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Prevalence of C*andida albicans* species among females with symptoms

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Abstract

*Candida albicans*isan opportunistic fungal pathogen found as part of the normal microflora in humans, it is one of approximately 200 species in the genus *Candida*.*Candidaalbicans* is the most common type of fungus to cause yeast infectionsand accounts for up to 75% of all candidalinfections**.**A study on the prevalence of vaginal infections was carried out on women at the effective Medical Laboratory, Port-Harcourt. The Clinical symptoms noted amongst women were itching, vaginal discharge, irritation of the vulvovaginal.A total of 100 samples were collected and analysed, a percentage occurrence of 64% growth and 36%no growth was gotten. Germ tubes test carried out shows that out of 64 samples that had growth, 42 of the samples isolated were positive and 22 were negative.Gram staining reactions of *Candida albicans* isolated shows that all were gram positive.This shows that *Candida albicans*was prevalent. Antifungal susceptibility test showed susceptibility of 42%, this was observed in Nystatin, which indicates that it's a better antifungal drug in the treatment of *Candida albican* infection above other antifungal drugs used for the analysis which include Griseoflucin, Fluconazole, Sivoketonazole.The fact that a patient presents symptoms of candidiasis does not mean that the infection is caused by *Candida albicans*. Proper diagnosis is required to ascertain the main cause of the infection.

Keywords: Prevalence; *Candida*; *albican;* Infection; Vagina

1. Introduction

Candidiasis is an infection caused by yeast (a type of fungus) called *Candida*. *Candida* normally lives inside the body (in places such as the mouth, throat, gut, and vagina) and on skin without causing any problems. Sometimes *Candida* can multiply and cause an infection if the environment inside the vagina changes in a way that encourages its growth. Candidiasis in the vagina is commonly called a "vaginal yeast infection." Other names for this infection are "vaginal candidiasis," "vulvovaginal candidiasis," or "*candida vaginitis*."Hundreds of *Candida* species can cause infection in humans; *Candida* yeasts can cause infections if they grow out of control or if they enter deep into the body (for example, the bloodstream or internal organs like the kidney, heart, or brain). Candidiasis that develops in the mouth or throat is called "thrush" or oropharyngeal candidiasis. Candidiasis in the vagina is commonly referred to as a "yeast infection." Invasive candidiasis occurs when *Candida* species enter the bloodstream and affect internal organs like the kidney, heart, or brain.The symptoms of vaginal candidiasis include: Vaginal itching or soreness, pain during sexual intercourse, pain or discomfort when urinating, abnormal vaginal discharge. (Goncalves *et al.,* 2016). Although most vaginal candidiasis are mild, some women can develop severe infections involving redness, swelling, and cracks in the wall of the vagina. (Sobel *et al* 2007).The aim of this work is to obtain the prevalence of *Candidaalbicans*amongst female with symptoms and validate the associated risk factors.*C. albicans* is a polymorphic fungi and a normal colonizer in mucosal sites such as the gut,vagina and the oral cavity of many healthy individuals along with many other non-pathogenicfungi and harmless bacteria (Naglik*et al.,* 2011; Mayer *et al*., 2013). The presence of*C. albicans* does not necessarily indicate

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infection by this organism (Naglik *et al.,* 2011).There are three major forms of disease: oropharyngealcandidiasis, vulvovaginalcandidiasis, and invasive candidiasis. Over 75% of women will suffer from a *C. albicans* infection, usually vulvovaginal candidiasis, in their lifetimes, and 40-50% of them will have additional occurrences(s). Interestingly, *C. albicans* are the 4th leading cause for nosocomial infections in patients' bloodstreams. This could result in an extremely life-threatening, systemic infection in hospital patients with a mortality rate of 30% (Pfaller*et al*,2007). For Osopharyngeal candidiasis, infection occurs in the mouth or throat, and is identified by white plaque growth on oral mucous membranes. Vulvovaginal candidiasis or a "yeast infection" is the overgrowth of *C. albicans* in the vagina, and results in rash, itchiness, and discharge from the genital region. Lastly, invasive candidiasis occurs when the fungal pathogen enters the bloodstream and can easily spread to organs throughout the body. Invasive candidiasis is best identified when antibiotics fail to cure a patient's fever. *C. albicans* infections are usually treatable with fluconazole, while severe infections require amphotericin B. (Kabir *et al,* 2012). *Candida albicans* is usually transmitted from mother to infant through childbirth, and remains as part of a normal human's microflora. Pathogenicity of *C. albicans* relies on successful production of virulence factors. Virulence factors are responsible for the transformation of *C. albicans* to a disease causing organism (pathogen) and makes it possible for *C. albicans* to survive, persist within the host and cause disease when the opportunity arises (Naglik *et al.,* 2011;Romani, *et al* .2000). Vaginal candidiasis is usually treated with antifungal medicine. For most infections, the treatment is an antifungal medicine applied inside the vagina or a single dose of fluconazole taken by mouth. For more severe infections, infections that don't get better, or keep coming back after getting better, other treatments might be needed. These treatments include more doses of fluconazole taken by mouth or other medicines applied inside the vagina such as boric acid, nystatin, or flucytosine.There are four classes of drugs available for the invasive fungal infections in humans. They are polyenes, fluorinated pyrimidines, azoles and echnocandins.

2. Material and methods

The research was done in Port Harcourt, Rivers State, Nigeria, a city that covers an area of about 369 square kilometers and located on the longitude 7`2'1''E and latitude 4`49'27''N. The analysis was carried out in Effective Medical Laboratory LTD, Water Lines Rivers State Nigeria. And the study was carried out among 100 randomly selected female patients presenting with vulvovaginal Candidiasis. The specimens were given laboratory identification number for analysis and retrieval of results.

2.1. Isolation of Candida albicans

The specimens were plated out by streaking on modified Sabouraudchloramphenical agar plates and incubated at 37°C for up to 24hours. The antibiotics 0.5% chloramphenicol, inhibited bacterial growth. On observation, the positive plates had entire edges, cream coloured colonies with pasty smell that is typical of *Candida spp*. Heavy growth of *Candida* with more than 30 colonies on SDA were considered this is to exclude normal flora.

2.2. The wet preparations

The wet preparations of the specimens were also examined after culture for presence of yeast cells. 1ml of normal saline was added to the swab sticks and mixed properly. A drop of suspension of each sample was transferred to a different grease free microscope slide. Cover slip was placed gently to exclude air bubbles and viewed microscopically under 10x and 40x objectives.

2.3. Identification of *Candida albicans*

Candida spp were differentiated from other yeasts and were identified to specie level using Gram stain, morphology, germ tube formation.

Gram staining was done from suspected yeast colonies only those with budding yeast cells and pseudohypae.

2.4. Gram stain examining

Overnight growth from an SDA were used for the Gram stain this differentiates Candida spp. This differential technique used to identify microorganisms based on their characteristics either as Gram positive or Gram negative organisms which is based on the ability of cell wall to retain a certain stain or not.

2.5. Procedure

The isolated organisms from the plates was smeared on a clean glass slide by placing a loopful of water and an inoculum on the slide and allowed to air dry. The smear was then heat fixed and allowed to dry. The prepared smear was flooded

with 1% crystal violet dye for 1 minute, after which the excess stain was rinsed off using distilled water, the smear was then flooded with Lugol's iodine for I minute, the iodine was rinsed off with distilled water, after which the smear was also flooded with 98% alcohol for 30 seconds, rinsed quickly with distilled water and then Safranin red was added. Allowed for 1 minute and rinsed off with distilled water. The various smear was air dried for few minutes and a drop of oil immersion was added and observed under the light microscope.

2.6. Germ Tube Test

Germ Tube Test 0.5mls (12 drops) of pooled human serum was added in a clean test tube. Light suspension of suspected yeast colonies was made on the serum. The mixture in the tube was incubated at 37 degrees centigrade for 3 hours. A drop of the suspension was placed on a clean glass slide using Pasteur pipette and cover with cover slip. The wet mount was microscopically examined at x40 microscope objective for production of germ tubes.

2.7. Standardization of the test microorganisms

The clinical isolates of the test *Candida albicans*used was standardized to 1x 10^6 cells/ml using Mcfarland standard.

2.8. Antimicrobial susceptibility test using agar diffusion method

Sterile Petri dishes were labelled in duplicates for the various test organisms. A 0.1ml of each of the microorganisms was added aseptically to the prepared Muella Hinton Agar pour in the universal bottle and properly mixed. The mixture was then poured into the corresponding Petri dish and allowed to solidify on the workbench. After the agar had solidified on the Petri dish, a sterile cork borer was used to remove 5 discs of agar from the agar layer in order to produce 5 wells in each agar plate. The Wells were labelled for the four (4) different antifungal drugs. Using a separate sterile Pasteur's pipette a drop of 50mg/ml concentration of each drug was carefully and aseptically added to the well already labelled. The Petri dishes were allowed to stay on the workbench for 15 minutes for proper diffusion of the extracts and standard antibiotic. The plates (Petri dishes) were incubated at 37° C for 24 hours.The diameter of the resulting Zones of inhibition were measured in millimetre (mm) through the base of the plates using a metrerule(CLSI, 2010).

3. Results and discussion

Analysis on isolation of *Candida albican* from HVS samples was undertaken. The isolates from HVS were inoculated on SDA slants,TM Media, incubated at 37 degrees centigrade for 24hours. Sixty –four isolates showed the characteristic growth typical of *Candida albicans*as the growth yielded entire, creamy colonies with smell akin to breweries. Thus, it was difficult to differentiate *Candida* to species level looking at the cultural features only. These procedures take long time and a lot of man power and results are not 100% reliable. Sabouraud dextrose medium was useful in detecting *Candida* when identification was inconclusive using Microscopy (Ugwa, 2015).100 samples were isolated with a percentage occurrence of 64 %. This shows that *Candida albican* was prevalent. The antifungal susceptibility test show a high susceptibility of 42%withNystatin at 5mg/ml which indicates that it's a better antifungal drug in the treatment of *Candida albicans* infection above other antifungal drugs used for the analysis which include Griseofluxcin, Gebefluc, sivoketonazole. The high occurrence of *Candida albican* indicates that the fungi is prevalent among women with vaginal infection which occurs as an opportunistic infection occurring in debilitate persons with symptoms of vaginal discharge and itches. This result also shows that vulvovaginal candidiasis is also caused by organisms other than *Candida species*. This agrees with the work done by Ugwa(2015) which he reported that *Proteus vulgaris* was also isolated from HVS specimens.

Conjectural detection of *Candida albican*is carried out by microscopic confirmation of germ tube test. In this study, the isolates from HVS specimens were incubated in pooled human sera for 3hours at 37 degrees centigrade. Out of 64 isolates, 21 (33.3%) showed short slender, tube like structures (germ tubes) when view under microscope. This indicates *Candida albicans*; 21(33.3%) showed no hyphal extension from a yeast cell, indicating non-albicans species and 21(33.3%) showed short hyphal extension with constriction at the point of origin (pseudo germ tube) indicating *Candida tropicalis.*Tapiwa*et al*., 2017 also reported that isolates from cultures suspected to be *C. albicans*were confirmed by germ tube formation. Although germ tube test is generally accepted as a screening procedure for identifying *Candida albicans*, in this study, the assertion has been annulled because there are different strains of *Candida albicans.*

Isolation of *Candida species* from 100 high vaginal swab (HVS) sample collected from Effective Laboratory The Percentage occurrence of *Candida albican*growth was 64% while 36% showed no growth. The result is seen in fig. 1.

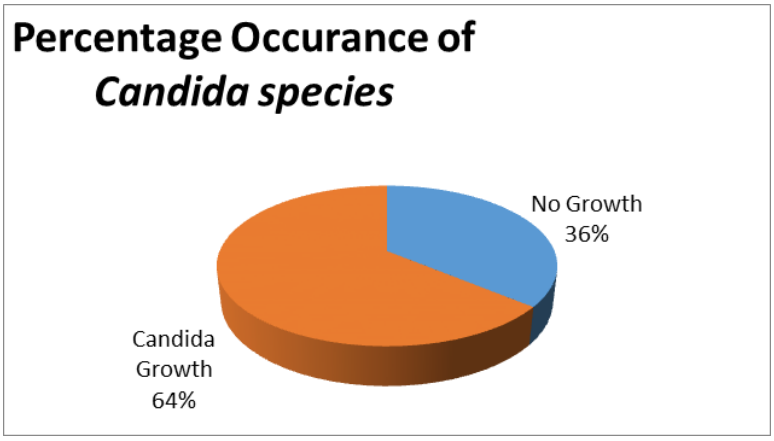
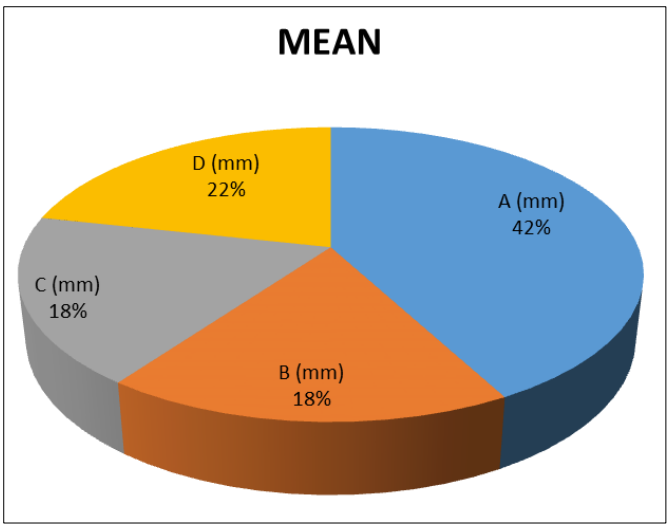


Figure 1 The percentage occurrence of *Candidaspecies* isolated

The table shows the gram reaction of *Candida albican*isolated with all being large gram positive cocci with bud. The result is shown in table 1.

Table 1 gram stain

Samples	Gram stain
2	Gram positive cocci with Bud
3	Gram positive cocci with Bud
6	Gram positive cocci with Bud
8	Gram positive cocci with Bud
9	Gram positive cocci with Bud
10	Gram positive cocci with Bud



KEYA= Nystatin (5mg/ml); B= Griseofluxcin(5mg/ml); C= Gebefluc (5mg/ml); D= Sivoketonazole(5mg/ml)

Figure 2 The mean values for Antifungal susceptibility test on Candida isolate on some antifungal drug

The table shows the result of Germ Tube Test carried out on the *Candida albican* isolate of which 42 of the samples isolated are positive to Germ tube and 22 of the samples isolated are negative. The result is shown in table 2.

Table 2 Summary of Germ tube Test

Germ tube test	Positive	Negative
	42	22

4. Conclusion

The fact that a patient presents symptoms of candidiasis does not mean that the infection is caused by *Candida albicans*. Proper diagnosis is required to ascertain the main cause of the infection. The prevalence of candidiasis calls for the need to routinely check for opportunistic infections especially in the case of an immune compromised individual. This will help to monitor disease progression and complications.

Compliance with ethical standards

Acknowledgment

John, G.N.

Disclosure of conflict of interest

None.

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