



Prevalence of *Candida albicans* among Female Patients in Two Selected Hospitals in Owerri Metropolis, Imo State Southeastern Nigeria

**M. O. Nwachukwu^{1*}, J. N. Azorji², P. C. Onyebuagu³, L. A. Adjeroh¹
and S. N. Nmezi¹**

¹Department of Biology, Federal University of Technology, Owerri, Imo State, Nigeria.

²Department of Biological Sciences, Hezekiah University, Umudi, Imo State, Nigeria.

³Department of Human Physiology, School of Basic Medical Sciences, Federal University of Technology, Owerri, Imo State, Nigeria.

Authors' contributions

This work was carried out in collaboration among all authors. Author MON designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors JNA, PCO and LAA managed the analyses of the study. Author SNN managed the literature searches. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/IJTDH/2020/v41i930313

Editor(s):

- (1) Dr. Ranthilaka R. Ranawaka, General Hospital Kalutara, Sri Lanka.
(2) Dr. Shankar Srinivasan, Rutgers - School of Health Professions, USA.

Reviewers:

- (1) Claudia Maricel Mattana, The National University of San Luis, Argentina.
(2) Ali Abdel-Hadi Mahoud Alsudani, University of Al-Qadisiyah, Iraq.
Complete Peer review History: <http://www.sdiarticle4.com/review-history/57507>

Original Research Article

**Received 24 April 2020
Accepted 30 June 2020
Published 17 July 2020**

ABSTRACT

The incidence of candidiasis in the female reproductive tract is a serious threat to public health. This study assesses the prevalence of *Candida albicans* among female patients in the two selected hospitals in Owerri metropolis. A systematic random sampling technique was used to select 120 female patients between the ages of 16-56 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. High Vaginal Swab (HVS) was aseptically collected from each of the patients, using a sterile swab stick. The collected samples were labeled appropriately and immediately sent

*Corresponding author: Email: nwachukwumichaelo@yahoo.com;

to the microbiology/mycology lab for analysis. The HVS samples were respectively streaked directly into sabouraud agar plates and incubated anaerobically at 37°C for 48 hours. Yeast growth characteristics were noted. Positive colonies were sub-cultured in Macconkey agar to obtain pure isolates. The *Candida albicans* were properly identified and confirmed by germ tube test, gram staining and biochemical tests. The results showed that the overall prevalence of *Candida albicans* among the women was (53.34%). The prevalence was highest among the patients in the age group 16-25 (44.17%), lower education qualification (n=65, 54.17%) and pregnant women (n=44, 36.67%). The possible risk factors for the infection include use of antibiotics (n=85, 70.83%), use of nylon underwear (n=78, 65.00), use of public toilet (n=90, 75.00%), use of squat WC (n=59, 9.17%) and use of oral contraceptive (n=59, 49.17%). The results call for preventive measures to protect women. Therefore public health education and campaign should be adopted.

Keywords: Prevalence; *Candida albicans*; selected Owerri metropolis.

1. INTRODUCTION

Candida albicans, a normal flora of nistic yeast (fungus) which causes a secondary infection known as candidiasis in individuals with some underlying immune- compromised conditions [1]. This organism is the most incriminated in candidiasis of the urinogenital tract. Other species include; *Candida tropicalis*, *C. stellatoides*, *C. parapsilopsis* and *C. crusei* [2].

The distribution of *Candida albicans* is widely in normal and healthy individuals and is usually found in mouth, skin, gastrointestinal and urinogenital tracts of females where they exist as normal flora.

Candida albicans infection or vaginal *Candidiasis*, is a common gynecological problem among women of child bearing age worldwide [3]; [4]. It has been reported that up to 75% of sexually active women will have experienced symptomatic vaginal candidiasis [5]. *Candida albicans* is one of the lower genital tract flora in 20-50% of healthy asymptomatic women [6]. It has been reported that carrier rates are higher in women treated with broad-spectrum antibiotics, in pregnant and diabetic women, and women with HIV/AIDS [7-10].

According [11], several factors can be associated with an increased rate of vaginal colonization by *C. albicans*: These include pregnancy, the use of high oestrogen content and oral contraceptives.

Uncontrolled diabetes mellitus [12,13], prolonged use of broad-spectrum antibiotics [14,15], which kill the good and beneficial bacteria, allowing yeast overgrowth, poor dietary habits and poor personal hygiene. Many practitioners believe that nylon underwear and tight insulating clothing predispose to vaginal candidiasis by increasing

the temperature and moisture content of perineum [16,17]. A study among African women wearing tight clothes reported a higher prevalence of *Candida albicans* in vulvovaginal candidiasis than those wearing loose clothing [18]. The same observation was made in the study by Wilson [19], where regular users of tight clothing had 88.2% of *Candida albicans* and occasional and no wearers had 68.6% of *Candida albicans*.

The carriers rate of *Candida albicans* among female patients has continued to be on the increase due to a lot of predisposing factors that cause changes in the vaginal environment [20]. Diabetes mellitus, pregnancy and the use of tight nylon underwear have been found to enhance overgrowth of *Candida albicans* in a manner that cannot be controlled by the body's defense mechanisms [21,22,23]. Other predisposing factors include excess stress, allergies, indiscriminate use of broad spectrum antibiotics, steroids, oral contraceptives, hormonal drugs, and nutritional deficiency [24,25]. Immune imbalances and changes in the dynamic environment inside the woman's vagina especially increased temperature and pH promote the pathogenesis of the yeast. Vaginal *candidiasis* predisposes women to other sexually transmitted infections such as HIV/AIDS [21,20].

There is a dearth of information and documentation regarding *Candida albicans* causing vaginitis among female patients in the study area. The prevalence rate of vaginal candidiasis in the study area has not been fully investigated, hence the need for this study. This study was undertaken to assess the prevalence of *Candida albicans* among female patients in two selected hospitals in Owerri Metropolis.

2. MATERIALS AND METHODS

The study was carried out between July and September, 2018 in Owerri metropolis, Imo State, South-Eastern Nigeria. This area is found on latitude 5°30'N and longitude 7°10'E Owerri Metropolis. The climate of the area is tropical and vegetation type is rainforest. There are 2 distinct seasons in the area namely: The rainy season (April – October), and the dry season (Nov–March). Rainfall is heavy during the rainy season and humidity is up to 80%. Temperature varies from 25°C to 32°C according to the seasons. The area is densely populated with urban slums and people from different ethnic and cultural backgrounds. There is a high level of environmental conditions in the area which are favorable for the growth, development and spread of fungal pathogens.

The people of Owerri are predominantly farmers, private business owners, Artisans, and petty traders. However, there are a few civil servants, social workers, and professionals like doctors, engineers, and lawyers.

2.1 The Study Subjects and Sampling Methods/Design

The study subjects comprised a total of 120 women (pregnant and non-pregnant) between the ages of 16-55 years.

Systematic random sampling techniques were used to select 60 women from each of the 2 hospitals (A = Eastern Summit Specialist Hospital and B = Enzy Specialist Hospital). Selection was based on informed consent to participate in the study voluntarily. 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.

2.2 Questionnaire Distribution for Socio-demographic Data Collection

All the participants (subjects) were first enlightened on the purpose and importance of the study and were educated on how to obtain a High Vaginal Swab devoid of contamination with the vaginal orifice. A simple, well structured questionnaire assessing the demographic information (age, marital status, occupation, religion and level of education.), symptoms and risk factors of candidiasis, were administered to the participants for completion. Only well

completed questionnaires were retrieved and used for analysis.

2.3 Collection of High Vaginal Swab (HVS) Specimen

With the help of laboratory scientists, the HVS was aseptically collected from each of the patients, using sterile swab sticks. The characteristic features of the probable symptoms presented by the patients include foul smelling odour, vaginal discharge (scanty, purulent), burning sensation and pain during urination, as well as itching and irritation of the vaginal orifice. All the collected samples were put in sterile containers, labeled appropriately, and immediately sent to the microbiology/mycology laboratory for analysis.

2.4 Microbiology/Mycology Analysis

All the swabs were sorted out for sample cultivation. Sabouraud Dextrose Agar (SDA) and MacConkey Agar were used as culture media.

2.5 Sample Cultivation and Isolation for Identification

After microscopic assessment for colour, appearance and odour, and microscopic examination of saline wet mount, the suspected yeasts colonies were cultured.

The HVS specimens/samples were respectively streaked directly onto sabouraud agar plates and incubated aerobically at 37°C for 48 hours. Yeast growth characteristics were sub-cultured on MacConkey agar to obtain pure isolates. The *Candida albicans* were properly confirmed and identified by germ tube test, gram staining and biochemical tests.

2.6 Germ Tube Test

Germ tube experiment was used as a rapid tool for identification of *Candida albicans* using a sterile wire loop, a small portion of a pure colony of *Candida albicans* was harvested and inoculated in a sterile test tube containing 0.5ml of human serum. The resulting suspension was incubated aerobically at 37°C for 3 hours. A drop of the yeast serum suspension was placed on a clean microscope slide with 1 drop of cotton blue lactophenol stain and covered with a coverslip, and examined microscopically, using the x10 and x 40 objective lenses of the microscope. The appearance of small sprouting tube-link

outgrowths or filaments projecting from the cell surface confirmed the production of germ tubes.

2.7 Gram Staining

A thin smear of the *Candida* culture on a clean grease free slide was heat-fixed, air-dried and then flooded with few drops of crystal violet and allowed for 1 minute. The slide was gently washed in an indirect stream of tap water for 2 seconds. The slide was flooded with a mordant (Gram's iodine) for 1 minute. The slide was flooded again with a gently indirect stream of tap water for 2 seconds. The slide was flooded with a decolorizing agent (Acetone-alcohol decolorizer) for 10-15 seconds, until decolorizing agent running for the slide runs clear. The slide was counterstained with *safranin* for 1 minute. The stain was washed out gently in an indirect stream of tap water until no colour appears in the effluents, and then blotted dry with absorbent paper. Oil immersion was applied and the slide was examined using x100 in a bright field microscope.

2.8 Biochemical Test (Carbohydrate Assimilation Test)

The carbohydrate assimilation test was carried out to determine the ability of the suspected *Candida* to use a particular carbohydrate substrate as its sole carbon source in a medium.

The *Candida* isolates were cultured in Yeast Potato Dextrose (YPD) Agar for 24 hours at 30°C. After, the cultures were centrifuged for 5 min at 2790rpm and the pellets were washed 3 times successively, suspended in sterile saline and adjusted to Mc Farland Standard. A volume of 300ml of Yeast Nitrogen Base (YNB, Difco) and 1.5ml of *Candida* species suspension were mixed to 30ml of sterile bacteriological agar in Falcon tubes. The suspension was placed in 15cm petridishes and after solidification of the medium, discs with the 2% carbohydrate (matrose, xylose, galactose, lactose, sucrose and glucose) were placed following a guide. The suspension was incubated in 30°C for 96 hours, and observed daily. The carbohydrate

assimilation was observed with a presence of a halo of growth around each carbohydrate.

2.9 Data Analysis

Data analysis was performed with the Statistical Package for Social Sciences (SPSS) software version 20, using analysis of variance procedure and simple descriptive and inferential statistics of tables, Frequencies, and percentage. Prevalence, figures were calculated for the study population and separately by their age group, level of education and pregnancy status, values for the risk factors of the *Candida albicans* were computed, analyzed and compared.

3. RESULTS

3.1 Identification and Isolated of *Candida albicans* in Pregnant and Non Pregnant Women

The results show that out of 120 women examined, 40 were infected, given 34 (85%) pregnant women and 6 (15.0%) non pregnant women. These results show a higher percentage of *Candida albicans* in pregnant women (Table 1).

3.2 Carbohydrate Assimilation Test and Morphological Features and Biochemical Properties

The result on the carbohydrate assimilation test shows the presence of glucose, dextrose, maltose and lactose while raffinose was absent (Table 2). Gram staining results show 4-6 mm in size of *Candida albicans* with oval shapes. The colour on chrom agar was green with chlamydo spores (Table 3).

3.3 Prevalence of *Candida albicans*

Table 4 shows that the prevalence of the *Candida* decreases with increasing age. The age group with the highest prevalence was 16-25 (29.17%) while the age group 46-55 had the least, patients with a high level of education had

Table 1. Identification and isolation of *Candida albicans* in women

Patients	Species	Number examined	Number infected	Number of isolated	Occurrence %
Pregnant	<i>C. albicans</i>	60	34	30	85.0
Non-pregnant	<i>C. albicans</i>	60	6	11	15.0
Total		120	40	41	100

Table 2. Carbohydrate assimilation test for *Candida albicans*

Substance	Detection
Glucose	+ve
Dextrose	+ve
Maltose	+ve
lactose	+ve
Reffinose	-ve

-ve = negative +ve = positive

Table 3. Morphological features and biochemical properties of the *Candida albicans* isolate

Laboratory Test	<i>Candida albicans</i>
Gram staining (blastoconia)	4-6µm, Oval
Germ tube	Yes
Carbohydrate assimilation	Glucose Dextrose Maltose Lactose
Colour on CHROM agar	Green
Chlamydospores	Yes

the lowest incidence of candidiasis 4 (3.33%) pregnancy is a risk factor of *Candida albicans* infection. In other words, pregnant women had more *Candida* infection 44 (36.67%) than their non-pregnant counterparts 20 (16.67%).

3.4 Knowledge of Respondents on Prevalence of *Candida albicans*

The respondents had a good knowledge of *Candida albican* infections. Out of 150 questionnaires distributed, 120 were returned and their knowledge was on the level of their education.(Tables 6 and 7). Primary education was the most dominant form of education (54.17%) among the study group. Majority were farmers (42.50%), Christians (88.33%) and 68 (56.67%) married. Table 7 presents the risk factors of *Candida albicans* infections. The use of broad spectrum antibiotics is associated with an increased incidence of infections. Also use of nylon/synthetic underwear, public toilet and squat wc, all are risk factors.

Table 4. Prevalence of *Candida albicans* among the women according to age

Age group	Number of participants	Number infected	Prevalence (%)
16 -25	53	35	29.17
26-35	30	15	12.50
36-45	23	10	8.33
66-55	14	4	3.33
Total	120	64	53.3

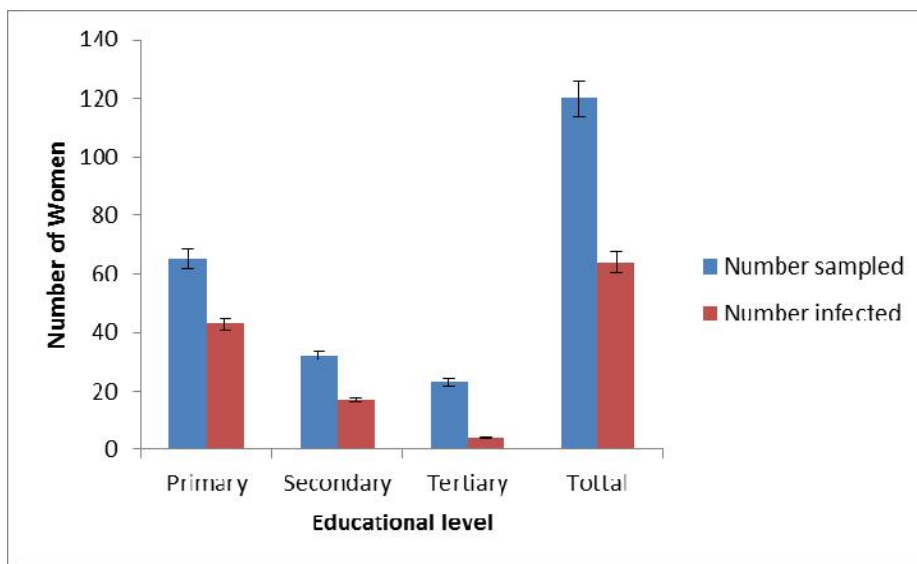


Fig. 1. Infection rate of *Candida albicans* among the women by level of education

Table 5. Prevalence of *Candida albicans* according to pregnancy status of the women

Status	Number sampled	Number infected	Prevalence (%)
Pregnant	60	44	36.67
Non-pregnant	60	20	16.66
Total	120	64	53.33

Table 6. Socio-demographic characteristics of the respondents

Parameters	Response (n =120)	
	Frequency	Percentage
Age		
16 - 25	53	44.17
26 - 35	30	25.00
36 - 45	23	19.17
46 – 55	14	11.66
Level of education		
Primary	65	54.17
Secondary	32	26.66
Tertiary	33	19.17
Occupation		
Housewife	20	16.67
Trader	23	19.17
Farmer	51	42.50
Teacher	23	18.33
Other	4	3.33
Religion		
Christianity	106	88.33
Muslim	5	4.17
Others	9	7.50
Marital Status		
unmarried	10	8.33
married	68	56.67
divorced	27	22.50
widowed	15	12.50
Number of Children		
1 – 3	29	24.17
4 – 6	67	55.83
7 and above	24	20.00

4. DISCUSSION

The results of the study showed that candidiasis is epidemiologically significant in the population of women visited in the hospitals in the study area. The overall prevalence of candidiasis among the female patients in the study area was 53.33. The prevalence of *Candida albicans* among the women is dependent on age, level of education, pregnancy status, and risk factors for its spread.

The age group with the highest prevalence of *Candida albicans* was 16-25 (44.17%). The higher with level of education, the lower the prevalence of the *Candida*. Those with primary education had the highest prevalence 65 (54.17%) of infection. pregnant women are more infected 44(36.67%) than the non-pregnant women 20(16.67%) use of antibiotics 85(70.83%) use nylon/synthetic underwear 78 (65.00%), public toilet (75.00%), squat WC (49.17%), dirty WC (43.33%) and oral contraceptive (59.17%) are not statistically significant.

Table 7. Factors predisposing the women to *Candida albicans* infection

Risk Factors	Response (n = 120)	
	Frequency	Percentage
Use of antibiotics		
Yes	85	70.83
No	35	29.67
Type of underwear		
Synthetic/nylon		
Cotton	78	65.00
Synthetic & Cotton	19	15.83
	23	19.17
Use of public toilet		
Yes	90	75
No	30	25
Type of WC used		
Squat	59	9.17
Sit	48	40.00
Stand	13	10.83
Nature of WC used		
Very clean	59	11.67
Quite clean	61	45.00
Dirty	52	43.33
Use of oral contraceptive		
Yes	59	49.17
No	61	50.83

The finding is however closer to the observation of [26], who reported a frequency of 38.0% prolonged use of antibiotics which kill the good and beneficial bacteria [27]; [28]. The prevalence may result to high risk of miscarriage, premature birth, fetal oxygen deficiency, low birth weight

and neonate mortality, wound infection after childbirth and systematic inflammation. Other possible risks include; pelvic inflammatory diseases (PID), infertility pelvic abscess, stress, discomfort and irritation [29].

In Table 4, it is clear that the age group with the highest infection of *Candida albicans* was 16-25 (29.17%) and mostly among those with a low level of education. As can be seen in Table 5 such as primary education (35.85%), this might be because they are youthful and sexually active or adventurous. The occurrence of the organism in this group is an indication that it is a leading causative agent of the reproductive tract yeast infection in women of childbearing age as also observed by [30]. The low occurrence of *Candida albicans* reported by [21] may be as a result of good personal hygiene and appropriate nutrition.

5. CONCLUSION

The result of this study showed a relatively low occurrence of *Candida albicans* in the study area. The overall results of this study emphasize the importance of the different factors that play a role in *Candida albicans* infections. The culture of vaginal discharge should be warranted because it is more sensitive than direct smear. The findings may help in drawing strategies in preventing and controlling vaginal candidiasis.

6. RECOMMENDATIONS

1. Early diagnosis and adequate treatment of vaginal candidiasis should be practiced.
2. The need for regular check-up at different gestation periods should be encouraged.
3. Consistent application of standard technique guidelines for the use of anti-fungal agents and control measures for predisposing factors of risk factors may potentially reduce the risk of drug-resistant life-threatening infections.
4. Application of standardized technology and its consistent use in diagnostic and research remains a major global challenge.

CONSENT AND ETHICAL APPROVAL

The verbal consent of the patients was sought and obtained after explaining the benefit of the study. The management of the hospital laboratories were informed of the project first by the chief lab technologist. Verbal consent was

given to the patients who volunteer to be used for the study.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Gow NAR. Microbe Profile: *Candida albicans*: A shape-changing, opportunistic pathogenic fungus of humans. *Microbiology*. 2017;163(8):1145–1147.
2. Hawksworth DL, Lücking R. Fungal diversity revisited: 2.2 to 3.8 Million Species. *Microbiology Spectrum*. 2017;5(4):79.
3. Hidalgo JA, Vazquez JA. Candidiasis: Workup". *Medscape*. WebMD; 2015.
4. Jurden L, Buchanan M, Kelsberg G, Safranek S. Clinical inquiries. Can probiotics safely prevent recurrent vaginitis?" *The Journal of Family Practice*. 2012;61(6):357-368.
5. Kendrick B. *The Fifth Kingdom: A New Look at Mycology*. Mycologue Publications; 2000.
6. Kerawala C, Newlands C. *Oral and maxillofacial surgery*. Oxford: Oxford University Press. 2010;446-447.
7. Kumamoto CA. *Candida* biofilms. *Current Opinion in Microbiology*. 2002;5(6):608–11.
8. Akah PA, Nnamani CE, Nnamani PO. Prevalence and treatment outcome of Vulvovaginal candidiasis in pregnancy in a rural community in Enugu State. *Nigeria Journal of Medical Sciences*. 2010;1(10); 447-452.
9. Ali JAO, Okonko IO, Odu NN, Kolade AF, Nwanze JC.. Detection and prevalence of *Candida* isolates among patients in Ibadan south Western Nigeria. *Journal of microbiology and Biotechnology Research*. 2011;(3):176–184.
10. Anderson M, Korasz A, Friedland S. Are vaginal symptoms ever normal. A review of the literature. *Med gen J*. 2004;6(4):49–55.
11. Aslam M, Hafeez R, Ijaz S, Tahir N. Vulvovaginal candidiasis in pregnancy. *Biomedica*. 2008;24:54-56.
12. Bouquot BW, Neville DD, Damm CM, Allen Jerry E. *Oral & maxillofacial pathology* (2. ed.). Philadelphia: W.B. Saunders. 2002;189–197.
13. Burrow RC, Bueshing UJ. Bacterial Vaginosis in virgins and sexually active

- females. Evidence against exclusive sexual transmission. *J. Obstet Gynecol.* 1999;8(5):97 – 99.
14. Calderone A, Clancy CJ. *Candida* and Candidiasis (2nd ed.). ASM Press; 2012. ISBN 978-1-55581-539-4
 15. Terri Warren RN. Is It a Yeast Infection?; 2010. (Retrieved 2011-02-23)
 16. University of Minnesota. Discovery of sexual mating in *Candida albicans* could provide insights into infections. *Science Daily*; 2013. Available:www.sciencedaily.com/releases/
 17. Uppuluri P, Khan A, Edwards JE. Current trends in candidiasis. In Prasad, Rajendra. *Candida albicans: Cellular and Molecular Biology.* Switzerland: Springer International Publishing AG. 2017;6.
 18. White D, Robertson C. United Kingdom National. Guideline on the Management of Vulvovaginal candidiasis clinical effectiveness group British. Association of Sexual Health and HIV. 2008;1:17.
 19. Wilson LS, Reyes CM, Stolpman MS, Allen K, Beney J. The Direct Cost and Incidence of Systemic Fungal Infections. *Value in Health.* 2002;5(1):26–34.
 20. Wise MG, Healy M, Reece K, Smith R, Walton D, Dutch W, Renwick A, Huong J, Young S, Tarrand J, Kontoyiannis D. *J. Med Microbiol.* 2007;56(6):778–787.
 21. Zinab AA, Salam A, Abbas A. Epidemiological and molecular study for *Candida* spp in vagina. *Medical Journal of Babylon.* 2014;11:1.
 22. Singh R, Chakrabarti A. Invasive candidiasis in the Southeast-Asian Region. In Prasad, Rajendra. *Candida albicans: Cellular and Molecular Biology* (2nd ed.). Switzerland: Springer International Publishing AG. 2017;27. [ISBN: 978-3-319-50408-7]
 23. Slutsky B, Buffo J, Soll DR. High-frequency switching of colony morphology in *Candida albicans.* *Science.* 1985;230(4726):666–9.
 24. Slutsky B, Staebell M, Anderson J, Risen L, Pfaller M, Soll DR. White-opaque transition: A second high-frequency switching system in *Candida albicans* (PDF). *J. Bacteriol.* 1987;1(169):189–197.
 25. Soll DR. High-frequency switching in *Candida albicans.* *Clin Microbiol Rev.* 1992;5(2):183–203.
 26. Srikumar C, Nagaraja HS. A comprehensive review of the occurrence and management of systemic candidiasis as an opportunistic infection. *Microbiology Journal.* 1(2):1–5.
 27. Parveen N, Munir AA, Dim I, Majeed R. Frequency of vaginal candidiasis in Pregnant women attending routine antenatal clinic. *Journal of Coll Physicians Surgpak.* 2008;18(3):154 -157.
 28. Pfaller MA, Diekema DJ. Epidemiology of invasive candidiasis: A persistent public health problem. *Clinical Microbiology Reviews.* 2007;20(1):133–63.
 29. Onyewole OA, Okoliegbe IN, Alkhalii S, IIsah P. Prevalence of vaginal candidiasis among pregnant women attending Federal University of Technology, Mina, Nigeria, Bossco Clinic. *Research Journal of Pharmaceutical, Biological and chemical sciences.* 2013;4(1):113.
 30. Pappas PG, Kauffman CA, Andes DR, Clancy CJ, Marr KA, Ostrosky-Zeichner L. Clinical practice guideline for the management of candidiasis: Update by the Infectious Diseases Society of America. *Clin Infect Dis.* 2016;62(4):e1-50.

© 2020 Nwachukwu et al.; This is an Open Access article distributed under the terms of the Creative Commons Attribution License (<http://creativecommons.org/licenses/by/4.0>), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Peer-review history:
The peer review history for this paper can be accessed here:
<http://www.sdiarticle4.com/review-history/57507>