# *Candida* species: Species distribution and antifungal susceptibility patterns

**Tracy Arendse, Heidi Orth**

**To the Editor:** *Candida* species cause serious infections in the immunocompromised and critically ill host. Studies have reported an emergence of non-albicans *Candida* (NAC) spp., particularly *C. tropicalis, C. glabrata, C. krusei* and *C. parapsilosis*.[1] The widespread use of fluconazole might have contributed to this increase in less-susceptible and intrinsically triazole-resistant *Candida* species.[2] While *C. albicans* remains the most common *Candida* species isolated at Tygerberg Hospital, especially in ICU patients, *C. tropicalis* and *C. parapsilosis* have emerged as predominant causes of candidaemia in children with haematological malignancies, and important pathogens in HIV-infected children. Fluconazole was active against all of our isolates and therefore continues to be the agent of choice for treating candidaemia. Because of changing trends, it is necessary to continuously or periodically monitor *Candida* species isolated in any specific setting, as well as antifungal activity, but this is not routinely done at many South African laboratories.

## Methods

We investigated the distribution and antifungal susceptibility of *Candida* species isolated from various clinical specimens and antifungal susceptibility patterns of bloodstream isolates at Tygerberg Hospital. All consecutive non-duplicate *Candida* species isolated from March to September 2005 were identified using the germ tube test and a commercial identification kit (Auxacolor 2, Bio-Rad, France). Susceptibility to amphotericin B and fluconazole was determined for all blood isolates (E-test method, AB BIODISK, Sweden). Results were read after 48 hours' incubation and interpreted according to NCCLS M27-A2, 2002. Quality control was performed using *Candida albicans* ATCC 90028 and *Candida tropicalis* ATCC 750. Patient data analysis included age, sex, specimen type, diagnosis and ward.

## Results

Of a total of 119 *Candida* isolates, 6 were excluded from the analyses as no patient details could be retrieved.

Seventy-five per cent of isolates were recovered from adults and 25% from children. *C. albicans* constituted 72%, and NAC 28%, of all *Candida* isolates from adult patients. In children, *C. albicans* accounted for 67%, and NAC for 33%, of isolates. The adult surgical intensive care unit (ICU) had the highest number of *C. albicans* isolates (15.2%). Other wards with a high number of *C. albicans* isolates included the labour ward and obstetric antenatal clinic (predominantly from urine specimens), the paediatric ICU, and the paediatric infectious diseases ward (predominantly HIV-infected patients). The paediatric infectious disease and oncology wards had the highest figure for NAC: 11.8% and 8.8% respectively.

*C. albicans* was the most common species isolated when comparing the isolation of *C. albicans* with NAC from urine (29% v. 17%), blood (16% v. 7%) and pus (8% v. 1%). All bloodstream isolates, including 17 *C. albicans*, 4 *C. parapsilosis*, 3 *C. tropicalis* and 1 *C. lucitaniae*, tested susceptible to both fluconazole and amphotericin B.

## Discussion

*C. albicans* (69%) is the most commonly isolated *Candida* species at Tygerberg Hospital, followed by *C. glabrata* (10%); *C. parapsilosis* (10%); *C. tropicalis* (4%); *C. lusitaniae* (2%); *C. krusei* (2%); and *C. lipolytica*, *C. guilliermondii*, *C. famata* (all 1%), which is consistent with international published data.[3]

Septicaemia was the most common diagnosis on the laboratory request forms. Positive blood culture isolates were mostly isolated from paediatric patients with malignancies where NAC predominated, which is consistent with studies citing that NAC occurs more frequently in leukaemia patients.[4] *Candida* species were also commonly isolated from urine. Urinary tract infections are usually associated with indwelling urinary catheters, particularly in patients on broad-spectrum antibiotics, although positive cultures may also represent contamination of urine specimens with vaginal flora, particularly in cases of vaginal thrush.

A high number of *C. albicans* were isolated from the surgical ICU. In contrast with trends among immunocompromised patients, *C. albicans* remains the predominant strain among ICU patients.[5] Risk factors for candidaemia include exposure to broad-spectrum antibiotics, invasive procedures and prolonged ICU stay.

All blood culture isolates tested susceptible to fluconazole, which is important since it has advantages over amphotericin B, including high oral bioavailability and low incidence of side-effects. Amphotericin B-susceptible results for *C. lusitaniae* should be interpreted with caution as this species has a higher propensity than other *Candida* species for developing resistance to amphotericin B.[6]

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# Pyruvate kinase deficiency in a South African kindred caused by a 1529A mutation in the PK-LR gene

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**To the Editor:** There is currently no investigation for pyruvate kinase (PK) deficiency in South Africa and nothing is known about local mutations. We describe the implementation of a PK assay and document the first mutation underlying PK deficiency in a South African patient who presented with a haemolytic episode following ARV (antiretroviral) prophylaxis.

PK deficiency is the most common inherited disorder of glycolysis in humans.[1] The enzyme catalyses the conversion of phosphoenolpyruvate (PEP) to pyruvate, producing adenosine triphosphate (ATP) from adenosine diphosphate (ADP) in the process. A PK deficiency therefore results in decreased intra-erythrocytic ATP, which cannot be compensated for by oxidative phosphorylation since erythrocytes lack mitochondria. This leads to membrane damage, haemolysis and premature destruction in the spleen.

Epidemiological studies indicated that the PK heterozygote allele frequency ranges from 1% to 5% in Caucasian populations, and one small study suggested the frequency might be twice as high in African-Americans.[2] Areas of south-east Asia may have a significantly higher prevalence,[2,3] although there have been no large-scale studies conducted in this region. The reason for the relatively high frequency of the abnormal PK allele has been debated. The majority of inherited erythrocyte disorders have been selected by their relative resistance to malaria,[4] and we have recently demonstrated that PK deficiency protects against malaria in humans (manuscript accepted for publication in *Haematologica*, May 2008), which provides a possible explanation.

The prevalence of the disease in South Africa is unknown, and few individual cases have been reported (personal communication, Dr N Alli, Chris Hani Baragwanath Hospital). There is currently no available assay for PK deficiency in South Africa, and nothing is known about the underlying mutations in the PK-LR gene in South Africans and Africans in general. More than 180 mutations cause the disease,[3] and it would be interesting to establish the mutation spectrum in southern Africans.

We report the implementation of an assay for PK activity. A patient with suspected PK deficiency was confirmed to have the disease, and the underlying mutation was identified.

## Methods

### PK assay implementation

The PK assay, based on Beutler's method[5] with a few minor modifications, was implemented in the Red Cell Membrane Unit, Department of Molecular Medicine and Haematology at the University of the Witwatersrand National Health Laboratory Service (NHLS) in Johannesburg. Qualitative (screening) and quantitative (confirmatory) assays were implemented, and the screening assay may be added to the routine tests offered by the NHLS. Guidelines for physicians are available from the authors.

The screening and quantitative assays use the same test principle: Plasma and leucocytes are removed from whole blood and the packed erythrocytes resuspended in 0.9% saline. A 1:20 haemolysate is made of the red cells and used to determine PK enzyme activity by the conversion of PEP to pyruvate. This reaction is coupled to a second reaction, which uses lactate dehydrogenase to convert pyruvate to lactate with nicotinamide adenine dinucleotide (reduced form) (NADH) as a co-factor. The oxidation of NADH is determined by a loss of fluorescence (screening assay) or spectrophotometrically at 340 nm (quantitative assay), and is used as a measure of PK activity.

Ethics clearance was obtained from the Human Research Ethics Committee (Medical), of the University of the



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