

PREVALENCE OF VAGINAL CANDIDIASIS IN WOMEN OF
REPRODUCTIVE AGE IN EKPOMA-NIGERIA

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ABSTRACT

To determine the prevalence of vaginal candidiasis in relation to age group douching, use of oral contraceptives and effects of the use of antibiotics, 300 women of reproductive age attending clinic in a primary health care centre were sampled. High vaginal swabs were collected with the aid of sterile swab sticks between the hours of 9.00am – 12noon on presentation at the health care centres. The samples were inoculated on Sabouraud Dextrose agar (SDA), wet preparation was done to examine for yeast cells microscopically. The germ tube test (GTT) was done to differentiate *Candida albicans* from other *Candida species*. Overall candidiasis infection rate was 105(35%). Infection was found to be lower in females ≤ 20 years. 45 (43%) than those within the age group 21-30 years 60

(57%) $P > 0.05$ which was statistically not significant. *Candida albicans* accounted for 60 (57%) of the positive isolates compared to *C. tropicalis* 25(24%) and *C. krusei* 20(19%). Germ tube test gave 55 (52%) positive isolates against the more sensitive CHROM agar of 60 (57%). Antimycotic sensitivity test revealed that *Candida* species were more sensitive to Nystatin (100%), Ketoconazole (97.1%), Miconazole(92.4%), others were Griseofulvin (84.8%) and clotrimazole (71.4%).

KEY WORDS: Vagina, candidiasis, women, Ekpoma-Nigeria.

INTRODUCTION

Vaginal candidiasis is a fungal infection caused by an organism called *Candida albicans*. Recent studies have shown that other *Candida* species are

becoming medically important because they are also frequently identified as an agent of vaginal candidiasis (Riley, 1986).

However, some factors or conditions may result in an overgrowth of these fungi and result in vaginal candidiasis. These factors include; diabetes mellitus, steroids, high dose of oestrogen, pregnancy hormones and prolonged use of contraceptives (Fidel and Sobel, 1996). Women with weakened immune systems such as those with HIV/AIDS or those taking steroid medications or chemotherapy, will all suppress the immune systems (Jindal *et al.*, 2007). Most women experience vaginal candida infection at some point in their lifetimes (Mabey *et al.*, 1996), about 75% of cases occur during the reproductive years (Mitchell, 2004). This is mostly due to factors such as pH changes which occur as a result of hormonal fluctuations, antibiotics usage, or prolonged use of oral contraceptive pills.

Conventional methods of contraception include the pill, condom and diaphragm but the most effective and widely accepted amongst young adults is the oral contraceptive pill which is also a steroid contraceptive, a combination of oestrogen and progestogen (Pumpianski and Ganor, 2007). The mechanism of action of the steroids involve the lowering of tissue level of pyridoxine, folic acid, ascorbic acid and zinc in the vagina, in addition to increasing cervical mucus and this results in bacterial changes. This changes favour the growth and proliferation of *Candida*. (Pumpianski and Ganor, 2007).

MATERIALS AND METHOD

Area of study

The study was carried out in Ekpoma Edo State-Nigeria. Edo State

lies between longitudes 05^o 4E and 06^o 43E and latitudes 05^o 44N and 07^o 34N. It shares boundary on the North with Kogi State. Ekpoma is located on latitude 06^o 45N, longitude 05^o 6E, the climate is characterized by two distinct seasons; the wet and dry seasons. The wet season (rainy) season occurs between April and October with a break in August and an average rainfall ranging from 150cm in the extreme North to 250cm in the South. The dry (harmattan) season lasts from November to April with a cold harmattan between December and January. Temperature average is about 25^oc. Ekpoma is the administrative headquarters of Esan West Local Government Area, with a projected population of 1,43778 and women of reproductive age is estimated to be 31,630. It has one general hospital, and eighteen primary health care centres.

Ethical consideration: Approval was obtained from the ethical committee of the Local Government Area for the study to be carried out in the primary healthcare centres. Informed consent was also obtained from the patients before questionnaires were administered. Clients presenting for the first time at the health facility without prior administration of antibiotics were included in the study while diabetic mellitus patients and pregnant women were excluded.

SAMPLE SELECTION AND COLLECTION

Women between the ages of 15 and 30 years were selected for the study while ages 31-40 years were not included due to the low turnout of women within that age group. Samples were collected from subjects between December 2010 and February 2011. Questionnaires were also administered. The formula of Dean *et al.* 1995 was used for sample size

selection. A total of 300 women living in Ekpoma and its environs who were apparently healthy individuals were chosen for the study. Samples were collected from the subjects with the aid of a sterile swab stick, and were taken immediately to the diagnostic laboratory for analysis.

Statistical analysis was done using the student's T-test to calculate probabilities and determine significance. A p-value of less than or equal to 0.05 was considered to be statistically significant ($p \leq 0.05$).

SAMPLE ANALYSIS

The samples were inoculated on Sabourand Dextrose Agar (SDA) and it was also used for wet preparation to examine for yeast cells. The sample was streaked out on (SDA) to obtain discrete colonies by incubating the plates aerobically at 37°C where they grow as white or cream coloured round colonies with stale odour, measuring about 2.5mm by 4mm in diameter. The colonies were then sub-cultured on CHROMagar plates and incubated at 37°C for 24hrs.

On CHROMagar, the colonies appear as lilac, light green or purple to differentiate between the *Candida* species.

Wet smear was made on a clean grease free slide by mixing the samples in a drop of normal saline, and examined microscopically. Wet preparation showed budding yeast, pus cells and epithelial cells.

The Germ Tube Test was carried out to differentiate *Candida albicans* from other *Candida* species. The test was carried out by emulsifying a colony of yeast cell into about 0.5mls of serum in a test tube. Incubation was at 37°C for 2hrs. a sterile pipette was used to transfer the mixture onto a clean grease free slide where it was examined microscopically at X10 and X40

objectives. Indirect Gram staining was done by using colonies growing on the (SDA) plates. A small drop of normal saline was placed on a clean grease free slide and then a discrete colony was touched slightly with a sterile wire loop and emulsified in the normal saline. A smear was made on the slide so that newsprint can be seen through it and it was allowed to air dry. It was heat fixed by passing through the pilot flame of the Bunsen burner and allowed to cool, gram positive slides showed large, purple, oval yeast cells.

RESULTS

Three hundred subjects were used for this study and they were all women within their reproductive age. Out of the 300 subjects, 105 (35%) showed positive growth of *Candida* species, while 195(65%) showed negative growth.

Table 1 shows the prevalence of vaginal candidiasis among women of different age groups. The prevalence of vaginal candidiasis among women within the age group ≤ 20 years is lower (30%) compared to age group 21-30 years (40%), and it is statistically not significant. The characterization of *Candida* species using the Germ tube test (GTT) and their distribution among the age groups shows that age group ≤ 20 years has a lower frequency of *Candida albicans* (GTT positive) of 22 (40%) than age group 21-30 years which has 33(60%) as shown in Table 2.

Table 3 shows the distribution of different *Candida* species isolated with CHROMagar, *C. albicans* had the highest occurrence rate of 60 (57%) followed by *C. tropicalis* 25 (24%) and *C. krusei* 20 (19%).

The incidence of vaginal candidiasis and various pre-disposing factors revealed that wearing of light clothing under wears was highest 47

(40%) followed by douching 36 (34%) and sexual activities 11 (10.5%) the least was oral contraceptives 7 (7%). Statistically oral contraceptive ($X^2 = 0.14$, $P > 0.05$) and antibiotics ($X^2 = 1.212$, $P > 0.05$) were not significant, while tight nylon under wear ($X^2 = 4.800$, $P < 0.05$) and douching ($X^2 = 5.958$, $P < 0.05$) was significant as shown in Table 4.

Table 5 shows the antimycotic pattern of drugs used, with the isolates showing a 100% sensitivity to Nystatin, followed by ketoconazole 97.1%, Miconazole 92.4% while the least was Clotrimazole 71.4%. the table also showed the resistant pattern of the isolates to the antimycotic drugs.

Table 1: The prevalence of vaginal candidiasis among women of different age group.

Age group	Positive	Negative	Total(n)
≤20 years	45(30%)	105(70%)	150(50%)
21-30 years	60(40%)	90(60%)	150(50%)
Total	105(35%)	195(65%)	300(100%)

$X^2 = 0.833$

$P. > 0.05$

Table 2: Characterization of *Candida* species using germ tube test and age group distribution

Age group	No. of	GTT Positive	GTT Negative	Total
years	Subjects	Presumed <i>C.</i>	Presumed Non.	Isolates (%)
		<i>albicans</i> (%)	<i>C. albicans</i>	
≤20 years	150	22(40%)	23(46%)	45(43%)
21-30 years	150	33(60%)	27(54%)	60(57%)
Total	300	55(52%)	50(48%)	105(100%)

GTT: Germ Tube Test. No. Number

Table 3: Distribution of different *Candida* species isolated with CHROMagar

Age group (years)	No. of subjects	CHROMagar			
		<i>C. albicans</i>	<i>C. tropicalis</i>	<i>C. krusei</i>	Total(%)
≤20 years	150	25(42%)	10(40%)	10(50%)	45(43%)
21-30 years	150	35(58%)	15(60%)	10(50%)	60(57%)
Total	300	60(57%)	25(24%)	20(19%)	105(100%)

Table 4: Distribution of vaginal candidiasis and predisposing factors

Predisposing Factors	Vaginal	Candidiasis	Total
	Positive		Negative
Oral contraceptives	7(67%)	24(12.3%)	31(10.3%)
Antibiotic use	9(8.6%)	32(16.4%)	41(13.7%)
Wearing of tight Clothing	42(40%)	63(32.3%)	105(35%)
Sexual activities	11(10.5%)	28(14.4%)	39(13%)
Douching	3.6(34%)	48(24.6%)	84(28%)
Total	105(35%)	195(65%)	300(100%)

DISCUSSION

A total of three hundred women of child bearing age were examined, and the prevalence of vaginal candidiasis was found to be 35% as compared to the women who were negative 65%. These women did not know whether or not they had candidiasis as at the period of this study.

The relationship between recent antibiotic use and vaginal candidiasis is not statistically significant in this study which is in contrast with the findings of

Xu *et al.*, (2008) who reported increased prevalence of *Candida* colonization of the vagina after a short course of oral antibiotics.

C. albicans was the highest occurring *Candida* species isolated. This is in consonance with the work of Enweani *et al.*, (1987) who reported *C. albicans* as the most frequently isolated *Candida* species from their work on vaginal infection amongst sexually active women in parts of Edo State . Oral contraceptive was not related to the growth of *Candida* species, which

was also reported by Schmidt *et al.*, (1997). Douching was also associated with the prevalence of vaginal candidiasis. It is in line with the study of Heng *et al.*, (2010) which stated that douching was significantly associated with vaginal candidiasis among subjects sampled. CHROMagar gave a 60% isolation rate for *C. albicans* (light green), *C. tropicalis*, was 25% (purple) and *C. Krusei*, was 20% (lilac) Odds and Berneats (1994).

Results of the antimycotic sensitivity patterns showed that Nystatin, ketoconazole and miconazole are quite effective in the treatment of candidiasis, though resistance among some isolates was observed with ketoconazole and miconazole. There was a 100% sensitivity of the all fungal isolates to Nystatin which is one of the oldest antifungal agent. This is in line with the work of Owhe-Ureghe, *et al.*, (2000).

CONCLUSION

Vaginal candidiasis caused by *C. albicans* as well as non-albicans species of *Candida* is quite prevalent in women of child bearing age of Edo State. Even though some of the subjects appeared healthy, they were positive for candidiasis. This emphasizes the fact that vaginal candidiasis could be asymptomatic, while CHROMagar appears to be very rapid and 99% accurate for distinguishing *Candida* species.

Nystatin, a polyene, could be used effectively for the treatment of vaginal candidiasis especially in any environment where there are no laboratories for proper diagnosis.

Public enlightenment/awareness should be created among subjects on the dangers of vaginal candidiasis.

indiscriminate use of antibiotics and the need to step up personal hygiene in order to keep the infection under check.

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