



GLOBAL JOURNAL OF MEDICAL RESEARCH: C
MICROBIOLOGY AND PATHOLOGY
Volume 20 Issue 1 Version 1.0 Year 2020
Type: Double Blind Peer Reviewed International Research Journal
Publisher: Global Journals
Online ISSN: 2249-4618 & Print ISSN: 0975-5888

Prevalence of Candidiasis amongst Undergraduate Students of COOU, Uli, Nigeria

By Umeaku, Chinyelu Nkiru; Ukoha, Chinwe Clarice; Ebe, Tochukwu Ezechi;
Ozo, Chinwe Njideka; Egbuna, Helen Ifeoma; Ibekwe, Maureen Ifeynwa;
Chukwuno, Esther Oluchukwu & Okeke, Ugochukwu Chibueze

Chukwuemeka Odumegwu Ojukwu University

Abstract- Most urinary tract infections are due to Candida species, *C. albicans* being most prevalent. Laboratory research study was used to examine the prevalence of candidiasis amongst undergraduate students of Chukwuemeka Odumegwu Ojukwu University, Uli. A total of 100 students were investigated. Clean catch midstream urine samples were used for the analysis. Standard microbiological procedures were utilized. A structured questionnaire was issued to each student to obtain their socio-demographic data. Our study found *Candida albicans* in 14(14%) of the urine samples. Significant candidiasis was strongly associated with being female as higher percentage of the isolates were from female students. Of the 14(14.0%) positive urine samples, 4(28.6%) were from symptomatic students, whereas 10(71.4%) were asymptomatic.

Keywords: *candida albicans, candidiasis, urine samples, students.*

GJMR-C Classification: NLMC Code: QW 1



Strictly as per the compliance and regulations of:



© 2020. Umeaku, Chinyelu Nkiru; Ukoha, Chinwe Clarice; Ebe, Tochukwu Ezechi; Ozo, Chinwe Njideka; Egbuna, Helen Ifeoma; Ibekwe, Maureen Ifeynwa; Chukwuno, Esther Oluchukwu & Okeke, Ugochukwu Chibueze. This is a research/review paper, distributed under the terms of the Creative Commons Attribution-Noncommercial 3.0 Unported License (<http://creativecommons.org/licenses/by-nc/3.0/>), permitting all non-commercial use, distribution, and reproduction in any medium, provided the original work is properly cited.

Prevalence of Candidiasis amongst Undergraduate Students of COOU, Uli, Nigeria

Umeaku, Chinyelu Nkiru^α; Ukoha, Chinwe Clarice^σ; Ebe, Tochukwu Ezechi^ρ; Ozo, Chinwe Njideka^ω; Egbuna, Helen Ifeoma[¥]; Ibekwe, Maureen Ifeynwa[§]; Chukwuno, Esther Oluchukwu^x & Okeke, Ugochukwu Chibueze^v

Abstract- Most urinary tract infections are due to *Candida* species, *C. albicans* being most prevalent. Laboratory research study was used to examine the prevalence of candidiasis amongst undergraduate students of Chukwuemeka Odumegwu Ojukwu University, Uli. A total of 100 students were investigated. Clean catch midstream urine samples were used for the analysis. Standard microbiological procedures were utilized. A structured questionnaire was issued to each student to obtain their socio-demographic data. Our study found *Candida albicans* in 14(14%) of the urine samples. Significant candidiasis was strongly associated with being female as higher percentage of the isolates were from female students. Of the 14(14.0%) positive urine samples, 4(28.6%) were from symptomatic students, whereas 10(71.4%) were asymptomatic. Although candida vulvovaginitis occurs commonly, the reasons for its occurrence and recurrence are often unclear. Several potential risk factors have been described, including the recent use of antibiotics and oral contraceptives, uncontrolled diabetes, increased estrogen levels, impaired immune system, gastrointestinal colonization by the organism, and specific immunological defects. However, the data supporting each of these factors are conflicting, and to date, none are predictive of the infection. This study evaluates the potential risk factors of *C. albicans* and discusses the implications for clinical practice. We, therefore, recommend that further studies be carried out to determine the definite risk factors of candidiasis.

Keywords: candida albicans, candidiasis, urine samples, students.

I. INTRODUCTION

Vulvovaginal candidiasis (VVC) is a fungal or yeast infection. It is found in the lower genital tract, the vulva, and the vagina of females (Sobel, 2007). When this disease is caused by *Candida* species, it is known as candidiasis or moniliasis. VVC can be recurrent or relapsing (Nyirjesy and Sobel, 2003). This occurs when a female experiences four or more episodes of VVC per year. Asymptomatic infections occur in about 5% of healthy women (Resett et al., 2000).

Author α σ χ v: Department of Microbiology, Chukwuemeka Odumegwu Ojukwu University, Uli, Anambra, Nigeria.
e-mail: chimeaku@yahoo.com

Author ρ: Department of Environmental Technology, Federal University of Technology, Owerri, Imo, Nigeria.

Author ω: Department of Science Laboratory Technology, Anambra State Polytechnic, Mgbakwu, Nigeria.

Author ¥ §: Department of Nutrition and Dietetics, Anambra State Polytechnic, Mgbakwu, Nigeria.

VVC remains a common problem worldwide, affecting all strata of the society. The absence of rapid, simple, and inexpensive diagnostic tests continues to result in both over diagnosis and under diagnosis of VVC. *Candida albicans*, non-albican species, and immune suppression have led to the development of recurrent diseases, some of which do not respond to conventional antifungal drugs (Nwadioha et al., 2013).

According to McClelland et al. (2009), *Candida* spp. are part of the lower genital tract flora in 20%–50% of healthy women. In line with the studies of Singh (2003), *C. albicans* is the most frequent colonizer and is incriminated in most cases of VVC. Over the last ten years, research evidence has demonstrated an increase in the frequency of cases caused by other species of *Candida*. *C. glabrata* is also a leading cause of VVC (Ray et al., 2007).

About 75% of women will experience at least one episode of VVC during their lifetime. 70 – 75% of healthy adult women have had at least one episode of VVC during their reproductive life, and half of the college women will by the age of 25 years have had one case of VVC diagnosed by a physician (Sobel, 2007). VVC is not a sexually transmitted disease, because it also affects children and women who abstain themselves from sexual relationships. However, it can be transmitted sexually (de Leon et al., 2002). Diagnosis of VVC should not be based solely on patient history and a genital examination because of its low specificity of symptoms and signs. In addition, other causes like leukorrhea and pruritus vulvae mimic VVC (Geiger and Foxman, 2006). Therefore, to have a definitive diagnosis of VVC, cultural isolation and identification of *Candida* spp. are crucial.

Previous findings have provided data on the prevalence of VVC. It is interesting to note that most previous studies focused on immune compromised subjects, especially pregnant women, diabetics, subjects on broad-spectrum antibiotic therapy, women on oral contraception with high estrogen content, and HIV-positive subjects, with few studies on otherwise immunocompetent women. Interrelationships between *Lactobacillus acidophilus* and other endogenous flora, estrogen, glycogen, vaginal pH, and metabolic by-products of these micro biomes determine a healthy vagina. *L. acidophilus* produces hydrogen peroxide

(as a by-product of metabolism), which is toxic to pathogens and keeps the healthy vaginal pH acidic. Alterations of the vaginal micro flora by invading pathogens or biochemical changes in the environment results in vaginitis (Odds, 2008).

Changes in the vaginal environment, *Candida* population, and their adherence to vaginal epithelial cells enhance the germination of daughter yeast cells (Sobel, 2007). These changes and attendant multiplication of *Candida* cells may transform asymptomatic colonization into symptomatic infection. VVC, like many vulva diseases, has the potential to cause psychological distress and negatively impact patient's quality of life.

a) Aim of the Study

Our study aims to assess the level of urethritis due to *Candida albicans* amongst undergraduate students of Chukwuemeka Odumegwu Ojukwu University, Uli.

b) Specific Objectives

- Determination of the prevalence of candidiasis amongst undergraduate students
- Correlation of the prevalence rates with age, sex, and other risk factors
- Evaluating the effects of predisposing factors on both symptomatic and asymptomatic persons.

c) Significance of the Study

Fungal infections of the urinary tract especially, those caused by *Candida albicans* are becoming increasingly common. Urethritis due to *Candida* is mostly misdiagnosed or undiagnosed, as most studies concentrate on the bacterial urinary tract infections. Studies on the epidemiology of fungal urinary tract infections are limited in apparently healthy individuals since most studies were carried out in the hospital settings amongst hospitalized patients.

There are few studies that provide good databases for guiding public health practitioners on the diagnostic criteria and therapeutic pathways.

d) Limitation of the Study

The study population was undergraduate students. This made the research participants selective. Collection of urine samples from students was burdensome due to the misconceptions and fear of societal ills. Some students refused to fill the questionnaires.

II. MATERIALS AND METHODS

a) Study Population

One hundred students of Chukwuemeka Odumegwu Ojukwu University, Uli were randomly selected for this research. Only undergraduate students in regular programs were used. Consent was obtained from the participants.

b) Sampling Procedures

i. Administration of questionnaires

We obtained baseline socio demographic data using well-structured questionnaires and ensured confidentiality amongst the respondents.

ii. Collection of urine samples

We gave well-labeled sterile wide-mouthed screw-capped plastic containers with the same unique numbers as written on the questionnaires to the respondents. Each student was instructed on how to collect clean-catch midstream urine sample. 10 ml was obtained from each student.

iii. Media used

Sabouraud dextrose agar (SDA) and cornmeal agar (CMA) were used.

c) Culture and Identification of *Candida albicans*

Sterile cornmeal agar plates were inoculated with the urine specimens and incubated at 25°C for 72 hours. Each plate was read daily, recording the colony size, color and shapes. The isolates were subsequently streaked on sterile Sabouraud dextrose agar plates and incubated at 30°C for 4 days. The pure cultures were Gram-stained and observed microscopically using x100 oil immersion objective (WHO, 2003).

i. Germ tube test

The pure cultures were suspended in test tubes containing 0.5ml human serum. These were incubated at 35°C for 2 hours. A drop of the yeast-serum suspension was placed on a microscope slide and overlaid with a coverslip. This was examined microscopically for the presence of Germ tubes (Winn *et al.*, 2006).

III. RESULTS

We present the socio-demographic characteristics of the study subjects in Table 1. Of the 100 students examined, 80(80.0%) were female and 20(20.0%) were male. Only 8 of the sampled students were married, none was pregnant. Of the sampled students 11(11.0%) knew about urinary tract infection, but only 7(7.0%) had history of urinary tract infection (previously suffered from it). 17% were symptomatic whereas the remaining 83% were asymptomatic. More so, 17(77.2%) students had used antibiotics either by prescription or self-medication, 5(22.7%) said they have not used it.

14 had *Candida* positive cultures making the prevalence of vulvovaginal candidiasis 14.0%. *Candida* positive cultures were observed mostly among ages 21-30 years [11(11.0%)]. The majority of students in this age group were in their third to final year and are sexually active. The prevalence of infection between the age groups was statistically not significant ($P > 0.05$). Therefore, there is no significant difference between the age groups.

Table 1: Socio-demographic characteristics (n=100)

Gender	No. of students	Prevalence(%)
Female	80	80.0
Male	20	20.0
Marital Status		
Single	92	92.0
Married	8	8.0
Knowledge of Urinary Tract Infection		
Yes	11	11.0
No	89	89.0
History of Urinary Tract Infection		
Yes	7	7.0
No	93	93.0
History of Antibiotics Use (for UTI or Other Infections)		
Yes	51	51.0
No	49	49.0

Table 2: Age distribution of candidiasis among undergraduate students of COOU, Uli

Age (years)	No. Examined	No. Positive	Prevalence(%)
15-20	7	3	3.0
21-25	54	6	6.0
26-30	37	5	5.0
Above 30	2	0	0.0
Total	100	14	14.0

Out of the 100 urine samples cultured, 14 showed *Candida* growth, and the 14 were from female students. The prevalence of infection between the sexes was statistically not significant ($P > 0.05$). Therefore, there is no significant difference between sexes.

Table 3: Distribution of *Candida* growth in urine culture according to sex of the students

Urine culture	No (%) of results		
	Male	Female	Total
Positive	0(00.0)	14(17.5)	14(14.0)
Negative	20(100.0)	66(82.5)	86(86.0)
Total	20(100.0)	80(100.0)	100(100.0)

Table 4: Distribution of *Candida* positive cultures across

Clinical presentation	No. examined	No. positive	Prevalence
Symptomatic	17(17.0%)	4(28.6)	23.5(66.2%)
Asymptomatic	83(83.0%)	10(71.4)	12.0(33.8%)
Total	100(100.0%)	14(100.0%)	35.5(100.0%)

IV. DISCUSSION

Our study found the prevalence of vaginal candidiasis amongst undergraduate students of Chukwuemeka Odumegwu Ojukwu University, Uli, Nigeria to be 14%. Our result is lower than that reported by Aringet *et al.* (2012). In their study the prevalence of candidiasis was 16.5%, 21.31%, and 19 % respectively.

The relatively low prevalence we observed may be attributed to adequate knowledge, good personal hygiene, and normal levels of estrogens and corticoids amongst undergraduate students. Our result is however, in agreement with the studies of Fernández *et al.* (2004).

We observed candidiasis in students between ages 20 – 30 [17 (8.5%)]. Students below the age of 20 had least infection prevalence. These findings do not align with the studies of Alo *et al.* (2012) who reported a higher prevalence of *C. albicans* (33.33%) within the age bracket of 36 – 40 years. In their study, age group between 20 and 25 years had the lowest prevalence (20.42%). This outcome agreed with the studies of Akortha *et al.* (2009), and Willacy and Jackson (2011), who reported peak vaginal infections between ages 20 and 40 years. Women within ages 26–30 represent the peak of childbearing in Nigerian societies, this group is also the sexually active one.

There was no statistically significant relationship between the prevalence of VVC with age ($P > 0.05$) or clinical symptoms of ill health ($P > 0.05$). This may be due to recurrent infections that might have contributed to the resistance of the vagina to candidiasis. Subjects with vulvovaginal discomfort had a higher percentage of *Candida*-positive cultures (29.1%) than those with no vulvovaginal discomfort (11.9%). This report is in agreement with the findings of Jombo *et al.* (2010). It is reasonable to believe that young women with genital discomfort consult health care centers more often than women without such symptoms (Jombo *et al.*, 2010).

All subjects with positive *Candida* culture results had already been on antibacterial therapy prior to their hospital visit – 28 (100%). This finding is in conformity with the fact that prolonged antibacterial use usually affects vaginal bacteria micro flora population and biochemical activity (mainly *L. acidophilus*), which thus increases vaginal pH as a result of reduced CO₂ production. This feature, alongside other factors (such as hormonal factors), encourages *Candida* overgrowth, consequently leading to vulvovaginitis (Bauters *et al.*, 2002).

Although the widespread use of antibiotics has been suggested as one of the major factors contributing to the rising incidence of VVC, (Foxman *et al.*, 2008) some case-control studies (Geiger *et al.*, 2006) found no evidence of an association between antibiotic agents and symptomatic VCC, whereas others reached the opposite conclusion (Spinillo *et al.*, 2009).

V. CONCLUSION

There is a need to create awareness of the involvement of *Candida* spp. in genital discomfort, especially vaginal candidiasis, amongst undergraduate students with or without notable signs and symptoms. It is worthwhile to consider culture test as adjunctive in combination with clinical symptoms in the definitive diagnosis of VVC. More work is required to build on findings generated from this study.

VI. RECOMMENDATIONS

We recommend the following:

- The presence of candidiasis among apparently healthy individuals should not be neglected.
- Follow-up studies on the appropriate management of asymptomatic candidiasis should be conducted periodically.
- Role of antibiotic usage should be reviewed to delineate the cause of antibiotic resistance in recurrent VVC.
- Factors that promote candidiasis among students should be addressed promptly through extensive public health enlightenment programs.

REFERENCES RÉFÉRENCES REFERENCIAS

1. Akinbiyi, A.A., Watson, R. and Feyi, W. P. (2008). Prevalence of *Candida albicans* and bacterial vaginosis in asymptomatic pregnant women in South Yorkshire, United Kingdom. *Archives of Gynecology and Obstetrics* 278: 463–466.
2. Akortha, E., Chikwe, O. and Nwaugo, O. (2009). Antifungal resistance among *Candida* species from patients with genitourinary tract infection isolated in Benin City, Edo state, Nigeria. *African Journal of Microbiological Research* 3(11): 694–699.
3. Alo, M.N., Anyim, C., Onyebuchi, A.K. and Okonkwo, E.C. (2012). Prevalence of asymptomatic co-infection of Candidiasis and vaginal *Trichomonas* among pregnant women in Abakaliki, South-Eastern Nigeria. *Journal of Natural Sciences Research* 2 (7): 2224–3186.
4. Andes, D.R., Safdar, N., Baddley, J.W., Playford, G., Reboli, A.C. and Rex, J.H. (2012). Impact of treatment strategy on outcomes in patients with candidemia and other forms of invasive candidiasis. *Clinical Infectious Diseases* 54 (8): 1110–1122.
5. Aring, B.J., Mankodi, P.J. and Jasani, J.H. (2012). Incidence of vaginal candidiasis in leucorrhoea in women attending in orthopedic of gynecology and obstetrics department. *International Journal of Biomedical and Advanced Research* 3(12): 867–869.
6. Bauters, T.G., Dhont, M.A., Temmerman, M.I. and Nelis, H.J. (2002). Prevalence of vulvovaginal candidiasis and susceptibility to fluconazole in

- women. *American Journal of Obstetrics and Gynecology* 187 (3): 569–574.
7. Calderone, R. A. and Fonzi, W. A. (2001). Virulence factors of *Candida albicans*. *Trends in Microbiology* 9: 327–335.
 8. Cauwenbergh, G. (2000). Vaginal candidiasis: evolving trends in the incidence and treatment of non-*Candida albicans* infection. *Current Problems in Obstetrics, Gynecology and Fertility* 8: 241.
 9. Chandrasekar, P.H. and Sobel, J.D. (2006). Micafungin: a new echinocandin. *Clinical Infectious Diseases* 42 (8): 1171–1178.
 10. Corsello, S., Spinillo, A. and Osnengo, G. (2003). An epidemiological survey of vulvovaginal candidiasis in Italy. *European Journal of Obstetrics, Gynecology, and Reproductive Biology* 110 (1): 66–72.
 11. Cullen, P. J. and Sprague, G. F. (2012). The regulation of filamentous growth in yeast. *Genetics* 190: 23–49.
 12. Das-Neves J., Pinto, E., Teixeira, B., Dias, G., Rocha, P. and Cunha, T. (2008). Local treatment of vulvovaginal candidosis: general and practical considerations. *Drugs* 68 (13): 1787–1802.
 13. de Leon, E.M., Jacober, S.J., Sobel, J.D. and Foxman, B. (2002). Prevalence and risk factors for vaginal *Candida* colonization in women with type 1 and type 2 diabetes. *Bio Med Central Infectious Diseases* 2.
 14. Erdogan, A. and Rao, S.S. (2015). Small intestinal fungal overgrowth. *Current Gastroenterology Reports* 17 (4): 16.
 15. Eschenbach, D.A. (2004). Chronic vulvovaginal candidiasis. *The New England Journal of Medicine* 351 (9): 851–852.
 16. Ferrer, J. (2000). Vaginal candidosis: epidemiological and etiological factors. *International Journal of Gynecology and Obstetrics* 71 (1): 21–27.
 17. Ferris, D.G., Nyirjesy, P., Sobel, J.D., Soper, D., Pavletic, A. and Litaker, M.S. (2002). Over-the-counter antifungal drug misuse associated with patient diagnosed vulvovaginal candidiasis. *Obstetrics and Gynecology* 99 (3): 419–425.
 18. Fidel, P.L. (2006). *Candida*-host interactions in Human Immune Deficiency Virus disease: relationships in oropharyngeal candidiasis. *Advances in Dental Research* 19: 80–84.
 19. Foxman, B., Marsh, J.V., Gillespie, B. and Sobel, J.D. (2008). Frequency and response to vaginal symptoms among white and African American women: results of a random digit dialing survey. *Journal of Women Health* 7 (9): 1167–1174.
 20. Geiger, A.M. and Foxman, B. (2006). Risk factors for vulvovaginal candidiasis: a case-control study among university students. *Epidemiology* 7 (2): 182–187.
 21. Hasan, F., Xess, I., Wang, X., Jain, N. and Fries, B.C. (2009). Biofilm formation in clinical *Candida* isolates and its association with virulence. *Microbes and Infection* (11): 753–761.
 22. Horn, D.L., Neofytos, D., Anaissie, E.J., Fishman, J.A., Steinbach, W.J., Olyaei, A.J., Marr, K.A., Pfaller, M.A., Chang, C.H. and Webster, K.M. (2009). Epidemiology and outcomes of candidemia in 2019 patients: data from the prospective antifungal therapy alliance registry. *Clinical Infectious Diseases* (48): 1695–1703.
 23. Ingham, C. J., Boonstra, S., Levels, S., de Lange, M., Meis, J. F. and Schneeberger, P. M. (2012). Rapid susceptibility testing and microcolony analysis of *Candida* species cultured and imaged on porous aluminum oxide. *Journal of Public Health* 1 (7): 318–338.
 24. Jombo, G.T., Opajobi, S.O., Egah, D.Z., Banwat, E.B. and Denen, A.P. (2010). Symptomatic vulvovaginal candidiasis and genital colonization by *Candida* species in Nigeria. *Journal of Public Health and Epidemiology* 2 (6): 147 – 151.
 25. Kauffman, C.A. (2013). Diagnosis and management of fungal urinary tract infection. *Infectious Disease Clinics of North America* 28 (1): 61–74.
 26. Kerawala, C. and Newlands, C. (2010). *Oral and maxillofacial surgery*. Oxford University Press, London, pp. 446–447.
 27. Kett, D.H., Shorr, A.F. and Reboli, A.C. (2011). Anidulafungin compared with fluconazole in severely ill patients with candidemia and other forms of invasive candidiasis. *Critical Care* 15 (5): 253.
 28. Lai, C.C., Wang, C.Y., Liu, W. L., Huang, Y.T. and Hsueh, P.R. (2012). Time to positivity of blood cultures of different *Candida* species causing fungaemia. *Journal of Medical Microbiology* (61): 701–704.
 29. Leegaard, M. (2004). The incidence of *Candida albicans* in the vagina of “healthy young women”. How often do they have symptoms? Possible etiological factors. *Acta Obstetrica Et Gynecologica Scandinavica* 63 (1): 85–89.
 30. Lewis, R. *Candida: New Rapid Blood Test Could Cut Mortality*. Medscape Medical News. Apr 25 2013. Available at <http://www.medscape.com/viewarticle/803135>. Accessed: Apr 30, 2013.
 31. Marrazzo, J. (2002). Vulvovaginal candidiasis. *British Medical Journal* 3 (4): 586–587.
 32. Martini, F.C., Ober, W.C., Garrison, C.W., Welch, K. and Hutchings, R.T. (2001). *Fundamentals of Anatomy and Physiology*. Fifth Edition, Prentice-Hall Eaglewood Cliffs, United States of America, pp. 143–162.
 33. Martins, N., Ferreira, I.C., Barros, L., Silva, S. and Henriques, M. (2014). “Candidiasis: predisposing factors, prevention, diagnosis and alternative treatment”. *Mycopathologia* 177 (6): 223–240.

34. McClelland, R.S., Richardson, B.A. and Hassan, W.M. (2009). Prospective study of vaginal bacterial flora and other risk factors for vulvovaginal candidiasis. *Journal of Infectious Diseases* 199 (12): 1883–1890.
35. Nandan, D., Gupta, Y.P., Krishnan, V., Sharma, A. and Misra, S.K. (2011). Reproductive tract infection in women of reproductive age group in Sitapur/ Shahjahanpur district of Uttar Pradesh. *Indian Journal of Public Health* 5 (1): 8–13.
36. Nelson, M., Wanjiru, W. and Margaret, M.W. (2013). Prevalence of vaginal candidiasis and determination of the occurrence of *Candida* species in pregnant women attending the antenatal clinic of Thika District Hospital, Kenya. *Open Journal of Medical Microbiology* (3): 264–272.
37. Nohmi, T., Abe, S., Dobashi, K., Tansho, S. and Yamaguchi, H. (2005). Suppression of anti-*Candida* activity of murine neutrophils by progesterone in vitro: A possible mechanism in pregnant women's vulnerability to vaginal candidiasis. *Microbiology and Immunology* (39): 405–409.
38. Nwadioha, S. I., Nwokedi, E. O., Egesie, J. and Enejuo, H. (2013). Vaginal candidiasis and its risk factors among women attending a Nigerian teaching hospital. *Nigerian Postgraduate Medical Journal* (20): 20–23.
39. Nyirjesy, P. (2001). Chronic vulvovaginal candidiasis. *American Family Physician* 63 (4): 697–702.
40. Nyirjesy, P. and Sobel, J.D. (2003). Vulvovaginal candidiasis. *Obstetrics and Gynecology Clinics of North America* 30 (4): 671–684.
41. Odds, F.C. (2008). *Candida and Candidiasis: A Review and Bibliography*. Second Edition. Bailliere Tindall Press, London, pp. 382–383.
42. Okungbowa, F.I., Isuehuemhen, O.S. and Dede, A. (2003). The distribution frequency of *Candida* species in the genitourinary tract among symptomatic individuals in Nigeria cities. *Revista Iberoamericana de Micologia* (20): 60–63.
43. Omar, A.A. (2001). Vulvovaginal candidiasis among women in makkah city, Saudi Arabia. *Research Journal of Medical Sciences* 7 (4): 86–89.
44. Pappas, P.G., Kauffman, C.A., Andes, D.R., Clancy, C.J., Marr, K.A. and Ostrosky, Z.L. (2016). Clinical Practice Guideline for the Management of Candidiasis. *Clinical Infectious Diseases* 62 (4): 1–50.
45. Pappas, P.G., Kauffman, C.A., Andes, D., Benjamin, D.K., Calandra, T.F. and Edwards, J.E. (2009). Clinical practice guidelines for the management of candidiasis. *Clinical Infectious Diseases* 48 (5): 503–535.
46. Pappas, P.G., Rex, J.H. and Sobel, J.D. (2004). Guidelines for treatment of candidiasis. *Clinical Infectious Diseases* 38(2): 161–189.
47. Paul, L.F., Jessica, C. and Chad, S. (2000). Effects of reproductive hormones on experimental vaginal Candidiasis. *Journal of Infection and Immunity* 68 (2): 651–657.
48. Ray, D., Goswami, R. and Banerjee, U. (2007). Prevalence of *Candida glabrata* and its response to boric acid vaginal suppositories in comparison with oral fluconazole in patients with diabetes and vulvovaginal candidiasis. *Diabetes Care* 30 (2): 312–317.
49. Reed, B.D. (2002). Risk factors for *Candida* vulvovaginitis. *Obstetrical and Gynecological Survey* 47 (8): 551–560.
50. Reed, B.D., Huck, W. and Zazove, P. (2009). Differentiation of *Gardnerella vaginalis*, *Candida albicans*, and *Trichomonas vaginalis* infections of the vagina. *The Journal of Family Practice* 28 (6): 673–680.
51. Rex, J.H., Walsh, T.J. and Sobel, J.D. (2000). Practice guidelines for the treatment of candidiasis. *Infectious Diseases Society of America. Clinical Infectious Diseases* 30 (4): 662–678.
52. Ringdahl, E. (2000). Treatment of recurrent vulvovaginal candidiasis. *American Family Physician* 61 (11): 3306–3312, 3317.
53. Silva, S., Negri, M., Henriques, M., Oliveira, R., Williams, D. W. and Azeredo, J. (2011). Adherence and biofilm formation of non-*Candida albicans* *Candida* species. *Trends in Microbiology* 19: 241–247.
54. Singh, S.I. (2003). Treatment of vulvovaginal candidiasis. *Clinical Reviews* 136(9): 26–30.
55. Sobel, J.D. (2004). Maintenance fluconazole therapy for recurrent vulvovaginal candidiasis. *N Engl J Med* 351(9): 876–883.
56. Sobel, J.D. (2007). Vulvovaginal candidosis. *The Lancet* 369 (9577): 1961–1971.
57. Spinillo, A., Capuzzo, E., Nicola, S., Baltaro, F., Ferrari, A. and Monaco, A. (2005). The impact of oral contraception on vulvovaginal candidiasis. *Contraception*. 51(5): 293–297.
58. Spinillo, A., Capuzzo, E., Acciano, S., de Santolo, A. and Zara, F. (2009). Effect of antibiotic use on the prevalence of symptomatic vulvovaginal candidiasis. *American Journal of Obstetrics and Gynecology* 180 (1): 14–17.
59. Willacy, H. and Jackson, C. (2011). Vaginal and vulval candidiasis. Retrieved from: <http://www.patient.co.uk/doctor/vaginal-and-vulval-candidiasis>. Accessed May 17, 2015.
60. Winn, W.C., Allen, S.D., Janda, W.M., Koneman, E.W., Procop, G.W., Schreckenberger, P.C. and Woods, G.L. (2006). *Koneman's Color Atlas and Textbook of Diagnostic Microbiology*. Sixth Edition, Lippincott Williams and Wilkins Press, United States of America. pp. 1216–1223.