ORIGINAL ARTICLE

ISSN: 0331 - 670X

https://doi.org/10.51412/psnnjp.2024.17

The Nigerian Journal of PHARMACY
AS MEN OF HONOUR WE JOIN HANDS
THE OFFICIAL ORGAN OF THE PHARMACEUTICAL SOCIETY OF NIGERIA FOUNDED 1927

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

Okonkwo, Eucharia Chinyere[1]., Onwa, Ndubuisi Collins[2]., Nwachi, Anthonia Chinyere[1]., Uraku, Anayo Joseph[3]

[1]Department of Applied Microbiology, Ebonyi State University, Abakaliki
[2]Department of Applied Microbiology, Alex Ekwueme Federal University, Ndufu-Alike Ikwo, Ebonyi State
[3]Department of Biochemistry, Ebonyi State University, Abakaliki

ARTICLE INFO

Article history:

Received 28th April 2024
Revised 9th July 2024
Accepted 19th July 2024
Online Published

Keywords:

**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 *Cryptococccus 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. neoformance* among pregnant women in relation to age and trimester revealed that the observed changes are likely to have occurred by chance at $\alpha = 0.0.5$ (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 animal[1]. 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 systems[2]. 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 meningitis[3]. 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 CO_2 levels, low iron availability, and a neutral to alkaline pH range[1]. 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 weakened[4]. 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 pregnancy[7]. 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 pregnancy[8].

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 infection*[11]. *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 tuberculosi*[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 bobbles 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 cells[13].

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 temperature[14].

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 shift[15].

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 reduction[15].

2.5.6 Antifungal susceptibility testing: Turbidity standard equivalent of 0.5 Mcfarland standards was prepared according to the method of Ochie and Kolhatar[17]. 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
Cryptococcus neoformans	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
Cryptococcus neoformans	6	• 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***	***α= 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	$X^2 = 4.73$	**CV** = 5.99		

Table 5: Antifungal susceptibility Test

					Status of pregnant women	
Antifungal agent	**Potency (µg)**	**No of isolates tested (N)**	**No susceptible *(N)***	***%***	**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 populations[19]. 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 system[21].

Pregnancy is described as a relative immune suppressor state, so it may be a risk factor for the development of Cryptococcosis[22]. 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 status[23].

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 predisposition[24], 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 Sabauraud 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 20 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, $x^2 = (4, N= 50)$ was 3.66 at $\alpha = 0.05$. The critical x^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, $x^2 = (2, N= 50)$ was 4.73 at $\alpha = 0.05$. The critical x^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 neofomans* is amphotericin B, which is consistent with many studies[19].

However, the use of amphotericin B during pregnancy is a major concern due to the drug's teratogenicity and variable pharmacokinetics[25]. 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 agents[19].

Conclusion

This is the first report on theprevalence and antifungal susceptibility profile of *Cryptococccusneoformans*among pregnant women in Abakaliki, Ebonyi State capital. The study revealed that the age group highly infected by *C. neofomans*is 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.

Acknowledgments

All authors are grateful to Almighty God for making this work a success.

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.

REFERENCES

1. Bahn YS, Sun S, Heitman J and Lin X (2020) Microbe profile: *Cryptococcus neoformans* species complex. *Microbiology* 166 (9): 797-799.
2. Costa MLB, Souza JPD, Olivera AFD and Pinto JL (2009) Cryptococcal meningitis in HIV-negative pregnant women: Case report and review of literature" *Revista do* Instituto *de Medicina Tropical* de *São Paulo* 51(5): 289-294
3. Sabiiti W and May RC (2012) Mechanism of infection by the human fungal pathogen *Cryptococcus neoformans*. *Future Microbiology* 7:1297-1313.
4. Levitz SM (1991) The ecology of Cryptococcus

neoformans and the epidemiology of cryptococcosis. *Reviews of Infectious Diseases* 13: 1163-1169

5. Caroline C, Anamelia LB and Arturo C (2014) The tools for virulence of *Cryptococcus neoformans*. *Advances in Applied Microbiolog* 87: 1-41.
6. Anjum S and Williamson PR (2019) Clinical aspect of immune damage in Ccrptococcosis. *Current Fungal Infection Reports* 13: 99-108.
7. Youbao Z, Leixin Y and Lingi W (2023) *Cryptococcus neoformanas*, a global threat to human health. *Infectious Diseases of Poverty* 20:9-11.
8. Singh N and Perfect JR (2007) Immune constitution Syndromes and exacerbation of infections after pregnancy. *Clinical Infectious Diseases* 45(9): 1192-1199.
9. Sinbanda EL, Weller IV, Hakim JG and Cowan FM (2013) The magnitude of loss to follow-up on HIV-exposed infants along the prevention of mother-to-child HIV transmission continuum of case: A Systemic Review and meta-analysis. *AIDS* 27:2787-2797.
10. Rajasingham R, Smith RM, Park BJ (2017) Global burden of disease of HIV-associated Cryptococcus meningitis: an update analysis. *Lancet infectious Diseases* 17: 873-881.
11. Maria LB, Joae PD and Joao LP (2009) Crytococcal meningitis in HIV negative pregnant women: case report and Review of Literature. *Revista do* Instituto *de Medicina Tropical* de *São Paulo* 51(5):289-94.
12. Bicanic T and Harrison TS (2005) Cryptococcal meningitis. *British Medical Bulletin* 72:99-118.
13. Deorukhkar SC, Saini S and Jadhov P (2012). Evaluation of different media for germ tube production of *Candida albican*s and Candida dubliniensis. *International Journal of Biomedical Advanced Research* 3: 704-707.
14. Moyrand F and Janbon G (2004) UGDi, encoding the *Cryptococcus neoformanss*UDP-glucose dehydrogenase, is essential for growth at 37°C and for capsule biosynthesis. *Eucharyot Cell* 3:1601-1608.
15. Macfaddin JF (2000) Urease Test. Biochemical Test for identification of medical Bacteria. (3rd Ed.). Philadelphia: Lippincott Williams and Wilkins, USA, pp424-438.
16. Deorukhkar SC and Roushani S (2018) Identification of Candida species: conventional methods in the era of molecular diagnosis. *Annals of Microbiology and Immunology* 1(1): 1-6.
17. Ochie J and Kolhatkar A (2000) *Textbook of medical Laboratory Science Theory and Practical*: 2ndedition. Tata McGraw – Hill. 331-349.
18. Perfect JR, Dismukes WE, Dromer F, Goldman DL, John RG (2010) Clinical practice Guidelines for the management of Cryptococcal Disease. Uptake by the infectious Diseases Society of America. *Clinical Infections Diseases* 50(3): 291-322.
19. Perfect JR and Cox GM (1999) Drug resistance in *Cryptococcus neoformans*. *Indian Journal Pathology* **2**(4): 259-269.
20. Lagrou K, Eldere J, Hagen F and Keulees S (2000) Zoonotic transmission of Cryptococcus neoformans from a magpie to an immunocompetent patient. *Journal* of *Internal Medicine* 257:380-388
21. Rajasingham R, Govender NP, Jordan A, Loyse A (2020) The global burden of HIV-associated cryptococcal infection in adults in 2020: a modelling analysis. Lancet *Infectious Disease* 15(6): 789-794
22. Philpot CR and Lo D (1972) Cryptococcus meningitis in pregnancy. *Medical journal of Australia* 2(18): 1005-1007.
23. Mar G and Gardenas I (2010) The immune system in pregnancy: a unique complexity" *American Journal of Reproductive Immunology* 63(6): 425-433
24. Akaihe CL and Nweze EL (2021) Epidemiology of Cryptococcosis in West Africa. *Mycosis* 64(1): 4-17
25. Food and drug administration (FDA) U.S.A (2011) Use of long term, high-dose diflucan (fluconazole) during pregnancy may be associated with birth defect in infants.