**Candida inconspicua, a Fluconazole-Resistant Pathogen in Patients Infected with Human Immunodeficiency Virus**

*Candida inconspicua*, first described in 1952, has remained an obscure organism that has not previously been recognized as a pathogen. In an extensive review of new and emerging yeast pathogens, Hazen [1] discusses 20 *Candida* species but does not mention *C. inconspicua*. This yeast is phenotypically similar to *Candida krusei*, and biochemical differentiation between these species can be difficult and unreliable. We identified 11 *Candida* isolates that were difficult to identify to the species level: nine of these isolates were from seven patients with advanced HIV disease, and 1 blood and 1 sputum isolate each were from two patients with acute leukemia. These isolates were all identified with use of ID32C (bioMérieux; Marcy l’Étoile, France) as *C. krusei* or *C. inconspicua*.

The inability to develop pseudohyphae on rice agar tween medium (RAT) and to assimilate N-acetylglucosamine is believed to be characteristic of *C. inconspicua* [2]. However, we have found *C. krusei* strains with one or both of these properties. We sent all 11 isolates to two mycology reference laboratories for identification based on phenotypic criteria. At one laboratory, all the strains were classified as *C. krusei*, and at the other, workers were unable to confidently identify all the strains to the species level. Accurate identification was achieved only by karyotyping [3].

With use of the primers C11(GCGCACGG) and R108(GTATTG-CCCT), four of the 11 isolates (from three patients) had RAPD (random amplification of polymorphic DNA) banding patterns that were similar to those of the *C. inconspicua* type strains, and seven had banding patterns characteristic of *C. krusei* (figure 1). These results correspond with those obtained for the same isolates with use of CHEF-DR II (contour-clamped homogenous electric field) (Bio-Rad; Richmond, CA) [3]. Another isolate from patient 4 (in Brussels) had a pattern of RAPD bands similar to those of *C. inconspicua* with both primers (not shown).

Herein we report the findings for four patients infected with *C. inconspicua*. Susceptibility testing for fluconazole and itraconazole was done by a broth macrodilution method as previously described [5]. Susceptibility testing for ketoconazole was similar to that for itraconazole; the medium was yeast nitrogen broth (Difco; Surrey, UK) with 1% glucose without citrate. Flucytosine susceptibility was determined by disk diffusion and broth macrodilution tests, as previously described [6]. An isolate for which the MIC of each drug was known was tested in parallel with the test isolates to ensure reproducibility.

All four patients from whom yeasts with *C. inconspicua* banding patterns were isolated were men with advanced HIV disease and CD4 lymphocyte counts of <50 × 10⁹/L. All had had extensive prior exposure to fluconazole; patients 1 and 2 were receiving long-term secondary prophylaxis. In two cases, *C. inconspicua* was isolated in mixed culture from sputum; one of the isolates was recovered with *Candida albicans* (patient 3), and one was recovered with *Candida glabrata* (patient 1). Evaluation of response to therapy was not possible. In the other two cases (patients 2 and 4), *C. inconspicua* was the sole oropharyngeal *Candida* isolate at the time the patients presented with clinical thrush.

Patient 2 was treated with fluconazole but died of intercurrent illness 7 days later while he was still receiving fluconazole and still had oropharyngeal candidiasis. On day 3 of his illness, *C. inconspicua* and *C. albicans* were isolated in mixed culture of his sputum.

Patient 4 had esophageal and oral candidiasis that did not respond to treatment with fluconazole or the experimental azole D0870 (Zeneca; Alderley Edge, Cheshire, UK). He responded to treatment with intravenous amphotericin B (50 mg/d). From the third to the fourteenth day of this illness, both *C. inconspicua* and *C. albicans* were consistently isolated in mixed culture of specimens from his mouth. By day 21, only *C. albicans* was present.

The recovery of *C. inconspicua* in the absence of any other known pathogen from two of the patients suggests that this yeast may be capable of causing clinical disease in the setting of HIV infection. However, since both patients had *C. albicans* isolated repeatedly at other times, it is difficult to exclude the possibility that *C. albicans* may have been present as well in low numbers on first presentation of these episodes.

Susceptibility testing of isolates from patients 1, 2, and 3 showed resistance to fluconazole, with MICs of >12.5 mg/L, and intermediate resistance to ketoconazole (MICs, 1–4 mg/L) and low and intermediate resistance to itraconazole (MICs, 0.06–0.5 mg/L). The MICs of flucytosine for these isolates ranged from 0.25 mg/L to 0.5 mg/L. As we have performed susceptibility tests on results correspond with those obtained for the same isolates with use only a small number of each drug was known was tested in parallel with the test isolates so that results are clear-cut and easy to interpret.

*C. inconspicua* cannot always be reliably distinguished from *C. krusei* by phenotypic methods. We have previously reported our attempt to clarify this difficult taxonomic area by karyotyping [3]. We have found that the much simpler method of RAPD typing analysis, with use of either of two primers, also clearly identifies these organisms. Both species exhibit considerable karyotypic variability [2]; there is much greater intraspecies homology of RAPD banding patterns, so that results are clear-cut and easy to interpret. *C. inconspicua* can also be reliably identified by restriction enzyme digestion (HhaI) of the internal transcribed spacer region [7].

As a result of prolonged selection pressure, fluconazole-resistant non-*albicans Candida* species are being isolated with increasing frequency from the upper and lower respiratory mucosa of patients with AIDS [6]. *C. inconspicua*, which is another example of this phenomenon, may also be capable of causing clinically important disease.

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Figure 1. RAPD (random amplification of polymorphic DNA) banding patterns for clinical isolates of *Candida* thought to be *C. inconspicua* as they did not form pseudohyphae on rice-agar-tween (RAT) plates, compared with those for *C. inconspicua* and *Candida krusei* type strains. DNA was extracted by the method of Scherer and Stevens [4] with minor adaptations. Two primers were used: C11 (GCG-CACGG) and R108 (GTATTGCCCT). The annealing temperature was 30°C for C11 and 35°C for R108. Denaturation was done at 94°C, and extension was done at 72°C. The mixture underwent 35 thermal cycles in a programmable heating block (Crocodile II; Ampligene, Strasbourg, France). Ten microliters of the resulting reaction mixture were subjected to electrophoresis in 1.8% agarose. **Top,** primer CII. **Bottom,** primer R108. Lanes for both gels: 1 and 18, λ *PstI* digests (markers); 2, *C. inconspicua* (ATCC [American Type Culture Collection] 16782); 3, *C. inconspicua* (bio-Mérieux 3187); 4, *C. inconspicua* (bio-Mérieux 18949); 5–15, clinical isolates from patients (5, patient 1 [sputum]; 6, patient 2 [sputum]; 7, patient 3 [sputum]; 8, patient 2 [oral specimen]; 9–15, other RAT plate–negative isolates; 16, *C. krusei* (ATCC 6258); 17, *C. krusei* (bio-Mérieux 18888). **Arrows on left:** bands occurring only with *C. inconspicua*. **Arrows on right:** heavy bands characteristic of *C. krusei*.

References

Therapy with RP 59500 (Quinupristin/Dalfopristin) for Prosthetic Valve Endocarditis Due to Enterococci with VanA/VanB Resistance Patterns

The increasing incidence of vancomycin-resistant Enterococcus faecium (VREF) has recently been of great theoretical and clinical concern [1–3]. RP 59500 (quinupristin/dalfopristin; Synercid; Rhône-Poulec Rorer, Collegeville, PA) is a semisynthetic streptogramin active against many species of gram-positive bacteria; the synergistic effect of its components inhibits bacterial protein synthesis by binding to ribosomes. Of particular interest is the inhibitory activity of RP 59500 against clinical isolates of E. faecium with various patterns of resistance to other antibiotics [1–4]. We report a case of prosthetic valve endocarditis and persistent VREF bacteremia that cleared with RP 59500 therapy.

Table 1. Susceptibilities of vancomycin-resistant Enterococcus faecium isolates recovered from a patient with prosthetic valve endocarditis and persistent bacteremia.

<table>
<thead>
<tr>
<th>Antimicrobial agent</th>
<th>Day 1*</th>
<th>Day 60(^\dagger)</th>
<th>Day 90</th>
<th>Day 150</th>
<th>Day 180(^\dagger)</th>
<th>Day 225</th>
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<tr>
<td></td>
<td>Strain</td>
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<td>Streptomycin</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
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<tr>
<td>Gentamicin</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
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<tr>
<td>Vancomycin</td>
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<td>R</td>
<td>R</td>
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</tr>
<tr>
<td>Teicoplanin</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
</tr>
<tr>
<td>Ampicillin/sulbactam</td>
<td>S</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
</tr>
<tr>
<td>Imipenem</td>
<td>S</td>
<td>R</td>
<td>R</td>
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<tr>
<td>Ciprofloxacin</td>
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<td>R</td>
<td>R</td>
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<tr>
<td>Tetracycline</td>
<td>S</td>
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<tr>
<td>RP 59500</td>
<td>0.5(^\dagger)</td>
<td>0.5(^\dagger)</td>
<td>0.125(^\dagger)</td>
<td>0.125(^\dagger)</td>
<td></td>
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</table>

NOTE: I = intermediate; R = resistant; S = susceptible.
* Date of aortic valve replacement.
\(\dagger\) Start of teicoplanin therapy (at time of readmission).
\(\dagger\) Start of RP 59500 (quinupristin/dalfopristin) therapy.
\(\dagger\) MIC = 0.5 \(\mu\)g/mL (breakpoint not defined).
\(\dagger\) MIC = 0.125 \(\mu\)g/mL (breakpoint not defined).

A 48-year-old woman with a history of chronic renal failure, diabetes mellitus, chronic bilateral pedal osteomyelitis, and an infected hemodialysis access graft was admitted to the hospital when she was found to be bacteremic with VREF. She had been treated as an outpatient with vancomycin, gentamicin, and oral ciprofloxacin. Shortly after admission, the patient developed acute pulmonary edema; a two-dimensional echocardiogram revealed an aortic valve vegetation as well as an acute aortic regurgitation due to perforation of an aortic valve leaflet. She underwent emergency aortic valve replacement and was treated with intravenous teicoplanin for 6 weeks postoperatively. Blood cultures were negative, and she was discharged to her home.

The patient was readmitted 2 weeks later with fever. She had a temperature of 101.5°F, a blood pressure of 140/80 mm Hg, and a heart rate of 100. She was lethargic but arousable. The lungs were clear to auscultation, and the heart murmur was stable. The spleen tip was palpable. Blood cultures were positive for two strains of Enterococcus, both susceptible to teicoplanin. The patient had an aortic valve vegetation that was seen on a transesophageal echocardiogram. These findings were diagnostic of prosthetic valve endocarditis.

The patient was treated with intravenous teicoplanin. Two E. faecium strains were consistently isolated from successive blood samples and differed from each other with respect to their patterns of antibiotic resistance (table 1). Four months of intravenous teicoplanin therapy (12 mg/kg q48 h) failed to clear the bacteremia.

Financial support: The drug RP 59500 (quinupristin/dalfopristin; Synercid) was provided by Rhône-Poulec Rorer (Collegeville, PA) for use by the patient described in this case report, under guidelines for compassionate/emergency use.

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