

Prevalence of *Candida Albicans* in Genital Tract of Pregnant Women Attending Antenatal Clinic of Nepalgunj Medical College Hospital

Rai M¹, Paudel TP², Gurung K³, Neupane GP⁴, BC D⁵

ABSTRACT

Introduction: Pregnancy is one of the important risk factor for occurrence of vaginal candidiasis which may lead to pregnancy complications like abortions, premature birth, low birth weight and other morbidities. It is essential to know pattern of distribution of the species of *Candida* that are pathogenic. **Aim and Objective:** To identify prevalence of *Candida albicans* in the genital tract of pregnant women. **Materials and Methods:** Total of 100 vaginal swabs were collected from 100 pregnant women attending at antenatal clinic of Gynaecology and Obstetrics department for a period of six months from March 2017 to August 2017. Gram's staining and inoculation onto Sabouraud dextrose agar (SDA) medium were done for general identification of yeast and to isolate the positive cases. The specifications of *Candida* species was done on the basis of sugar fermentation, carbohydrate assimilation, production of germ tube in fresh human serum, formation of chlamyospore on Cornmeal agar and colour of colony on CHROMagar. **Results:** Out of 100 high vaginal swabs 40(40%) samples were positive for *Candida*. Of these positive samples, *Candida albicans* was isolated in 52.5% of pregnant women. *Candida albicans* was most commonly isolated in the age group of 25-29(38.09%), in third trimester of pregnancy and in the multigravidae. **Conclusions:** High prevalence of *Candida albicans* species among pregnant women were documented in this study. Our results indicate that gravidity, as the risk factor for occurrence of infection, has the significant role in the occurrence of vaginal candidiasis.

Key words: *Candida species, pregnant women, vulvovaginal candidiasis, vaginal swab, yeast*

The female genital tract is the portal of entry for numerous sexually and non-sexually transmitted diseases. Different kinds of bacterial, fungal and viral infections exist that affect the female reproductive tract¹. *Candida* species are widespread in distribution, ranging from endosymbionts of animals, to being pathogenic in many animals and humans. It is usually part of the normal flora in the mouth, vagina, skin and gut of the human. *Candida* is found in the vagina of 35% to 50% of healthy women. But *Candida* also can cause opportunistic infections in persons with decreased cell mediated immune state may be due to prolonged antibiotic therapy, use of corticosteroids and chemotherapy, malnutrition, pregnancy, use of contraceptives and in diabetes².

Pregnant women are more susceptible to vaginal candidiasis due to immune suppression and hormonal imbalance. The hormone progesterone and estrogen are the known hormones that elevate during pregnancy. These two hormones suppress the normal functioning of the body's immune system.

1. Dr. Maya Rai
2. Dr. T. P. Poudel
3. Dr. K. Gurung
4. Dr. G. P. Neupane
5. Dr. Durga B.C.

Address for correspondence:

Dr. Maya Rai
Department of Microbiology
Nepalgunj Medical College,
Chisapani, Banke, Nepal
E-mail: ganeshpn2003@yahoo.com

Progesterone suppresses neutrophil function by inhibiting the production of IL-8. The IL-8 is required for the migration of neutrophil³. Estrogens decreases immunoglobulin (IgA, IgG) by decreasing the number of plasma cells as well as increases glycogen content in vagina which favours *Candida* infection⁴. In addition, estrogens enhances adherence of yeast to vagina by reducing antifungal activity of epithelial cells. Estrogen causes transition of vaginal epithelial cells from columnar to stratified squamous epithelial cell which makes them more permissive for adherence and growth of *Candida*. Estrogens also cause inhibition of antigen presentation by vaginal epithelial cells^{5,6}.

Candida infection in pregnancy does not usually harm the unborn child but causes great discomfort to the mother. If the disease is not treated, the baby will get infected (oral thrush) at birth which can be a very serious health problem in premature baby⁷.

Candida albicans is the most frequently isolated invasive fungal pathogen in humans, with the majority of infections being localized to the urogenital or oropharyngeal tracts of the patient. In addition to localized infections, *Candida albicans* is also able to establish a systemic infection in its host. *Candida albicans* is both the most frequent colonizer and is responsible for most cases of vulvo vaginal candidiasis¹. *Candida* infection among pregnant women is increasing due to wearing tight under-garments, poor hygiene, altered dietary and sanitary practices. Since vaginal *Candida* infection is common in pregnant women, it is necessary to identify the species of *Candida* for the better pharmacotherapy. The aim of this

research work was to determine the prevalence of candida albicans among pregnant women attending Nepalgunj Medical College Teaching Hospital, Kohalpur.

MATERIAL AND METHODS

Total of 100 vaginal swabs were collected from 100 pregnant women between ages of 15-45 years attending at antenatal clinic of Gynaecology and Obstetrics Department of Nepalgunj Medical college Kohalpur for a period of six months from March 2017 to August 2017. A verbal consent was obtained from each participant who also completed a questionnaire. Pregnant women with or without symptoms of vaginal candidiasis attending for antenatal check-up who were giving consent were included for study. Pregnant women with history of HIV-infection, age less than 15 years and more than 45 years pregnant women suffering from tuberculosis and with severe life threatening condition such as myocardial infarction, stroke, diabetic ketoacidosis etc. and women not giving consent for study were excluded. After completion of recording the working proforma two vaginal swabs were collected from each patient and transported to the microbiology laboratory of Nepalgunj Medical College for processing.

Sample collection:

Samples of vaginal swabs were collected under aseptic condition from pregnant women attending antenatal clinic. Collections of vaginal samples were done by exposing the posterior fornix with a sterile vaginal speculum. The sterile cotton swab sticks were inserted and gently rotated against the wall of the endocervix. The swab sticks were immediately replaced in its casing and labeled appropriately. Two sets of swabs were collected from each patient⁸. The samples were labeled and taken to the laboratory of microbiology.

Microscopic examination of specimen: Out of two swabs, one swab was used for Gram's stain to demonstrate budding yeast cells. Other swab was used for culture into Sabouraud dextrose agar⁸.

Gram's stain: Smear from the vaginal swab was prepared for gram staining. The smear was examined microscopically under oil immersion objective lens to demonstrate the Candida morphology⁹. On examination, Gram positive budding yeast cells were seen in Candida positive cases.

Culture:

Another vaginal swab was aseptically inoculated on Sabouraud dextrose agar (SDA) medium. The inoculated plate was incubated at 37°C for 24 hours. The plate with no growth was further incubated for another 48 hours and was discarded after 72 hrs. in which growth was not seen. The colony characteristics of growth were recorded. After that Gram's stain was done to see yeast cells. The colonies were subcultured on Sabouraud dextrose agar slants for further tests¹⁰.

Lactophenol Cotton Blue mount: Few drops of Lactophenol Cotton Blue reagent were put on the clean glass slide. A small portion of the colony was mixed with that solution. Then the cover slip was placed and observed on low power followed by high power objectives lens to demonstrate budding yeast cells and pseudohyphae¹⁰.

Gram's stain, Culture on Sabouraud dextrose agar (SDA) medium, Lactophenol Cotton Blue mount, were done for general identification Candida yeast and Sugar fermentation test, Carbohydrate assimilation test, Growth on CHROMagar medium, Germ tube formation test, and Chlamyospore formation test were done for identification of Candida albicans. Collected data were entered in Microsoft Office Excel 2007 and analyzed using SPSS Inc. Statistical Software Version 16.0

RESULTS

The result revealed that out of 100 high vaginal swabs 40(40%) samples were positive for Candida while 60(60%) samples were negative Figure 1.

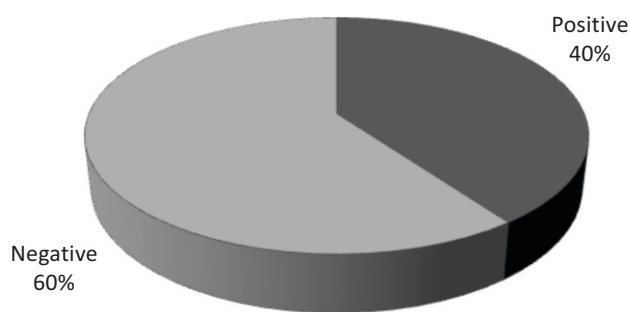


Figure 1: Prevalence of vaginal Candida among pregnant women

Out of 40 positive samples Candida albicans was isolated in 52.5% of pregnant women table I.

Species	No of isolates	%
Candida albicans	21	52.5%
Other species	19	47.5%
Total	40	100

Table I: The percentage occurrence of Candida species in the pregnant women

Candida albicans was most commonly isolated in the age group of 25-29 (38.09%) Table II. Candida albicans was most commonly isolated in the third trimester of pregnancy 76.19%. Table III. Candida albicans was more commonly isolated in multigravidae women (76.19%) in comparison to primigravidae.

Age groups	Number of Candida albicans Isolates n (%)	%
15-19	3	14.28
20-2	4	419.04
25-29	8	38.09
30-34	5	23.83
35-39	1	4.76
40-45	0	
Total	21	100

Table II: The distribution of Candida species according to age group of pregnant women

Trimester of Pregnancy	Number of Candida albicans Isolates	%
First	2	9.53
Second	3	14.28
Third	16	76.19
Total	21	100

Table III: The distribution of Candida species according to trimester of pregnancy

Gravidae	Number of Candida albicans Isolates	%
Multigravidae	16	76.19
Primigravidae	5	23.81
Total	21	100

Table IV: The distribution of Candida species according to gravidae

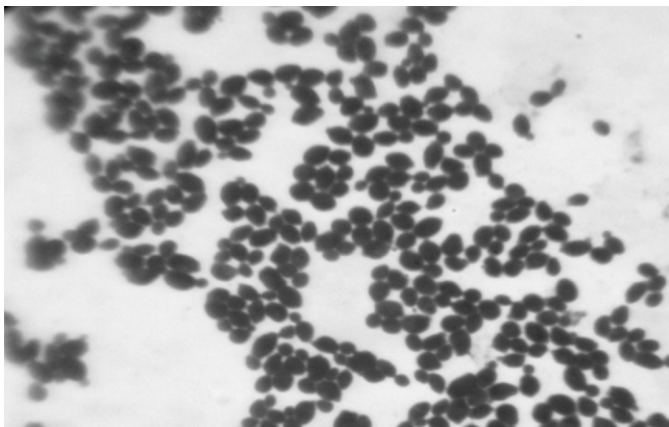


Figure 2 : Gram's staining of Candida species from the colonies showing Gram positive budding yeast cells

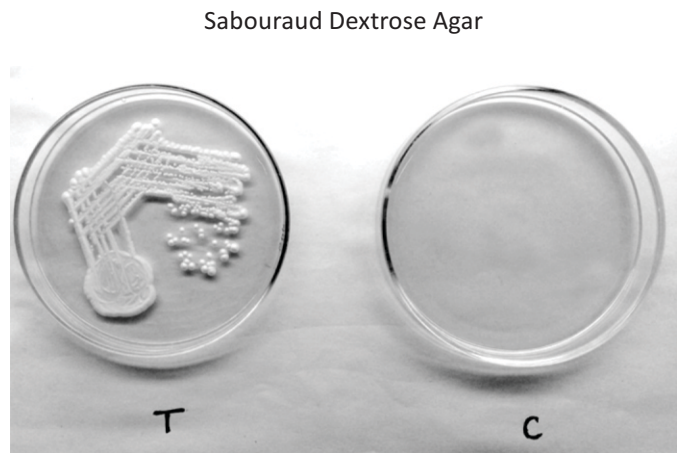


Figure 3: Growth of Candida on SDA Medium with control

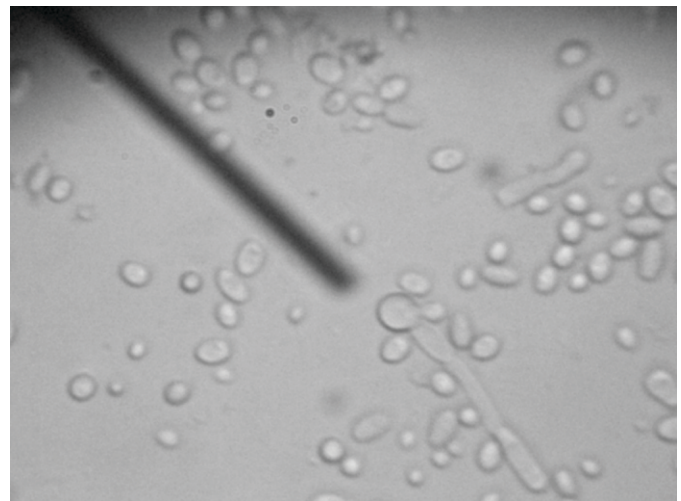


Figure 4: Arrow showing Chlamydospore of Candida albicans on Cornmeal agar

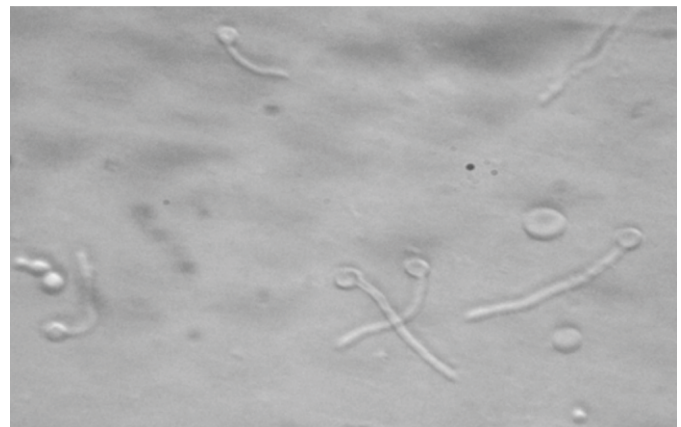


Figure 5: Germ-tube showing by Candida albicans in fresh human serum at 37°C centigrade within 3 hours

DISCUSSION

Vulvovaginal candidiasis in pregnant women is usually ignored in our country. The data on vaginal Candida and on identification of different species among pregnant women in developing countries is very scanty¹¹. The study showed out of 100 samples, 40 were Candida positive. This is similar to finding of Kanagal DV et al¹¹, Feyi Waboso et al¹² and Oviasogie et al¹³ who reported an occurrence of 42.3%, 42.9% and 44.8% respectively. However higher occurrence was shown in study of Nelson et al¹⁴ (90.3%), Oyewole et al¹⁵ (70%) and lower occurrence was seen in study of Donbraye Emmanuel et al¹⁶ (26%) and in Nepal, Shrestha et al¹⁷ (29.5%). The differences could be due to geographic, ethnic and socioeconomic factors as well as differences in sampling and culture technique. Variations may also reflect differences in sexual practices and environmental factors such as hygiene and nutrition¹⁶.

The result of this study revealed that Candida albicans is the most common (52.5%) vaginal Candida species among pregnant women. Results of this present study are consistent with previous study by Oyewole et al¹⁵ who reported the occurrence of 50% Candida albicans. The highest occurrence rate of Candida albicans observed in this study is an indication that it is a leading causative agent of the reproductive tract yeast infections in pregnant women. This may be due to its virulent factors which include dimorphism and phenotypic switching. Candida albicans produces protease and phosphatase which enhance its attachment to human epithelium. It can also be deduced that the high incidence rate of Candida albicans could be due to increased physiological changes, estrogen hormone and rich glycogen content of the vaginal mucosa thereby providing an adequate supply of utilizable sugar that favour its growth during pregnancy¹⁵. Candida albicans is also able to adhere to vaginal epithelium more readily than other Candida species¹⁸.

In present study Candida albicans was isolated in all age groups. This observation is consistent with reports of other researchers Nelson et al¹⁴, Olarian et al¹⁹. This may be as a result of Candida albicans predominating over other species in the females¹⁴. The distribution of Candida species according to trimester of pregnancy showed that Candida albicans was isolated in all the three trimester of pregnancy and most commonly in the third trimester. These findings are consistent with the studies of Samuel et al.²⁰ and Nelson et al¹⁴. This may be due to the fact that the Candida albicans is the most common vaginal Candida species in a population.

The highest isolation of Candida species in 3rd trimester may be due to increased emotional stress as a pregnant woman is expecting a child as well as decreased level of the vaginal defence mechanisms against Candida species, higher levels of placental estrogens, progesterone and corticosteroids in advanced pregnancy which encourage the growth of yeast cells^{14,21}.

In this study the distribution of Candida species according to gravidae revealed that Candida albicans was the commonest species isolated in multigravidae. The results of our study are consistent with research of Olanriyan et al¹⁹ This high prevalence among the multigravidae may be due to the use of contraceptive and antibiotics²².

LIMITATIONS

The limitations of the study are small sample size because of high cost of the study and study was conducted in single center only.

CONCLUSIONS

In conclusion, from this study it had been seen that Candida albicans had the highest occurrence. Diagnosis of vaginal Candida infections should be made only with laboratory confirmation. Presumptive identification followed by confirmation of yeast species helps to initiate early appropriate antifungal therapy thereby reducing the associated morbidity. Therefore routine medical examination, adequate antenatal services and appropriate treatment of the infected women are inevitable.

Culture of vaginal swab is highly recommended for pregnant women to ensure detection of vulvovaginal infection by Candida species. It is important for national health policy maker in Nepal to introduce health education programme in pregnant women to reduce candidal infection and to increase awareness. Awareness can also be increased through health worker at personnel visit of pregnant women during antenatal check-up. Further for identification of Candida species properly, newer laboratory techniques are required in developing country like Nepal.

REFERENCES

1. Uzoh CV, Iheukwumere IH, Umezurike KC, Onyewenjo SC. Prevalence of Candida albicans among women attending federal medical centre Asaba, Delta State, Nigeria. International Journal of Biochemistry and Biotechnology. 2017; 6(1): 735-39.
2. Okungbowa FI, Isikhuemhen OS, Dede APO. The distribution frequency of Candida species in the genitourinary tract among symptomatic individuals in Nigerian cities. Rev iberom micol. 2003; 20: 60-63.
3. Kelly RW, Illingworth P, Baldie G, Leask R, Brouwer S, Calder AA. Progesterone control of interleukin-8 production in endometrium and chorio-decidual cells underlines the role of the neutrophil in menstruation and parturition. Hum Reprod. 1994; 9(2): 253-58.
4. Wira CR, Sullivan DA. Estradiol and progesterone regulation of immunoglobulin A and G and secretory component in cervicovaginal secretions of the Rat. Biology of Reproduction. 1985; 32: 90-95.
5. Barousse MM, Espinosa T, Dunlap K, Fidel PL. Vaginal Epithelial Cell Anti-Candida albicans Activity Is Associated with Protection against Symptomatic Vaginal Candidiasis. Infection and immunity. 2005 Nov; 73(11): 7765-67.
6. Fidel PL, Cutright C, Steele C. Effects of reproductive hormones on

- experimental vaginal candidiasis. *Infection and immunity*. 2000 Feb;68(2): 651-57.
7. Eggimann P, Garbino J, Pittet D. Epidemiology of Candida species infections in critically ill non-immunosuppressed patients. *Lancet Infect Dis*. 2003; 3: 685–702.
 8. Oyewole OA, Okoliegbe IN, Alkhalil S, and Isah P. Prevalence of Vaginal Candidiasis among Pregnant Women Attending Federal University of Technology, Minna, Nigeria, Bosso Clinic. *Research Journal of Pharmaceutical, Biological and Chemical Sciences*. 2013;4(1):113-20.
 9. Cheesbrough M. *Medical laboratory manual for tropical countries*. 1st edition. Great Britain:University press; 1984. Chapter 34, Microscopical examination of specimens; p. 26-39.
 10. Chander J. *Text book of medical mycology*. 3rd edition. New Delhi (India): Mehata publishers;2009. Chapter 20, Candidiasis; p. 266-90.
 11. Kanagal DV, Vineeth VK, Kundapur R, Shetty H, Rajesh A. Prevalence of Vaginal Candidiasis in Pregnancy among Coastal South Indian Women. *J Womens Health*. 2014; 3(6):1-3.
 12. Feyi-Waboso PA, Amadi AN. The Prevalence and Pattern of Vaginal Candidiasis in Pregnancy in Aba. *Journal of Medical Investigation and Practice*.2001;2: 25-27.
 13. Oviasogie FE, Okungbowa FI. Candida species amongst pregnant women in Benin city, Nigeria: effect of predisposing factors. *African journal of clinical and experimental microbiology*. 2009 May; 10(2):92-98.
 14. Nelson M, Wanjiru W, Margaret MW. 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*. 2013;3: 264-72.
 15. Oyewole OA, Okoliegbe IN, Alkhalil S, and Isah P. Prevalence of Vaginal Candidiasis among Pregnant Women Attending Federal University of Technology, Minna, Nigeria, Bosso Clinic. *Research Journal of Pharmaceutical, Biological and Chemical Sciences*. 2013;4(1):113-20.
 16. Donbraye-Emmanuel OOB, Donbraye E, Okonko IO, Alli JA, Ojezele MO, Nwanze JC. Detection and prevalence of Candida among pregnant women in Ibadan, Nigeria. *World Applied Sciences Journal*. 2010; 10 (9): 986-91.
 17. Shrestha S, Tuladhar NR, Basnyat S, Acharya GP, Shrestha P, P Kumar., Prevalence of vaginitis among pregnant women attending Paropakar Maternity and Women's Hospital, Thapathali, Kathmandu, Nepal. *Nepal Med Coll J*. 2011; 13(4): 293-96.
 18. Dias LB, Melhem MSC, Szesz MW, Filho JM, Hahn RC. Vulvovaginal candidiasis in Mato Grosso, Brazil: pregnancy status, causative species and drugs tests. *Brazilian Journal of Microbiology*. 2011;42:1300-07.
 19. Olaniran O, Adefusi OF, Idowu OJ, Oladipo OA, Afolayan DO, Aderibigbe IA et al. Isolation and evaluation Candida species of among pregnant women in obafemi awolowo university teaching hospital, ile-ife. Nigeria. *Clinical Microbiology & Case Reports*. 2015;1(1):1-6.
 20. Samuel O, Ifeanyi O, Ugochukwu O. Prevalence of Candida Species among Vaginitis Symptomatic Pregnant Women Attending Ante-natal Clinic of Anambra State University Teaching Hospital, Awka, Nigeria. *Bioengineering and Bioscience*.2015; 3(2): 23-27.
 21. Lisiak M, Klyszejko C, Pierzchalo T, Marcinkowski Z. Vaginal candidiasis: frequency of occurrence and risk factors. *Ginekol Pol* 2000;71:964-70.
 22. Al-Aali KY. Prevalence of Vaginal Candidiasis among Pregnant Women Attending Al-Hada Military Hospital, Western Region, Taif, Saudi Arabia. *International Journal of Science and Research (IJSR)*. 2015 May; 4(5):1736-43.