

Prevalence and Antifungal Susceptibility Profile of *Cryptococcus neoformans* among Pregnant Women in Abakaliki, Ebonyi State, Nigeria

Okonkwo, Eucharika Chinyere¹, Onwa, Ndubuisi Collins², Nwachi, Anthonia Chinyere¹, Uraku, Anayo Joseph³

¹Department of Applied Microbiology, Ebonyi State University, Abakaliki

²Department of Applied Microbiology, Alex Ekwueme Federal University, Ndufu-Alike Ikwo, Ebonyi State

³Department of Biochemistry, Ebonyi State University, Abakaliki

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*Corresponding Author:

Uraku, Anayo Joseph
Email: anayo.uraku@ebsu.edu.ng
Tel: +2348068073037
LiveDNA*: 234.25095
Orcid ID: 0000-0002-6518-4627

ABSTRACT

Background: The prevalence and antifungal susceptibility profile of *Cryptococcus neoformans* was investigated among pregnant women hospitalized in a tertiary Hospital in Abakaliki, Ebonyi State capital.

Methods: A total of (50) pregnant women were enrolled in the study and a subsequent 50 urine samples were cultured on Sabouraud dextrose agar (SDA). Culture plates which showed significant growth were further processed for the identification of *Cryptococcus* using standard microbiology procedures. Antifungal agents with known potency were used to determine susceptibility profile of the isolates according to Kirby-Bauer disc diffusion method.

Results: The prevalence of isolation was 12% and the drug of choice was found to be amphotericin B (83% susceptibility). Isolates were resistant to nystatin (%) followed by voriconazole (%). The prevalence of *C. neoformans* among pregnant women in relation to age and trimester revealed that the observed changes are likely to have occurred by chance at $\alpha = 0.05$ (N=6).

Conclusion: Effective screening of pregnant women for neglected invasive fungal diseases should be considered an additional step in ensuring safe maternal and child health care.

1. Introduction

Cryptococcus neoformans, a yeast encased in a capsule and categorized within the Tremellomycetes class, is an obligate aerobe capable of thriving in various hosts, both plant and animal¹. This particular organism is known to be an invasive fungus that spreads through the inhalation of spores, leading to the development of cryptococcosis, a potentially fatal condition characterized by an initial stage of pneumonia that may progress to severe meningoencephalitis, especially in individuals with

compromised immune systems². Clinical manifestations of this infection typically involve symptoms such as fever, headache, malaise, as well as photophobia and neck stiffness as the disease advances to meningitis³. A distinguishing trait of *Cryptococcus* is its polysaccharide capsule, the formation of which is induced by environmental conditions mimicking the human body, such as high CO₂ levels, low iron availability, and a neutral to alkaline pH range¹. According to the hypothesis, a significant percentage of the human population hosts *C.*

neoformans, possibly in a latent state situated in the pulmonary system for prolonged periods and could reappear to initiate illness if the person's immune responses are weakened⁴. Despite its intricate intracellular survival mechanisms, this fungus is capable of existing freely without relying on mammalian hosts for virulence, frequently affecting the respiratory and central nervous systems^{5,6}.

The escalation of fungal pathogens and resultant infections present a growing menace to global public health. Individuals most susceptible to developing invasive fungal disease (IFD) are those with a compromised immune system, stemming from conditions such as HIV infection, chemotherapy, immunotherapy for cancer, and solid organ transplantation. Additional risk factors encompass diabetes mellitus, liver or kidney ailments, and pregnancy⁷. As outlined by Sabiiti *et al*³, *Cryptococcus meningitis* warrants consideration during pregnancy in instances of unexplained headache, altered vision, modified mental status, nausea, and fever. Within pregnant individuals, symptoms typically emerge postpartum due to immune reconstitution inflammatory syndrome and may evade detection during pregnancy⁸.

Cryptococcus neoformans is responsible for 15% of AIDS-related mortality, with an estimated 70% mortality rate in Sub-Saharan Africa^{9, 10}. In Africa, women of childbearing age account for approximately 41-55% of *Cryptococcal* patients, highlighting the significance of *Cryptococcus* as a crucial factor in the AIDS epidemic and a notable opportunistic infection¹¹. *Cryptococcus* has now emerged as the most prevalent cause of meningitis in adults, consequently becoming one of the primary causes of death among immune compromised individuals in Sub-Saharan Africa, where it claims more lives annually than tuberculosis^{10,11,12}.

2. Methods

2.1 Study Area and Population: The study area of this research was Federal Teaching Hospital at Abakaliki. The hospital is a tertiary institution that runs ante-natal clinic for pregnant women. Those who are sick or near delivery are often admitted. Samples were collected from participants between October and November, 2023.

2.2 Ethical approval: Ethical approval (AEFUTH /2023/102) was obtained from the Hospital Administration and individual consent was obtained before collecting samples from each patient. Management and the pregnant

women were assured of confidentiality of personal information.

2.3 Sample collection: Participating pregnant women were each given a sterile urine bottle and instructed to collect mid-stream urine in the morning. Structured questionnaire was used to collect information on gestation age and other information of participants. All collected samples were labelled and transported to the microbiology unit of Ebonyi State University Abakaliki within one hour of collection for analysis.

2.4 Sample Analysis: A wire-loopful of each urine sample was carefully streaked unto freshly prepared Sabouraud Dextrose agar (SDA). The fungal colonies were sub-cultured on fresh Sabouraud agar to obtain pure culture. All plates were incubated at 37°C for 2 days.

2.5 Identification of *Cryptococcus neoformans*:

2.5.1 Direct microscopy; A smear from the fungal culture was made on clean, grease-free glass slides using a wire loop. A drop of lactophenol cotton blue (Indian ink) was added to the smear and gently mixed. A cover-slip was gently pressed unto the smear to remove air bubbles and then examined under low and high- power objective. Organism was identified as non-septate single or budding yeast cells with a zone of clearance (halo) surrounding the cells¹³.

2.5.2 Growth Test: Pure colonies were freshly streaked unto SDA and incubated at 37°C for 3-4 days. A robust growth indicates *C. neoformans* ability to thrive at human body temperature¹⁴.

2.5.3 Urease Tests: A loopfull of 72-hour culture was inoculated on urea agar medium supplemented with phenol red indicator. Set up was allowed for 4-5 days at 30°C and observed for colour change indicating alkaline pH shift¹⁵.

2.5.4 Glucose Fermentation Test: Glucose agar was inoculated with fungal colonies and incubated at 30°C for 4-5 days. Colour change due to acid production and pH changes were observed. (*Candida* ferments glucose but acidifies the medium)¹⁶.

2.5.5 Nitrate Test: Fresh isolates were inoculated into nitrate broth and incubated at 30°C for 4-5 days. Sulfanilic acid, naphthylamine solution was added to the broth.

Possible colour changes were observed due to possible nitrite reduction¹⁵.

2.5.6 Antifungal susceptibility testing: Turbidity standard equivalent of 0.5 Mcfarland standards was prepared according to the method of Ochie and Kolhatar¹⁷. A suspension of *Cryptococcus neoformans* isolates was prepared in peptone water to match 0.5 Mcfarland Standard as earlier described. Isolates were inoculated into freshly prepared Mueller Hinton agar plates. The antifungal disk was placed on Mueller Hinton plates and incubated at 37°C

for 24 hours. The zones of inhibition were measured with meter rule and compared with the Clinical and Laboratory Standard Institute (CLSI, 2009) guidelines as described by Perfect *et al*¹⁸.

2.6 Statistical Analysis: Data was analyzed using descriptive analysis with the Graph-pad prism version 6.01 (Graph-pad software, San DIEGO, CA).

3. Results

Table 1: Morphological observation and biochemical test

Fungi	No of Sample 'n'	No of Isolates n (%)	Morphology	Biochemical Reaction	Sugar Fermentation
<i>Cryptococcus neoformans</i>	Urine (50)	6 (12)	Mucoid, circular, smooth/irregular colonies on SDA	Urease positive, nitrate positive	Fermented glucose

Table 2: Microscopic appearance and growth of *Cryptococcus neoformans* at 37°C

Fungi	No of Isolates 'n'	Microscopic appearance	Growth at 30°C
<i>Cryptococcus neoformans</i>	6	<ul style="list-style-type: none"> • Yeast in in capsules • Narrow- based budding cells • Absence of hyphae 	Robust growth

Table 3: Age distribution of pregnant women infected with *Cryptococcus neoformans*

Age	No of Samples	No of infected(N)	%	No of non-infected (N)	%
15-19	2	-	0	2	100
20-24	11	1	10	10	90
25-29	12	2	17	10	83
30-34	15	2	14	13	86
>35	10	1	10	9	90
Total	50	6		44	
	$X^2 = 3.66$	$CV = 9.49$	$\alpha = 0.05$	$df = 4$	

Table 4: Distribution of *Cryptococcus neoformans* according to gestational age

Trimester	No of Samples	No of infected (N)	%	No of non-infected (N)	%
First	12	1	9	11	91
Second	20	3	15	17	85
Third	18	2	12	16	88
Total	50	6		44	
	df = 2	X² = 4.73	CV = 5.99		

Table 5: Antifungal susceptibility Test

Antifungal agent	Potency (µg)	No of isolates tested (N)	No susceptible (N)	%	Status of pregnant women	
					No of non-infected (N)	%
Fluconazole	25	6	4	67	2	33
Voriconazole	10	6	2	33	4	67
Amphotericin B	10	6	5	83	1	17
Nystatin	100	6	-	0	6	100

1. Discussion

Cryptococcus neoformans is a fungal infection that mainly affects people with compromised immune systems. Over the past few decades, the burden of fungal diseases has increased significantly and continues to be a global threat to the health of human, plant, and animal populations¹⁹. The fungus is commonly found in soils, decomposing wood, trees, or bird droppings. Inhalation has been described as a source of infection and is not infectious, but zoonotic identification of birds and humans has been observed^{4,20}. In this study, the prevalence and antifungal sensitivity pattern of *Cryptococcus neoformans* in pregnant women hospitalized in the Tertiary Clinics in Abakaliki were studied. The prevalence was 12%, clearly different from Maria *et al*¹¹ reports which stated about 41-55% of the total infection of *Cryptococcus* throughout Africa. Report indicates that *C. neoformans* infections are low among

people with a relatively healthy immune system²¹.

Pregnancy is described as a relative immune suppressor state, so it may be a risk factor for the development of Cryptococcosis²². The regulation of the immune system during pregnancy is very complex. The immune system has complex modulations that involve different reactions to invasive pathogens depending on pregnancy status²³. Despite this, no recent review article has focused on the publication of the results of Cryptococcosis in West Africa among pregnant women, especially those living with HIV. Therefore, overall data on the clinical prevalence of fungi in pregnant women are scarce and vary in this region. However, few reports from Nigeria show a range of 2.3% to 22% in terms of environmental predisposition²⁴, which is consistent with the study.

Thus, more studies are needed to fill the observed gaps in the epidemiology of cryptococcus. From this study, the

results in table 1 showed that *C. neoformans* appears as mucoid, circular, smooth/irregular colonies on Sabouraud Dextrose Agar. It also showed a prevalence rate of 12% from 50 samples examined. Also, table 2 showed the microscopy of *Cryptococcus neoformans* as encapsulated yeast cells, budding yeasts with narrow-base. A robust growth was observed when grown on SDA at 37°C.

The highest incidence (17%) of *Cryptococcus* is recorded in pregnant women aged 25 to 29 years while 14% fall within 30-34 years and 10% fall within 20-24 and >35 years. Though, no infection has been observed in women aged 15 to 19 (Table 3). The observed differences in the incidence of *Cryptococcus* among pregnant women in relation to age range did not differ from what would have been expected theoretically, $\chi^2 = (4, N= 50)$ was 3.66 at $\alpha = 0.05$. The critical $\chi^2 (0.05, 4, N=50)$ was 9.49. Therefore, the variation was NOT too large enough to be explained by mere chance alone.

The distribution of infections by quarter indicated that women in the second quarter of pregnancy had the highest number of infections. This was followed by those in third trimester with 12% while the first trimester recorded the lowest with 8%. Although only one sample was positive for women in the first quarter, the least number of women in this group participated, which could have led to the lowest incidence of infection compared to other stages (table 4). The observed differences in the incidence of *Cryptococcus* among pregnant women in relation to age range did not differ from what would have been expected theoretically, $\chi^2 = (2, N= 50)$ was 4.73 at $\alpha = 0.05$. The critical $\chi^2 (0.05, 2, N=50)$ was 5.99. Therefore, the variation was NOT too large enough to be explained by mere chance alone.

Often diagnosing, aggressively managing and closely coordinating patients are needed to reduce the potential high mortality rate and risks of untreated cryptococcal meningitis in pregnant women. The results of the Antifungal susceptibility tests in this study showed that the *Cryptococcus* isolates were highly tolerant to amphotericin B (83%) and fluconazole (67%). All isolates are essentially resistant to Nystatin (100%) (Table 5). The recommended treatment for *Cryptococcus neoformans* is amphotericin B, which is consistent with many studies¹⁹.

However, the use of amphotericin B during pregnancy is a major concern due to the drug's teratogenicity and variable pharmacokinetics²⁵. The treatment guidelines for cryptococcosis during pregnancy are then based on expert opinions.

Approaches and strategies for the prevention and treatment of antifungal resistance have been identified, including host

immune modulation, dose optimization, prophylaxis/empirical regimes, improved drug delivery systems such as amphotericin B lipid preparations, the combination of antifungal treatments and the development of new antifungal agents¹⁹.

Conclusion

This is the first report on the prevalence and antifungal susceptibility profile of *Cryptococcus neoformans* among pregnant women in Abakaliki, Ebonyi State capital. The study revealed that the age group highly infected by *C. neoformans* 25-29 followed by 30-34 years. Also, it unveiled that pregnant women in the second trimester are more prone to the microbes and that Nystatin antibiotics have no potency against the organism.

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Conflict of Interest

All authors declared no conflict of interest

Authors' Contributions

OEC conceptualized the initial idea and designed the scope and directions of the study. OEC, ONC, NAC and UAJ made equal contributions to different parts of the review. OEC contributed to the review and managed the references. UAJ designed, retrieved, and analyzed the survey and data obtained from the survey. OEC and UAJ thoroughly overhauled the manuscript and made valuable inputs. All authors read and edited the final copy of the manuscript. OEC gave the final authorization for the submission of the manuscript.

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