. albicans* biotypes were encountered only five times among 545 yeast-positive samples. In instances where *Candida* spp. were isolated at successive times from the same site in a patient, the same yeast type was encountered on 97 (87%) of 112 occasions when the interval between samples was less than 15 weeks, and on 19 (66%) of 29 occasions when the interval was 15 weeks or more. These data indicate a tendency to carriage of phenotypically consistent types of *Candida* among most women attending genitourinary clinics.

Introduction

Physiological tests to differentiate biotypes of *Candida albicans* have been used widely in epidemiological studies of genital candida infections. Although no evidence has been obtained to associate particular *C. albicans* biotypes with symptomatology in vulvovaginitis (Warnock *et al.*, 1979*b*; Meinhof, 1982; Odds *et al.*, 1983, 1987), biotyping procedures have corroborated the concept of sexual transmissibility of the fungus (Warnock *et al.*, 1979*b*; Malyszko *et al.*, 1980; Odds *et al.*, 1983) and shown that recurrence in vaginal candidosis is most often the result of relapse due to the same strain of *C. albicans* rather than reinfection with a different strain (O'Connor and Sobel, 1986; Sobel, 1986).

In several studies, most female patients who harbour *C. albicans* were found to carry the same biotype in different anatomical sites, and usually the same biotype was retained over a period of weeks or months (Warnock *et al.*, 1979*a, b*; Odds, 1982). These conclusions were based on tests involving series of only 25–30 patients. Warnock *et al.* (1979*a, b*) also found that patients frequently harboured more than one *C. albicans* biotype. The present paper makes retrospective use of data

collected from 198 patients in the course of biotyping surveys in two genitourinary clinics (Odds *et al.*, 1987, 1988) to confirm and extend previous observations on the natural history of *Candida* spp. in the setting of genitourinary medicine.

Patients and methods

Patients

The data given were compiled from records of 198 patients among more than 2000 women attending genitourinary clinics in London, Loughborough and Leicester and who were sampled for yeasts by culture in 1983 and 1984. The criterion for inclusion of data in the present survey was a record of yeast isolation from two or more anatomical sites (mouth, anus, vagina, urethra, finger nails), or isolation of yeasts from one of these sites on more than one occasion, or both. The great majority of women included were of child-bearing age and most were self-referred to the clinics. Nine of the women were pregnant at the time of the survey.

Isolation of yeasts

In almost all the patients, a vaginal sample was taken with a dry, cotton-tipped swab and other sites (mouth, anus, urethra and finger nails) were similarly sampled

with swabs according to individual circumstances (all five sites were sampled in only 60 of the original population of more than 2000 studied). In the Leicester-shire clinics, swabs were immediately rubbed on plates of Sabouraud's Glucose Agar (Oxoid) and the plates were then incubated for 48–72 h at 37°C. In the London clinics, the plates were similarly inoculated immediately, but were then stored at 4°C for up to 3 weeks before incubation at 37°C for 48 h. These differences in protocol had no apparent effect on the overall isolation rate of yeasts from the vagina, which was approximately 20% in both groups of clinics. For some analyses of the data, patients were placed into one of two subgroups, according to the diagnosis made at the time of sample: (1) patients in whom an exclusive diagnosis of vulvovaginal candidosis was made on the basis of clinical signs, symptoms and detection of a *Candida* sp. in the vagina; (2) all other patients.

The protocol for identification and biotyping of yeast isolates was designed to maximise the probability of detection of multiple yeast types in a single sample. Three colonies were selected from each culture (three colonies from a streaked-out subculture in instances where fewer than three yeast colonies grew on the original plate). When different colonial forms (determined by their size, hue, texture and morphology) were seen on the plate, examples of each form were included among the three selected. Where the colony characteristics appeared to be identical, three colonies were picked at random. The triplicate isolates from each clinical sample were processed for presumptive species determination and for differentiation of *C. albicans* biotype in physiological tests (Odds and Abbott, 1980, 1983). In brief, isolates were tested for growth at pH 1.55, resistance to cetrimide and lack of growth on MacConkey's Agar (Difco) 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 that gave negative or equivocal results in the presumptive tests for identification of *C. albicans* were identified on the basis of their microscopic morphologies and their profiles in API 20C tests.

The physiological biotyping system used to differentiate *C. albicans* isolates was originally described as reproducible to within one test difference among the nine tests involved (Odds and Abbot, 1980). Subsequent experience has confirmed that most isolates of *C. albicans* give replicate test results that fall within this range of error. However, occasional isolates have been found to differ by two results in replicate tests; therefore, isolates in the present study were recorded as "different" only when they differed in three or more test results.

Results

Yeasts were isolated from two or more sites on 200 occasions from 179 of the 198 patients. The

total number of yeast types isolated was 550 from 545 yeast-positive samples. Thus, different yeast types were found in the same sample on only five occasions: in one sample, two *C. albicans* biotypes were found together; in the other four samples, *C. albicans* and a second *Candida* sp. were found. The number of positive and negative isolations, respectively, from individual sites were 181 and 19 from the vagina, 142 and 46 from the urethra, 139 and 61 from the anus, 78 and 117 from the mouth, 10 and 24 from finger nails. From these data it can be seen that the vagina and anus were sampled on all 200 occasions. Four of the five sites were sampled on 157 occasions, and all five in 30 instances.

Of the 550 yeast types isolated, 500 were identified as *C. albicans*, 15 as *C. (Torulopsis) glabrata*, one as *C. guilliermondii*, 20 as *C. parapsilosis*, two as *C. tropicalis* and 12 as *Saccharomyces cerevisiae*. The high prevalence of isolates of *C. parapsilosis* and *S. cerevisiae* is partly explained by their occurrence in multiple sites in two patients who each attended the clinics on three occasions.

Consistency of yeast types in different sites in the same patient

Table I indicates how often the same yeast type occurred when yeasts were isolated at the same time from pairs of anatomical sites. It can be seen that concurrent isolation of yeasts of similar species or biotype was the most common finding overall. However, there was a much greater concurrence of yeast types between the three sites that are closely related anatomically (urethra, vagina and anus) than between any of these sites and the mouth or finger nails. None of the differences in strain type concordance between patients with and without a diagnosis of candidosis was statistically significant.

Consistency of yeast types at different sample times

The results summarised in table II indicate that most patients who harbour *Candida* spp. over a period tend to retain the same type of yeast in any given site. However, the proportion of yeast isolations from the same patient that differed over a period of time rose as the time elapsed between samples increased. For samples taken from the same patient after an interval of 15 weeks or more (including samples taken more than 30 weeks apart in 11 instances), the yeast species or biotype found was the same in approximately two-thirds of all instances. This tendency towards changes of biotype with prolonged intervals between samples was similar for all four anatomical sites studied.

Table I. Instances in which the *Candida* sp. or *C. albicans* biotype isolated from two anatomical sites was identical (Results obtained from records of 200 patient visits when yeasts were cultured from two or more sites)

Sites of isolation	Patients with			
	vaginal candidosis		other or no genital infection	
	number of pairs of yeast isolates	number of identical pairs	number of pairs of yeast isolates	number of identical pairs
vagina, urethra	103	101 (98%)	37	37 (100%)
vagina, anus	88	83 (94%)	36	33 (92%)
vagina, mouth	44	36 (82%)	19	11 (58%)
vagina, nails	9	6 (67%)	---	---
urethra, anus	66	62 (94%)	28	26 (93%)
urethra, mouth	27	23 (85%)	13	8 (62%)
urethra, nails	8	5 (63%)	---	---
mouth, anus	29	24 (83%)	24	17 (71%)
mouth, nails	6	3 (50%)	---	---
anus, nails	8	5 (63%)	---	---

--- = nails were not sampled in patients without clinical evidence of vaginal candidosis.

Discussion

The sample of patients included in this survey represents a broad cross-section of women attending the genitourinary clinics involved. Most were unselected, randomly chosen individuals who were routinely sampled for yeasts in four sites as part of the protocol for another survey. Only in those few individuals whose finger-nails were swabbed was there a selective factor since in all such patients there was prior clinical suspicion of genital *Candida* infection. Therefore, the results may be taken as a reasonable indication of trends in candida carriage among women who attend the clinics studied.

The main difference between the results of the present survey and previous surveys of *Candida* types in similar patient populations concerns the prevalence of multiple yeast types in single swab

samples. Warnock *et al.* (1979*a, b*) reported the simultaneous occurrence of two *C. albicans* biotypes in vaginal samples from two of eight patients and in 14 of 30 patients in their biotyping surveys, whereas Odds (1982) noted two *C. albicans* biotypes occurring simultaneously in only one of 25 patients. In the present survey, simultaneous occurrence of two *C. albicans* biotypes was noted only once among 500 samples containing this species. Reliability of the statistics on the simultaneous occurrences of different types depends on the test method used and the criterion for deciding that two isolates are phenotypically different strains. If isolates differing in the present biotyping tests by just one or two results had been recorded as different strains, the proportion of cases in which two or three "different" biotypes had occurred in the same sample would have been substantially higher. However, in a

Table II. Instances in which the *Candida* sp. or *C. albicans* biotype isolated from a given anatomical site over various periods was identical (Results obtained from records of 69 patients who were yeast-positive in at least one site on two or more occasions)

Time between samples (weeks)	Vagina		Urethra		Mouth		Anus	
	number of pairs of samples	number of identical pairs of yeast isolates	number of pairs of samples	number of identical pairs of yeast isolates	number of pairs of samples	number of identical pairs of yeast isolates	number of pairs of samples	number of identical pairs of yeast isolates
1-5	25	23 (92%)	12	10 (83%)	14	12 (86%)	18	14 (78%)
6-14	19	16 (84%)	7	6 (86%)	10	9 (90%)	7	7 (100%)
15 or more	13	9 (69%)	2	1 (50%)	8	5 (63%)	6	4 (67%)

system where replicate concordance is usually reliable to one test difference in nine but where two differences in nine are sometimes observed, a more strict definition of strain differences was used in this study to avoid over-interpretation of the results.

The remainder of the present findings confirm and amplify previous reports that the *C. albicans* biotypes and *Candida* spp. found in the genito-anal region of an individual woman are the same in nearly every case, but that the yeast types found in this area may differ from those isolated from the mouth in approximately 20–30% of instances, and may differ from those found under the finger nails in 30–40% of instances. This finding is essentially similar in patients with and without clinically overt genital candidosis.

Most women who carry a *Candida* sp. carry the same type for an extended period, though in approximately one-third the yeast type is likely to have changed after 15 weeks or more. This

observation agrees well with the findings of O'Connor and Sobel (1986) that recurrences of vaginal *Candida* infection within 3 months of an initial screening sample involved the same biotype in 65% of cases.

The consistency of physiological biotypes between different anatomical sites and from time to time must be contrasted with the study of Soll *et al.* (1987) who found that strains of *C. albicans* in suspensions of yeasts made directly from vaginal samples were genetically identical but often generated colony variants at high frequency when cloned on to a special medium and incubated for 1 week or more at 25°C. It is possible that rapid phenotypic variation may occur in individual *C. albicans* cells *in vivo*, but the design of the present study did not investigate such a possibility.

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