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Isolation and evaluation candida species of among pregnant women in obafemi awolowo university teaching hospital, ile-ife. Nigeria

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Abstract

Background: Pregnant women are more susceptible to both vaginal colonization and infection by yeast. Untreated Candida infection can lead to very serious health and medical problems and is worsened while pregnant.

Objective: This study aimed at isolating and evaluating the carriage rates of Candida species among pregnant women in Ile-Ife southwestern Nigeria.

Methods: The High vaginal swabs collected were inoculated onto Sabroud dextrose agar (SDA) and incubated aerobically at 37°C for 24hrs. Candida species were checked for on the SDA medium. Germ-tube test was performed on the yeast isolates to confirm *Candida albican*, the morphologic and biochemical characteristics of other Candida species were also carried.

Results: Ninety-nine antenatal clinic attendees at Obafemi Awolowo University Teaching Hospital were recruited for this study. Out of the 99 with mean age of 30.17 women recruited, 49.5% with mean age of 30.59 were positive for *Candida albican* infection. Other species implicated are *C. pseudotropicalis 9(9.1%), C. krusei 15(15.2%), C.parapsolosis 1(1.0%), C. stellatoides 4(4.0%), C. tropicalis 8(8.1%),* and unknown 13(13.1%). The age range 30-39years had the highest prevalence of *C.albican 30(30.3%) and C.krusei 10(10.1%)* but *C.tropicalis 5(5, 1%), C.stellatoides 3(3.0%) C. pseudotropicalis 5(5.1%)* were prevalent in age range 20-29years. Multigravid had the highest the highest rate of *C.albican* of 14(50.0%) whereas, parity range 1-2 within multigravid had highest occurrence of *C*. albican in age range 30-39years with 24(33.8%). Zero (0) parity within primigravid had *C.albican* with 23(31.9%).

Conclusion: The percentage of *Candida albican* among 99 infected pregnant women was 49.5% emphasizing the importance of routine screening of pregnant women thereby assisting in prevention of invasive neonatal candidal infection. Thorough medical examination and culture of HVS is highly recommended for pregnant women to ensure detection of vulvovaginal infection by candida species among the immunosupressed person. Appropriate health and proper and well coordinated sex education is needed to reduce candidal infection.

Keywords: Isolation and evaluation, Candida species, Pregnant women, Nigeria

Introduction

The adverse effect of infectious diseases in many developing countries, in particular in sub-Saharan Africa is considerable and within those countries economically disadvantaged diseases and least likely to access appropriate treatment [1]. Candida is the fourth most common cause of nosocomial bloodstream infection in the United States [2]. Approximately three-quarters of all women experience at least one episode of vulvovaginal candidiasis during their lifetime nearly half of them suffer from multiple episode [3]. Vaginal candidiasis is a vaginal mucosis infection caused by species of the genus Candida. It is one of the most common vaginal infections in women, in the fertile period, and also the most frequent and most important fungal disease of vaginal content. Women around the world get diagnosed of vaginal candidosis. It is estimated that 75% of women during the fertile period have at least one episode of vaginal candidosis. Approximately 40-50% of women have repeated infection. Less than 5% of adult female population receives repeated infection. Point- prevalence studies indicates that Candida species may be isolated from the genital tract of approximately 20% (range 10-55%) of asymptomatic healthy women in the child bearing age [2,4-6].

Vulvovaginal candidiasis is more serious and more dangerous during pregnant

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because the body changes due to conditions in the body (such as hormonal changes) and this can lead to the infection becoming chronic, or recurring. Previous use of antibiotics or the birth control pill can increase the chances of getting candidiasis during pregnancy (or at any other time) and hormones changes increase the risk of getting it while pregnant. Untreated Candida infection can lead to very serious health and medical problems and is worsened while pregnant. Bacterial such as lactobacillus acidophilus balance Candida and prevent yeast overgrowth and pathogenic infection. Conditions that disrupt the balance of normal vaginal flora include antibiotic use, oral contraceptive devices, high estrogen levels and immunocompromised states such as diabetes mellitus and HIV [7]. Another risk factor for vulvovaginal candidiasis may be intrauterine contraceptive device [8].

Symptoms classically are described as pruritus, vaginal irritation and dysuria. Thick, curd-like discharge is often present, but scant discharge may also characterize infection. Vaginal edema and erythematic are present on examination. Epidemiologically, Vaginal Candida infections are important as they may increase viral shedding in HIV- infected women [2].

Pregnancy brings yeast infections, often multiple ones per pregnancy. Candida and pregnancy often go hand in hand. Pregnancy is a very beautiful and natural thing but it often weakens the immune system of the mother making it easier for them to come down with other illnesses or disease. Some symptoms of Candida infection in pregnancy include; painful urination, pain during sex, enlarges or swollen vulva, white discharge from the vaginal and itching or discomfort in the vaginal area.

The Candida fungus is both normal flora and an invasive pathogen. The range of infection with *candida* species varies from a benign local mucosal membrane infection to disseminated disease. Severe disease is typically associated with an immunocompromised state including those vulnerable to iatrogenic pathogens in the intensive care unit or those with predisposing immunological conditions such as malignancy, organ dysfunction or immunosuppressive therapy. *Candida albican* is the most common pathogenic species identified. Other species that are commonly found include *C. glabrata, C. parapsilosis, C. tropicalis, and C. krusei* [7]. Non *Candida albicans* accounted for 70% of candidemia in a northern Indian pediatric intensive care unit. Other Candida species that have emerged are *C. parapsilosis and C. dubliniensis* [9] C. glabrata and C. krusei have been identified as the leading causes of candidemia in patients with malignancy of hematologic origin [10,11]. *C. parapsilosis* has been identified as the leading cause of candidemia secondary to medical instrumentation such as central venous catheters, prosthetic devices and nosocomial spread [12] *C. dubliniensis* has been identified in an immunocompromised patient with multifocal osteomyelitis in Germany [12] and in a patient with meningitis in Australia [13,14].

Neonatal invasive candidiasis occurs with an incidence inversely proportional to birth weight. Candida colonization is found in approximately 30% of infants weighing less than 1500 grams at birth weight. Sources of invasive infection in one study included blood (70%), urine (cerebrospinal fluid (10%) and peritoneal fluid (5%). *C. albican and C. parasilosis* are the most common 15%), species found in neonates [7,15]. Neonates can also develop candidemia even after cesarean delivery due to premature rupture of amniotic membranes [16]. Therefore the aim of this study therefore, was to evaluate the vaginal carriage rate of Candida species among pregnant women in Ile- Ife, Southwestern, Nigeria.

Materials and Methods

Study area

The study was conducted at the Obafemi Awolowo University Teaching Hospital Complex (OAUTHC), Ile-Ife, southwest of Nigeria. This is a 600 bedded hospital that serves as referral center to about 5 neighbouring states of the country.

Study population

A total of Ninety-nine (99) HIV infected pregnant women of different ages and socioeconomic status attending antenatal clinic of the OAUTHC Ile-Ife were enrolled in this study. The study was conducted between March and July 2010 by recruiting consecutive consenting women. Other relevant information of all participants such as Gestational age, Parity, Gravid e.t.c were obtained using proforma specially design for this purpose.

Specimen collection

Sample from High vaginal were collected using evapon sterile swab sticks under aseptic condition using a speculum with the help of gynecologist. The specimens were immediately transported to the Medical Microbiology and Parasitology laboratory O.A.U where they were seeded on appropriate culture medium.

Wet preparation, Culture isolation and identification

A sample of the exudates was transferred to a microscopic slide. A drop of sterile physiological saline was added and mixed. It was covered with a cover slip and examined under microscope using x10 and x40 objective lens. The vaginal swabs collected were inoculated onto Sabroud dextrose agar (SDA) and incubated aerobically at 37°C for 24hrs. Candida species were checked for on the SDA medium. Germ-tube test using serum inoculated with the isolated yeast and incubated for at least four hours was performed to confirm *Candida albican*. The morphologic and biochemical characteristics of other Candida species were also carried out using carbohydrate fermentation and assimilation tests such as Galactose, lactose, maltose, sucrose, e.t.c, according to Frankel and Reitman 1970. The ethical clearance was obtained from the hospital and oral informed consent was obtained from all participating patients and there are no competing interests. The results were analyzed using SPSS package (X^2 test).

Results

There were 99 infected pregnant women with mean age of 30.17 (± 4.68) used for this study, out of which 49 (49.5%) with mean age of 30.59 (± 4.27) showed positively *for Candida albican* infection. Other species of Candida implicated are *C. pseudotropicalis* 9(9.1%) with mean age of 29.00 (± 3.500), *C. Krusei* 15 (15.2%) with mean age of 30.87 (± 4.533), *C. parapsolosis* 1(1.0%),mean age of 36.00, *C. stellatoides* 4 (4.0%) with mean age of 26.00 (± 8.83), *C.tropicalis* 8 (8.1%) with mean age of 27.25 (± 5.00) and Unknown 13 (13.1%) with mean age of 30.17 (± 4.68) as shown on Table 1.

The age range 30-39years had the highest prevalence of *C. albican* infection with 30 (30.3%), mean age of 32.7 (± 2.58), *C. krusei* 10(10.1%) mean age of 33.70 (± 1.889) and Unknown 8 (8.1%) mean age of 33.25 (± 2.493) whereas, *C.tropicalis 5 (5.1%) mean age of 23.80 (± 1.924) , C. stellatoides 3(3.0%) mean age of 21.67 (± 2.082), C. pseudotropicalis 5(5.1%) mean age of 26.40 (± 1.673)* were more prevalent in age range 20-29 years as shown on Table 2.

Age range distribution in Primigravid and Multigravid showed that multigravid had the highest prevalence of *Candida albican* with age range 30-39years having 24 (33.8%) and age range 20-29 years had 10 (14.1%), followed by C. krusei 10(14.1%) in age range 30-39 years, then *C.pseudotropicalis* with 4(5.6%) in the age range 20-29 years. The total C.albican for multigravid is 35(49.3%) compare to that of the primigravid as shown on Table 3. In the distribution of *C. albican* in relation to gravid and parity, Zero (0) parity range within primigravid had the highest *C. albican* infection of 14 (50.0%) whereas, parity range 1-2 within multigravid had highest *C. albican* with 23 (31.9%) and *C. krusei* with 10 (20.4%). Generally, Multigravidae had the highest *C. albican* and *C. krusei* infection rates of 35 (48.6%) and 14(19.4%) respectively. On parity distribution as shown on

Organism isolated	N(%)	Mean Age	Mean Gestational age	Parity
Candida albican	49 (49.5)	30.59 ± 4.271	36.17 ± 1.555	0.98 ± 1.127
Candida pseudotropicalis	9 (9.1)	29.00 ± 3.500	36.33 ± 1.581	0.78 ± 0.833
Candida krusei	15(15.2)	30.87 ± 4.533	37.07 ± 1.280	1.67 ± 1.496
Candida parapsolosis	1 (1.0)	36.00 ± 0.000	38.00 ± 0.000	1.00 ± 0.000
Candida stellatoides	4 (4.0)	26.00 ± 8.832	37.75 ± 0.957	0.50 ± 1.000
Candida tropicalis	8 (8.1)	27.25 ± 5.007	37.00 ± 2.000	0.62 ± 0.744
Unknown	13 (13.1)	32.00 ± 4.472	37.23 ± 1.739	1.15 ± 1.214
Total	99 (100)	30.17 ± 4.682	36.88 ± 1.554	1.04 ± 1.160

Table 1: Of general characteristics the *Candida species* with Vulvo-vaginal candidosis.

Age(years)	N (%)	Mean Age	Mean Gestational Age	Parity
20-29years				
Candida albican	18 (18.2)	25.89 ± 1.811	36.50 ± 1.383	0.61 ± 1.243
Candida pseudotropicalis	5 (5.1)	26.40 ± 1.673	36.60 ± 2.074	0.60 ± 0.548
Candida krusei	5 (5.1)	25.20 ± 1.924	37.40 ± 1.517	0.80 ± 0.447
Candida stellatoides	3 (3.0)	21.67 ± 2.082	38.00 ± 1.000	0.00 ± 000
Candida tropicalis	5 (5.1)	23.80 ± 1.924	35.80 ± 0.837	0.80 ± 0.837
Unkown	4 (4.0)	27.25 ± 2.062	36.50 ± 2.082	0.50 ± 1.000
Total	40 (40.4)	25.42 ± 2.241	36.65 ± 1.511	0.60 ± 0.955
30-39years				
Candida albican	30(30.3)	32.77 ± 2.582	36.80 ± 1.669	1.13 ± 0.973
Candida pseudotropicalis	4 (4.0)	32.25 ± 1.893	36.00 ± 0.816	1.00 ± 1.155
Candida krusei	10 (10.1)	33.70 ± 1.889	36.90 ± 1.197	2.10 ± 1.663
Candida parapsilosis	1 (1.0)	36.00 ± 0.000	38.00 ± 0.000	1.00 ± 0.000
Candida stellatoides	1 (1.0)	39.00 ± 0.000	37.00±0.000	2.00±0.000
Candida tropicalis	3 (3.0)	33.00 ± 1.000	39.00 ± 1.732	0.33 ± 0.577
Unknown	8 (8.1)	33.25 ± 2.493	37.62 ± 1.685	1.50 ± 1.309
Total	57 (57.6)	33.14 ± 2.445	37.02 ± 1.598	1.32 ± 1.198
40-49years				
Candida albican	1 (1.0)	40.00 ± 0.000	38.00 ± 0.000	3.00 ± 0.000
Unkown	1 (1.0)	41.00 ± 0.000	37.00 ± 0.000	1.00 ± 0.000
Total	2 (2.0)	40.50 ± 0.707	37.50 ± 0.707	2.00 ± 1.414

Table 2: The Age distribution, Mean age, Gestational age and Parity of the study population.

GRAVID	AGE RANGE	C. albican	C. pseudotropicalis	C.krusei	C. parapsillosios	C. stellatoides
PRIMI	20-29yrs	8.0(28.6)	1.0(3.6)	2.0(7.1)	Nil	Nil
	30-39yrs	6(21.4)	2(7.1)	0.0(0.0)	Nil	Nil
	Total	14(50.0)	3(10.7)	2(7.1)	Nil	Nil
MULTI	20-29yrs	10(14.1)	4(5.6)	3(4.2)	0.0(0.0)	Nil
	30-39yrs	24(33.8)	2.0(2.8)	10(14.1)	1.0(1.4)	Nil
	40-49yrs	1.0(1.4)	0.0(0.0)	0.0(0.0)	0.0(0.0)	Nil
	Total	35(49.3)	6(8.5)	13(18.3)	1.0(1.4)	Nil

Table 3: The Age distribution of Candida species in relation to Gravid.

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Parity range	Candida species	Number	Mean Age (%)	Mean Gestational age(%)	Mean Parity
0	*C. albican*	22	27.95(22.2)	36.18(20.0)	0.00
	C. pseudotropicalis	4	28.00(4.0)	36.75(4.0)	0.00
	C. krusei	2	30.00(2.0)	38.00(2.0)	0.00
	C. stellatoides	3	21.67(3.0)	38.00(3.0)	0.00
	C. tropicalis	4	27.75(4.0)	38.00(4.0)	0.00
	Unknown	5	28.20(5.1)	36.40(5.0)	0.00
	Total	40	27.60(40.4)	63.68(40.0)	0.00
1-2	*C. albican*	23	32.17(23.2)	37.22(23.0)	1.48
	C. pseudotropicalis	5	29.80(5.1)	36.00(5.0)	1.40
	C. krusei	10	29.70(10.1)	37.27(10.0)	1.36
	C. parapsilosis	1	36.00(1.0)	38.00(1.0)	1.00
	C. stellatoides	1	39.00(1.0)	37.00(1.0)	2.00
	C. tropicalis	4	26.75(4.0)	36.00(4.0)	1.25
	Unknown	7	34.29(7.1)	37.57(7.0)	1.57
	Total	51	31.53(51.5)	37.08(52.0)	1.44
3-4	*C. albican*	3	35.00(3.0)	36.33(3.0)	3.00
	C. krusei	2	35.00(2.0)	37.00(2.0)	00.0
	Unknown	1	35.00(1.0)	39.00(1.0)	4.00
	Total	6	35.00(6.1)	37.00(6.0)	3.17
5-6	*C. albican*	1	29.00(1.0)	38.00(1.0)	5.00
	C. krusei	1	36.00(1.0)	38.00(1.0)	6.00
	Total	2	32.50(2.0)	38.00(2.0)	5.50

Table 4: Distribution of *Candida species* in relation to Parity.

Table 4, C. albican had the highest prevalence of 22.2% followed by C. pseudotropicalis 4(4.0%) and C. tropicalis 4(4.0%) in Zero range while in range 1-2, C.albican had 23(23.2%) followed by C. krusei 10(10.1%), the unknown was high in this range (Figures 1 and 2).

Discussion

Candida albican and other candida species had been isolated from clinical specimen from different parts of Nigeria [17] and different parts of the world [7,18].

This study was carried out to determine the vaginal carriage rate of Candida species among pregnant women in ile -Ife. In this
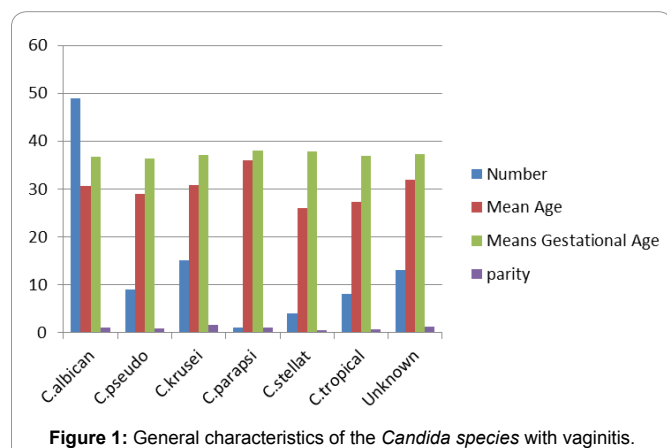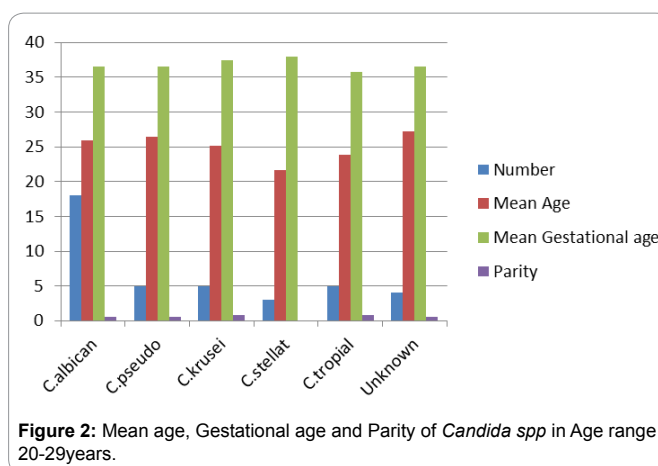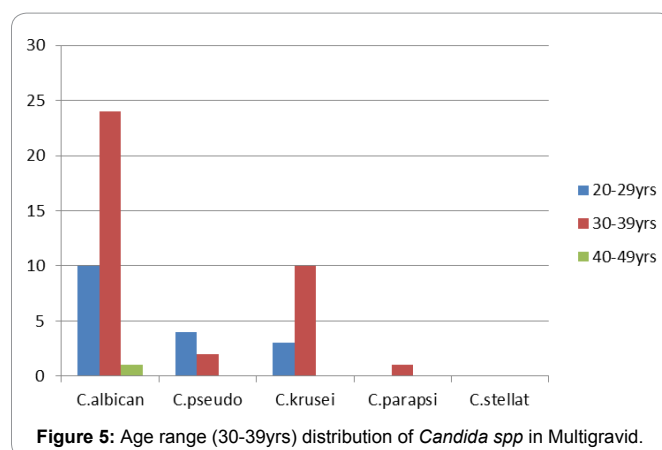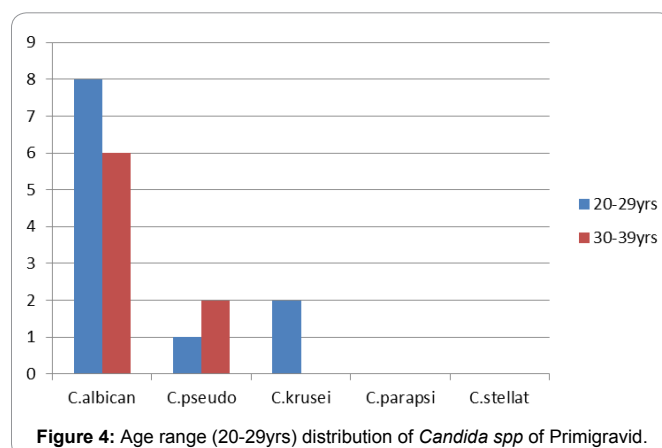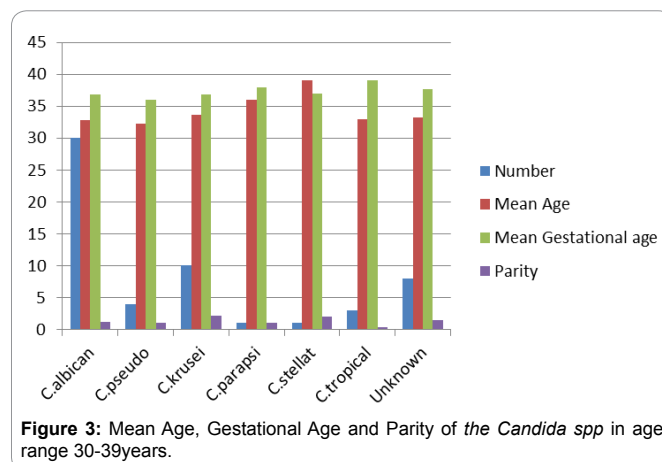

Figure 2: Mean age, Gestational age and Parity of *Candida spp* in Age range 20-29years.

study, overall prevalence of *Candida albican* was 49.8 per cent which is similar to the work done by *Mirela and Mirsada 2010* with the prevalence of *C. albican* to be 46.8% and consistent with candida colonization stands as a risk factor for preterm birth [5]. Similarly, the work done by *Donbraye et al., 2010 and Paul et al., 2000* also had the prevalence of *C. albican* infection to be 65.4% and 30.7% respectively. The variations could be due to geographic, ethnic, socioeconomic factors, differences in sampling, culturing techniques and sexual practice as well as environmental factor such as nutrition and hygiene [19,20] (Figures 3-5).

The isolation of non-candida albican species from the study was also documented by Mirela and Mirsada with *C. krusei*

Figure 1: General characteristics of the *Candida species* with vaginitis.

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Figure 3: Mean Age, Gestational Age and Parity of *the Candida spp* in age range 30-39years.

Figure 4: Age range (20-29yrs) distribution of *Candida spp* of Primigravid.

Figure 5: Age range (30-39yrs) distribution of *Candida spp* in Multigravid.

(3.2%), *C. parapsillosis* (3.2%) and Christine et al., 2011 with *C. parapsillosis* (3.1%), *C. krusei* (1.0%), *C. tropicalis* (1.0%),* these species has been identified as the leading causes of Candidemia in patients with Malignancy of haematologic origin [10]. C. parapsillosis has been identify as the leading cause of Candidemia secondary to medical instrumentation such as central venous catheters, prosthetic devices and nosocomial spread [21]. The high prevalence of Candida albican in age range 30-39 years in this study is in contrast to the work done by *Akinbiyi et al., 2008* with the prevalence of *C. albican* to be 65.7% in the age range

of 21-30 years. Although age range 20-32years had more of non-*candida albican* species and there is an increasing shift toward infections caused by these species with 40-60% of them currently being reported [22].

The high prevalence of *C. albican* infection in the primigravid with Zero parity could be as a result of pregnancy that have not been able to result into the birth of an infant capable survival and invariably, this high rate could be the result of the birth that could not be able to survive or could cause still birth but the high rate in multigravid within the parity range 1-2 could be as a result of the many experiences of pregnancies. In conclusion, from this study, it could be seen that *C. albican* had the highest prevalence followed by *C. krusei* and then others. Therefore, the need for appropriate health education to reduce candidal infection is imperative and essential because most of the subjects used is this study during our contact still don't understand how and when to prevent this infection, some believe that the infection during pregnancy is normal despite the inconveniencies. Proper and well coordinated sex education should be organized for adolescents and pregnant women to prevent the infections. A screening protocol could be readily incorporated into routine antenatal care and treatment should be available which will reduce preterm birth from candidiasis.

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