

Prevalence and Antibiogram of *Candida albicans* Obtained from Post–Natal Mothers Attending Rivers State University Teaching Hospital, Nigeria

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ABSTRACT

Due to the increasing resistance of *Candida albicans* to commonly used antifungal agents, it has thereby enhanced its virulence in humans. In this study, the prevalence and antibiogram of clinical isolates of *C. albicans* was determined and investigated. A systematic random sampling technique was carried out using High Vaginal Swab (HVS) of females attending the Ante-Natal ward of the study area; 115 female patients between the ages 16 and 59 were selected comprising of Post Natal Mothers and other Female patients which served as controls. *Candida albicans* were isolated from collected HVS through inoculation on Saboraud dextrose agar and incubation at 37°C for 48hrs. Ensuing yeast colonies were subjected to germ tube and biochemical testing. The overall prevalence of *Candida albicans* among the female patients was 32.2%; consisting of 17 (14.8%) Post Natal Mothers and 20 (17.4%) Non Pregnant women. The prevalence of *C. albicans* among Non Pregnant Women was highest (7.0%) in age 26-30 years whereas among Post Natal Mothers, the highest prevalence (6.1%) was found in 36 and above years of age. Antifungal susceptibility testing revealed that the recovered isolates were multidrug resistant with a decreasing resistance trend in the order Clotrimazole 13(35.1%) > Fluconazole 12(32.4%) > Nystatin 11 (29.7%) > Itraconazole 8 (21.6%). Eleven 11(29.7%) of the isolates from Post Natal Mothers had a MAR_i greater than 0.2; whereas 16(43.2%) of isolates from the Non Pregnant Women Population had a MAR_i above 0.2. Although, the study may have revealed a low prevalence in the Post Natal Mothers, their prevalence is still significant to the public health and need for strict monitoring of antifungal usage is therefore vital.

Keywords: Post natal mothers; high vaginal swab; *Candida albicans*; antibiogram; prevalence, vulvovaginal candidiasis.

Introduction

The total number of eukaryotic species on Earth has recently been estimated at 8.7 million, with fungi making up approximately 7% (611,000 species) of this number. Of all fungi, only around 600 species are human pathogens (François *et al.*, 2013).

Candida spp. are the fourth most common cause of hospital-acquired systemic infections in the United States with crude mortality rates of up to 50% (Pfaller and Diekema, 2010). *Candida albicans*, a dimorphic commensal yeast, has two reservoirs: the patients' normal flora and the environment. Both interact making it difficult to block transmission of the pathogen between patients (Barada *et al.*, 2008).

Infections range from superficial, affecting the skin, mouth and vagina, to systemic associated with high morbidity and mortality rates in immunosuppressed

Individuals, HIV patients, chemotherapy patients, and organ transplant patients (Mayer *et al.*, 2013).

Based on the pathogenicity of *Candida albicans*, the ability of *C. albicans* to infect diverse host niches is supported by a wide range of virulence factors and fitness attributes. The pathogenicity of *C. albicans* depends upon two major factors; the immune status of the host and the virulence factors of the pathogen. The immune status of the host can also be related to the fitness attribute of the host; these attributes include; rapid adaptation to fluctuations in environmental pH, host's metabolic flexibility, powerful nutrient acquisition systems and robust stress response machineries. While the virulence factors of *Candida albicans* (Pathogen) will include; the morphological transition between yeast and hyphal forms, expression of adhesins and invasins on the cell surface, thigmotropism, secretion of hydrolytic enzymes, phenotypic switching and formation of biofilms

(François *et al.*, 2013). The incidence of candidiasis in the female reproductive tract has become a serious threat to public health. Vaginal candidiasis, is a common gynecological problem among women of child bearing age worldwide (Douglas, 2003).

The study therefore aims to assess the prevalence and the antibiotic susceptibility pattern, of *Candida albicans* isolates strains obtained from the study population.

Materials and Methods

Specimen Collection

A Total of 115 High Vaginal swab samples were aseptically collected using sterile swab sticks from female patients attending the Ante-Natal ward of Rivers State University Teaching Hospital. The One Hundred and fifteen (115) female patients that were selected comprised of Post Natal Mothers and other Female patients which served as controls. The subjects were all between the ages of 16 and 59 years. Only patients who showed no symptoms of urinary tract infections and who were not on anti-fungal therapy at the time of the study were included in the study. A well-structured interview questionnaire was used to source information on socio-demographic characteristics of the respondents as well as the risk factors of *Candida albicans* infections.

Isolation of Organism

Candida albicans were isolated from collected HVS through inoculation on Saboraud dextrose agar using the streak plate method and incubation at 37°C for 48hrs. Ensuing yeast colonies were subjected to further testing (Kim *et al.*, 2016).

Preservation of Isolates

Pure cultures of the yeast isolates were preserved in bijoux bottles containing 10% freshly prepared glycerol, the bottles were kept frozen in the refrigerator (Missiakas and Schneewind, 2013).

Characterization and Identification of Isolates

After incubation, plates were observed for yeast colony growth. Colonies appeared as smooth, creamy, pasty colonies.

Isolates were characterised based on their cultural morphology, biochemical properties and germ tube forming ability. The biochemical test includes; Sugar fermentation, Cornmeal plate test and Germ tube test.

Sugar Fermentation

10ml of the sugar medium was inoculated with the test isolate and incubated at 37°C for 10 days. A change in colour from light purple to yellow indicates acid production due to fermentation of the incorporated sugar. A retention of the light purple colour indicates a negative reaction, i.e, no sugar fermentation (Cheesbrough, 2006).

Cornmeal Plate Test

Cornmeal is used in distinguishing the different species of *Candida*, as it stimulates sporulation in many fungi. For studying the morphology of yeast-like fungi, a quarter of a plate was used for each organism. One hand streak down the centre of the area was made with wire loop containing the inoculum of the test organism (slightly cutting the agar) and three or four soft streaks across the first one. The area was covered with a 22 x 22mm cover slip and incubated at room temperature for 3 days. Examination was done by placing the plate, without its lid on the microscope stage and using a low-power (10x) and high-dry (40x) objectives. Characteristics formation of pseudohyphae, thick-walled terminal chlamydospores and the pattern of the arrangement of blastospores were used for preliminarily identification of *Candida spp.* Formation of septate pseudohyphae with clusters of round blastospores at the septa and large, thick-walled terminal chlamydospores are characteristics of *Candida albicans* (Adhikary and Joshi, 2011).

Germ Tube Test

A light suspension of a yeast-like organism was made in 0.5-1.0ml of sterile serum. The suspension was incubated at 37°C for 3 hours. After incubation, a drop of the yeast-serum was placed on a slide with a coverslip and examined microscopically for germ-tube production. Germ-tube production is a characteristic of *Candida albicans* and therefore is used for diagnostic purpose. Microscopic examination was done using the low-power (10x) and high-power dry (40x) objectives (Adhikary and Joshi, 2011).

Antifungal Susceptibility Test

Antifungal agents nystatin (NYS) (100U), fluconazole (FLU) (10µg), Itraconazole (ITR) (10µg) and clotrimazole (CLT) (10µg) were prepared as stock solutions in sterile distilled water and at different concentrations of the stock solution was added with sterile pipettes under aseptic conditions to different flasks containing different milliliters of molten SDA agar (52°C). This resulted in different concentration of SDA agar medium respectively. The medium was dispensed into sterile Petri dishes under aseptic conditions and was allowed to solidify. The Forty-eight-hour growth of the organisms in Sabouraud's dextrose broth was streaked on various segments of the antibiotic agar plates using a 1mm diameter calibrated wire loop capable of delivering 0.05ml of yeast suspensions.

The plates were incubated for 24-48 hours at 37°C. Zones of inhibition were assessed after the period of incubation (CLSI, 2020). After incubation, zones of inhibition in diameter (mm) were measured using a meter rule and values were interpreted using the CLSI Zone diameter Interpretative standards as susceptibility (S), intermediate susceptibility (I) or resistance (R) (CLSI, 2020).

The Multiple Antibiotics Resistance (MAR) index for each isolate was calculated using the formula;

MAR = a/b, where a, denotes the number of antibiotics to which the test isolate demonstrated resistance and b, denotes the overall number of antibiotics to which the test isolate's susceptibility has been evaluated (Adenaike, 2016).

Results

The result of prevalence of isolates of *Candida albicans* among general study population is presented in Figure 1. Out of the 115 swab samples screened, *Candida albicans* isolates were present in 37 (32.2%) cases. It also shows that out of the 37 (32.2%) that tested positive for *C. albicans*. 17 (14.8 %) were Post-Natal Mothers while 20 (17.4%) were Non-pregnant women as presented in Figure 2. Of the total recovered *C. albicans* isolates, 5 (4.3%) were from 21-25 age group, 14 (12.2%) were from 26-30 age group, 8 (7.0%) were from 31-35 age group and 10 (8.7%) were from 36 years and above sampled.

The prevalence of *C. albicans* was found to be highest 7 (6.1%) in women within 36 and above age group and lowest 2 (1.7%) in 21-25 age group population of Post Natal mothers while among the Non pregnant Women population the prevalent rate was found highest 8 (7.0%) within 26-30 age group and lowest 3 (2.6%) in 21-25 age group (Figure 3).

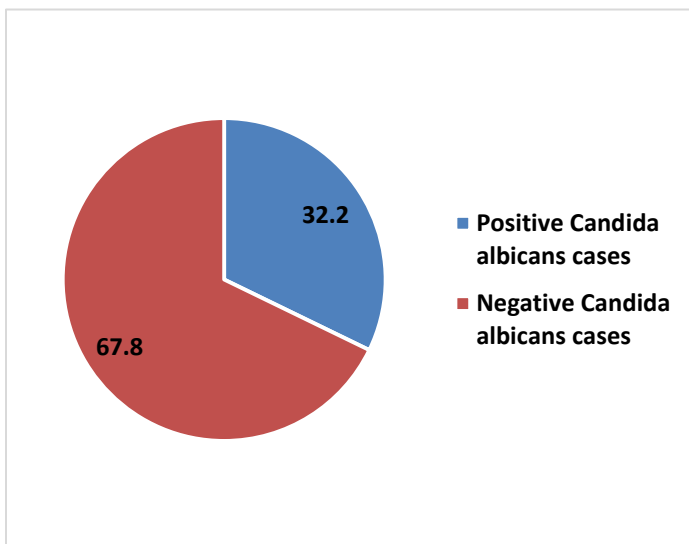


Fig. 1: Prevalence of Isolates of *Candida albicans* among General Study Population

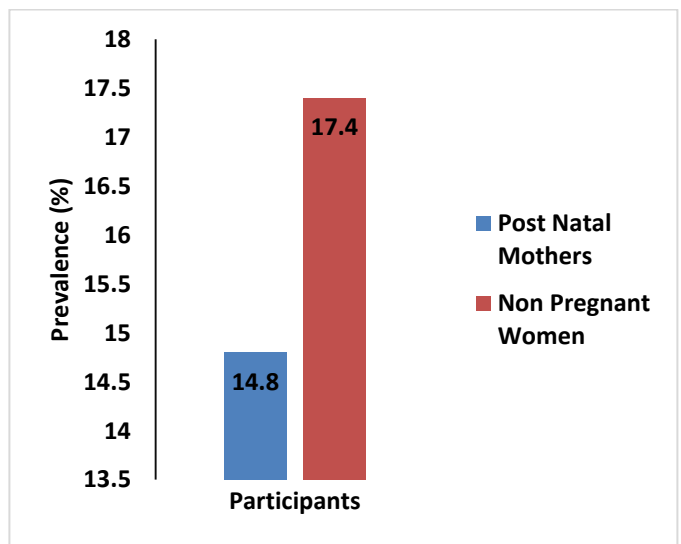


Fig. 2: Prevalence of Isolates of *Candida albicans* among Population

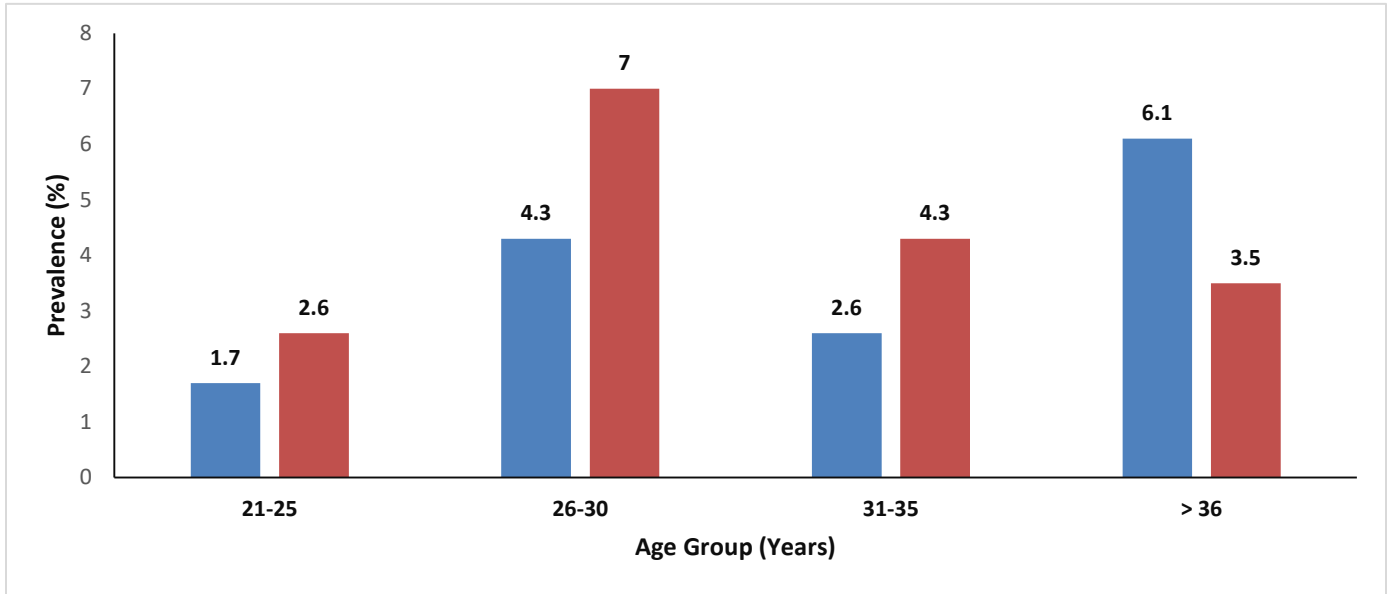


Fig 3: Prevalence of *Candida albicans* in Post Natal and Non Pregnant Women Population According to Age

The result of antifungal sensitivity pattern of the *Candida* isolates is as shown in Table 1. Susceptibility of fluconazole was determined as follows: 27.1% of the *Candida albicans* isolates were sensitive, 40.5% were Intermediate and 32.4% were resistant. Susceptibility of clotrimazole was analyzed, and it was sensitive in 27.1% of isolates, Intermediate in 37.8% of isolates and resistant in 35.1% of isolates.

Itraconazole susceptibility was recorded to be sensitive in 48.6% of isolates, Intermediate in 29.7% of isolates and resistant in 21.6% of isolates suffering from Vaginal candidiasis. Susceptibility results for Nystatin in patients with Vaginal candidiasis were as follows: 43.2% of isolates were sensitive, 27.1% of isolates were Intermediate, and 29.7% of isolates were resistant. The antifungal susceptibility test results as presented in Table 1 shows that a number of

Candida albicans isolates were resistant to all of the antifungal agents used (Fluconazole, Clotrimazole, Itraconazole and Nystatin) and a decreasing trend was seen in the order; Clotrimazole 13(35.1%) > Fluconazole 12(32.4%) > Nystatin 11 (29.7%) > Itraconazole 8 (21.6%) while susceptibility of the isolates were observed more in Itraconazole 18 (48.6%), followed by Nystatin 16 (43.2%) and the least was Fluconazole 10(27.1%) and Clotrimazole 10(27.1%). Susceptibility pattern based on the source of isolate is presented in Table 2. A higher frequency of *C. albicans* isolates was observed to be resistant to Fluconazole in the Post Natal mothers than the Non Pregnant women. While for antifungal agents; Clotrimazole, itraconazole and nystatin, *C. albicans* isolates presented to be more resistant in Non-Pregnant women than Post Natal mothers.

Table 1: Antifungal Susceptibility Pattern of *Candida albicans* (n = 37)

Antifungal agents Conc. (µg/ml)	Resistant n (%)	Intermediate n (%)	Susceptible n (%)
Fluconazole (10µg)	12 (32.4%)	15 (40.5%)	10 (27.1%)
Clotrimazole (10µg)	13 (35.1%)	14 (37.8%)	10 (27.1%)
Itraconazole (10µg)	8 (21.6%)	11 (29.7%)	18 (48.6%)
Nystatin (100U)	11 (29.7%)	10 (27.0%)	16 (43.2%)

Table 2: Susceptibility Pattern of *Candida albicans* Isolates obtained from Post Natal Mothers and Non Pregnant Women Population

Antifungal Agents Conc. (µg/ml)	Post Natal Mothers <i>C. albicans</i> isolates n = 17			Non Pregnant Women <i>C. albicans</i> isolates n = 20		
	R	I	S	R	I	S
	n(%)	n(%)	n(%)	n(%)	n(%)	n(%)
Fluconazole (10µg)	6 (35.3)	6 (35.3)	5 (29.4)	6 (30)	9 (45)	5 (25)
Clotrimazole (10µg)	6 (35.3)	5 (29.4)	6 (35.3)	7 (35)	9 (45)	4 (20)
Itraconazole (10µg)	3 (17.6)	7 (41.2)	7 (41.2)	5 (25)	4 (20)	11(55)
Nystatin (100U)	2 (11.8)	6 (35.3)	9 (52.9)	9 (45)	4 (20)	7 (35)

Table 3 shows the Multiple Antibiotic Resistance Index for *Candida albicans* isolates obtained from all the positive sampled patients (general population) and for the post natal mothers population and non pregnant women population. The value of the Multiple Antibiotic Resistance Index ranged between 0.0, 0.3, 0.5 and 0.8. The most common MAR index for the general population was 0.3 as 15 (40.5%) of the isolates were resistant to 1 out of the 4 antifungal agents tested, while the least common MAR index was

0.8 as 5 (13.5%) of the isolates were resistant to 3 out of the 4 antifungal agents tested. 27(72.9%) of the total isolates has a MAR index greater than 0.2. The MAR indices from the post natal mothers population and non pregnant women population showed that, *Candida albicans* isolates from non pregnant women had a higher MAR index (45% of 0.3 MAR_i, 15% of 0.5 MAR_i, 20% of 0.8 MAR_i) than *C. albicans* isolates from Post Natal Mothers which revealed only (35.3% of 0.3 MAR_i and 29.4% of 0.5 MAR_i).

Table 3: MAR Indices of *Candida albicans* of General Population, Post Natal Mothers and Non Pregnant Women

MAR Index	Multiple Antibiotic Resistance (MAR) index of <i>Candida albicans</i> during the study n (%)		
	General population (n=37)	Post Natal Mothers (n =17)	Non-Pregnant Women (n=20)
0.0	10 (27.0)	6 (35.3)	4 (20.0)
0.1	0 (0.00)	0 (0.00)	0 (0.00)
0.2	0 (0.00)	0 (0.00)	0 (0.00)
0.3	15 (40.5)	6 (35.3)	9 (45.0)
0.4	0 (0.00)	0 (0.00)	0 (0.00)
0.5	7 (18.9)	5 (29.4)	2 (10.0)
0.6	0 (0.00)	0 (0.00)	0 (0.00)
0.7	0 (0.00)	0 (0.00)	0 (0.00)
0.8	5 (13.5)	0 (0.00)	5 (25.0)
0.9	0 (0.00)	0 (0.00)	0 (0.00)
1.0	0 (0.00)	0 (0.00)	0 (0.00)

Discussion

Candida albicans isolates cultured from the lower genital tracts of female patients attending the Rivers State Teaching Hospital Clinic; this organism still remains the most incriminated in candidiasis of the urinogenital tract (Hawksworth and Lücking, 2017). Although studies have showed that *Candida albicans* is a normal flora yeast which causes a secondary infection known as candidiasis in individuals with some underlying immune - compromised conditions

(Gow, 2017), of which one of these conditions would include pregnancy. It is suspected that two weeks Post Natal mothers could still be at risk of VVC as some cases of the infection could persist after childbirth.

The results of the study showed that, to an extent candidiasis is epidemiologically significant in the population of women visiting the hospital in the study area. The overall prevalence of candidiasis among the female patients in the study area was 32.2%.

The overall prevalence pattern was similar to the ones reported by (Abdullahi and Danyaya, 2021) in Northwestern Nigeria where prevalence of vulvovaginal candidiasis was 38%. However, this study on vulvovaginal candidiasis has an overall prevalence that is lower than 20% reported in Owerri, South-Eastern Nigeria by Nwachukwu *et al.* (2020) and this may be due to variation in time study. Although, Non-pregnant population shows a higher rate (17.4%) of *Candida albicans* colonization when compared to postpartum population (14.8%) as revealed by the study. This high rate is suspected to be as a result of random Non-Pregnant women attending the health facility for treatment of their unknown infections.

This report of high prevalence existing among the 26-30 age group is a replication of that of another study (Okungbowa *et al.*, 2003) that showed that the occurrence of VVC peaks in the third decade of life, declining in women older than 40 years. It is, however, contrary to study by Ako-Nai *et al.*, (1993) where highest incidence was among women 20–25 years of age.

Antifungal resistance among *Candida* species involved in Vulvovaginal candidiasis (VVC) continues to thrive as a serious public health concern. Vulvovaginal candidiasis is a conventional gynecological opportunistic mycological infection caused by *Candida* species in the lower genital tract among females globally (Sobel 2007). It has been ascertained that approximately 75% of sexually active females have a minimum of one-time experienced symptomatic VVC (Lisiak *et al.*, 2003). The rise in incidence of fungal infection has resulted in an extensive use of antifungals (Gupta and Tomas, 2003).

The results of the antifungal sensitivity patterns, as interpreted using the Reference scheme for yeast susceptibility testing (National Committee for Clinical Laboratory Standards [NCCLS] Guideline M44A document, showed that a significant amount of *Candida albicans* isolate was susceptible to Itraconazole (48.6%) and Nystatin (43%) demonstrating that they are the most effective drug on *Candida albicans* from this work and it is consistent with the work of (Khan *et al.*, 2018) which showed that *Candida albicans* is most sensitive to Voriconazole, Itraconazole and Nystatin isolated from clinical samples.

The antifungal susceptibility pattern of the study also showed that more than 50% of the isolates of *C. albicans* were susceptible to the antifungal agent Nystatin (100U) among the Post Natal Mothers population and more than 50% of the isolates of *C. albicans* were susceptible to the antifungal agent Itraconazole (10µg) among the Non pregnant women population. Fluconazole and clotrimazole had the lowest susceptibility to *Candida albicans* with susceptibility of 27%.

Non Pregnant women population revealed higher percentage of resistant isolates than Post Natal population this could be as a result of higher prevalence of vaginal infection rate among the Non Pregnant Women. The azoles are fungistatic and have a broad spectrum of activity with few side effects with Fluconazole, Itraconazole and voriconazole, being the most frequently utilized (Cowen *et al.*, 2002). Fluconazole is the preferred azole because of its high oral bioavailability, good safety profile and broad efficacy against most pathogenic yeast (Xu *et al.*, 2000), while the polyene (nystatin) is fungicidal and have the broadest spectrum of activity of any clinically useful antifungal drug but can cause nephrotoxicity (Geogopapadakou, 1996).

Although, the study may have revealed a low prevalence in the Post Natal Mothers, their prevalence may still have resulted in wound infection after childbirth and systematic inflammation in the women whose VVC infection persist after pregnancy.

Antifungal resistance has posed a major concern in clinical practice. The advance of therapy has increased the survival rate but the risk factors have accumulated with an increase in cases of infectious diseases. 27 (73%) *Candida albicans* isolates obtained had a MAR index greater than 0.2. However, a significant portion of the MAR indices of *Candida albicans* identified in this investigation demonstrated various antifungal resistance and indiscriminate use of these antifungal agents for infections (Davis *et al.*, 2016).

This suggests that there is significant antibiotic abuse in this setting due to frequent self-medication, which is frequently linked to insufficient dosage and noncompliance with therapy, as well as the accessibility of antibiotics to consumers over the counter with or without a prescription (James *et al.*, 2017).

In conclusion, the present study demonstrated the importance of susceptibility testing for antifungals used among female patients in the clinical unit. *C. albicans* proved to be the predominant cause of Vulvovaginal candidiasis in the female participants in this study. The result of this study showed a relatively low occurrence of *Candida albicans* in Post Natal Mothers but as well revealed a significant concern to the public health. The overall results of this study emphasize the relevance of pregnancy, previous treatment of the infection, antifungal completion as different factors that play a role in *Candida albicans* infections. The study therefore, establish the need for strict monitoring of antifungal usage and antifungal resistance surveillance to ensure that antifungal potency is retained and to prevent fungi from developing total resistance to the last resort antifungal agent.

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