MANAGEMENT OF AZOLE-REFRACTORY CANDIDA SPECIES USING BORIC ACID PREPARATIONS: A CASE STUDY IN DAR ES SALAAM, TANZANIA

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ABSTRACT
The aim of this study was to determine the antifungal agents and boric acid susceptibility to the azole-resistant Candida species, as well as clinical outcomes following treatment with antifungal agents that are commonly prescribed in Dar es Salaam and boric acid which is not available in Tanzania market. Microscopic examination of the vaginal discharge during prolonged therapy with three antifungal agents ( clotrimazole, miconazole, and nystatin) and boric acid were carried out. Samples were collected from 150 women of reproductive age group (13 to 45 years) with chronic vaginal candidal infections. The samples were cultured in Sabouraud’s dextrose agar (supplemented with 0.005% chloramphenicol and 0.05% cycloheximide) followed by aerobic incubation for 48 hours at 37°C in order to obtain pure cultures. Identification was done by Gram stain, while the test for ability to ferment and assimilate different sugars was done on API Candida and API 20C AUX. The stock cultures of Candida albicans (ATCC 32354), Candida glabrata (ATCC2001) and Candida guilliermondii (ATCC6260) were used as controls. Patients were dispensed together with 10 ml syringes for self douching of boric acid solution, and three commonly used antifungal vaginal drugs for Candida vaginitis. The results revealed a total of 167 Candida species dominated by C. albicans 116 (69.46%), followed by C. glabrata 21 (12.57%), C. kruusei 8 (4.8%), C. tropicalis 7 (4.2%), C. famata 6 (3.59%), C. lusitaniae 5 (3.0%), C. parapsilosis, Trichosporon 2 (1.2%) and C. guilliermondii 1(0.6%). The results further showed that out of 116 C. albicans isolates, 23 (19.83%) were resistant to clotrimazole while 14 (12.1%) were resistant to miconazole in vitro test. Interestingly, all C. albicans isolates and the non-albicans candida species were very sensitive to boric acid at a very low MIC values (0.025µg/ml). Generally, the overall success rate for clotrimazole in treating C. albicans infections was 41.7%, miconazole 56.5%, nystatin 77.3% and boric acid 100%. This study shows that, compared to other commonly used drugs in the country, the best performance of boric acid envisage the need to update the national treatment guidelines for the treatment of Candida vaginitis.

Key words: Non-albicans Candida species, azole resistance, boric acid.

INTRODUCTION
Management of resistant Candida species remains to be a challenge in clinical practices in Tanzania and other tropical countries where warm and humid climate favors their survival. Epidemiologic and susceptibility studies indicate that non-albicans Candida species are more resistant to treatment with conventional antifungal drugs especially with the azole antifungal groups and are considered as causative pathogens of vaginal candidiasis (White et al., 1993; Sobel, 1998a; Abu-El-teen, 2001; Klepser, 2001; Namkinga et al., 2005a). Treatment of vaginal candidiasis is normally done with antifungal agents such as polyenes (nystatin or amphotericin-B); imidazoles ( clotrimazole, miconazole, econazole, and ketoconazole); triazoles (fluconazole, itraconazole & voriconazole).
and other antimetabolites (White et al. 1998, Namkinga et al. 2005a).

Despite this wide array of antifungal drugs, there have been several reports indicating that vaginal candidiasis is refractory to treatment and the development of recurrence cases is common (Sheehan et al. 1999, Abu-El-teen 2001). Several reasons for these trends have been attributed to increased incidences of vaginal candidiasis associated with non-\textit{albicans} Candida species such as \textit{C. glabrata}, \textit{C. krusei}, \textit{C. tropicalis} and \textit{C. lusitaniae} (Hazen 1995, Sobel 1998b, Abu-El-teen 2001); uncontrolled consumption of antifungal preparations resulting in secondary, resistance, i.e. increasing usage of short course antifungal therapies and inadequate treatment or poor patients compliance (Jovanovic et al. 1991, Johnson et al. 1995, Rex et al. 1995, Silverman et al. 2001). Other factors include extensive use of broad spectrum antibiotics (Nasibwa et al., 1994) and immune-suppression e.g., increased prevalence of HIV infection (Spinillo et al. 1994, Sullivan et al. 1996, Baily et al. 1997, Pfaller et al. 1997, Nwokolo and Boag 2000); contraceptive use (Baeten et al. 2001) and to a lesser extent corticosteroids and other immunosuppressive drugs (Baeten et al., 2001). Putative factors associated with vaginal candidiasis include; pregnancy and uncontrolled diabetes (Sobel 1992, Ringdahl 2000, De Leon et al. 2002), poor hygiene and promiscuity (Ginter et al. 1992, Spinillo et al. 1993; Marin et al. 2000, Ringdahl 2000); iron deficiency anaemia (Higgs and Wells 1972) and allergies from condom usage or type of clothes used for underwear (Eckert et al. 1998). However, there are cases of vaginal candidiasis without a recognizable predisposing factor (Sobel, 1998a). It has been postulated that allergy (Moraes, 1998), or certain blood groups (Chaim et al. 1997), or the usage of commercially available solutions for vulvo-perineal cleaning or vaginal douching could be the cause for occurrence or recurrence of vaginal candidiasis in such women (Spinillo et al. 1993).

Typical symptoms for vaginal candidiasis include; pruritus vulvae often during premenstrual period, vulval soreness, erythema and production of non-odorous thick discharge (cheese-like caseous white to yellowish purulent adherent plaques on the vaginal, vulval, or cervical epithelium), dysuria and dyspareunia (Gough et al. 1985, Sobel 1981, 1992, 1993, Denning 1995, Namkinga et al. 2005a,b). Although pruritus and inflammation of the vaginal introitus are typical symptoms, only less than 50% of women with genital pruritus suffer from vaginal candidiasis. It is also important to note that, co-infections with almost similar signs or symptoms such as trichomoniasis or bacterial/or viral vaginosis may occur, therefore, most of the above mentioned signs and symptoms are not specific for the diagnosis of vaginal candidiasis (Namkinga et al. 2005a).

Candida vaginitis is considered to be recurrent when at least four specific episodes occur in one year or at least three episodes unrelated to antibiotic therapy or any other putative factor occur within one year (Ringdahl 2000). Recurrent VC can be classified as primary or secondary depending upon established underlying causes (Fidel and Sobel 1996). Secondary sporadic RVC refers to those infrequent vaginal infections precipitated by pregnancy, or by exogenous factors such as antibiotics or wearing of tight undergarments (Fidel and Sobel 1996). Secondary recurrent vaginal candidal infections commonly occur as a result of uncontrolled diabetes mellitus, immunosuppressive therapy, hormone replacement therapy and possibly AIDS (Fidel and Sobel 1996). Primary sporadic VC and recurrent VC are idiopathic with no known causes. However, 5-10% of women with a primary sporadic episode of vaginitis
will subsequently develop recurrent VC (Hurley 1977, Hurley 1981, Fidel and Sobel 1996). Women with recurrent VC can avoid all potential causes of acute vaginitis and still experience repeated episodes of vaginitis. In women with idiopathic recurrent VC, antifungal agent therapy is highly effective for individual attacks but frequently fails to prevent future recurrence. In fact, recurrent episodes of vaginitis will appear as early as a few days to 3 months after cessation of successful treatment in approximately 50% of women with recurrent VC (Fleury 1981, Sobel 1989, Fidel and Sobel 1996, Singh et al. 1997, Sobel et al. 2003).

Molecular studies of VC have attributed the occurrence of recurrences to either relapse (by the same strain of *C. albicans*) or reinfection with a different strain or a new species of *Candida* (Powderly et al. 1993). Relapses caused by a similar strain may be indicative of the failure of therapy to eradicate initial infection (colonization), whereas those due to a new strain or new species indicate subsequent colonization with less susceptible organisms. Non-albicans *Candida* species such as *Candida glabrata, C. dubliniensis, C. krusei* and *C. tropicalis* have been associated more frequently with recurrence of the disease than *Candida albicans* (Spinillo, et al. 1995, 1999; White, et al. 1998), probably due to the fact that they are more resistant to treatment with antifungal agents especially azoles (White, et al. 1998). As azole antifungal agents have become important in the treatment of mucosal candidiasis in AIDS patients, reports of resistance have increased (Law et al. 1994, White et al. 1998). In fact, azole resistance has now been found in patients not infected with HIV and, in some situations, in patients not previously exposed to antifungal agent agents (Singh et al. 1997, Sobel et al. 2003).

Several studies have documented increased infections due to the intrinsically azole-resistant non-*albicans* Candida species (Nguyen, et al. 1996). At one institution, *C. albicans* comprised 87% of the isolates recovered from blood prior to the use of fluconazole (1987 to 1991) but accounted for only 31% of the isolates in 1992, when fluconazole was used frequently for prophylaxis and treatment. Similarly, an increased incidence of non-albicans Candida species with increased azole MICs arose over a 3.5-year study period at another institution (Nguyen, et al. 1996). Primary resistance to 5-FC is common in certain yeasts and molds. Non-*albicans* *Candida* spp., as well as *Apergillus* spp., *C. neoformans* and the dimorphic fungi have high rates of 5-FC resistance (Francis and Walsh, 1992). In addition, secondary resistance is a common development, especially in patients receiving 5-FC monotherapy. There is some indication that the severity of immune-suppression and fungal burden especially infections with non-albicans Candida species may be important risk factors leading to the development of resistance (Johnson et al. 1995; Shinohara and Tasker, 1997). For example, *in vitro* susceptibility studies of sucrose negative Candida species showed *Candida novergensis* to be more resistant to clotrimazole, miconazole and ketoconazole than clinical isolates of *C. albicans* (Ahearn et al. 1984). Likewise, *Candida lusitaniae* and *Candida tropicalis* show resistance to 5-fluorocytosine and to the azoles than other *Candida* species (Ahearn et al. 1984). Because 5-FC resistance develops frequently, the drug should never be used as a single agent to treat either yeast or mold infections.

*Candida* vaginitis (CV) is a common condition and usually straight forward to treat. In contrast, complicated CV can be intractable and cause considerable psychological morbidity. Complicated CV
includes recurrent or severe disease, or when there are adverse factors in the host. This includes persistent infection with species other than Candida albicans, (‘non-C albicans’) and the more common recurrent albicans CV. The importance of distinguishing the two conditions is that non-C albicans chronic vaginal yeast infection is potentially resistant to treatment with azole antifungal agents such that newer approaches or drugs may be needed in treatment. This study attempted to correlate the in vitro susceptibility with treatment success of the azole refractory Candida species using boric acid.

Materials and methods
A total of 150 women of reproductive age group (13 to 45 years of age) with chronic vaginal candidal infections were followed up with microscopic examination of the vaginal discharge during prolonged therapy with antifungal agents and boric acid.

Preparation of isolates
The isolates were inoculated on Sabouraud’s dextrose agar (SDA) (Oxoid Ltd. Hampshire, England), supplemented with 0.005% chloramphenicol and 0.05% cycloheximide and incubated aerobically for 48 hours at 37°C in order to obtain pure cultures. The isolates were identified by Gram stain, germ tube production on horse serum (Oxoid, Hampshire, England), conidia enhanced morphology on corn meal agar (Oxoid Ltd. Hampshire, England), ability to ferment and assimilate different sugars on API Candida (BioMerieux SA, France) and by API 20C AUX (BioMerieux SA, France). Stock cultures of Candida albicans (ATCC 32354), Candida glabrata (ATCC2001) and Candida guilliermondii (ATCC6260) were used as controls.

Boric acid susceptibility/resistance test
The susceptibility to boric acid was assessed by incorporating the boric acid into an agar plate at a concentration of 1%. The inocula for this test were prepared by sub-culturing the isolates onto Sabouraud’s dextrose agar (Oxoid Ltd. Hampshire, England), supplemented with 0.005% chloramphenicol and 0.05% cycloheximide and incubated aerobically for 48 hours at 37°C, observing after every 24 hours in order to obtain pure cultures. From these cultures, suspensions of approximately 10⁶ cells ml⁻¹ (according to Mc Farland concentrations) was prepared. A synthetic swab was used to inoculate the suspension onto Mueller Hinton agar containing 1% boric acid. A control plate which contains only the basal medium without boric acid was inoculated simultaneously. Control organisms were also inoculated. The test plates and control plates were then inoculated aerobically for 48 hours at 37°C, after which each isolate was scored as being resistant (R), moderately resistant (MR), moderately sensitive (MS) or sensitive (S) to boric acid, as indicated by the presence or absence of confluent growth.

Preparation of boric acid solution for in vivo test for patients
A sufficient amount of boric acid powder (Sigma Chemical co., St. Louis, USA) enough to make a 1% solution was weighed on a OHAUS-triple beam balance 700 series of capacity 2610 g (USA). The powder was then dissolved in a bacteria-free water of 70°C previously sterilized by autoclaving. The boric acid solution was then filled and capped into 100 ml pet cumber amber glass bottles. Preparation of the boric acid solution was done in a factory (AA-Dispensing & Manufacturing Company) with permission from Tanzania Food and Drug Authority (TFDA) and requested by the Board of Registrar (Tanzania) following good manufacturing practice (GMP) and standard operating procedures (SOPs). The bottles were then labeled indicating the name of preparation, strength, amount, dosage, batch number, manufacturing and expiry dates, and company name and address. Patients
were dispensed together with 10 ml syringes for self douching of boric acid solution.

**Azole antifungal susceptibility/resistance test**

Three commonly used antifungal vaginal drugs for Tanzania market, namely; Clotrimazole, miconazole and nystatin in different brands in a form of pessaries and ovules were used in the study. Women with Candida vaginitis were separated into four groups depending on the antifungal agent drug administered. Dosage used were; clotrimazole pessaries (100 mg) inserted deeply into the vagina in the evening on 6 consecutive days; miconazole ovules (400 mg) inserted daily into vagina at bed time for 3 consecutive days; and nystatin vaginal pessaries (100,000 IU) inserted into the vagina for 14 consecutive nights regardless of any intervening menstruations.

**Treatment evaluation**

All patients were requested to return for assessment two weeks after treatment was completed. On this occasion, symptoms and clinical signs of vaginitis (the vagina mucosa was reddened, or granular; the vulva inflammation was reddened, swollen, fissured or ulcerated), were noted and high vaginal swabs were taken for mycological investigation. Patients were monitored for two consecutive months on the type of drugs taken, and laboratory analysis of the Candida species carriage. Patients with poor response to the azoles were then given nystatin vaginal preparation on the second month of visit to the clinic. Again if the response was poor to nystatin, the patient was subjected to treatment with boric acid solution. Assessment of treatment was based on mycological and clinical criteria. Mycological cure was defined as negative or low (less than 10 colonies on Mueller Hinton agar plates) culture result (Odds *et al.* 1987), while clinical cure was regarded as absence of clinical signs and symptoms associated with Candida vaginitis (Milne and Warnock 1979). Treatment was considered to be successful if both clinical and mycological cure were achieved.

**Standard powders**

Miconazole (Janssen Research Laboratory, Belgium), clotrimazole (BUFA, B. V. Pharm. Product, Uitgeest, Holland) and nystatin (BUFA, B. V. Pharm. Product, Uitgeest, Holland) together with boric acid were tested for *in vitro* susceptibility against Candida species. The isolates used in the analysis were *Candida albicans*, *Candida glabrata*, *Candida tropicalis*, *Candida krusei*, *C. parapsilosis*, *C. famata* and *Trichosporon*.

**Standard organisms**

*Candida albicans* (ATCC 32354), *C. krusei* (ATCC 6258), *C. parapsilosis* (ATCC 22019) and *C. glabrata* (ATCC 2001) were used as standards in this study.

**Patient’s specimens**

A gynaecological examination was performed by a physician at Masana Hospital, Dar es Salaam, Tanzania and women were told to come after two weeks for their results. High vaginal swabs were collected and placed in Stewart’s transport media for laboratory diagnosis of Candida vaginitis. All specimens were processed in the Department of Molecular Biology and Biotechnology at the University of Dar es Salaam, Tanzania. The wet mounts were prepared and examined microscopically for the presence of yeast cells. Gram stained smears were also prepared. Provisional detection of *Candida spp.* was based on colonial morphology on Sabouraud dextrose agar (SDA) media containing 0.005% chloramphenicol and 0.05% cycloheximide.

**Ethical clearance**

This is a continuation of projects from a PhD study which was ethically approved by the College Research and Publications Committee of the Muhimbili University
College of Health Sciences, (MU/PGS/AEC/III/126 of November 11, 1997). Older samples (1997 – 2003) resistant to azoles were used as control while the current samples (2012) were used as test organisms.

RESULTS
A total of 167 Candida species were isolated from 50 samples out of which C. albicans were 116 (69.46%), C. famata 6 (3.59%), C. tropicalis 7 (4.2%), C. glabrata 21 (12.57%), C. krusei 8 (4.8%), C. lusitaniae 5 (3.0%), C. parapsilosis and C. guilliermondii 1 (0.6%) each, and Trichosporon 2 (1.2%). Out of 116 C. albicans isolated, 23 (19.83%) were resistant to clotrimazole and 14 (12.1%) were resistant to miconazole in vitro test. The total of 18 (15.5%) isolates of C. albicans were moderately resistant to azole antifungal agents (clotrimazole and miconazole), and 78 (67.24%) were susceptible to azoles tested (Fig. 1e). From 6 isolates of C. famata tested, 5 (83.33%) were resistant to azoles (Fig. 1b). As for C. lusitaniae, C. guilliermondii and C. krusei (Fig. 1d), all were resistant to azoles in vitro testing. Out of seven C. tropicalis isolates tested 4 (57.14%) were resistant to azoles, 1 (14.3%) moderately resistant, 1 (14.3%) moderately susceptible and only 1 (14.3%) was susceptible to azoles (Fig. 1c). Candida parapsilosis and Trichosporon were resistant to clotrimazole only. All isolates (C. albicans and the non-albicans candida species) were very sensitive to boric acid at a very low MIC values 0.025 µg/ml (Fig. 1a - g). Also, the old samples (1998-2003), which were resistant to azoles, were now all susceptible to boric acid.

![Figure 1a.](image-url)
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Figure 1e

Figure 1f

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Figure 1g: Susceptibility Pattern of diverse Candida species to antifungal drugs commonly available in Tanzania market and boric acid which is not in the market. The vertical line represents the number of isolates against types of drugs used; Clotrimazole, Miconazole (azoles), Nystatin and Boric acid. (S= Susceptible; MS = moderately susceptible; R = resistant; MR = moderately resistant).

**Treatment in vivo success rates**

The overall success rate of clotrimazole in treating *C. albicans* infections was 41.7%, being 40.0% and 50.0% at MIC values ≤0.8 µg/ml and 1.6 µg/ml, respectively. Miconazole was effective in treating 56.5% of *C. albicans* infections. The overall success rate of nystatin was 77.3%; all isolates had MIC values of ≤0.8 µg/ml. Azoles had less success rate in treating *C. albicans* infections. For *Candida glabrata* infections, the overall success rate for miconazole was 16.7%, being 0.0% at MIC values of ≤3.2 µg/ml, and to 50% at MIC values of ≥6.4 µg/ml. The same fungus, when subjected to clotrimazole, recorded a success rate of 0.0% at all MIC values, while for nystatin it was 67.0% (overall), 66.7% (MIC value of ≤0.8 µg/ml), and 0.0% at MIC value of 1.6 µg/ml. For *Candida tropicalis* infections, the overall success rate with miconazole was 50%, being 33.3% at MIC ≤0.8 µg/ml and 100% at MIC 3.2 µg/ml. With clotrimazole the success rates were 25% and 0.0%, respectively. The overall success rate of treating *C. tropicalis* infection with nystatin was 50% being 66.7% at ≤0.8 µg/ml and 50% at ≥6.4 µg/ml.

For *C. krusei* infections, the overall success rates were 56.6%, 10%, and 0% for nystatin, miconazole and clotrimazole, respectively. *C. parapsilosis, C. norvegensis* and *C. famata* infections, were rather few and all their isolates had very high MIC values (≤3.2 µg/ml), and their treatment success with azoles was very poor. The success rates for *C. parapsilosis* and *Trichosporon* were 0.0% and 100% with clotrimazole and nystatin, respectively. Treatment success rate for *C. norvegensis* were 100% for nystatin and 0.0% for clotrimazole, while for *C. famata* success rate was 5% for clotrimazole and Miconazole and 100% for nystatin and boric acid.
DISCUSSION
The in vitro susceptibility patterns of the isolates were determined by three methods namely; disk-diffusion, agar dilution and the reference broth macro dilution technique. The agar dilution and disk diffusion methods were included since they are feasible in our routine laboratory and could potentially serve as alternatives to the reference broth macro dilution method. The disk diffusion method showed good agreement with the tube macro dilution method, which is in keeping with other findings, (Yucesary et al. 2001; Lee et al. 2001; Barry, et al. 2002), and appears to be a useful, rapid and reliable screening technique for testing the susceptibility of clinical isolates to imidazoles, polyenes and even boric acid.

The incidence of resistance to treatment of vaginal fungal infections in women has increased dramatically in Tanzania as has the number and variety of infecting fungal species or strains. Results of this study concur with several other investigators’ reports encountering increased numbers of women with vaginal fungal infections caused by non-albicans Candida species (Sobel, 1985; Sobel et al. 1995, 1998). Although Candida vaginitis is a very common condition in women, and usually straight forward to treat, complicated CV that leads to recurrences can be intractable and cause considerable psychological morbidity.

Treatment failure, attributable to the development of azole-resistant C. albicans strains, appears to become more common, but still seems to be confined to patients receiving long-term treatment. Majority of HIV positive women in Tanzania receive Fluconazole prophylaxis which might be the cause for the development of Candida vaginitis by the non-albicans Candida species. The rapid development of drug resistance and the persistence in patients with the non-albicans Candida species such as C. glabrata, C. parapsilosis, C. tropicalis, C. lusitaniae, C. famata or C. krusei infections to treatment with azole antifungal drugs is of concern and high lights the need for accurate identification of organisms before commencing treatment. Results of this study showed that treatment with boric acid vaginal preparation was of value in recalcitrant cases (Figure 1 a-g) which is in line with other studies (Redondo-Lopez et al. 1990, Sobel and Chaim 1997, Shinohara and Tasker, 1997; Guaschino et al. 2001; Lavazzo et al. 2011), which suggested that boric acid is safe, cheap and is the best alternative in the management of non-albicans Candida species.

The non-albicans Candida species and some few C. albicans 23 (19.83%) showed rather high MIC values (≥ 12.8 µg/ml), indicating that the isolates were resistant. Such isolates are likely to have been isolated from either patients who had received prior courses of anti-fungal therapy (or could as well be associated with the intrinsic resistance of these species to azoles as it has been also noted by Rex et al. (1995), White et al. (1998), Sheehan et al. (1999), Davies et al. (2012) are likely to be associated with recurrences of Candida vaginitis. Effective treatment of isolates with high MICs, which is in this study belonged mostly to C. glabrata, C. lusitaniae, C. famata, C. krusei and C. tropicalis, is likely to base either on combination of anti-fungal agents with different mechanisms of action or new antifungal agents such as voriconazole (Kronvall and Karlsson, 2001) or boric acid. The notable significant species differences in vitro susceptibility to antifungal agents seem to suggest the usefulness of speciating Candida as a first step towards determining appropriate anti fungal regimen.

This study attempted to correlate in vitro susceptibility with treatment success. The MIC values included in the correlation are those obtained using the broth macro
dilution technique (NCCLS M27), which is the reference method for antifungal agent susceptibility testing (Pfaller et al. 1997, Rex et al. 2001). This method has been studied extensively to examine the roles of variables such as inoculum preparation, inoculum size, medium composition, incubation temperature and incubation time. As a result, it produces results that are comparable in quality with those of antibacterial susceptibility testing (Pfaller et al. 1997).

A notable observation was the fact that boric acid had better treatment success for non-albicans infections than the azoles. Also, the control samples (of the year 1998-2003) which were resistant to azoles were now susceptible to boric acid. It is postulated that the fungistatic or fungicidal effects of boric acid may be a result of its inherent properties as a weak acid. The acid penetrates the cell wall and disrupts the cell membrane, whereas current antifungal agents bind and inhibit ergosterol synthesis. However, the vaginal preparations of boric acid are not available in Tanzania markets yet.

The relative resistance of the organisms to azoles may be due to several reasons such as extensive use of these compounds. This is due to the fact that many cheap brands of azoles exist in the market, a fact that may influence not only prescription tendency but also availability over the counter. In addition, it is known that non-albicans Candida species such as C. glabrata, C. krusei, C. parapsilosis and C. tropicalis are more resistant to treatment with azoles. This is in line with studies done by White et al. (1993, 1998, Singh et al. (1997), Sobel et al. (2003), Ray et al. (2007) and Davies et al. (2012). It should also be noted also that some species such as C. norvegensis, C. famata, C. krusei, C. parapsilosis, C. lusitaniae, C. tropicalis, C. guilliermondii and Trichosporon had very few isolates (ranging from 1-8 isolates), which contributed to the limited amount of data obtained.

CONCLUSION
The evolving pattern of the non-albicans Candida isolates, some of which with high MIC values againstazole antifungal drugs, underlines the need for continuous surveillance of the responsible Candida species and determination of their susceptibility to antifungal agents. Treatment of VC was more successful with boric acid followed by nystatin than azoles antifungal agents for most Candida species. There is a need to update the national treatment guidelines for the treatment of Candida vaginitis.

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