Effect of pH on In Vitro Susceptibility of Candida glabrata and Candida albicans to 11 Antifungal Agents and Implications for Clinical Use

Claire S. Danby, a Dina Boikov, b Rina Rautema-Richardson, c and Jack D. Sobel b

Department of Obstetrics and Gynecology, Maine Medical Center, Portland, Maine, USA; Division of Infectious Diseases, Wayne State University School of Medicine, Detroit, Michigan, USA; and Manchester Academic Health Science Center, School of Translational Medicine, University of Manchester and University Hospital of South Manchester, Manchester, United Kingdom

The treatment of vulvovaginal candidiasis (VVC) due to Candida glabrata is challenging, with limited therapeutic options. Unexplained disappointing clinical efficacy has been reported with systemic and topicalazole antifungal agents in spite of in vitro susceptibility. Given that the vaginal pH of patients with VVC is unchanged at 4 to 4.5, we studied the effect of pH on the in vitro activity of 11 antifungal agents against 40 C. glabrata isolates and compared activity against 15 fluconazole-sensitive and 10 reduced-fluconazole-susceptibility C. albicans strains. In vitro susceptibility to fluconazole, fluconazole, voriconazole, posaconazole, itraconazole, ketoconazole, clodrimazole, miconazole, ciclopirox olamine, amphotericin B, and caspofungin was determined using the CLSI method for yeast susceptibility testing. Test media were buffered to pHs of 7, 6, 5, and 4. Under conditions of reduced pH, C. glabrata isolates remained susceptible to caspofungin and fluconazole; however, there was a dramatic increase in the MIC50 for amphotericin B and every azole drug tested. Although susceptible to other azole drugs tested at pH 7, C. albicans strains with reduced fluconazole susceptibility also demonstrated reduced susceptibility to amphotericin B and all azoles at pH 4. In contrast, fluconazole-sensitive C. albicans isolates remained susceptible at low pH to azoles, in keeping with clinical observations. In selecting agents for treatment of recurrent C. glabrata vaginitis, clinicians should recognize the limitations of in vitro susceptibility testing utilizing pH 7.0.

Vulvovaginal candidiasis (VVC) accounts for up to one third of all vaginitis cases presenting to gynecologists (1, 2, 3). VVC is most commonly caused by Candida albicans but can also be caused by non-albicans Candida species, with Candida glabrata being the most common (24, 26). Symptomatic C. glabrata vaginitis poses a significant problem for clinicians because effective treatment and eradication of C. glabrata from the vagina have proven difficult (23, 25, 26). The organism has variable intrinsic resistance to azole drugs (23, 25, 26). C. glabrata vaginitis has been moderately successfully treated with boric acid, but this is not curative in one third of patients (8, 23, 25, 26). Other therapies have been advocated, such as topical fluconazole, oral itraconazole, and nystatin suppositories (8, 18). Amphotericin B suppositories in patients with non-albicans Candida resistant to azoles were studied by Phillips and found to be promising; however, symptomatic C. glabrata vaginitis is often unresponsive to these regimens (11, 18). VVC is also occasionally caused by fluconazole-resistant C. albicans, posing a similar treatment dilemma in that susceptibility of these organisms to other azole and non-azole drugs is not clinically predictive (26).

Drug treatment of vaginal infections may be unique in that the normal pH of the vagina is 4 to 4.5, which remains unchanged during VVC (13). Previous studies have found that the test medium pH in in vitro susceptibility testing can alter the azole MIC for Candida species and that an acidic pH tends to increase the MICs of fluconazole for selected Candida species (16). However, it was concluded that more acidic conditions did not change the designation of the isolates from susceptible to resistant, neither were clinical implications evident. The purpose of this study was to determine whether a change in test medium pH had an effect on in vitro susceptibility of C. glabrata and both fluconazole-susceptible and reduced-susceptibility C. albicans to seven azole and four non-azole antifungal agents, in order to explain the frequent in vivo failure of these agents in women with vaginitis caused by C. glabrata.

MATERIALS AND METHODS

Vaginal isolates of C. glabrata and C. albicans were chosen from the Wayne State Vaginitis Clinic microbiology laboratory organism bank. The definition of fluconazole-susceptible C. albicans was the presence of an MIC of ≤2 µg/ml, and reduced susceptibility was defined as an MIC of ≥4 µg/ml (4). Vaginal isolates were randomly chosen from the years 2000 to 2010 and plated on CHROMagar to verify purity of culture. These plates were incubated for 48 h at 37°C in ambient air. Susceptibility testing was then performed using a broth microdilution method, according to CLSI document M27-A3 (2008) guidelines utilizing pH 7 (4). Antifungals and concentrations tested were fluconazole and fluconazole (at MIC ranges of 0.125 to 64 µg/ml), and voriconazole, posaconazole, itraconazole, ketoconazole, clodrimazole, miconazole, ciclopirox olamine, amphotericin B, and caspofungin (all with MIC ranges of 0.03 to 16 µg/ml). C. albicans isolates known to be fluconazole susceptible (MIC, ≤2 µg/ml) were not tested against itraconazole, ketoconazole, clodrimazole, and miconazole. A 0.1-ml yeast inoculum of 1.5 ×10^3 cells/ml in RPMI 1640 medium was added to each microdilution well. The trays were then incubated at 35°C for 48 h in ambient air. The MICs were read as the lowest

Received 7 June 2011 Returned for modification 26 July 2011 Accepted 24 December 2011 Published ahead of print 9 January 2012 Address correspondence to J. D. Sobel, jsobel@med.wayne.edu Copyright © 2012, American Society for Microbiology. All Rights Reserved.
doi:10.1128/AAC.05025-11

0066-4804/12/$12.00 Antimicrobial Agents and Chemotherapy p. 1403–1406 aac.asm.org 1403
Elevated MICs for fluconazole were observed for fluconazole-resistant isolates of both C. glabrata and C. albicans. These isolates were susceptible to posaconazole and voriconazole but resistant to fluconazole. This suggests that isolates with elevated MICs to one azole may also be resistant to other azoles.

**DISCUSSION**

The results of this study reveal that different classes of antifungals and the two species of Candida studied in vitro behaved differently with decrease in pH. The results confirm the susceptibility of fluconazole-sensitive C. albicans isolates to all azoles and the variable resistance of C. glabrata to fluconazole, and they may also offer insight as to why some antifungal medications may not be as effective in vivo with a more acidic physiologic vaginal pH. Previous studies similarly found that the medium pH can alter azole MICs for Candida species, and specifically an acidic pH was reported to increase the MICs of fluconazole for selected Candida species (16). The clinical implications of this observation were not, however, recognized.

C. glabrata vaginal infection is by no means infrequent, but case numbers are insufficient to perform a randomized controlled trial in order to establish optimal treatment (23, 25, 26). The resistance of C. glabrata to fluconazole, at all pH levels, observed in the present study is consistent with numerous in vitro studies (19, 20) and reflects experience when treating vulvovaginal candidiasis. No MICS for fluconazole were observed in this study for any isolate; however, the clinical implications of this observation were not, however, recognized.

C. albicans isolates were susceptible to all azoles tested at all pH levels. The trends observed for MICs of fluconazole for selected C. albicans isolates were similar to those observed for C. glabrata isolates. The clinical implications of this observation were not, however, recognized.

**TABLE 1 MIC<sub>50</sub> and MIC<sub>90</sub> susceptibility results**

<table>
<thead>
<tr>
<th>Type (no.) of isolates</th>
<th>pH</th>
<th>FPC</th>
<th>FLU</th>
<th>AMB</th>
<th>VORI</th>
<th>POSA</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>MIC&lt;sub&gt;50&lt;/sub&gt;</td>
<td>MIC&lt;sub&gt;90&lt;/sub&gt;</td>
<td>MIC&lt;sub&gt;50&lt;/sub&gt;</td>
<td>MIC&lt;sub&gt;90&lt;/sub&gt;</td>
<td>MIC&lt;sub&gt;50&lt;/sub&gt;</td>
</tr>
<tr>
<td>Fluconazole-resistant 7</td>
<td>0.125</td>
<td>0.125</td>
<td>0.125</td>
<td>32</td>
<td>8</td>
<td>2–64</td>
</tr>
<tr>
<td>C. glabrata (40)</td>
<td>6</td>
<td>0.125</td>
<td>0.125</td>
<td>0.125–2</td>
<td>&gt;64</td>
<td>&gt;64</td>
</tr>
<tr>
<td>Fluconazole-resistant 5</td>
<td>0.125</td>
<td>0.125</td>
<td>0.125–1</td>
<td>&gt;64</td>
<td>&gt;64</td>
<td>2–64</td>
</tr>
<tr>
<td>C. albicans (10)</td>
<td>4</td>
<td>0.125</td>
<td>0.125–0.5</td>
<td>&gt;64</td>
<td>&gt;64</td>
<td>2–64</td>
</tr>
<tr>
<td>Fluconazole-sensitive 7</td>
<td>2</td>
<td>0.125–4</td>
<td>0.125–4</td>
<td>4</td>
<td>2</td>
<td>0.125–16</td>
</tr>
<tr>
<td>C. albicans (15)</td>
<td>6</td>
<td>0.125</td>
<td>0.125–0.5</td>
<td>0.5</td>
<td>0.25</td>
<td>0.25–1</td>
</tr>
<tr>
<td>Fluconazole-sensitive 5</td>
<td>0.25</td>
<td>0.125–2</td>
<td>0.25</td>
<td>0.125–2</td>
<td>32</td>
<td>8</td>
</tr>
</tbody>
</table>

<sup>a</sup> FPC, flucytosine; AMB, amphotericin B; CASPO, caspofungin; CPO, ciclopirox olamine; FLU, fluconazole; VORI, voriconazole; POSA, posaconazole; ITRA, itraconazole; KTZ, ketoconazole; CLO, clotrimazole; MIC, miconazole.
Topical amphotericin B has in small studies demonstrated effectiveness for treatment of non-
*Candida* vaginitis, but emerging resistance to this antifungal has been documented (11, 18). Topical amphotericin B has also been used in combination with other antifungals, such as fluconazole. If fluconazole is as effective as previously described and stable at a low pH, perhaps it is contributing much more than amphotericin B to successful treatment (25). It was found in the present study that for both *Candida* species, amphotericin B activity was profoundly affected by pH, with at least a 16-fold increase in MIC90 with decrease in pH.

Ciclopirox olamine is an agent applied topically and well known for its potency against dermatomycoses, tinea pedis, pityriasis versicolor, and seborrheic dermatitis. Its use in treating vaginal candidiasis has also been studied, with limited success (9, 17, 21), and it has shown clinical promise against azole-resistant *Candida* species, including *C. glabrata*. It has demonstrated good topical and systemic tolerance in rats and rabbits when vaginal tissue was examined (5, 15) and has been studied in settings with a lower pH (9, 10). However, in this study, a 4-fold rise in MIC90 from 0.5 to 2 μg/ml with a decrease in pH was seen. One factor that limits the clinical application of these data is that the breakpoint of ciclopirox olamine is unknown and the clinical relevance of increased MICs is questionable.

Caspofungin is an echinocandin that has demonstrated activity against *Candida* species both in vitro and in vivo for systemic infections (6). None of the echinocandins are available as topical agents, and they have not yet been studied for vulvovaginal candidiasis or at decreased pH levels. The results of this study demonstrated stable MICs with a decrease in pH, with all *C. albicans* isolates having an MIC90 of less than 2 μg/ml, and continued activity against *C. glabrata* isolates at lower pH. Additional studies would need to be performed to evaluate echinocandin response in vivo as a topical compound.

This *in vitro* study demonstrates the potential limitations of conventional *in vitro* testing in predicting antifungal clinical success when faced with the challenge of treating recurrent vulvovaginal *C. glabrata* infections as well as fluconazole-refractory *C. albicans* vaginitis. Although the importance of medium pH in standardizing susceptibility testing is widely recognized in recommending routine testing at pH 7, the profound effect of pH on *C. glabrata* susceptibility has not been appreciated but is probably relevant only to patients with yeast vaginitis. The exact mechanism of pH-induced reduced susceptibility has not been established. In contrast, fluconazole-susceptible *C. albicans* strains responsible for the majority of vaginitis episodes are less vulnerable to the pH influence. Finally, *C. albicans* vaginal isolates already demonstrating reduced azole sensitivity at pH 7 are further compromised by lowering pH, resembling the effect seen with *C. glabrata*. This study also emphasizes the need for new alternate agents for treatment of *C. glabrata* vaginitis as well as to consider measuring *C. glabrata* drug susceptibility *in vitro* at pH 4 to 5 before recommending antymycotic therapy; however, validation studies are essential.

**REFERENCES**

characterizing the association between previous fluconazole use and fluconazole resistance. Am. J. Infect. Control 38:456–460.