

CLINICAL REPORT

Efficacy of Fluconazole and Nystatin in the Treatment of Vaginal *Candida* Species

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The aim of this study was to determine and compare the efficacy of treatment with fluconazole and nystatin in Brazilian women with vaginal *Candida*. In a population of 932 women, vaginal cultures were performed for yeasts, whether or not the women showed signs and symptoms of vulvovaginal candidiasis. Yeasts were isolated from 12.2% of the women (114/932): 53.2% of the yeasts were *Candida albicans*, 27.0% *C. glabrata*, 13.5% *C. tropicalis* and 6.3% *C. parapsilosis*. Treatment was carried out with both drugs. The overall mean cure rates with fluconazole (87.0%) and nystatin (74.0%) were similar; among women with non-*albicans*, the cure rate with fluconazole was 100%, whereas that with nystatin was 44.4%. The cure rate for women with *C. albicans* was high with both fluconazole and nystatin; however, for those with non-*albicans* species the cure rate was excellent with fluconazole and very low with nystatin, differing from the majority of *in vitro* studies. Key words: efficacy; fluconazole; nystatin; treatment; vaginal *Candida* spp.

(Accepted May 4, 2011.)

Acta Derm Venereol 2012; 92: 78–82.

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Vaginitis is one of the most common conditions treated by physicians in the USA, and results in 10 million medical appointments annually (1). Approximately 30% of all cases of vaginitis are caused by infection with *Candida* species, and such cases are termed vulvovaginal candidiasis (VVC) (1, 2). VVC is caused predominantly by species of the genus *Candida*, mainly *C. albicans*. According to some investigators, the prevalence of this yeast can reach 85–95% (1). However, infections by non-*albicans* species (*C. tropicalis*, *C. glabrata*, *C. krusei*, *C. parapsilosis*, *C. kyfer* and *C. lusitaniae*) are increasing, and in some populations, *C. albicans* has been found to be responsible for only 50% of cases (3, 4).

VVC is clinically characterized by intense itching, burning, dyspareunia, dysuria, oedema, vulvovaginal

erythema and a grumous creamy vaginal discharge. The symptoms worsen during the pre-menstrual period, when vaginal acidity increases. The lesions may extend to the perineum, perianal and inguinal regions. In typical cases, yellowish-white spots appear on the vaginal walls and cervix. Itching is considered the most important symptom when VVC is compared with vulvovaginitis of other aetiologies (1, 2, 9).

It is estimated that approximately 75% of adult women experience at least one episode of VVC during their lifetime, while 40–50% of these will experience further occurrences and 5% will reach recurrent infection status (RVVC), defined as the occurrence of four or more symptomatic episodes within a 12-month period (1, 5, 6).

The therapeutic arsenal available for the treatment of fungal infections is limited to polyene and azole antifungals (7). For the polyene drugs, nystatin (NYS) in the form of an ointment or vaginal pessary has been used for almost three decades. However, the high frequency of vaginal isolates with dose-dependent susceptibility (DDS) to this drug, as observed in some studies, has caused concern, since the recommended dose may be insufficient to achieve the desired therapeutic efficacy. Amphotericin B is an excellent therapeutic option because of its high efficacy; however, because it is highly toxic, only topical formulations exist for use in VVC (8).

Among the azole drugs, fluconazole (FLU) is one of the most widely used in VVC, although in addition to its higher cost, resistance to FLU has been reported in both *C. albicans* and non-*albicans* species (1, 9). Together, these studies show that there are differences with regard to the species of vaginal yeasts isolated as well as in their profiles of susceptibility to antifungals. Therefore, knowledge of the therapeutic success of the most widely used antifungals in different populations is very important. The aim of the present study was to determine and compare the efficacy of treatment with FLU and NYS of women in the state of Paraná, Brazil, with vaginal *Candida* species.

PATIENTS AND METHODS

The study involved women aged between 15 and 83 years who participated in the Cervical Cancer Triage Program, regardless

of whether they showed signs and symptoms of VVC. These women were seen during the period between 1 January and 31 December 2009 at the Paicandú Municipal Health Center, which is part of the public health system (Sistema Único de Saúde; SUS) of Paraná. Pregnant women or those with a history of immunosuppressant disease, including AIDS, were excluded from the study. The women answered a standardized questionnaire with information regarding symptoms of VVC and epidemiological data. All the women gave their written informed consent to participate in the study. This research was approved by the Committee for Ethics in Research with Humans (COPEP) at UEM (CAAE no. 0006.0.093.000-07, report no. 185/2007).

Prior to the collection of smears for cervical-vaginal cytology and bacterioscopy, material was collected from the rectouterine pouch with the aid of a sterile swab and a disposable vaginal speculum, inoculated into sterile saline, and immediately seeded on plates containing Sabouraud dextrose agar (SDA) (Difco, Detroit, USA), with the addition of 100 mg/ml chloramphenicol, and incubated at 25°C for up to 5 days. A pool of the colonies grown on each plate was subcultured in CHROMágar *Candida*[®] (Probac, France). Beginning with the pure culture, the yeasts were identified by classical phenotypic methods (10).

Women with vaginal yeasts were classified according to their clinical profile, which was determined from information obtained at the time of sample collection (5), as follows: asymptomatic or colonized, those without symptoms of VVC; with VVC, those with an acute episode, consisting of women who presented with at least two symptoms (discharge, itching, dysuria and dyspareunia) and no previous episode within a period of 12 months; with RVVC, women presenting two or more of the symptoms, and four or more episodes within a period of 12 months.

Those women whose cultures were positive for yeasts were invited to undergo treatment with fluconazole (FLU) or nystatin (NYS), and were told that they would be requested to return for a follow-up visit after the treatment, in order to monitor the cure rate. A total of 60 women agreed to participate in this part of the study. The antifungals FLU and NYS were selected for the treatment because they are available free of charge through the Brazilian SUS; each represents a class of antifungals, polyenes and azoles, respectively, which are most often used worldwide. NYS is available only in topical formulations, and FLU is administered orally. The women were divided into two treatment groups: Group 1 (FLU): treatment with oral fluconazole (Fluconeo, Neoquímica, São Paulo, SP, Brazil), single dose of 150 mg weekly, for 2 weeks; Group 2 (NYS): treatment with nystatin vaginal cream (Nistatina, Teuto, Anápolis, GO, Brazil), 25,000 IU/g, in a tube containing 60 g and a 7 ml applicator for daily application on 7 consecutive days. Thirty women were randomly assigned to each treatment group, independently of the clinical profile and the species of *Candida* isolated. They were instructed to return between 20 and 30 days after the completion of the treatment, to receive a second clinical evaluation including collection of material for culture, so that cure rates could be established for each group. Mycological cure was considered to have been achieved when the result of culture for yeasts after treatment was negative. On their return visit, the patients were asked about the presence of side-effects during treatment, mainly vulvovaginal irritation or pruritus in the NYS group, and nausea, abdominal pain, diarrhoea, flatulence, rash and headache in the FLU group.

The data obtained were subjected to analysis of variance (ANOVA), using the program Graph Pad Prism (Graph Pad[®] Soft, San Diego, CA, USA). A level of $p < 0.05$ was considered significant.

RESULTS

Of the total of 932 women included in this study, yeasts were isolated from the vaginal exudate of 114 (12.2%). Complete data on the isolation and identification of the yeasts were obtained for 111 patients, whose isolates were distributed as follows: *C. albicans* ($n=59$; 53.2%) and non-*albicans* species ($n=52$; 46.8%) (Table I). Among the latter, *C. glabrata* was the most frequent isolate ($n=30$; 27.0% of all yeasts isolated), followed by *C. tropicalis* ($n=15$; 13.5%) and *C. parapsilosis* ($n=7$; 6.3%).

Of the women with vaginal yeasts, 38.7% showed colonization, of which 65.0% consisted of non-*albicans* species; 48.7% had VVC, of which 61.1% was caused by *C. albicans*; and 12.6% had RVVC, 78.6% caused by *C. albicans* (Table I). The rates of isolation for *C. albicans* and non-*albicans* species in the women with different clinical profiles were similar ($p=0.5844$). The rates of isolation of the different non-*albicans* species in the women with different clinical profiles were also similar to each other ($p=0.1353$).

The mean age of the women with vaginal yeasts was 38.0 years, which was lower than that of women with a negative culture (43.3 years) ($p=0.0185$). The mean age of women with *C. albicans* and non-*albicans* species did not differ among the groups with different clinical profiles ($p=0.4483$), although the mean age was lower for those with *C. albicans* (31.5 years) than for those with non-*albicans* species (44.6 years) ($p=0.0115$). Among the women with non-*albicans* species, those with *C. tropicalis*, *C. glabrata* and *C. parapsilosis* had mean ages of 46.1, 47.7 and 40.1 years, respectively ($p=0.0955$).

A total of 23 women in the FLU group and 23 in the NYS group completed the treatment, including the follow-up examination and yeast culture within the required period. These consisted of 16 women with *C. albicans* and 7 with non-*albicans* species who were treated with FLU, and 14 with *C. albicans* and 9 with non-*albicans* species who were treated with NYS. These 46 women comprised 40.3% of the original group with vaginal cultures who were positive for yeasts.

Table I. Relative distribution of *C. albicans* and non-*albicans* species according to the clinical profiles of 111 women in the state of Paraná, Brazil

Clinical profiles**	<i>C. albicans</i> ** n (%)	Non- <i>albicans</i> ** n (%)	Total n (%)
Colonized	15 (35.0)	28 (65.0)	43 (38.7)
VVC	33 (61.1)	21 (38.9)	54 (48.7)
RVVC	11 (78.6)	3 (21.4)	14 (12.6)
Total	59* (53.2)	52* (46.8)	111 (100)

There was no difference in the rate of isolation of *C. albicans* and among the non-*albicans* species found ($*p > 0.05$) or with the different clinical profiles ($**p = 0.5844$).

VVC: vulvovaginal candidiasis; RVVC: recurrent vulvovaginal candidiasis.

The mean cure rate for the women in the FLU group was 87.0%, 82.4% for those with *C. albicans* and 100% for those with non-*albicans* species, with no significant difference between the groups of yeasts isolated (*C. albicans* or non-*albicans* species) ($p=0.8608$) (Fig. 1). The mean cure rate for the women in the NYS group was 74.0%, similar to that of the FLU group ($p>0.05$). The cure rate for the women with *C. albicans* treated with NYS was 93.0%, higher than that of the women with non-*albicans* species, which was 44.4% ($p=0.02666$). With respect to the cure rates with NYS for the different non-*albicans* species, 40.0% of the women with *C. glabrata* and 66.7% of those with *C. tropicalis* were cured, while the only patient with *C. parapsilosis* who was treated was not cured (Fig. 1). None of the women in either group reported side-effects.

Fig. 2 provides an overview of the study and the results.

DISCUSSION

The objective of the present study was to determine the therapeutic efficacy of FLU and NYS in women in the state of Paraná, Brazil, with a vaginal culture positive for *Candida* spp. In this Brazilian state, as in the rest of the country, research on antifungal activity in vaginal yeasts takes the form of *in vitro* susceptibility studies (3, 4). Considering the difficulty in extrapolating *in vitro* results to the *in vivo* environment (11), studies such as ours can aid in the understanding of important aspects of the management of women with VVC and RVVC.

In the present study, species of vaginal *Candida* were isolated from 12.2% of the women. It is well established

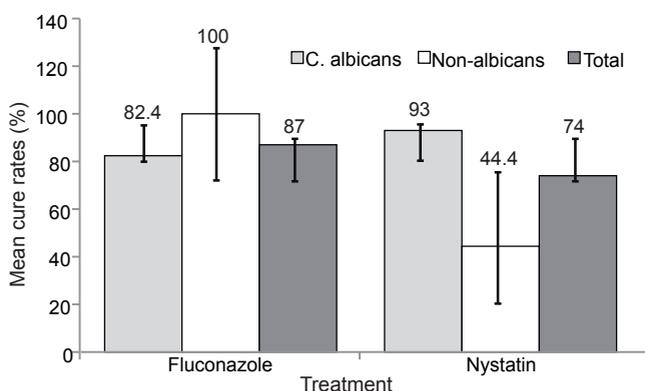


Fig. 1. Mean cure rates (%) for women in the state of Paraná, Brazil, who had a positive culture for *C. albicans* and non-*albicans* species and were treated with fluconazole or nystatin. The mean cure rate with fluconazole was 87.0%: 82.4% for those with *C. albicans*, and 100% for those with non-*albicans* species, with no significant difference between the groups of yeasts isolated (*C. albicans* or non-*albicans* species) ($p=0.8608$). The mean cure rate with nystatin was 74.0%, similar to that with fluconazole ($p>0.05$). The cure rate among women with *C. albicans* who were treated with nystatin was 93.0%, superior to the 44.4% rate obtained with non-*albicans* species ($p=0.02666$).

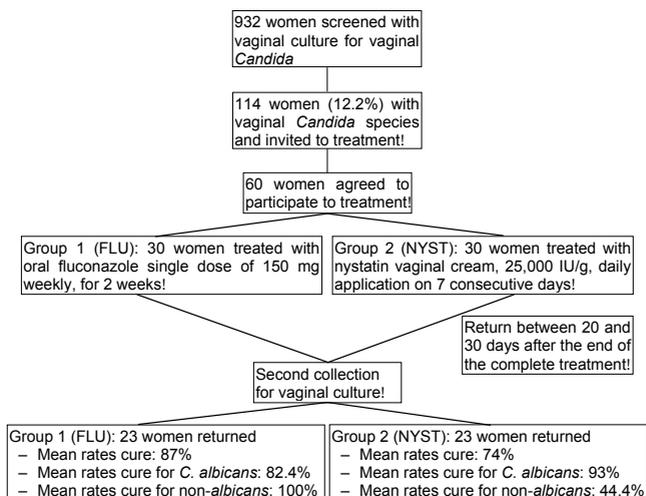


Fig. 2. Flow-chart of the study.

that the frequency of isolation of vaginal yeasts varies widely between different populations. For example, Wei et al. (4) observed rates of 7.7% and 4.5% for vaginal *Candida* species in Chinese and Tibetan women, respectively. Some authors have suggested that, in addition to geographical differences, cultural and ethnic factors can also influence the rate of isolation of vaginal yeasts (4, 11). An investigation by Lepes Consolaro et al. (5) in women from another location in the state of Paraná revealed a rate of 21.7%, reinforcing the importance of geographical distribution in the frequency of isolation of vaginal yeasts.

Once again our results are in agreement with this evidence, since the mean age of women whose vaginal cultures were positive for yeasts was significantly lower (38.0 years) than the mean age of those with a negative culture (43.3 years). Although they were probably not menopausal, the older women in this latter group may be in a pre-menopausal state, when changes in the normal hormone secretion pattern may already be influencing the rates of vaginal infection by *Candida* species.

The proportions of *C. albicans* and non-*albicans* species isolated were similar, as also observed by other investigators (4, 12). *C. glabrata* was the second most frequently isolated species, representing 27.0% of all women with vaginal yeasts and 57.7% of women with non-*albicans* species, similar to the rates described by Fan et al. (13), who reported a proportion of 51.0%. All of these data are in agreement with studies that have shown high rates of non-*albicans* species, mainly *C. glabrata*, in some populations (3–5, 12). However, according to the findings of several other investigators (1, 14) this does not appear to be a general trend. Populations with a vaginal-yeast isolation profile such as ours cause greater concern, since the management of women with non-*albicans* species, mainly *C. glabrata*, is difficult because of the reduced sensitivity of non-*albicans* to both the azoles (8, 9) and polyenes (12).

Table I shows an apparent trend, although not statistically significant, toward more frequent isolation of *C. albicans* in symptomatic cases (VVC and RVVC) and non-*albicans* species in colonization, similar to that observed by Lopes Consolaro et al. (5). Spinillo et al. (13) reported that vaginal infection by non-*albicans* species characteristically produces fewer symptoms than *C. albicans*. Studies that determine the treatment efficacy of antifungals in asymptomatic women are few, and are necessary because vaginal yeast colonization predisposes to infection (1, 2), and some physicians prefer to treat this condition. In the present study, women with vaginal *Candida* were treated because this is the routine practice of the doctor managing their care, which strengthened the data with respect to the antifungal susceptibility profile in these women.

The mean age of the women was lower for those with *C. albicans* (31.5 years) than for those with non-*albicans* species (44.6 years) ($p=0.0115$). These findings are consistent with data obtained in a study by Vermitsky et al. (15), who found an increase in the prevalence of non-*albicans* species and a decrease in *C. albicans* with advancing age. However, the factors underlying this phenomenon remain to be determined.

The mean cure rates with FLU and NYS were increased and were similar to each other, in spite of the different mechanisms of action of these drugs, which are fungistatic and fungicidal, respectively. With FLU, the cure rates did not differ between women with *C. albicans* and those with non-*albicans* species, which is intriguing since non-*albicans* species, mainly *C. glabrata*, have been reported as less susceptible to the azoles (8, 9, 13). Although the cure rate in women with *C. albicans* treated with FLU was increased, the observed therapeutic failure cannot be ignored, since this agent is one of the antifungals of choice to treat VVC (1, 9). However, few studies similar to ours have evaluated cure rates in women with vaginal yeasts treated with FLU, although some *in vitro* studies have demonstrated the development of resistance to FLU in vaginal *C. albicans* (1, 9, 16) and non-*albicans* species (1, 9).

Taking this into account, our results for NYS are even more intriguing, since although some *in vitro* studies have shown elevated rates of vaginal isolates with DDS and even some resistance (3, 8), this drug showed excellent activity against *C. albicans*, with a mean cure rate of 93.0%. On the other hand, the cure rates in women with non-*albicans* species were very low (44.4%), in contrast to the reports from *in vitro* studies. The results obtained with NYS for non-*albicans* species are surprising, since NYS is a fungicidal drug that is the main option for the treatment of women with non-*albicans* species. These findings are particularly important considering that NYS is used in low-cost topical formulations, which is an important characteristic for access to treatment by

people with limited resources, mainly in underdeveloped or developing countries (3, 5). NYS has been used for several decades as one of the principal treatments for vaginal *Candida* spp. in Brazil (3, 5, 15). This history may partly explain the low cure rates with this drug for the non-*albicans* species, which may be developing resistance, although this did not occur for *C. albicans*. Large-scale use of FLU began more recently, which may also partly explain the better therapeutic activity of this drug found in the present study.

For the women with *C. glabrata*, the mean cure rate was only 40.0%, which is compatible with the report by Holland et al. (12) that this yeast also shows reduced sensitivity to polyenes. On the other hand, 100% therapeutic success in the elimination of vaginal non-*albicans* species has been reported with amphotericin B and with flucytosine (14).

With regard to the cure rates achieved with the remaining non-*albicans* species, *C. tropicalis* returned a rate of 66.7%, while the only woman with *C. parapsilosis* who was treated was not cured. The actual importance of the presence of the latter yeast in the vaginal environment has been questioned. Nyierjesy et al. (17) found that *C. parapsilosis* was frequently associated with vaginal symptoms and showed a good response to a variety of antifungal agents. However, Dalben-Dota et al. (15) obtained an isolate of *C. parapsilosis* that was resistant to NYS *in vitro*, among five isolates tested.

In general, cure rates with FLU are superior to those achieved with NYS. Nevertheless, to improve the interpretation of results obtained with FLU and NYS treatments, the therapeutic efficacy of these drugs should be further investigated, mainly in women with non-*albicans* vaginal species, to establish whether the higher cure rate of FLU and the lower cure rate of NYS are a general characteristic of the response *in vivo*, or if they occur only in women from certain populations. The present study investigated mainly older women, and in future studies it will be important to include women in younger age groups so that their response to treatment with these antifungals can also be established.

The results of *in vitro* tests of susceptibility do not always faithfully reflect what occurs *in vivo*, because of inter-individual variation, drug characteristics, and the variable behaviour of microorganisms in each individual (16). Even so, these studies may aid therapy since high values for the minimum inhibitory concentration may be associated with resistance, which would indicate that a different treatment is necessary. Thus, although our treatment results are not entirely in agreement with *in vitro* studies of susceptibility, both types of investigation should be performed in multiple populations, to identify the *in vitro* and *in vivo* susceptibility of vaginal yeasts to the antifungals that are most widely used in these populations.

Conclusion

Both FLU and NYS are efficient in women with vaginal *C. albicans*, while in women with nonalbicans species only FLU seems to have an acceptable efficiency. This is in contrast to reports from *in vitro* studies.

ACKNOWLEDGEMENTS

Fundação Araucária de Apoio ao Desenvolvimento Tecnológico e Científico do Paraná/Brazil provided financial support for this study.

The authors declare no conflicts of interest

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