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Identification and Susceptibility Profile of Vaginal *Candida* Species to Antifungal Agents among Pregnant Women Attending the Antenatal Clinic of Thika District Hospital, Kenya[*]

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ABSTRACT

Fungal infections have emerged as a world-wide health care problem in recent years, owing to the extensive use of broad-spectrum antibiotics. We screened 104 pregnant women with symptoms of vaginal candidiasis in the antenatal clinic of Thika District Hospital, Kenya in order to identify vaginal *Candida* species and determine their susceptibility profile to commonly used antifungal drugs for treatment of the infection. The drugs tested were fluconazole, ketoconazole, itraconazole, clotrimazole and topical nystatin. Vaginal swabs were collected and subjected to mycological and biochemical tests for *Candida* species identification. Susceptibility profile of the identified vaginal *Candida* species to the antifungal drugs was carried out using broth micro-dilution minimum inhibiting concentration method based on the approved National Committee for Clinical Laboratory Standards (NCCLS, 2002) guidelines. *Candida albicans* was susceptible to most of the azoles drugs while the other species had varying responses. *Candida krusei* and *Candida glabrata* species isolated were resistant to fluconazole and ketoconazole. *Candida albicans* isolates had a high susceptibility to itraconazole (88.33%). Five percent (5%) of the isolates were susceptible in dose dependent (S-DD) with Minimum Inhibitory Concentrations (MICs) of 0.25 - 0.5 µg/ml while 11.67% of *C. albicans* isolates were resistant (MICs ≥ 1 µg/ml). Itraconazole resistance was highest among C. *glabrata* isolates (50%) while 32.14% were S-DD (MICs 0.25 - 0.5 µg/ml). Only 17.85% of the *C. glabrata* isolates were susceptible (MICs of ≤ 0.125 µg/ml). All isolates of *Candida* isolates were susceptible to itraconazole and clotrimazole except *C. krusei* which was 100% resistant to clotrimazole. All *Candida* species isolates had low susceptibility to topical nystatin except *Candida parapsilosis* that was 100% susceptible. Data also showed an emerging resistance of *Candida krusei* to most of the drugs used except itraconazole. The results of this study support the continued use of these antifungal drugs for the treatment of vaginal candidiasis in the pregnant women except topical nystatin.

Keywords: Pregnant Women; Vaginal Candidiasis; *Candida* Species; Antifungal Drugs; Susceptibility Profile

1. Introduction

Multidrug resistant microorganisms are becoming a major challenge worldwide because of irrational use of antibiotics and the increasing population of immunocompromised individuals [1]. In the last two decades, there has been a steady increase in the incidence of systemic opportunistic fungal infections especially in Sub-Sahara Africa [2]. This rise is associated with Acquired Immunodeficiency Syndrome (AIDS) pandemic, prolonged antimicrobial therapy, invasive procedures and immunosuppressive therapy [3]. Vaginal Candida species are emerging as significant opportunistic organisms that have increased over the past few decades [4].

The available treatment for vaginal candidiasis in-

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cludes azoles (fluconazole, ketoconazole, itraconazole, voriconazole and clotrimazole) and polyene antifungals such as nystatin and amphotericin B. However, in Thika District Hospital where the study was conducted, azole antifungal drugs (fluconazole, ketoconazole, itraconazole and clotrimazole) and topical nystatin are the drugs of choice prescribed to pregnant and non-pregnant women for the treatment of vaginal candidiasis (according to the Hospital health records).

In Kenya, there are few data on *Candida*'s role in causing vaginal candidiasis in pregnant women and its antifungal susceptibility [5]. In the recent past, reports of research done in Kenya on oral candidiasis indicated that there is an emerging resistance among *Candida* species to some of the antifungal drugs [1]. It is therefore necessary to investigate antifungal resistance of *Candida* species from clinical sources in order to determine any emerging resistance. The present study was therefore undertaken to evaluate the susceptibility profile of identified vaginal *Candida* species isolated from vaginal samples of pregnant women.

2. Materials and Methods

2.1. Study Design

Cross sectional study was adopted and susceptibility tests were carried out at Thika District Hospital Laboratory, Thika, Kenya.

2.2. Sampling Technique

Purposive sampling technique was used among the pregnant women with symptoms of vaginal candidiasis attending the antenatal clinic of Thika District Hospital.

2.3. Study Approval and Ethical Consideration

This study was approved by Kenyatta University and Thika District Hospital. The objectives of the study were explained to the pregnant women attending Thika District hospital antenatal clinic. Those who volunteered to participate in the study signed consent form after it was read and explained to them. They were assured that all the information regarding their participation would be confidential.

2.4. Hypotheses

All pregnant women attending the antenatal clinic of Thika District Hospital do not have vaginal candidiasis.

The vaginal *Candida* species isolated from pregnant women attending the antenatal clinic of Thika District Hospital are not resistant to the antifungal agents used.

2.5. Sample Collection

Vaginal swabs were collected from pregnant women with

symptoms of vaginal candidiasis. The symptoms included itching, difficult in walking, dysuria and presence of thick adherent plaques on the vulval, vaginal or cervical epithelium. Samples collected were taken to the Hospital Laboratory for *Candida* species isolation, identification and drug susceptibility testing.

2.6. Gram Stain

The test was carried out essentially according to the procedure of Chander [6]. Gram stained smears were used to examine the presence of gram positive budding yeast cells with pseudohyphae. Specimen was considered as acceptable when 25 or more polymorphonuclear leukocytes were seen per low power field (100×) with few (less than 10) squamous epithelial cells.

2.7. Culture Procedure

The samples were cultured on Saboraud dextrose agar (SDA) containing two percent chloramphenicol. Inoculated plates were incubated at 37˚C and examined after 48 hours for cream coloured pastry colonies and budding yeast cells suggestive of *Candida* species. Isolates from SDA were inoculated on CHROMagar (France) using an inoculating needle and incubated at 37˚C for 72 hours to ensure detection of mixed cultures by colour changes. The method is based on the differential release of chromogenic breakdown products from various substrates by *Candida* species following differential exoenzyme activity [7]. This test was used for presumptive identification of *C. albicans*, *C. tropicalis* and *C. parapsilosis*

2.8. Germ Tube Test

This method was used as a confirmatory test for identification of *Candida albicans*. A single colony of the test yeast cells from a pure culture was inoculated in human serum and incubated at 37˚C for 2 - 4 hours. A drop of the incubated serum was placed on a microscope slide and covered with a cover slip. The wet mounts were examined under the microscope to look for the presence of germ tube using the 40× objective according to Dalmau morphology method [7]. The isolates were classified as either germ tube positive or germ tube negative.

2.9. Sugar Assimilation Test

The test was carried out essentially according to the procedure of Lodder *et al.* [8]. The assessment of the ability of yeast to utilize carbohydrates was based on the use of carbohydrate-free yeast nitrogen base agar. Observation for the presence of growth around carbohydrate impregnated filter paper discs was done after incubation for 18 hours at 30˚C.

Carbohydrates used were glucose, galactose, lactose,

maltose, sucrose, raffinose, trehalose and cellobiose. Presence of growth in the medium indicated the ability of the isolate to assimilate a sugar. The *Candida* species were identified using the sugar assimilation patterns for individual species by Lodder *et al.* [8].

2.10. Antifungal Susceptibility Testing

The test was carried out on the identified isolates using broth microdilution method of Hace *et al.* [9], and based on the proposed National Committees for Clinical Laboratory Standards (NCCLS) guidelines for the broth microdilution method as contained in document M27-A2 [10]. The antifungal drugs tested were fluconazole, ketoconazole, itraconazole, clotrimazole and topical nystatin.

Different concentrations of fluconazole and ketoconazole were prepared and tested as follows; fluconazole (0.125 - 64 µg/ml and ketoconazole (0.007 - 8 µg/ml) as proposed by NCCLS, 2002 [10]. The concentrations of itraconazole ranged from 0.015 to 8 µg/ml [30] and 0.03 - 16 µg/ml for clotrimazole [2]. For the purposes of this study the final concentrations of topical nystatin ranged from 0.06 - 16 µg/ml [11].

The different antifungal drug dilutions were dispensed into a U-shaped microtiter plates (Greiner Bio-one, Holland). The test isolates were suspended in Roswell Park Memorial Institute (RPMI-1640 medium usually matched to 0.5 McFarland standard suspensions. The mixture 0.1 ml was inoculated into each microtiter wells containing different concentrations of the test drug. The inoculated microtiter plates were incubated at 35°C for 48 hours. The MICs for the azole drugs were read as the lowest antifungal concentration with substantially lower turbidity (~50%) compared to growth in the antifungal free growth control. Topical nystatin MICs were read as the minimal antifungal concentration with complete inhibition of growth (optical clarity). The test was carried out in duplicate in each concentration of the drugs and the mean was recorded. Quality control was ensured by testing the NCCLS-recommended quality control strains *Candida parapsilosis* ATCC 22019 and *Candida krusei* ATCC 6258.

We used the proposed standards for MIC interpretation criteria by NCCLS, 2002 [10] for fluconazole and ketoconazole. The isolates of *Candida* species with MICs of <8 µg/ml for fluconazole were regarded susceptible (S); 16 - 32 µg/ml were susceptible in dose dependent (SDD) and > 64 µg/ml were resistant (R). *Candida* species isolates with MICs of <0.125 µg/ml were susceptible; 0.25 - 0.5 µg/ml were S-DD while >1 µg/ml were resistant for ketoconazole. According to Gonzalez *et al.* [12], isolates with MICs of ≤0.125 µg/ml for itraconazole were regarded susceptible while MICs of 0.25 - 0.5 µg/ml were considered susceptible but in a dose dependent manner; MICs of ≥1 µg/ml were considered resistant [12]. This

interpretation was used for this study too. *Candida* species isolates with MICs of <0.5 µg/ml for clotrimazole were considered susceptible while those with MICs of >0.5 µg/ml were considered resistant as suggested by Pelletier *et al.* [12]. The topical nystatin MICs of ≤1 µg/ml was adopted as susceptible breakpoint while >1 µg/ml was regarded as resistance breakpoint as suggested by Sandra *et al.* [11].

2.11. Quality Control Strains

Prior to antifungal susceptibility testing each isolate was sub-cultured at least twice on SDA for 24 hours before use. This was to obtain a pure culture of each isolate. The Quality Control strain, *Candida parapsilosis* ATCC 22019 and *Candida krusei* ATCC 6258.

2.12. Data Analysis

All collected data were introduced into Microsoft Excel data sheet. The susceptibility of vaginal *Candida* species to the antifungal drugs was calculated using ANOVA to establish any variations. Chi-square test was used to test the susceptibility association of the two groups of the antifungal drugs used to the vaginal *Candida* species isolated. All the statistical analysis was carried out using MINITAB and SPSS version 13.0 computer package.

3. Results

In the present study, five (5) vaginal Candida species were isolated and identified. These species included *Candida albicans*, *Candida glabrata*, *Candida krusei*, *Candida tropicalis* and *Candida parapsilosis*. *Candida albicans* was the most frequently isolated species accounting for 63.83% of all the isolates, followed by *Candida glabrata* (29.79%), *Candida tropicalis* (3.19%), *Candida krusei* (2.1%) and *Candida parapsilosis* with the least percentage isolates (1.06%). Results on susceptibility of these vaginal *Candida* species to different antifungal agents are shown in **Tables 1-5**.

Fifty seven (95%) *Candida albicans* isolates were susceptible (MICs of <8 µg/ml); 3.33% isolates were susceptible in dose dependent manner (16 - 32 µg/ml) and 1.67% were resistant (MICs >64 µg/ml) to fluconazole. *Candida glabrata* (42.85%) isolates had an MIC of <8 µg/ml and were regarded susceptible to fluconazole. However, 50.01% of the isolates were susceptible in dose dependent (MICs of 16 - 32 µg/ml) while 7.14% were resistant (MICs > 64 µg/ml). High resistance (100%) was recorded for *Candida krusei* since all the 2 isolates had an MIC of >64 µg/ml. *Candida tropicalis* and *Candida parapsilosis* were all susceptible to fluconazole since all their isolates had MICs of <8 µg/ml (**Table 1**). The MIC_{90} to fluconazole was 64.0 µg/ml while MIC_{50} was 4.0 µg/ml.

Table 1. Susceptibility of *Candida* species to different concentrations of Fluconazole.

Fluconazole (µg/ml)	*C. albicans* n (%)	*C. glabrata* n (%)	*C. krusei* n (%)	*C. tropicalis* n (%)	*C. parapsilosis* n (%)
0.125	14 (23.33)	6 (21.43)	NIL	NIL	NIL
0.25	24 (40)	1 (3.57)	NIL	NIL	1 (100)
0.5	8 (13.33)	2 (7.14)	NIL	NIL	NIL
1.0	5 (8.33)	NIL	NIL	3 (100)	NIL
2.0	2 (3.33)	1 (3.57)	NIL	NIL	NIL
4.0	1 (1.67)	2 (7.14)	NIL	NIL	NIL
8.0	3 (5)	NIL	NIL	NIL	NIL
16	1 (1.67)	10 (35.72)	NIL	NIL	NIL
32	1 (1.67)	4 (14.29)	NIL	NIL	NIL
64	1 (1.67)	2 (7.14)	2 (100)	NIL	NIL
Total	60 (100)	28 (100)	2 (100)	3 (100)	1 (100)

Nil = No organism was detected at that concentration.

Table 2. Susceptibility of *Candida* species to different concentrations of Ketoconazole.

Ketoconazole (µg/ml)	*C. albicans* n (%)	*C. glabrata* n (%)	*C. krusei* n (%)	*C. tropicalis* n (%)	*C. parapsilosis* n (%)
0.007	4 (6.67)	7 (25)	NIL	NIL	NIL
0.014	27 (45)	1 (3.57)	NIL	NIL	NIL
0.028	6 (10)	4 (14.2)	NIL	2 (66.67)	NIL
0.06	2 (3.33)	9 (32.1)	NIL	NIL	1 (100)
0.125	16 (26.67)	3 (10.71)	NIL	1 (33.33)	NIL
0.25	2 (3.33)	NIL	NIL	NIL	NIL
0.5	NIL	NIL	1 (50)	NIL	NIL
1.0	1 (1.67)	1 (3.57)	NIL	NIL	NIL
2.0	2 (3.33)	NIL	1 (50)	NIL	NIL
4.0	NIL	2 (7.14)	NIL	NIL	NIL
8.0	NIL	1 (3.57)	NIL	NIL	NIL
Total	60 (100)	28 (100)	2 (100)	3 (100)	1 (100)

Nil = No organism was detected at that concentration.

Fifty five (91.67%) *Candida albicans* isolates were susceptible (MICs < 0.125 µg/ml); 3.33% were susceptible in dose dependent (MICs of 0.25 - 0.5 µg/ml) while 5% of the isolates were resistant to ketoconazole (MICs > 1 µg/ml). *Candida glabrata* isolates (85.72%) were susceptible to the drug (MICs < 0.125 µg/ml) and the remaining 14.28% were resistant (MICs > 1 µg/ml). *Candida krusei* (50%) isolates were susceptible in dose dependent (MICs of 0.25 - 0.5 µg/ml) while 50% were resistant (MICs > 1 µg/ml). Both *Candida tropicalis* and *Candida parapsilosis* were 100% susceptible to keto-conazole since all of their isolates had MICs of > 1 µg/ml (**Table 2**). The MIC_{90} to ketoconazole was 0.125 µg/ml while MIC_{50} was 0.014 µg/ml.

Candida albicans isolates had a high susceptibility to itraconazole. Eighty three point three (88.33%) percent of *Candida albicans* isolates had an MIC of ≤0.125 µg/ml and were regarded susceptible while 5% of the isolates were susceptible in dose dependent (MICs of 0.25 - 0.5 µg/ml). Eleven point six seven percent (11.67%) of *Candida albicans* isolates was resistant (MICs ≥ 1 µg/ml) as shown in **Table 3**. All isolates of *C. krusei*, *C. tropicalis* and *C. parapsilosis* were susceptible to itraconazole (MICs of ≤0.125 µg/ml) **Table 3**. Itra-conazole resistance was highest among *Candida glabrata* isolates. Fifty percent (50%) of the *Candida glabrata* isolates had MIC of ≥1 µg/ml and were regarded resistant while 32.14% were susceptible in dose dependent (MICs of 0.25 - 0.5 µg/ml). Only 17.85% of the *Candida glabrata* isolates had an MIC of ≤0.125 µg/ml and were susceptible. The MIC_{90} to itraconazole was 4.0 µg/ml while MIC_{50} was 0.03 µg/ml.

Candida albicans isolates were susceptible to clotri-mazole. Sixty three point three percent (63.3%) to *C.*

Table 3. Susceptibility of *Candida* species isolates to different concentrations of Itraconazole.

Itraconazole (µg/ml)	*C. albicans* n (%)	*C. glabrata* n (%)	*C. krusei* n (%)	*C. tropicalis* n (%)	*C. parapsilosis* n (%)
0.015	27 (45)	NIL	NIL	NIL	1 (100)
0.03	3 (5)	3 (10.71)	NIL	2 (66.67)	NIL
0.06	5 (8.33)	2 (7. 14)	NIL	NIL	NIL
0.125	15 (25)	NIL	2 (100)	1 (33.33)	NIL
0.25	2 (3.33)	1 (3.57)	NIL	NIL	NIL
0.5	1 (1.67)	8 (28.5)	NIL	NIL	NIL
1.0	1 (1.67)	NIL	NIL	NIL	NIL
2.0	2 (3.33)	NIL	NIL	NIL	NIL
4.0	3 (5)	14 (50)	NIL	NIL	NIL
8.0	1 (1.67)	NIL	NIL	NIL	NIL
Total	60 (100)	28 (100)	2 (100)	3 (100)	1 (100)

NIL: No organism was detected at that concentration.

Table 4. Susceptibility of *Candida* species isolates to different concentrations of Clotrimazole.

Clotrimazole (µg/ml)	*C. albicans* n (%)	*C. glabrata* n (%)	*C. krusei* n (%)	*C. tropicalis* n (%)	*C. parapsilosis* n (%)
0.03	4 (6.67)	9 (32.14)	NIL	NIL	NIL
0.06	10 (16.67)	3 (10.71)	NIL	NIL	NIL
0.125	3 (5)	12 (42.78)	NIL	2 (66.67)	1 (100)
0.25	20 (33.33)	1 (3.57)	NIL	NIL	NIL
0.5	1 (1.67)	NIL	NIL	NIL	NIL
1.0	2 (3.33)	NIL	1 (50)	NIL	NIL
2.0	7 (11.67)	NIL	NIL	1 (33.33)	NIL
4.0	2 (3.33)	2 (7.14)	1 (50)	NIL	NIL
8.0	3 (5)	NIL	NIL	NIL	NIL
16	8 (13.33)	1 (3.57)	NIL	NIL	NIL
Total	60 (100)	28 (100)	2 (100)	3 (100)	1 (100)

NIL: No organism was detected at that concentration.

albicans isolates had MICs of ≤0.5 µg/ml and were regarded susceptible while the rest 36.7% had an MIC of > 0.5 µg/ml and were considered resistant (**Table 4**). *Candida glabrata* isolates had the highest in susceptibility to clotrimazole. Eighty nine point two nine percent (89.29%) of the isolates had MICs of ≤0.5 µg/ml and were susceptible while 10.71% of the isolates were resistant (MICs > 0.5 µg/ml). The other non *Candida albicans* species isolates were fairly susceptible to clotrimazole except *Candida krusei* where all the isolates had MICs of >0.5 µg/ml and were regarded resistant. Out of the three (3) isolates of *Candida tropicalis*, 2 were susceptible (MICs ≤ 0.5 µg/ml) while 1 isolate was resistant (MICs > 0.5 µg/ml). The only isolate of *Candida parapsilosis* was susceptible to Clotrimazole (**Table 4**). The MIC_{90} of clotrimazole was 16 µg/ml while MIC_{50} was 0.25 µg/ml.

Candida albicans isolates (41.67%) were susceptible to topical nystatin. The isolates had MICs of ≤1 µg/ml while the rest 58.33% isolates had MICs of >1 µg/ml and were resistant (**Table 5**). Forty two point eight six percent (42.86%) of *Candida glabrata* isolates were susceptible (MICs ≤ 1 µg/ml). Fifty seven point one four (57.14%) of *Candida glabrata* isolates had MICs of >1 µg/ml and were regarded resistant. One (1) isolate of *Candida krusei* was susceptible (MIC ≤ 1 µg/ml) while the other one was resistant (MIC >1 µg/ml) as shown in **Table 5**. One (1) out of the three *Candida tropicalis* isolates was susceptible to topical nystatin (MIC ≤ 1 µg/ml) while the two isolates (66.67%) were resistant (MIC >1 µg/ml). The only isolate of *Candida parapsilosis* was susceptible to topical nystatin (MIC ≤ 1 µg/ml). The MIC_{90} of topical nystatin was 16 µg/ml and MIC_{50} was 2 µg/ml.

Table 5. Susceptibility of *Candida* species isolates to different concentrations of topical Nystatin.

Topical Nystatin (µg/ml)	*C. albicans* n (%)	*C. glabrata* n (%)	*C. krusei* n (%)	*C. tropicalis* n (%)	*C. parapsilosis* n (%)
0.06	NIL	2 (7.15)	NIL	NIL	NIL
0.125	4 (6.67)	1 (3.57)	NIL	NIL	NIL
0.25	5 (8.33)	1 (3.57)	NIL	1 (33.33)	NIL
0.5	7 (11.67)	5 (17.87)	NIL	NIL	1 (100)
1.0	9 (15)	3 (10.71)	1 (50)	NIL	NIL
2.0	10 (16.67)	7 (25)	NIL	NIL	NIL
4.0	8 (13.33)	2 (7.14)	1 (50)	2 (66.67)	NIL
8.0	10 (16.67)	4 (14.29)	NIL	NIL	NIL
16	7 (11.67)	3 (10.71)	NIL	NIL	NIL
Total	60 (100)	28 (100)	2 (100)	3 (100)	1 (100)

NIL: No organism was detected at that concentration.

4. Discussion

Increased multidrug resistant microorganisms are becoming a major challenge worldwide. The problem is attributed to inappropriate use of antibiotics and the increasing population of immune-compromised individuals [1]. Although multidrug resistant bacterial pathogens has been the main focus in the past, infection by yeasts account for 10% - 15% of hospital infections [14]. In Thika District Hospital, azole antifungal drugs (fluconazole, ketoconazole, itraconazole and clotrimazole) and topical nystatin are usually prescribed to pregnant and non pregnant women for the treatment of vaginal candidiasis. It is also a common belief among some of women that the constant use of antifungal agents will prevent yeast infection [1]. Pregnant and non pregnant women usually use topical nystatin because it is cheaper than the azole drugs and commonly found over the counters. Emerging resistance among *Candida* species from clinical specimen to some of the antifungal drugs have been documented [1]. The present study evaluated the susceptibility of the identified vaginal *Candida* species isolates recovered from vaginal samples of pregnant women to fluconazole, ketoconazole, itraconazole, clotrimazole and topical nystatin.

The results of this study indicated that most *Candida albicans* (95%) isolates were susceptible to fluconazole. The high fluconazole susceptibility in *Candida albicans* found in this study is consistent with other reports by Ogunbayo and Akortha [15,16]. Ogunbayo [15] reported a Fluconazole susceptibility of 96.3% among *Candida albicans* isolates in Nigeria while Akortha [16] reported a susceptibility of 95.7% among the *Candida albicans* isolates in the same country. This is probably due to the fact the drug is more tolerated with a wider spectrum of efficiency against *Candida albicans*. No fluconazole resistance was reported among yeast isolates in earlier studies on vulvovaginitis conducted in the United States,

England and Brazil [17,18]. *Candida albicans* isolates (3.33%) were resistant to fluconazole (**Table 1**). This is consistent with other research findings for example; a U.S study reported fluconazole resistance of 3.6% [17]. Akortha *et al.* [16] also reported a 4.3% *Candida albicans* resistance in Edo state in Nigeria while a 2.1% *Candida albicans* resistance was reported in New York [19].

As expected, higher MICs were recorded among *Candida glabrata* species isolates to fluconazole. Only 42.85% of *Candida glabrata* isolates were susceptible while the remaining 57.15% if the isolates were resistant (**Table 1**). This is due to the fact that *Candida glabrata* is innately less susceptible to fluconazole than most of other species of *Candida* [20]. This is in agreement with past studies by Pfaller *et al.* [5,20] and Kangogo *et al.* [2] that there has been an emerging resistance of *Candida glabrata* to fluconazole.

Candida krusei is intrinsically resistant to fluconazole even at high doses [11,21,22]. In this study, a 100% resistance was observed for *Candida krusei*, which is consistent with another research report, by Klastersky [22]. This is in agreement with past studies by Pfaller *et al.* [23] and Kangogo *et al.* [2] that there has been an emerged resistance of *Candida krusei* to fluconazole. The other *non-albicans* species isolated in this study; *Candida tropicalis* and *Candida parapsilosis* were all susceptible to fluconazole. This is in agreement with past studies by Pfaller *et al.* [23] that most of the *non-albicans* except *Candida krusei* are susceptible to fluconazole.

A similar susceptibility pattern to that of fluconazole was observed in ketoconazole (**Table 2**). This is because the two antifungal drugs are in the same group of antifungal drugs (azole drugs) and have a similar mode of action. The 91.67% susceptibility and 8.33% resistance of the drug to *Candida albicans* observed for ketoconazole in this study were also consistent with other reports [17,24]. Majority of the *non-albicans Candida* species

were susceptible to ketoconazole. However, a marked resistance was observed among *Candida glabrata* and *Candida krusei* isolates. This is similar to past findings by Pfaller *et al.* [23] that there has been an emerging resistance to ketoconazole between the two species.

Itraconazole was the other azole antifungal drug tested in this study. Most of the *Candida albicans* were susceptible (83.33%) to itraconazole with MICs ≤ 0.125 μg/ml (**Table 3**). Itraconazole is said to be among the first line antifungal drugs used against *Candida* species and it has a wider spectrum of efficiency against *Candida albicans* [25]. However, 16.67% of the isolates were resistant; 5% susceptible in dose dependent and 11.67% resistant. Incomplete therapy, overgrowth of resistant strains and subsequent infections with resistant *Candida albicans* strains could probably be among the factors that contributed to the resistance of 16.67% isolates to this drug. Prophylactic use of azole drugs in the treatment of *Candida* infections could also have contributed to the development of resistance to itraconazole. The finding is in line with other studies whereby itraconazole resistant *Candida albicans* accounted for 6% - 12% of the isolates from women with vaginal candidiasis [26]. A U.S. study reported itraconazole resistance of 6% to *Candida albicans* isolates [17]. In this study, itraconazole resistance was highest among *Candida glabrata* isolates (50%); 32.14% of the isolates were reported as susceptible in a dose-dependent manner and only 17.85% of the *Candida glabrata* isolates. This could be associated with clinical overt failure of antifungal azole therapy to the *Candida* species infection. The findings are similar with that of Gonzalez *et al.* [12] who reported *Candida glabrata* isolates resistance of 43.75%, 37.5% were susceptible in a dose dependent and only 18.7% were susceptible to Itraconazole among blood stream isolates.

Candida krusei was 100% susceptible to itraconazole. This is because *C. krusei* is intrinsically susceptible to itraconazole than the other azole drugs [17]. This finding is in agreement with a past study by Sobel *et al.* [17]. The other non-*albicans Candida* isolates (*Candida parapsilosis* and *Candida tropicalis*) were also 100% susceptible to itraconazole. This observation is consistent with other report by Dixon *et al.* [27] that *Candida tropicalis*, *Candida parapsilosis* and most of the non *Candida albicans* species are susceptible to Itraconazole.

A *Candida albicans* isolates were susceptible to clotrimazole (63.33%). However, the other remaining 36.67% isolates had an MIC of >0.5 μg/ml, which is an indication of drug resistance. Clotrimazole has been shown to inhibit the major fungi causing systemic infection especially vaginal candidiasis at a concentration of 1 μg/ml with efficacy against *Candida* [28]. Although, favorable results from systemic treatment of candidiasis and vaginal candidiasis have been described, some *Can-*

dida albicans strains still show high MICs to clotrimazole. Despite the high MICs to clotrimazole, the drug is extensively used in Kenya for management of vaginal candidiasis and dermatological conditions [1]. The resistance of *Candida albicans* isolates (36.67%) reported in this study could have been contributed by clinical overt failure of antifungal azole therapy resulting to the development of resistance of *Candida albicans* to clotrimazole [13]. These findings are in line with a report by Kangogo *et al.* [2] where *Candida albicans* susceptibility of 61% and a resistance of 39% to Clotrimazole.

Candida glabrata isolates had the highest in susceptibility to clotrimazole compared to the other *Candida* species isolates (**Table 4**). Eighty nine point two nine (89.29%) of the isolates were susceptible. However, a marked resistance of 10.71% of the *Candida glabrata* isolates was recorded. This could have been contributed by subsequent infections with resistant strains of *Candida glabrata* and the frequent use of azole drugs for treatment of candidiasis which pose the risk of development of resistance of some of the *Candida* species especially the non *albicans Candida* species. A high resistance was observed among *Candida krusei* isolates in this study (100%) probably because *Candida krusei* is only susceptible to Itraconazole and not to the other azole drugs [17]. These findings are in agreement with a past study by Pfaller *et al.* [23] that reported an emerging resistance among *Candida krusei* and *Candida glabrata* species to clotrimazole. On the other hand, two non *albicans* species (*Candida tropicalis* and *Candida parapsilosis*) isolated in this study were 66.67% and 100% susceptible to clotrimazole.

Topical nystatin was the only polyene antifungal drug prescribed to pregnant women in Thika District Hospital. Thus, it was the only one tested. Both *Candida albicans* and *Candida glabrata* had low susceptibility to topical nystatin (41.67% and 42.88%) respectively in this study. However, 58.34% and 57.14% isolates respectively of the species were resistant to topical Nystatin (**Table 5**). Likewise, the misuse of the drug by the pregnant women due to its easily availability could have contributed to the resistance of *Candida albicans* and *Candida glabrata* isolates to the drug. This is in agreement with a previous observation that emerging resistance to topical nystatin by the two species is on the increase [29]. *Candida krusei* and *Candida tropicalis* were 50% and 33.33% susceptible to topical nystatin respectively. This could have probably been contributed by the frequent use of the drug by the pregnant women for the treatment of vaginal candidiasis that resulted to development of resistance of *Candida* species to the drug. The only isolate of *Candida parapsilosis* had an MIC of <1.0 μg/ml and it was regarded susceptible (100%). This finding is consistent with that of Kangogo *et al.* [2] who reported *Candida*

parapsilosis susceptibility of 83% to the drug.

Susceptibility between the azole drugs and the only polyene drug used in this study showed that vaginal *Candida* species were less susceptible to the polyene drug used than the azole drugs. This is probably because of the difference in the mode of action between the azole and the polynes drugs. The mode of action of azoles is less altered by *Candida* species compared to the polyenes drugs. These findings are in agreement with the report by Okungbowa [30] that *Candida* species are more susceptible to azole antifungal drugs than polyenes drugs commonly used. Fungal infections are often challenging to manage; caution has to be exercised in the use of antifungal drugs to arrest any further increase in resistance. They have to be taken only under a clinician's prescripttion.

5. Conclusion

Candida albicans was the most prevalent species causing vaginal candidiasis in pregnant women. Majority of vaginal *Candida* species isolates exhibited high susceptibility to the azole drugs tested in this study. However, a tendency for lower susceptibility of some isolates to topical nystatin was observed and compared to azole drugs. An emerging resistance of *non-albicans* species especially *Candida krusei* which is resistant to all drugs used except itraconazole was also observed. Nonetheless, the susceptibility of vaginal *Candida* species to the azole drugs observed in this study supports the continued use of the antifungal drugs for the treatment of vaginal candidiasis in pregnant women except topical nystatin in Thika District Hospital. However, continuous surveillance on antifungal resistance to *Candida* species should be done in order to detect any emerging drug resistance.

6. Acknowledgements

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