Short communication

**In vitro activity of five antifungal agents against clinical isolates of Saccharomyces cerevisiae**

FRANCESCO BARCHIESI, DANIELA ARZENI, PATRIZIA COMPAGNUCCI, LUIGI FALCONI DI FRANCESCO, ANDREA GIACOMETTI & GIORGIO SCALISE

Institute of Infectious Diseases and Public Health of the University of Ancona, Ancona, Italy

We evaluated the *in vitro* activity of fluconazole, itraconazole, ketoconazole, 5-fluorocytosine and amphotericin B against 30 clinical isolates of *Saccharomyces cerevisiae* by a broth microdilution method, following the NCCLS recommendation. Testing was performed either in RPMI-1640 or yeast nitrogen base (YNB). YNB supported the growth of all isolates tested, while results in RPMI-1640 were not obtained for six isolates (20%). The MIC of all three azoles in YNB were one or two dilutions higher than those obtained in RPMI-1640 (*P* = 0.001 for fluconazole and itraconazole, *P* = 0.03 for ketoconazole). Elevated MICs were observed for all three azoles, while all the isolates were susceptible to 5-fluorocytosine and amphotericin B. All MIC values were confirmed by spectrophotometric reading. Six strains of *S. cerevisiae* isolated from the faeces and consecutive blood cultures from an AIDS patient over a 7-month period were typed by electrophoretic karyotyping (EK). EK showed the maintenance of the same karyotype over time suggesting that the faecal isolate changed from a colonizing to infection-causing strain. The relative resistance of *S. cerevisiae* toazole drugs as well as its ability to cause widespread infections may promote the emergence of this species as a pathogen in immunosuppressed patients.

**Keywords** *in vitro* susceptibility testing, *Saccharomyces cerevisiae*

**Introduction**

*Saccharomyces cerevisiae* is a yeast used in baking, brewing, viniculture and for genetic and molecular biology research. Although commonly considered a non-pathogen, it has been reported recently in a number of cases of sepsis in patients with predisposing factors such as prolonged hospitalization, immunosuppression, broad-spectrum antibiotic therapy and foreign devices such as central venous catheters or prosthetic cardiac valves [1–3]. Recently, Arzeni *et al.* studied the prevalence of vaginal yeasts in 222 outpatients attending a gynaecological centre in Ancona, Italy, and found that *S. cerevisiae* was the most commonly isolated yeast after *Candida albicans* and *C. glabrata* [4]. In addition, it has been reported that some virulence characteristics, such as the ability to grow in suboptimal temperatures, formation of pseudohyphae, frequency of morphology switching and increased pathogenicity in mouse infection models, are more pronounced in clinical than in laboratory isolates of *S. cerevisiae* [5–7].

Thus far, an optimal treatment for infections due to *S. cerevisiae* has not been established and little information is available regarding the susceptibility of *S. cerevisiae* clinical isolates to the most common antifungal drugs [8,9].

The main aim of this study was to evaluate the *in vitro* activity of five systemic antifungal agents: fluconazole, itraconazole, ketoconazole, amphotericin B and 5-fluorocytosine against 30 clinical isolates of *S. cerevisiae*. 

Correspondence: Dr Francesco Barchiesi, Istituto di Malattie Infettive e Medicina Pubblica, Università degli Studi di Ancona, Ospedale Umberto I°, Largo Cappelli 1, 60121, Ancona, Italy. Tel.: +39 71 5963467; Fax: +39 71 5963468; E-mail: cmalinf@popcsi.unian.it

© 1998 ISHAM
Materials and methods

Thirty recent clinical isolates of *Saccharomyces cerevisiae* were used in the study. They were obtained from 25 individuals: 16 AIDS patients and nine HIV-negative subjects. The strains were isolated from faeces (*n* = 8), oral cavity (*n* = 5), blood (*n* = 5), vagina (*n* = 5), sputum (*n* = 4) and skin (*n* = 1). Putative identification of each isolate to the species level was carried out by morphological and biochemical conventional methods. *Candida krusei* ATCC 6258 and *S. cerevisiae* ATCC 9763 were used as control organisms in all experiments.

The following five antifungal agents were used: fluconazole (Pfizer), itraconazole (Janssen), ketoconazole (Janssen), amphotericin B (E. R. Squibb & Sons) and 5-fluorocytosine (Hoffmann-La Roche). Stock solutions were prepared in water (fluconazole and 5-fluorocytosine), polyethylene glycol (itraconazole and ketoconazole) or dimethyl sulphoxide (amphotericin B). Susceptibility testing was performed by a broth microdilution method, following the NCCLS recommendations [10]. Since in pilot experiments we found that RPMI-1640 did not support the growth of some isolates of *S. cerevisiae*, we conducted all the experiments by using either RPMI-1640 medium (Gibco) buffered to pH 7·0 with 0·165 m morpholinepropanesulphonic acid (MOPS) buffer (Sigma) and yeast-nitrogen-base (YNB) medium (YNB 6·7 g, glucose 10 g, asparagine 1·5 g 100 ml⁻¹, Difco) buffered to pH 7·0 with 0·165 m MOPS. Fifty µl of the inoculum, prepared spectrophotometrically and further diluted in test medium to obtain a concentration ranging from 1·0 to 5·0 × 10⁵ cfu ml⁻¹ (2 · inoculum), were added into the wells of each row of the microplates bringing the drug dilutions to the final test concentrations: 0·125–64 mg l⁻¹ for fluconazole and 5-fluorocytosine, 0·03–16 mg l⁻¹ for ketoconazole and amphotericin B and 0·0078–4·0 mg l⁻¹ for itraconazole. The microplates were incubated at 35 °C and read at 48 h. Visual reading (V) was performed with the aid of a reading mirror; the growth in each well was compared with that in the growth control (drug-free) well. Azole and 5-fluorocytosine MIC were defined as the lowest concentrations at which a prominent decrease in turbidity was observed (MIC-2; turbidity in the well which corresponds to 50% less than that in the control well [10]), while amphotericin B MIC was defined as the lowest concentration at which a complete absence of growth was observed. Spectrophotometric reading (SP) was performed with an automatic plate reader (Dynatech MR 700) set at 490 nm after the microplates had been agitated for 5 min by an automatic plate shaker. SP MIC endpoints were determined as the first concentration of the antifungal agent at which turbidity in the well was 50% (azoles and 5-fluorocytosine) or 90% less (amphotericin B) than that in the control well. The MIC data were logarithmically transformed to approximate a normal distribution prior to statistical analysis. Continuous variables were compared with the Mann–Whitney test. Differences were considered significant if *P* was less than 0·05.

Molecular typing of selected isolates was accomplished by electrophoretic karyotyping (EK). Briefly, agarose plugs containing yeast DNAs were prepared as described previously and inserted in 1% agarose gel [11] (Ultra Pure DNA Grade Agarose; Bio-Rad). Chromosomes were resolved with a counter-clamped homogeneous electric-field system (CHEF-DR).
Table 1  \textit{In vitro} susceptibility of 30 clinical isolates of \textit{S. cerevisiae} to five antifungal agents

<table>
<thead>
<tr>
<th>Drugs</th>
<th>Readings</th>
<th>MIC (mg l(^{-1})) determined in RPMI(^b)</th>
<th>MIC (mg l(^{-1})) determined in YNB(^b)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Range</td>
<td>50%</td>
</tr>
<tr>
<td>Fluconazole</td>
<td>V</td>
<td>0–5–16</td>
<td>2–0</td>
</tr>
<tr>
<td></td>
<td>SP</td>
<td>0–25–16</td>
<td>2–0</td>
</tr>
<tr>
<td>Itraconazole</td>
<td>V</td>
<td>≤0–007–0–5</td>
<td>0–125</td>
</tr>
<tr>
<td></td>
<td>SP</td>
<td>≤0–007–1–0</td>
<td>0–06</td>
</tr>
<tr>
<td>Ketoconazole</td>
<td>V</td>
<td>≤0–03–1–0</td>
<td>0–25</td>
</tr>
<tr>
<td></td>
<td>SP</td>
<td>0–06–2–0</td>
<td>0–06</td>
</tr>
<tr>
<td>5-Fluorocytosine</td>
<td>V</td>
<td>≤0–125–1–0</td>
<td>≤0–125</td>
</tr>
<tr>
<td></td>
<td>SP</td>
<td>≤0–125–4–0</td>
<td>0–5</td>
</tr>
<tr>
<td>Amphotericin B</td>
<td>V</td>
<td>0–25–4–0</td>
<td>1–0</td>
</tr>
<tr>
<td></td>
<td>SP</td>
<td>0–125–4–0</td>
<td>0–5</td>
</tr>
</tbody>
</table>

\(^a\) V, visual reading; SP, spectrophotometric reading; \(^b\) 50\% and 90\%, MIC at which 50\% and 90\% of the isolates tested were inhibited.

Results and Discussion

While YNB supported the growth of all isolates tested, results in RPMI-1640 were not obtained for six isolates (20\%) because of poor growth in this test medium. The MIC of all three azoles obtained in YNB were constantly one or two dilutions higher than those obtained in RPMI-1640 (\(P=0.0001\) for fluconazole and itraconazole; \(P=0.03\) for ketoconazole), while no differences were found for 5-fluorocytosine and amphotericin B MICs. Elevated MIC were observed for all three azoles. Fluconazole MIC at which 90\% of the strains were inhibited (MIC\(_{90}\)) were 4–0 mg l\(^{-1}\) in RPMI-1640 and 8–0 mg l\(^{-1}\) in YNB; itraconazole MIC\(_{90}\) were 0–25 mg l\(^{-1}\) in RPMI-1640 and 1–0 mg l\(^{-1}\) in YNB; ketoconazole MIC\(_{90}\) were 0–5 mg l\(^{-1}\) in RPMI-1640 and 1–0 mg l\(^{-1}\) in YNB (Table 1). In general, no differences were found in fluconazole, itraconazole and ketoconazole MIC for the strains isolated from HIV-negative individuals and those isolated from AIDS patients. Fluconazole MIC ranged from 0–5 to 4–0 mg l\(^{-1}\) and from 0–5 to 16 mg l\(^{-1}\) for the strains isolated from the HIV-negative and -positive individuals, respectively (\(P=0.26\)); ketoconazole MIC ranged from 0–06 to 0–5 mg l\(^{-1}\) and from 0–03–1–0 mg l\(^{-1}\) for the strains isolated from the HIV-negative and -positive individuals, respectively (\(P=0.84\)). Similarly, no differences between the two groups were found for azoles MIC obtained in YNB (data not shown). All the isolates were susceptible in \textit{vitro} to 5-fluorocytosine and to amphotericin B (Table 1). MICs for all five antifungal agents were confirmed by the spectrophotometric reading (Table 1).

Six strains of \textit{S. cerevisiae} isolated from the faeces and from consecutive blood-cultures of an AIDS patient over a 7-month period were typed by EK (Fig. 1). The first strain was isolated from the faeces in June 1995 (fluconazole MIC: 16 mg l\(^{-1}\)), four other strains were isolated from four blood cultures obtained on 17 January 1996 (range fluconazole MIC: 4–0–8–0 mg l\(^{-1}\)), while the last strain was isolated from a blood culture obtained on 18 January 1996 (fluconazole MIC: 8–0 mg l\(^{-1}\)). EK showed the maintenance of the same DNA type over time (Fig. 1).

Our findings underline several important features of the antifungal susceptibility of \textit{S. cerevisiae} clinical isolates. First, RPMI-1640 did not support the growth of all isolates of \textit{S. cerevisiae}. Five isolates (20\%) in our series showed an undetectable growth in RPMI-1640. Recently, \textit{S. cerevisiae} was reported that only two of \textit{S. cerevisiae} showed poor growth in this test medium [12]. On the other hand, our data showed that YNB is a suitable medium for testing these five antifungal agents against isolates of \textit{S. cerevisiae}. It must be emphasized, however, that azoles MICs obtained in YNB are one or two dilutions higher than those obtained in RPMI-1640.
Secondly, elevated MICs were observed for all three azoles. These data are in agreement with that reported recently by other authors [8,9]. It is interesting to note that we did not find any difference in the distribution of azole MICs between the strains isolated from the HIV-positive and -negative individuals. Almost all the HIV-positive patients in this series were treated withazole drugs, while none of the HIV-negative individuals had been treated with azoles. This fact suggests an intrinsic fungal resistance of \textit{S. cerevisiae} to azoles [12]. These MIC values were further confirmed by the spectrophotometric reading.

In order to analyse the maintenance or replacement of strains of \textit{S. cerevisiae} we performed EK on six strains isolated sequentially from an AIDS patient. Our data showed the maintenance of the same genotype during the time period considered and suggest that the faecal isolate changed from a colonizing to an infecting strain.

To this point, few data are available on DNA typing methods of clinical isolates of \textit{S. cerevisiae}. Recently, Zerva et al. investigated the genotypes of \textit{S. cerevisiae} clinical isolates by restriction endonuclease analysis of genomic DNA (REAG) in 70 bone marrow transplant patients [12]. They found that only one of the three patients who had multiple isolates typed was colonized with a single genotype, while the other two were colonized by multiple genotypes (2 or 3).

Further studies of surveillance in immunocompromised patients should be conducted to confirm the role of \textit{S. cerevisiae} as emerging pathogen.

**Acknowledgements**

This work was in part supported by a grant from Istituto Superiore di Sanità, Rome, Italy (X AIDS project).

**References**