Biomedical science in brief

Candida species from genital sites: their identification and susceptibility to fluconazole

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The most common cause of vaginal candidiasis is infection with *Candida albicans*.[1] Traditional therapies, such as topical Canesten®, are both prolonged and inconvenient, often leading to poor compliance and recurrence of symptoms. The recent introduction of fluconazole, available without prescription to the general public at pharmacies (Diflucan®, Pfizer Ltd), has proved a convenient and popular alternative. However, other *Candida* species are involved in vaginal candidiasis,[1] and many of these have an intrinsic resistance to azole drugs.[2] With this in mind, we decided to determine both the *Candida* species isolated from the female genital tract and their susceptibility to fluconazole.

Unselected routine genital swabs were inoculated onto Sabaroud dextrose agar (Oxoid Ltd) with chloramphenicol (0.4 g/L) added to suppress bacterial growth. Cultures were incubated at 37°C for 48 h. Any yeasts isolated were sub-cultured onto CHROMagar (M-Tech Diagnostics Ltd) and a germ-tube test was performed.[3] Isolates that were germ tube-positive and produced green colonies on CHROMagar were identified as *C. albicans*. Pink colonies on CHROMagar that were germ tube-negative were presumptively identified as *Candida krusei*. Other isolates were not identified further. For the purpose of this study, strains that were germ tube-negative and produced colourless colonies on CHROMagar were designated 'other *Candida* species'. Minimum inhibitory concentration (MIC) tests were performed using fluconazole E-test® strips (AB Biodisk) on Casitone agar, and incubated at 30°C for 24 h, following the manufacturer's instructions. *C. albicans* NCTC 3179 was used as a control.

One hundred and twelve yeast isolates were collected from routine genital swabs over a period of four weeks; the breakdown into *C. albicans*, *C. krusei* and other *Candida* species is shown in Table 1.

Isolates were obtained from specimens received from general practitioners, the genitourinary medicine (GUM) clinic and hospital sources (both in-patient and out-patient), and the distribution is shown in Table 2. Of the isolates designated other *Candida* species, two were from hospital in-patients and one was from a patient attending the GUM clinic.

Table 1. Identification of *Candida* species isolated

Organism	Number isolated (%)
C. albicans	102 (91)
C. krusei	7 (6.3)
Other *Candida* species	3 (2.7)

Table 2. Distribution of isolates

Patient group	Number of *Candida* isolates (%)	Number of *C. krusei*, (%) of total in group
General practice	50 (44)	3 (6)
GUM clinic	22 (20)	2 (9)
In-patient	20 (18)	1 (5)
Out-patient	20 (18)	1 (5)

With *C. albicans*, the MIC of fluconazole ranged from 0.5 to 8.0 μg/mL (mean: 2.5 μg/mL) (Fig. 1); for all *C. krusei* strains, MICs were >265 μg/mL; and MICs for the three other *Candida* species were 1, 12 and 24 μg/mL.

With the reported emergence of infection due to *Candida* species other than *C. albicans*, and their general resistance to azole drugs, it was noted that *C. albicans* remains the predominant genital pathogen in this population. The proportion of *C. krusei* isolates was higher in GUM patients and may be a factor in their frequent reference to this clinic with recurrent thrush infection.

With *C. albicans*, the MICs of fluconazole were all <8.0 μg/mL, which is well within the ranged quoted for sensitive isolates.[4] Thus, treatment with a 150 mg tablet of Diflucan® should be effective against these

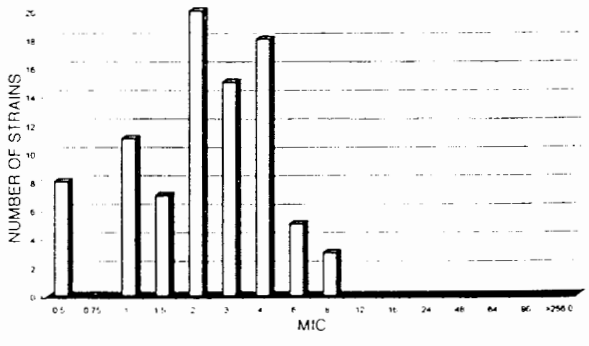

Fig. 1. MIC values of fluconazole with *C. albicans*.

C. albicans infections. However, as *C. krusei* has been shown to be resistant to fluconazole, we feel that it would be useful to identify genital yeast isolates, as patients with non-*C. albicans* infection would be at potential risk of treatment failure if given Diflucan®.

The cost of using a chromogenic agar routinely to isolate and identify yeasts from genital swabs is prohibitive to most laboratories.[5] However, the use of a medium such as CHROMagar to sub-culture germ tube-negative isolates for preliminary identification has minimal cost implications,[5] and would readily alert the laboratory to any increase in the proportion of non-*C. albicans* isolates.

References

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Haemorheological changes in sickle cell disease: measurement and significance

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Patients with homozygous sickle cell (HbSS) anaemia are heterogeneous with respect to clinical, rheological and haematological parameters, and attempts to demonstrate a correlation between these variables have produced conflicting results due to the difficulty in defining clinical severity in these patients.[1]

Rheological tests, such as erythrocyte sedimentation rate (ESR), plasma viscosity (PV) and fibrinogen levels,[2–4] are useful in monitoring sickle cell anaemia, and most are based on the mechanical and physical properties of the intact red cell. Although rapid and simple in concept, they require careful attention to technical detail. Therefore, we assessed a simple viscometer technique, described by Reid and Ugwu,[5] based on rate of flow. The method, which has been used successfully to assess both acute and chronic phases of the disease,[6–9] is simple, inexpensive, reproducible, correlates with standard methods, and uses only a small volume of blood or plasma.

Despite the importance of blood viscosity in clinical medicine, and its investigation in Caucasians,[10] few studies have been carried out on Africans of negroid stock. Therefore haemorheological changes were investigated in 25 patients with homozygous sickle cell (HbSS) anaemia (age range: 17–41 years, 12 male, 13 female), 29 with heterozygous sickle cell (HbAS) disease (age range: 19–39 years, 15 male, 14 female), and 44 normal (HbAA) controls (age range: 20–50 years, 22 male, 22 female). Patients recovering from a sickle cell crisis within the previous two weeks were excluded from the study. Sickle cell status was established by cellulose acetate electrophoresis using the method of Dacie and Lewis.[11]

Blood (5 mL) was obtained from each patient and control, with a minimum of venous occlusion, and collected into 3.8% sodium citrate (0.5 mL) on melting ice. Plasma fibrinogen concentration was measured on the day of collection using the method of Ingram.[12] Packed cell volume (PCV) was determined using microhaematocrit tubes and a microhaematocrit centrifuge. Whole blood viscosity (WBV) and PV were assessed, using the method of Reid and Ugwu,[5] on a sample (5 mL) collected into EDTA (1.5 mg/mL). A graduated syringe (1 mL, Gillette) was used, to which a hypodermic needle (21G, 0.8 × 40 mm, Nr. 2) was fitted. The composite syringe with its plunger and needle was held vertically in a retort stand, and was calibrated using clean distilled water and freshly pooled plasma at 37°C (± 0.5°C) in an incubator. The apparatus and test samples were allowed to equilibrate to 37°C before the tests were performed.

The fluid to be tested was drawn up into the vertical syringe, taking care to avoid air bubbles, until the end of the plunger passed the 1 mL graduation mark. The plunger was then removed carefully and a stop-watch started when the lower meniscus of the fluid fell to the 1 mL graduation mark. The time required for 1 mL of fluid to flow down the syringe was noted. The same syringe and needle combination was used for the whole series of measurements. The WBV and PV were expressed as the ratio of the flow-time for 1 mL of whole blood (Tb) or plasma (Tp) to the same volume of distilled water at 37°C. The mean flow-time for distilled water (xTw) was derived from 30 successive runs at 37°C, and this was found to be 17.01 s. Viscosity was calculated as follows:

$$\text{WBV} = \frac{\text{Tb (s)}}{17.01} \qquad \text{PV} = \frac{\text{Tp (s)}}{17.01}$$

Results were expressed as mean values (± standard error of the mean [SEM]), and statistical comparisons were carried out using Student's *t*-test for unpaired data.

The mean values (±SEM) for PCV, WBV, PV and fibrinogen obtained for the three groups are shown