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Isolation of *Candida albicans* from Urine Sample of Women in some Hospitals in Idah Metropolis with Vaginosis

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ABSTRACT

Candidiasis is an infection caused by *Candida albicans* the organism normally lives in the body and they exist as saprophytes. Five hundred (500) urine samples were obtained from the General Hospital Idah, Udoma Hospital and Sharon Clinic Idah. Analysis was carried out using urine microscopy and urine culture. After analysis, 363 were positive to *C. albicans*. Among the age groups, 18-24 years age group record the highest prevalence with 47% and 39-45 years group record 5.6% being the least. from the analysis carried out shows that *C. albican* produces soft/smooth cream colour colony after incubating for 24hours at a room temperature of 37°C. The high prevalence rate among the age group 18-24 years could be due to some predisposing factors such as wearing of tight leggings, use of synthetic under wears, tight jeans, hormonal or physiological disturbance and stress. Minimizing these factors could help in reducing the incidence of Candidiasis, so women are advised to visit hospital for medical advice any time there is a strange feeling or symptoms in their body.

Keywords:Candidiasis, *Candida albicans*, infection, prevalence

INTRODUCTION

Candidiasis is an infection caused by *Candida albicans* [1]. The organism normally lives in the body. The part of the body where the organism lives is what the infection is name. there are different types of *candidiasis*. The various forms of *candidiasis* include: oral thrush, *oesophageal* thrush, cutaneous (skin) candidiasis, vaginal *Candidiasis*, *balanitis* and systematic *candidiasis* [2]. Candida can effect different parts of the body causing either infections or overwhelming illness, depending on the individual general state of health [3]. *Candida albicans* is a wide spread organism with worldwide distribution. It is normally found in small amount in the vaginal, in the mouth, the disease or symptoms (approximately 25% of women) without disease symptoms have this organism present [4]. The various forms of *candidiasis* are often included among the sexually transmitted infections because they may occur with other type of reproductive system infection [5]. Candida species are yeast which exist as saprophytes. They are also unicellular fungi that can live in human under certain condition. Such infection are invariable secondary to some pre-existing disease such as malignancy or diabetes or to a defective immune system [6]. Infection is common among pregnant women who use oestrogen drugs. This is due to the increase level of oestrogen in the body. The increase in hormone level causes changes in the vaginal environment that makes it suitable for fungal growth and nourishment [7].
Symptoms appear when the balance between the normal microorganisms of the vaginal is lost and the C. albican population becomes large in relation to other microorganism population. This happens when the environment (the vaginal) has certain favourable conditions that allow growth and nourishment of *C. albicans*. Yeast infection may

follow a cause of antibiotics (particularly tetracycline) that were prescribed for another purpose. The antibiotic changes the normal "balance" between the organisms in the vaginal by suppressing the growth of protective bacteria that normally have antifungal effect. This project isolated and characterized *C. albicans* from urine sample from women in some hospitals in Idah metropolis presenting with vaginosis.

Materials and Methods

Study Area

The sample was collected from some hospitals in Idah metropolis presenting with vaginosis and analyzed in the microbiology laboratory of fedral polytechnic idah.

Study Design/Scope

Idah is a town in Kogi State, Nigeria on the eastern bank of the Niger River in the middle belt region of Nigeria. It is the headquarter of the Igala kingdom, and also a smaller local government area with an area of 36km^2 around the town, with a population of 79,815 at the 2006 census. The town is the traditional capital of the Igala by name HRN Michael Ameh Oboni II. The town is a major food supplier of Kogi State. It has commercial routes of the River Niger linking to Lokoja, the Kogi State capital, to the North of the country and Onitsha in Anambra State to the south, Agenebode Edo State across to the Niger to the west. It is population is primarily Igala. Idah is located in latitude 7^{o}N and 8^{o}N and longitude 6^{o}E and 7^{o}E the major occupation of this people are; farming, fishing and trading.

Sample Collection

For the purpose of this work, 500 urine samples were collected from women in some hospitals in Idah metropolis presenting with vaginosis. Using a sterile urine container and was transferred to the Microbiology Laboratory of Department of Science Laboratory Technology, Federal Polytechnic, Idah for microbiological essay for isolation and identification of *Candida albicans.*

Diagnostic Examination of Urine Sample

Medical Professionals use two Primary method to diagnosed yeast infections [8]; [9].

Microscopic examination and culturing

a. Microscopic Examination
Tissue biopsies centrifuged spinal fluid and other specimens may be examined in Gram stained smears for pseudohyphae and budding cells.

b. Culture
S All specimens are cultured on fungal or bacteriological media (sabouraund dextrose agar). at room temperature of about 37^{0}C. Yeast colonies are examined for the presence of Pseudohyphaes *C. albican* is identified by the protection of germ tubes or chlamydosphore. Other Candida isolates are speciated with a battery of biochemical reactions.

Isolation of *Candida albicans*

The organism was isolated by growing on a sabouraund dextrose salt agar medium after which they were observed carefully for colony, morphology with respect to colour, shape, size, nature of colony and pigmentation. The isolates were Gram stained and observed under the light microscope. Yeast colonies are examined for the presence of pseudohyphea. *C. albicans* is identified by the production of germ tubes or chlamydosphore. Other candida isolates are C. Africana, *C. tropicalis C. dublinensis, C. krusei, C. glabrata, C. lusitaniae* and *C. parapsilosis.*

Biochemical Characterization

Identification of Isolates

The isolate were identified using biochemical characteristics they are: catalase, coagulase, urease, citrase, oxidase and indole test. *Candida albicans* after the test was observed to be catalase positive, coaglase positive, urease positive, citrase positive, oxidase negative and indole negative [10].

Catalase Test

A drop of hydrogen peroxide (H_2O_2) was placed on a clean slide and emulsified with suspected colony of *Candida albicans* from the medium with a sterile wire loop for each Petri dish. It was showed to be foaming with effervescent gas coming out. Showing that it is catalase positive.

Coagulase Test

A colony of suspected isolated of *Candida albicans* was placed on a clean slide and a drop of normal saline and rabbit plasma were added and emulsified with sterile wire loop for each Petri dish and they were all rocked for 5 minutes and were observed for coagulation.

Urease Test

A colony from the pure culture was suspended which is to be investigated in MR/VP medium. It was incubated at a temperature of about 35^{0}C in 18 to 24 hour. 0.2mL of 40%koH was added and 0.6mL of phenol red (indicator) and was added and the colour changes from it's original orange yellow colour to bright pink, showing the presence of *Candida albicans*

Citrate Test

A tube containing citrate medium was inoculated with a small amount of the suspected *Candida albicans*. It was incubated at 30-37^{0}C during 24-48 hours growth in the citrate medium. The growth with colour change to blue in Simmon's citrate tube shows the presence of *Candida albicans*.

Oxidase Test

A drop of freshly prepared one percent solution of oxidase reagent was put on a piece of filter paper and a sterile loop was used to pick a test colony and rubbed it on the paper in the area impregnated with oxidase reagent. There was no colour change showing that it is oxidase negative.

Indole Test

The test organism was inoculated into a peptone water broth and was incubated at the temperature of 37^{0}C for 48-96 hour. 0.5m of kovac's reagent was added to it and was shaked gently. There was no colour change even after the addition of appropriate reagent showing that *Candida albicans* is indole negative.

Gram staining was carried out [11]; [12]

- A drop of normal saline was placed on clean slide and a colony of *Candida albicans* from the isolated one was emulsified on it for each Petri dish.
- They were all heat fixed by passing them through Bunsen flame three times.
- The slide were all flooded with crystal violet for 1-2 minutes and washed with tap water.
- The slide were also flooded with lugols iodine, mordant for 1 minute.
- They were decolourized with 50% of acetone or alcohol.
- The slides were rinsed with tap water.
- All specimen was counter stain with safranin for 3 minute.
- Rinsed with tap water and allowed to be air dry.
- A drop of immersion oil was placed on each of the slide and
- They were all viewed under the light microscope using x 100 oil immersion objective lens. *Candida albican* appear purple showing that it is gram positive.

Antibiotic Sensitivity Testing

Disc Diffusion Method

Because of convenient, efficiency and cost, the disc diffusion method is probably the most widely used method for determining antimicrobial resistance in private veterinary clinic [13]. A growth medium, (Muller Hinton agar) was evenly seeded throughout the plate with the isolate of interests that has been diluted at a standard concentration (approximately 1 to 2 x 10^8 colony forming unit per ml) then a commercially prepared discs, each of which are pre-impregnated with a standard concentration of particular antibiotics (Nystatin) are then evenly dispended and lightly pressed onto the agar surface. The test antibiotic immediately begins to diffuse outward from the discs, creating a gradient of antibiotic concentration in the agar such that the highest concentration is found close to the disc with decreasing concentration further away the disc. After an overnight incubation for 24 hour, the fungi growth around each disc was observed if the test isolate is susceptible to a particular antibiotic, a clear area of "no growth" will be observed around that particular disc. The zone around an antibiotic disc that has no growth is referred to as zone of inhibition since this approximates the minimum antibiotic concentration sufficient to prevent growth of the test isolate. This zone is then measure in mm and compared to a standard interpretation chart.

RESULTS

Candida albicans were isolated for 363 urine samples, the colony appeared as creamy colonies with characteristic smell on sabouraund dextrose salt agar table1 and on urine microscopy it appeared as bud of yeast. There is high prevalence in 18-24 age group with 47% and the least (5.6%) in age 39-45 years (table1).

Table 1: Occurrence of Candida albican among Female of Various Age group in Idah metropolis.

Age Groups (Years)	Numbers of Urine Screened	Number of + ve	Percentage of Occurrence (%)
18-24	200	180	47
25-31	130	100	30
32-38	120	65	17.4
39-45	50	18	5.6
Total	**500**	***363***	**100**

Biochemical Characterization of *Candida albicans* isolated from Women attending some Hospitals in Idah Metropolis

The suspected *Candida albicans* was found to be catalase, coagulase, urease positive and citrate positive, but oxidase and indole negative (table 2).

Table 2: Biochemical Characterization of *Candida albicans* Isolated from Women attending some Hospitals in Idah Metropolis

S/N	Test Type	Result
1.	Catalase	Positive (+)
2.	Coagulase	Positive (+)
3.	Urease	Positive (+)
4.	Citrate	Positive (+)
5	Oxidase	Negative (-)
6.	Indole	Negative (-)

Total Plate Count of Fungi on Urine Sample

From table 3 the practical test carried out sample code plate 10^{-6} tend to have more growth than all the sample code plate recorded as 10^{-3} to 10^{-4} and 10^{-5}. Sample code plate 10^{-3} has total plate count after incubating it for 24 hour at the room temperature of 37^0C as $8x10^4$, sample code plates 10^{-4} has total plate count as $5x10^6$, sample code plate 10^{-5} has total plate count of $1x10^6$ and sample code plate 10^{-6} has the highest total plate count of $3x10^9$. After it was calculated using

$$\frac{\text{average colony x reciprocal dilution (CFU)ml}}{\text{Inoculums size}}$$

Table 3: Total Plate Count of Fungi on Urine Sample

Distribution	Total Plate (cfu)ml Count (dilution)	Log_{10} (cfu)ml
Plate 10-3	8×10^4	4.90
Plate 10-4	5×10^6	5.69
Plate 10-5	1×10^6	7.00
Plate 10-6	3×10^9	8.48
Mean x $\Rightarrow$	77645,000	6.5175

Figure of culture Plate

DISCUSSION

From table 1 above Finding from this study shows the occurrence of *Candida albicans* in urine culture and urine micp=roscopy according for 47% among age group 18 – 24 years however, there have been reports of increasing incidence of urinary yeast infection over the past decade [14]. Reported *C. albican* to be the most frequent isolates. *C. albican* and *C. stellatoidea* are commonly associated in the vaginal flora. It is observed from the table that the highest prevalence is among females of the age group 18-24 years (47%) followed by 25-31 years (30%), the least being 39-45 years (5.6%). Form the result there is a high prevalence of Candida among age group 18-24 years with 47% because of the external use of irritants (such as detergent or douches) or internal disturbance (hormonal or physiological), which can perturb the normal flora. It is also prevalence among this age group if they are under stress. Have an inadequate diet, have lack of sleep or ill. Certain types of clothing may predispose for Candida in this age group such as wearing tight fitting clothes and synthetic under wear. Base on this people under this age group are engaged in this activities thus, there is a high prevalence among this age group [15]. The age group 25-31 years is the next on the table with 30%. This may be due to pregnancy, use of oral contraceptive diabetes and the use of antibiotics. The high prevalence of candidiasis in pregnant women is not surprising because of the elevation of oestrogen and progesterone, which contribute to high incidence of Candida organism. The link with oestrogen is perhaps more. Obscure, however it has been demonstrated that most cases of yeast related vaginitis occur in women with oestrogenized vaginal [16]. Antibiotic therapy is another predisposing factor to incidence of *Candida albicans* in urine has being investigated based on medical report however, anyone who has been on long-time antibiotics therapy or has been taking antibiotic often probably has a local acidity or secretion yeast infection often develop where a moist, warm environment encourage fungal growth. Prime areas include the webs of finger and toes, nails, genitals and folds of skin. This is particularly the case of diabetes.

The age group 32-38 years and 39-45 years have small values because they are not exposed to the use of vaginal douches and they do not experience any physiological changes in their body. People at this age group hardly wear tight fitted clothes and synthetic under wears compare to age group 18-24 years thus the prevalence of the infection is low in this age group.

The isolate were identified using biochemical characteristics they are: catalase, coagulase, urease, citrase, oxidase and indole test. *Candida albicans* after the test was observed to be catalase positive, coaglase positive, urease positive, citrase positive, oxidase negative and indole negative [17] (table 2). Findings from this study shows that *Candida albican* produces a soft/smooth cream colour colony after incubating for 24 hours at a room temperature of 37^0C and when it was sub-cultured the same colony was produce and there is no growth in both control of culture and the sub-culture as well (table 3).

CONCLUSION

From the result of this study; there is high prevalence in age-group 18-24 yeast (47%) while age group 39-45 years have the least (5.6%). The most important preventive measure is to avoid distributing the normal balance of the microbial flora and host defense. Candidiasis is not communicable, since virtually all persons normally harbours the organism. Women are advised to visit hospital for medical advice anytime there is a strange feeling or symptoms in their body. Limit wearing of tight leggings, nylon underwear and tight jeans. Discouraging the use of deodorant spray especially when having feeling or signs of infection. Women are advised to wear loose, natural fibre clothing and underwear with a cotton crotch.

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APPENDIX

FIGURE 1 : ANTIBIOTIC SENSITIVITY TESTING OF CANDIDIASIS

FIGURE 2 : Gram Staining of *Candida albican*

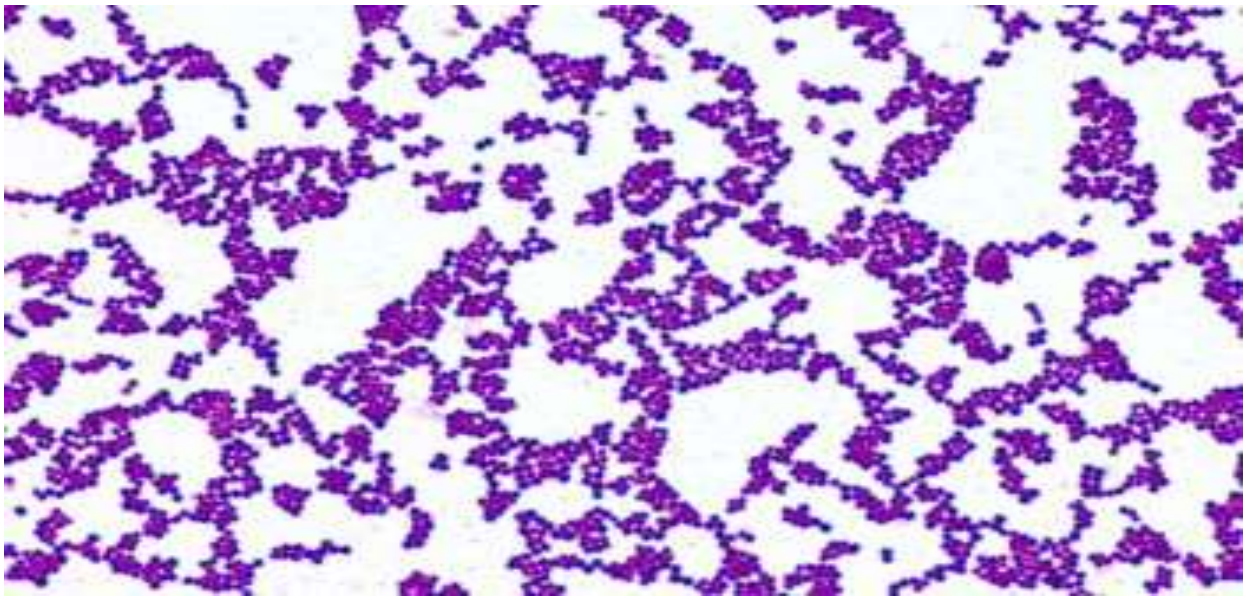

FIGURE 3 : Image of catalase test of *Candida albican*

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