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. krusei 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-*albicans* Candida species such as *C. glabrata, C. krusei, C. tropicalis* and *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-perneal 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 vagnosis 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 re-infection with a different strain or a new species of C*andida* (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 C*andida* 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 mono-therapy. 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 C*andida 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-fluorocytesine 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 37ºC 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 37^0C, observing after every 24 hours in order to obtain pure cultures. From these cultures, suspensions of approximately 10^6 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 incubated 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 70^0C 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.

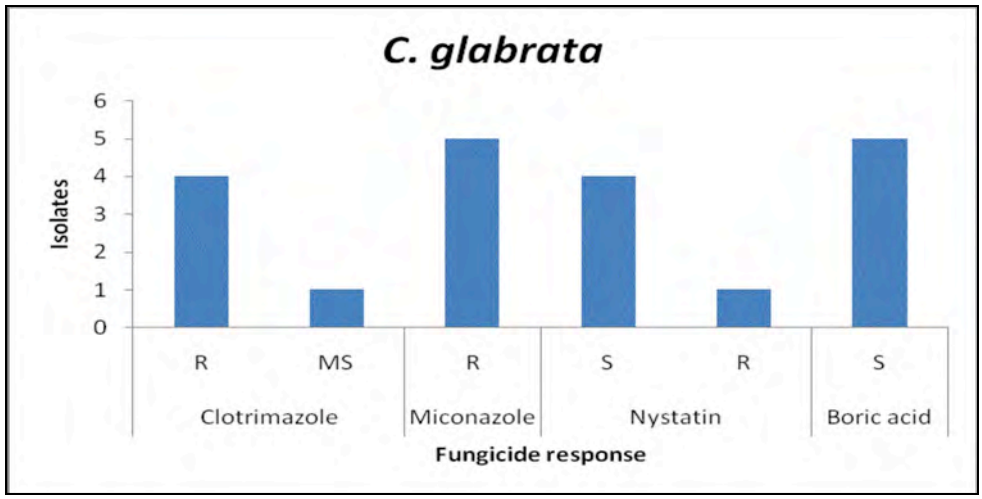


Figure 1a.

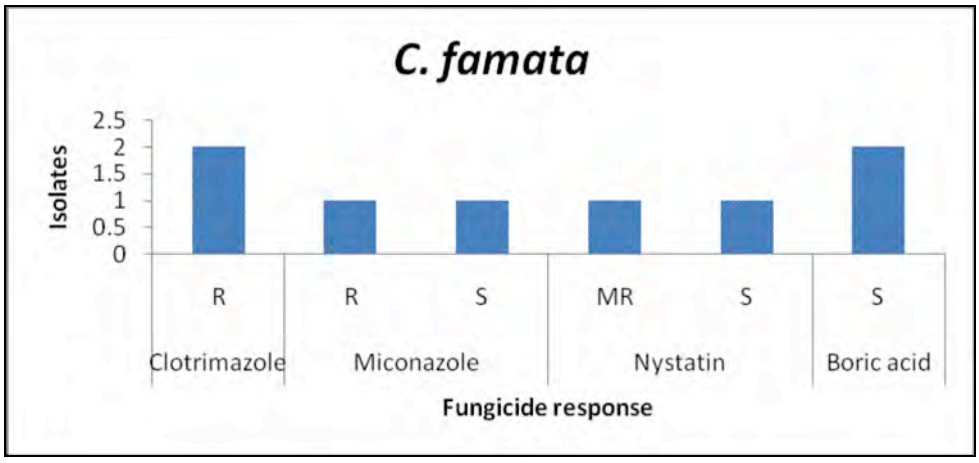


Figure 1b.

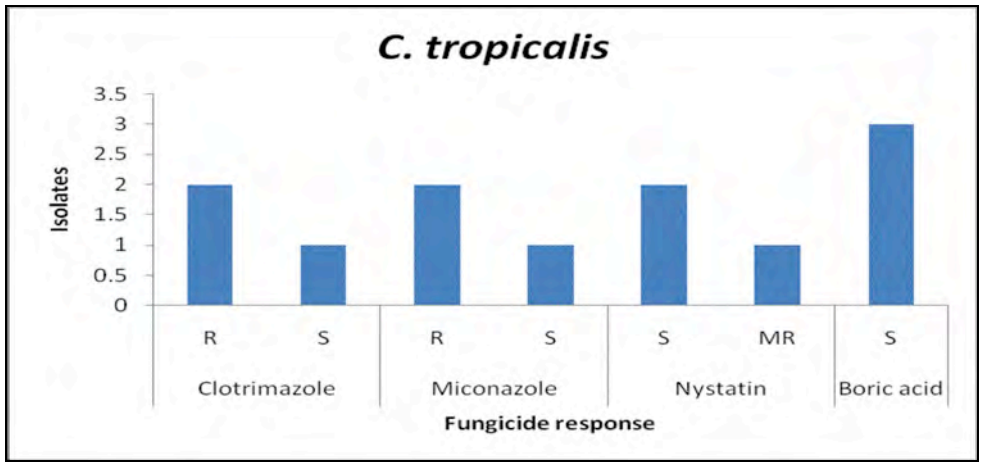


Figure 1c

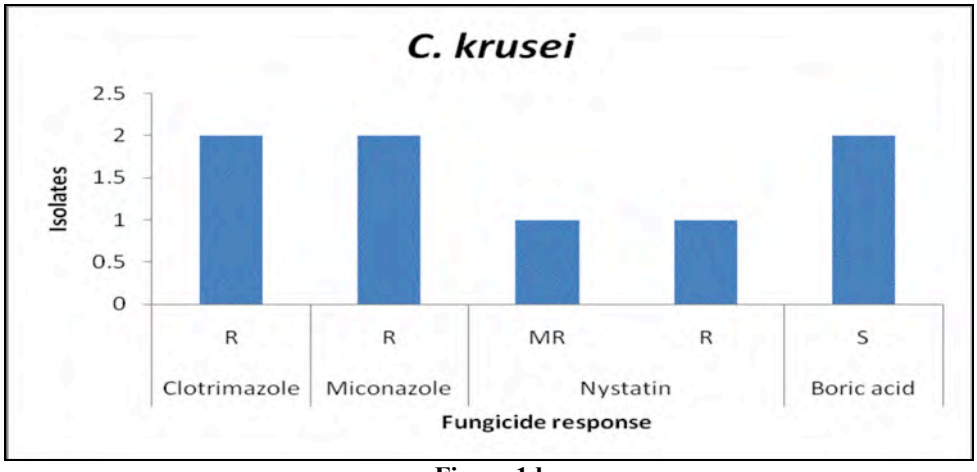
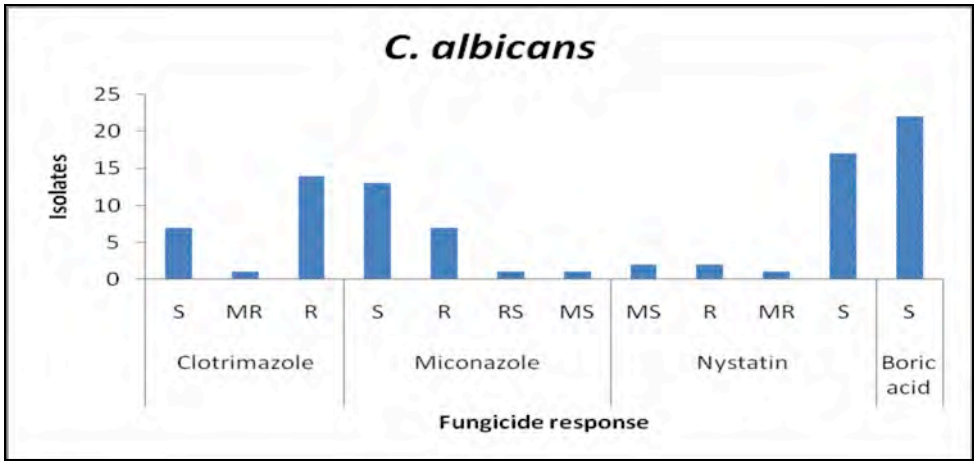


Figure 1d



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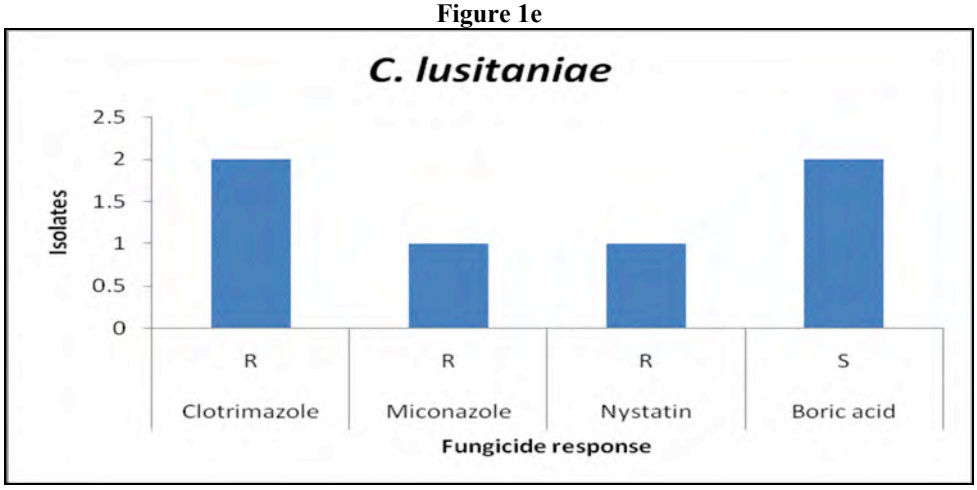

Figure 1f

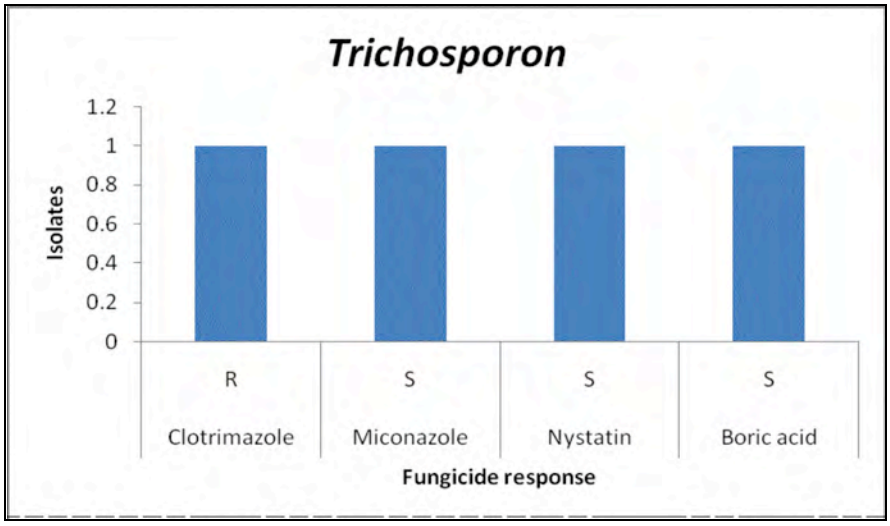

Figure 1g

Figure 1(a-g): 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 against azole 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.

ACKNOWLEDGEMENT

The author is grateful to the technical staff of the Department of Molecular Biology and Biotechnology, University of Dar es Salaam; Gynecologists and technical staff of Massana Hospital; AA-Dispensing and Manufacturing Company; and the Department of Microbiology and Immunology, Muhimbili University of Health and Allied Sciences, for their technical support.

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