Antibodies against *Trichosporon beigelii* in vaginal washings from asymptomatic women

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Summary. *Trichosporon beigelii* was isolated from vaginal washings from three asymptomatic women. All three women had IgG or IgA anti-*T. beigelii* antibody titres ≥ 20 when tested by an indirect immunofluorescence assay against the three strains isolated. Titres ≥ 160 were found when each patient was tested against her own isolate. Patients with *Candida albicans* vulvovaginitis, or from whom *C. albicans* or *Torulopsis glabrata* was isolated from vaginal washings, or who had negative cultures for yeasts, had titres ≤ 20 .

Introduction

In the past decade awareness of the importance of infections caused by opportunistic fungi, including *Trichosporon beigelii* (syn. *T. cutaneum*) has increased. *T. beigelii* is usually non-pathogenic but it can cause a mild infection on hair shafts, known as white piedra. Since 1970, when it was first reported to be capable of causing systemic infection (Watson and Kallichurun, 1970), several reports have confirmed that it can cause deep-seated infections, mainly in surgical patients, intravenous drug abusers, bone marrow transplant recipients, and other immunosuppressed patients; Hoy *et al.*, 1986; Walsh *et al.*, 1986).

Despite the isolation of *T. beigelii* from vaginal swabs (Pritchard and Muir, 1985) and from patients with penile ulcers (Chapel *et al.*, 1978) its pathogenic status in these locations is still unclear. Its presence may represent colonisation, asymptomatic infection, or a true infection with few symptoms, that may be attributed to another cause.

Here we report three cases in which *T. beigelii* was isolated from vaginal washings and attempt to elucidate the clinical relevance of the findings.

Materials and methods

Subjects

One hundred women attending a gynaecological clinic were investigated. They were examined clinically and signs of vulvitis, vaginitis and discharge were recorded as 0 (absent), 1 (moderate) and 2 (severe).

Vaginal samples

Sterile saline (10 ml) was instilled into the posterior fornix and aspirated. A washing of about 8 ml was recovered from each patient. A gram-stained smear from the same location was examined for the presence of blastospores, mycelia or pseudomycelia. Each washing was centrifuged at 3000 rpm for 10 min and the sediment was inoculated on to Sabouraud's dextrose agar (glucose 40 mg/ml, peptone 10 mg/ml, agar 20 mg/ml) containing chloramphenicol 0.05 mg/ml, and incubated at 37°C for 48 h. If no growth had occurred the culture was then incubated at 25°C for a further 7 days. All the yeasts isolated were identified by the serum germ tube test and the API 20C AUX yeast identification system (API System SA, Montalieu Vercieu, France). Identification as *T. beigelii* was based on the following criteria: the presence of well-developed hyphae, pseudohyphae, and rectangular arthroconidia and blastoconidia; aerobic growth on Sabouraud's agar, producing smooth, shiny colonies that become cream-coloured and wrinkled after one week; absence of ability to ferment carbohydrates; assimilation of glucose, galactose, sucrose, maltose and lactose and hydrolysis of urea; assimilation of nitrate; and production of a film in broth (Kreger van Rij, 1984; Hoy *et al.*, 1986).

Immunoglobulin assays

Immunofluorescence assays were done as previously described (Quindós *et al.*, 1987). Briefly, 10 μ l of cell suspensions (10^6 /ml) in phosphate buffered saline (PBS) of three different isolates of *T. beigelii* were applied to teflon-coated microscope slides; 1 ml of vaginal washing supernate was lyophilised and resuspended in 0.5 ml of PBS for study by immunofluorescence assay; fluorescein-conjugated goat anti-human immunoglobulins A and G were diluted 1 in 320 in PBS supplemented with Evans blue 0.001% and Tween 20 0.001% for use.

Results

Of the 100 women included in this investigation, 23 were culture positive for yeasts. The organisms isolated as shown in the table. Microscopy of gram-stained vaginal smears was found to be positive in only a reduced number (18·2%) of the patients with a positive culture. It is noteworthy that the three isolations of *T. beigelii* were obtained on successive days. No common source of *Trichosporon* transmission by personnel or equipment was discovered and control cultures performed on them failed to grow *Trichosporon*.

The results obtained by immunofluorescence in the three patients with *T. beigelii* were compared with two control groups: patients with a positive growth of yeasts other than *T. beigelii*, and patients from whom yeasts were not grown (figure). Both IgG and IgA anti-*T. beigelii* antibody titres were raised (20–60) in *T. beigelii* carriers. Titres were highest when each patient was tested against her own *T. beigelii* isolate. Antibody titres were either much lower (≤ 20) or absent in the control groups.

Table. Yeasts isolated from vaginal washings from 23 patients with positive cultures

Species	Number of isolates
<i>Candida albicans</i>	12
<i>Trichosporon beigelii</i>	3
<i>Torulopsis glabrata</i>	3
<i>Candida parapsilosis</i>	2
<i>Candida tropicalis</i>	1
<i>Candida krusei</i>	1
<i>Candida guilliermondii</i>	1
Total	23

Discussion

Trichosporon beigelii is a common fungus associated with animals and has a wide distribution in nature (Kreger van Rij, 1984). In studies of immunocompromised patients, this organism has been demonstrated in samples of skin, sputum, stool and urine (Sandford *et al.*, 1980). It is considered that the most likely portals of entry in

Figure. Antibody response of IgA (▲ ■ ●) and IgG antibodies (△ □ ○) to *T. beigelii* observed in vaginal washings from 11 patients with and without *T. beigelii* isolation. *T. beigelii* strains used as antigens were isolated from patients 1 (▲, △), 2 (●, ○) and 3 (■, □). *Other yeasts: patients 4 and 5 had *C. albicans* vulvovaginitis; *C. albicans* and *T. glabrata* were isolated from vaginal washings from patients 6 and 7.

disseminated trichosporonosis are the alimentary tract and the lungs (Walsh *et al.*, 1986). Moreover, although the organism has been found to occur in high frequency in the rectal cultures of homosexual men (Torssander *et al.*, 1985), infections with *T. beigelii* have not yet been reported in patients with AIDS.

Although *T. beigelii* has been previously reported in vaginal cultures (Pritchard and Muir, 1985), it is not considered a common coloniser of the vagina (Goldacre *et al.*, 1979). However, the evidence of a humoral response in our subjects may suggest the presence of a subclinical infection. *T. beigelii* has been isolated from penile ulcers together with other micro-organisms (Chapel *et al.*, 1978). This raises the question of its role as a secondary pathogen, as well as of its possible sexual transmission.

The isolations of *T. beigelii* took place during a short time period. This corroborates the findings of Walsh *et al.* (1986) who reviewed the cases of disseminated trichosporonosis of their hospital and

observed that four of 15 occurred during the same month.

Although others have found cross reactions with *Cryptococcus neoformans* (Campbell *et al.*, 1985; MacManus and Jones, 1985; MacManus *et al.*, 1985), in this study there was no cross reactivity with *C. albicans*. The antibody titres to *T. beigelii* found in the vaginal washings of our control patients (both asymptomatic and with candida vaginitis) were very low or absent. These findings seem to contradict those reported by Matthews *et al.* (1986) who found anti-*T. beigelii* antibodies in the sera of all patients without clinical evidence of *T. beigelii* infection. Further investigations are required to determine the pathogenic significance of *T. beigelii* in the human vagina.

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