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Impact of pH on the Minimum Inhibitory Concentration (MIC) for Antifungal Agents against Different Yeast Strains

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Abstract

To better assess efficacy in serious blood and soft tissue infections, antifungal susceptibility testing is standardized to a neutral pH level of 7. However, these tests may not accurately reflect antifungal activity in the acidic environment of the vagina, contributing to the discrepancy between *in vitro* and *in vivo* performance of antifungal agents in the treatment of vulvovaginal candidiasis (VVC). The objective of this study is to explore the effect of acidic pH on the antifungal activity of four different agents. To do this, we compared the minimum inhibitory concentrations (MICs) of miconazole, fluconazole, tioconazole, and terconazole against seven species of yeast in two pH environments (pH of 4.0 and 7.0). Strain-specific MIC values increased for nearly all antifungal agents at a pH of 4.0 as compared with a pH of 7.0. An exception to this trend was tioconazole activity against *Saccharomyces cerevisiae*, where MIC improved with a lower pH. Of the antifungal agents examined, miconazole had the lowest MICs across almost all strains at either pH. Its MICs against *Candida glabrata*, *Candida parapsilosis*, and *S. cerevisiae* remained relatively stable at the lower pH compared with the other three agents. Thus, the MIC values of antifungal agents against yeast strains are impacted by a decrease in pH, which may account for the inconsistencies in clinical performance when treating VVC. Furthermore, obtaining a vaginal pH and identifying the yeast strain present may have clinical utility in tailoring the selection of antifungal agents to the actual clinical environment.

Keywords

Candida, Vulvovaginal Candidiasis, Antifungal, Susceptibility Testing, Vaginal pH

1. Introduction

Vulvovaginal candidiasis (VVC) will impact approximately 70% of women at least once in their lifetime, with significant implications on quality of life [1]. This translates into a frequent concern for the obstetrics-gynecology community, and it represents nearly $368 million in treatment expenses and roughly 1.4 million outpatient visits each year in the United States [2].

Although *Candida albicans* is traditionally implicated in VVC, the prevalence of non-*albicans Candida* species is rising. The *non-albicans Candida* species included in this study, *C. glabrata*, *C. krusei*, *C. tropicalis*, *C. lusitaniae*, and *C. parapsilosis*, are the most common species isolated in VVC in recent investigations, sometimes representing over 50% of infections. *S. cerevisiae* has recently been implicated as a species of non-candida vulvovaginal infections, presumably due to its common use in foods, beverages, and probiotics [3]. These non-*albicans Candida* yeast are more likely to be resistant to fluconazole, a first-line agent, representing a growing challenge in the treatment of VVC [3] [4]. Alternative antifungal treatments include topical azole antifungals such as miconazole, tioconazole, and terconazole. These antifungal agents have demonstrable *in vitro* activity against *C. albicans* and non-*albicans Candida* species. Even so, clinical treatment of VVC has failed to mirror these *in vitro* sensitivity results, with *in vivo* treatment yielding mixed and often disappointing clinical results. Recurrence is common, with recurrent VVC defined as ≥3 episodes of VVC within one year [5]. Roughly 138 million women are impacted annually with recurrent infections, with a predicted increase to 158 million by 2030 [1].

This discrepancy between *in vivo* and *in vitro* results may be due in part to the impact of pH on susceptibility testing. Antifungal susceptibility testing is routinely performed at a pH level of 7. This is useful in the context of life-threatening bloodstream infections, where the neutral pH more accurately depicts the targeted antifungal environment. However, the pH level of the vagina ranges from 3.8 - 5.0 and is often within this normal range with VVC [6]. Several studies have posited the utility of antifungal susceptibility testing at a lower pH level, noting the increase in the antifungal minimum inhibitory concentration (MIC) with a decrease in the pH environment [7] [8]. However, these studies focused on antifungal activity against *C. albicans* or limited non-*albicans Candida* species, including *C. glabrata*. This study attempts to expand on the work as it explores the impact of pH on the MIC of antifungal agents against six *Candida* species and *Saccharomyces cerevisiae*. In doing so, this study attempts to highlight the potential applicability of pH-tailored VVC treatment.

2. Methodology

The seven yeast strains selected for this study are as follows: *C. albicans*, *Candida glabrata*, *Candida tropicalis*, *Candida lusitaniae*, *Candida krusei*, *Candida parapsilosis*, and *S. cerevisiae*. Cultures were prepared via direct colony suspension from Sabouraud dextrose agar. Miconazole cream 4% (200 mg), 100-mg flucona-

zole tablets, tioconazole ointment 6.5% (300 mg), and terconazole cream 0.4% (20 mg) were used to prepare stock solutions of miconazole, fluconazole, tioconazole, and terconazole, respectively. These stock solutions were prepared in dimethyl sulfoxide to a minimum concentration of 10 times the highest concentration necessary for testing (6400 micrograms per microliter). Next, the solutions were serially diluted (10×) with test medium RMPI-1640 or a complete supplement mixture to ensure the solvent concentration did not exceed 1%. Any pH adjustment needed was done prior to starting the study using NaOH or HCl. The media used, RPMI-1640, has a buffering system consisting of sodium bicarbonate, which helps to stabilize the pH. The plates were sealed well during incubation and not checked again at the end of the incubation period. The antifungal solutions were loaded onto microdilution plates that were inoculated with 100 microliters of inoculum prepared from the yeast suspensions. The microdilution plates were incubated without agitation at 35 degrees Celsius for 24 - 48 hours. The growth within each microdilution plate well was compared with that of the growth in the control tubes (no antifungal) to determine the MIC. The MIC was defined as the antifungal concentration at which yeast growth is inhibited by 50% as compared with control growth (with no drug exposure). The study was repeated three times for each unique combination of pH (4.0 vs 7.0), yeast strain, and antifungal agent.

3. Results

Figure 1 displays the average MIC for each combination of pH, yeast strain, and antifungal agent. Figure 2 illustrates the impact of pH on the MIC, expressed as the difference between the MIC at a pH of 4.0 and a pH of 7.0. Therefore, a more positive value indicates the MIC was increased at a pH of 4.0 compared to 7.0, implying that antifungal activity may be significantly impeded by a decrease in pH level.

Strain-specific MIC values increased for nearly all antifungal agents at a pH of 4.0 compared with a pH of 7.0. An exception to this trend was tioconazole activity

		Average MIC (averaged over three trials)							
	Antifungal Agent	Miconazole		Tioconazole		Terconazole		Fluconazole	
	pH	4	7	4	7	4	7	4	7
Yeast Strains	C. albicans	0.25	0.073	1.7	0.46	>16	0.083	1.7	0.67
	C. glabrata	0.83	1.0	16	0.83	>16	1.3	>16	16
	C.tropicalis	0.33	0.83	2.0	0.67	16	2.7	1.0	0.10
	C. lusitaniae	0.25	0.17	2.0	0.88	>16	0.33	3.3	3.3
	C. krusei	2.0	0.50	>16	5.3	>16	0.75	>16	>16
	C.parapsilosis	0.50	0.42	8.0	5.3	>16	0.10	4.0	0.42
	S. cerevisiae	0.25	0.25	2.0	16	>16	>16	>16	>16

Figure 1. Average minimum inhibitory concentrations (MIC) for each antifungal agent, yeast strain, and pH.

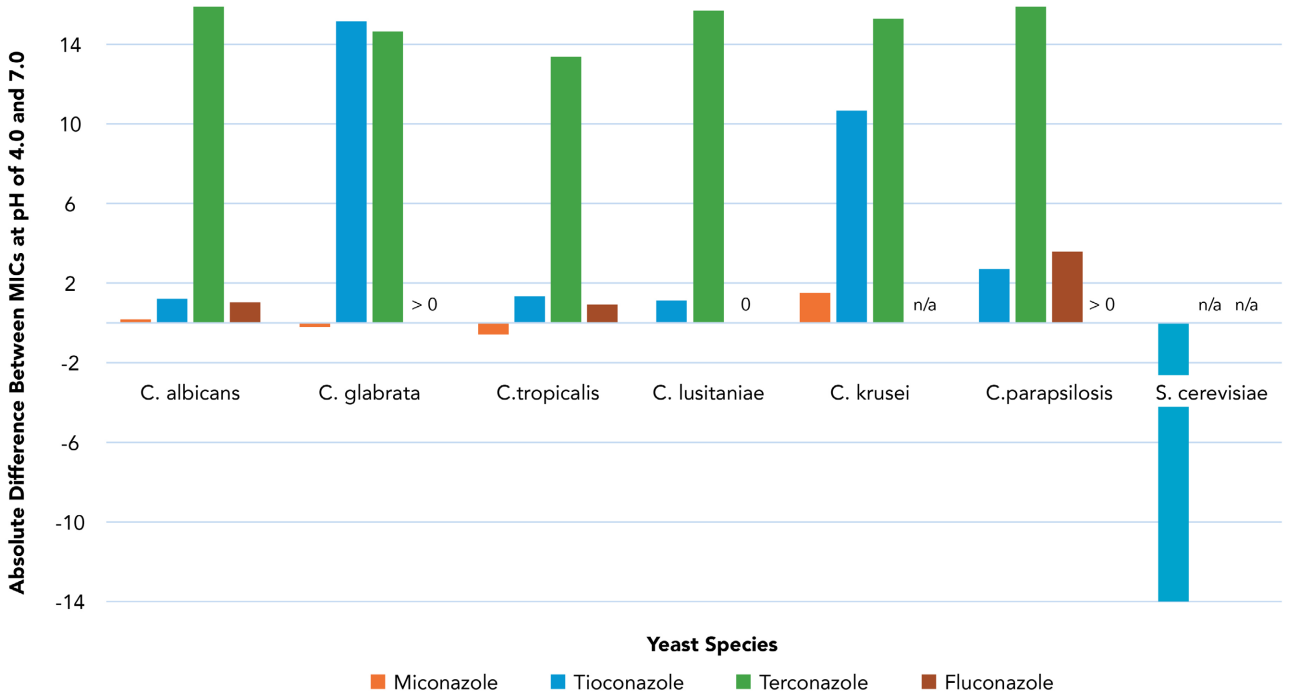

Figure 2. Impact of pH on the minimum inhibitory concentration (MIC) of antifungal agents against different yeast strains.

against *S. cerevisiae*, where MIC improved with a lower pH. Of the antifungal agents examined, miconazole had the lowest MICs across almost all strains, regardless of the pH. Its MICs against *C. glabrata*, *C. parapsilosis*, and *S. cerevisiae* remained relatively stable despite a drop in pH compared with the other three agents. In fact, miconazole's average MICs against *C. glabrata* and *C. tropicalis* were lower at a pH of 4.0 as compared to pH 7.0.

4. Conclusions

The MIC values of antifungal agents against yeast strains are impacted by a lower pH level, consistent with the naturally occurring vaginal environment for most yeast infections in most reproductive-aged women. This may account for the inconsistencies in clinical performance when treating VVC.

This study highlights that the impact of pH on MIC is specific to both the yeast strain and antifungal agent. Fluconazole is a unique orally administered agent that must be absorbed and transported in the blood to the vaginal epithelium, representing a novel pathway that exposes the agent to varying pH levels. The topical antifungal agents have been found to act differently with changing pH levels due to alterations in yeast cell adherence to the vaginal epithelium, drug solubility, and fungal membrane interactions. If corroborated, a more personalized or precise application of these findings may incorporate determining the vaginal pH level at the time of fungal testing (polymerase chain reaction or culture) with the use of an antibiogram for antifungal agents, similar to what is determined in hospital susceptibility reports. Once the species is identified, the antifungal agent most likely to be effective at that patient's specific pH level could be prescribed. The

lack of antifungal susceptibility testing at the pH level of the infected site (vagina) and the failure to determine the pH level of the patient's infected site should put into question most clinical trial results to date. For example, a clinical trial that had a disproportionate number of menopausal patients (pH closer to 7.0) or was limited to *C. albicans* species may have falsely skewed the clinical success rate [9]. In another case, a clinical trial that looked at reproductive-aged women (usually pH close to 4.0), screened out vaginal bacteria or protozoal infections (pH 5.0 - 7.0), and included non-*albicans Candida* species would possibly have had lower success rates [10].

Therefore, this study highlights the potential utility of tailoring antifungal treatment in VVC according to the yeast species and pH level. While most infections with *Candida* occur at the lower pH of the vagina (3.8 - 4.2), there are also times when yeast infections exist at a higher pH level. For example, a mixed infection with bacterial vaginosis or trichomoniasis or following treatment with an antibiotic whereby the lactobacilli have been eradicated would often have a pH level > 5.0.

This study supports antifungal susceptibility testing at both higher and lower pH values. Doing so allows one to explore which antifungal agents may outperform others at a given pH value, with implications for selecting a VVC treatment specific to a patient's vaginal pH environment and implicated yeast strain. However, in most cases, pH determination is not performed, and antibiograms of antifungal agents have not been performed, so some general trends can be extrapolated from the current data.

Generally, it appears that fluconazole and terconazole have the greatest increase in MICs with the reduction in the pH to normal physiologic levels, with fluconazole entering the resistance range in over half the species and terconazole entering the resistance range for every species, including *C. albicans*.

Miconazole has small if any, increases in the MICs and would appear from this research to potentially be the preferable choice for empiric therapy or directed therapy if the pH is in the normal premenopausal physiologic range.

Further prospective research is necessary to develop and confirm the efficacy of a pH- and strain-tailored antifungal protocol in the treatment of VVC and to examine its clinical impacts.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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