SUSCEPTIBILITY PATTERN OF ANTIFUNGALS AGENTS ON CANDIDA AND ASPERGILLUS IN SPUTUM SAMPLES FROM HIV POSITIVE PATIENTS IN ESUTH.

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ABSTRACT

Candida and Aspergillus are two of the most common fungal pathogens causing a range of diseases in individuals with HIV, these fungi have varying susceptibility for antifungal agents. **Aim:** This study determined the susceptibility patterns of antifungal agents on Candida and Aspergillus isolated from sputum of HIV patients at the ESUT Teaching Hospital, Parklane. **Method:** A cross-sectional study design was employed between July 2024_ Sep 2024.. A total of 150 sputum samples were collected. Fungal isolates were identified using standard microbiological procedures, including microscopy and culture on Saboured dextrose agar at 35°C for 24–48 hours. Antifungal susceptibility testing was conducted using the Clinical and Laboratory Standards Institute (CLSI) disk diffusion method. Data were analyzed using the Chi-square test .Of the participants, 84 (56%) were female, and 66 (44%) were male. **Result**: The prevalence of Candida and Aspergillus isolates was 30% and 18.67% respectively. Candida isolates showed sensitivity to fluconazole (20%), itraconazole (34%), nystatin (40%), ketoconazole (37%), and clotrimazole (32%), while resistance rates were 16%, 8%, 4%, 6%, and 10%, respectively. Aspergillus isolates exhibited sensitivity to itraconazole (17%), nystatin (24%), ketoconazole (15%), and clotrimazole (24%), but none were sensitive to fluconazole, with resistance rates ranging from 1% to 28%. **Conclusion:** This study highlights the increasing antifungal resistance due to widespread antifungal use, emphasizing the critical role of antifungal susceptibility testing in guiding effective treatment. Routine susceptibility testing should be adopted by healthcare providers to improve patient outcomes.

KEYWORDS. Fungal pathogens, HIV patients, antifungal susceptibility , Candida, Aspergillus.

INTRODUCTION

The HIV/AIDS pandemic has led to an increase in the number of immune-compromised patients in recent decades1. Morbidity and mortality in these individuals are due to opportunistic bacterial, fungal and viral agents1. This has led to a significant increase in opportunistic diseases, especially Candida infections2l. Most opportunistic infections are respiratory infections. Respiratory infections caused by Candida species are one of the health problems caused by Candida species3.

Candidiasis and aspergillosis are an increasing medical problem that requires prompt diagnosis and treatment. The definitive diagnosis of pulmonary candidiasis and aspergillosis is based on histological evidence of yeast in lung tissue at autopsy, as well as visible inflammatory changes. Increased rates of antifungal resistance have also been reported.

In vitro susceptibility testing provides a means of measuring antifungal activity and correlating in vivo activity to how a patient will respond to treatment. They can also be used to monitor resistance in susceptible species and to test the efficacy of new antifungal agents5-6.

Two methods of antifungal susceptibility testing are used: the broth dilution method and the disk diffusion method6. These methods are influenced by a number of factors, namely: medium composition

, inoculum size, hydrogen ion concentration, incubation time, incubation temperature, and MIC determination method[5-7.]

Antifungal susceptibility testing is a very active area of research. It has been used for drug discovery and epidemiological studies[7]. Many

antifungals with different spectrums of activity have been used to combat the growing number of fungal infections. Therefore, it is necessary to determine the sensitivity of fungal strains to existing antifungal drugs8.

Material and Methods

This study was a cross-sectional study conducted at the University Teaching Hospital Enugu.

Sample size:

150 sputum samples were collected from HIV-infected patients attending ESUTH

Inclusion criteria:

HIV-infected patients aged 20 years and above, both sexes, with respiratory symptoms and the ability to expectorate sputum.

Exclusion criteria:

Patients who were taking antifungal medications and refused to participate were excluded from the study.

Candida and Aspergillus tests were performed: [9,14,16.]

1) Direct examination:

a. Wet mount

b. Gram stain

a. Wet mount:

For direct microscopic examination, the specimen is placed on a slide, 10% KOH (potassium hydroxide) is added and a coverslip is placed on the specimen. The slide is warmed slightly and a light pressure is applied to the coverslip to expel trapped air. Microscopic examination is performed, first at low magnification, then at high magnification.

BC Gram stain:

Smear smears are made from clinical specimens on a clean, grease-free glass slide and then heat-fixed by simply exposing the slide to a flame.

The smear is then stained using the Gram method and viewed under an oil immersion objective and examined for the presence of Gram-positive, oval yeast-like budding cells (2-4 µm) and/or pseudohyphae.[15],

2) Culture:

[14, 17]. For culture, Sabouraud dextrose agar (SDA) containing chloramphenicol is used. The sample is inoculated onto an SDA plate and incubated at 25 0C. Plaques are observed daily for 2 days.

Colonies were identified based on colony characteristics.

3) Microscopy:

Isolates were examined under a microscope using lactophenol blue cotton.

Antifungal Susceptibility Testing:

The antifungal susceptibility testing of isolates was performed using the disk diffusion method according to CLSI guidelines.

The antifungals used for the disk diffusion method were:

1.itraconazole
2. Fluconazole
3. Nystatin
4. Ketoconazole
5. Clotrimazole

To determine whether the isolates tested with itaconazole, nystatin, fluconazole, ketoconazole, clotrimazole were susceptible, intermediate or resistant; The diameters of the inhibition zones obtained 13 were compared with the interpreted breakpoints of the standard zones published by CLSI M44-A2[18]

CHAPTER FOUR
RESULTS
RESULT ANALYSIS

The distribution of fungal isolates among the study population revealed that *Candida* was the most frequently isolated fungus, representing 30% of the total samples, followed by *Aspergillus* at 18.67%. Other fungal isolates collectively accounted for 18% of the samples, with *Penicillium* contributing 10% and *Mucor* 8%. Additionally, 33.33% of the samples showed no fungal isolates. This indicates a diverse range of fungal pathogens with Candida being the predominant isolate.

Table 1: Distribution of Fungal Isolates among the Study Population

Isolates	Frequency N=150	(%)

Candida	**45**	**30.00%**
Aspergillus	**28**	**18.67%**
Others	**27**	**18.00%**
Negative isolates	**50**	**33.33%**
Total	**150**	**100.00%**

Table 1b: Others fungi isolated from the study subjects

Isolates	Frequency N=150 (%)
Penicillium	**15 (10.00%)**
Mucor	**12 (8.00%)**
Total	**27 (18.00%)**

The distribution of fungal isolates according to gender indicates that *Candida* isolates were more prevalent among females (29 isolates, 76%) compared to males (16 isolates, 24%), but this difference was not statistically significant (X^2 = 1.623, p = 0.202). Similarly, *Aspergillus* isolates were slightly more common among females (17 isolates, 60.71%) than males (11 isolates, 39.29%), but the difference was also not statistically significant (X^2 = 0.068, p = 0.795). These findings suggest that gender does not significantly influence the distribution of *Candida* or *Aspergillus* isolates in the study population.

Table .2 Distribution of fungal isolates according to gender among the study population

GENDER	Frequency	No. of *Candida* isolates	X^2	p-value
Male	**66 (44%)**	**16 (24%)**	**1.623**	**0.202**
Female	**84 (56%)**	**29 (76%)**		
Total	**150 (100%)**			

Table 2b Distribution of fungal isolates according to gender among the study population

GENDER	Frequency	No. of *Aspergillus* isolates	X^2	p-value
Male	**66 (44%)**	**11 (39.29%)**	**0.068**	**0.795**
Female	**84 (56%)**	**17 (60.71%)**		
Total	**150 (100%)**			

The distribution of *Candida* isolates according to age shows that the highest frequency of *Candida* isolates was observed in the 30-39 age group (17 isolates, 38%), followed by the 40-49 age group (11 isolates, 24%). However, the difference in *Candida* distribution across age groups was not statistically significant (X^2 = 5.763, p = 0.222). Similarly, for *Aspergillus* isolates, the highest frequency was also found in the 30-39 age group (13 isolates, 28.89%), followed by the 40-49 age group (8 isolates, 17.78%). The distribution of *Aspergillus* across age groups was also not statistically significant (X^2 = 4.578, p = 0.334). This suggests that age does not significantly influence the distribution of *Candida* or *Aspergillus* isolates in the study population.

Table 3 Distribution of *Candida* according to age among the study population

Age Group	Frequency	No. of *Candida* isolates	X^2	p-value
20-29	**20 (13.33%)**	**9 (20%)**	**5.763**	**0.222**
30-39	**45 (30%)**	**17 (38%)**		
40-49	**50 (33.3%)**	**11 (24%)**		
50-59	**25 (16.67%)**	**6 (12%)**		
60-60	**10 (6.7%)**	**2 (4%)**		
Total	**150 (100%)**			

Table 3 Distribution of *Aspergillus* according to age among the study population

Age Group	Frequency	No. of *Aspergillus* isolates	X^2	p-value
20-29	20 (13.33%)	3 (10.71%)	4.578	0.334
30-39	45 (30%)	13 (28.89%)		
40-49	50 (33.3%)	8 (17.78%)		
50-59	25 (16.67%)	3 (10%)		
60-60	10 (6.6%)	1 (3.57%)		
Total	150 (100%)			

The use of antifungal drugs in the study population reveals that *Fluconazole* was the most commonly used antifungal (86.67%), followed by *Nystatin* (73.33%) and *Itraconazole* (60%). In contrast, the use of *Ketoconazole* was quite low, with only 14% of participants using it. This suggests a preference for certain antifungal medications, particularly *Fluconazole* and *Nystatin*, among the study population.

Table 4 Use of Antifungal drugs

Anti-fungal drugs	Yes Frequency (%)	No Frequency (%)
Fluconazole	130 (86.67%)	20 (13.33%)
Itraconazole	90 (60%)	60 (40%)
Nystatin	110 (73.33%)	40 (26.67%)
Ketoconazole	21 (14%)	129 (86%)
Clotrimazole	49 (32.67%)	101 (67.33%)

The antifungal susceptibility of *Candida* isolates shows that the highest percentage of sensitivity was observed with *Nystatin* (40%), followed by *Itraconazole* (34%). The resistance rate was highest for *Fluconazole* (16%), with 9% of isolates showing intermediate resistance. These results indicate that *Candida* isolates were generally more susceptible to *Nystatin* and *Itraconazole*, while *Fluconazole* showed relatively higher resistance.

Table 5 Anti-fungal susceptibility on Candida isolates

Anti-fungal drugs	Sensitive Frequency (%)	Intermediate Frequency (%)	Resistant Frequency (%)
Fluconazole	20 (20%)	9 (9%)	16 (16%)
Itraconazole	34 (34%)	3 (3%)	8 (8%)
Nystatin	40 (40%)	1 (1%)	4 (4%)
Ketoconazole	37 (37%)	2 (2%)	6 (6%)
Clotrimazole	32 (32%)	3 (3%)	10 (10%)

The antifungal susceptibility of *Aspergillus* isolates shows that 100% of *Aspergillus* isolates were resistant to *Fluconazole*. The sensitivity was highest with *Itraconazole* (17%), *Nystatin* (24%), and *Clotrimazole* (24%). These findings suggest that *Aspergillus* isolates exhibited significant resistance to *Fluconazole*, and were relatively more susceptible to *Itraconazole*, *Nystatin*, and *Clotrimazole*, albeit to a lesser extent.

Table 6 Anti-fungal susceptibility on Aspergillus isolates

Anti-fungal drugs	Sensitive Frequency (%)	Intermediate Frequency (%)	Resistant Frequency (%)
Fluconazole	0 (0%)	0 (0%)	28 (100%)
Itraconazole	17 (17%)	5 (5%)	6 (6%)
Nystatin	24 (24%)	2 (2%)	2 (2%)
Ketoconazole	15 (15%)	6 (6%)	7 (7%)
Clotrimazole	24 (24%)	3 (3%)	1 (1%)

DISCUSSION

The distribution of fungal isolates among the study population, as presented in Table 4.1, provides valuable insights into the prevalence of various fungal species in this cohort. Out of the 150 study participants, 45 (30.00%) were found to have Candida isolates, making it the most common fungal species identified in this population. This is not surprising, given that Candida is a common commensal fungus that can be found on the skin and mucous membranes of healthy individuals. Aspergillus was the second most common fungal isolate, detected in 28 (18.67%) of the study participants. Aspergillus is a ubiquitous fungus that can be found in soil, water, and air, and is known to cause a range of diseases, from allergic reactions to invasive aspergillosis. The "Others" category, which comprised 27 (18.00%) of the study participants, likely includes a diverse range of fungal species that were not specifically identified. This highlights the complexity of fungal infections and the need for further research to better understand the epidemiology of fungal diseases. 50 (33.33%) of the study participants had negative fungal isolates, suggesting that a significant proportion of the population did not have any detectable fungal infections. A study conducted by Al-Mousa et al. (2017) in Saudi Arabia found that Candida was the most common fungal isolate, accounting for 34.6% of all isolates, followed by Aspergillus, which accounted for 23.1% of all isolates. The findings of this study are consistent with previous studies that have reported a high prevalence of Candida and Aspergillus isolates in various populations.

The results presented in Table 4.2 reveal the distribution of fungal isolates according to gender among the study population. The study population consisted of 150 individuals, with 66 (44%) males and 84 (56%) females. The distribution of Candida isolates, which was the most common fungal isolate in the study population, was found to be slightly higher in females (29 isolates, 76%) compared to males (16 isolates, 24%). However, the difference in the distribution of Candida isolates between males and females was not statistically significant, as indicated by the X^2 value of 1.623 and a p-value of 0.202. These findings suggest that gender may not be a significant factor in the distribution of Candida isolates in this study population.

The results presented in Table 4.2b reveal the distribution of Aspergillus isolates according to gender among the study population. The study population consisted of 150 individuals, with 66 (44%) males and 84 (56%) females. The distribution of Aspergillus isolates was found to be slightly higher in females (17 isolates, 60.71%) compared to males (11 isolates, 39.29%). However, the difference in the distribution of Aspergillus isolates between males and females was not statistically significant, as indicated by the X^2 value of 0.068 and a p-value of 0.795. These findings suggest that gender may not be a significant factor in the distribution of Aspergillus isolates in this study population. This is consistent with previous studies that have found no significant difference in the prevalence of Aspergillus infections between males and females (Al-Mousa et al., 2017). Aspergillus is a ubiquitous fungus that can be found in a variety of environments, including soil, water, and air. Infection with Aspergillus can occur through inhalation of spores, and can cause a range of diseases, from allergic reactions to invasive aspergillosis.

The results presented in Table 4.3 reveal the distribution of Candida isolates according to age among the study population. The study population consisted of 150 individuals, with ages ranging from 20 to 60 years. The distribution of Candida isolates was found to be highest in the 30-39 age group, with 17 (38%) isolates. This was followed by the 20-29 age group, with 9 (20%) isolates, and the 40-49 age group, with 11 (24%) isolates. The lowest number of Candida isolates was found in the 60-60 age group, with only 2 (4%) isolates. The X^2 value of 5.763 and a p-value of 0.222 indicate that the difference in the distribution of Candida isolates among the different age groups is not statistically significant. This suggests that age

may not be a significant factor in the distribution of Candida isolates in this study population. These findings are consistent with previous studies that have found no significant association between age and the prevalence of Candida infections (Al-Mousa et al., 2017). However, other studies have reported a higher prevalence of Candida infections in older adults (Nyirjesy, 2016). The reasons for these differences are not fully understood and may be influenced by a range of factors, including changes in immune function, increased exposure to healthcare settings, and underlying health conditions.

The distribution of Candida isolates among the study population, as presented in Table 4.3, reveals an interesting trend. The highest number of Candida isolates was found in the 30-39 age group, with 17 (38%) isolates. This was followed by the 20-29 age group, with 9 (20%) isolates, and the 40-49 age group, with 11 (24%) isolates. The frequency of Candida isolates decreased with increasing age, with the lowest number of isolates found in the 60-60 age group, with only 2 (4%) isolates. This trend suggests that Candida infections may be more prevalent among younger adults, particularly those in their 30s. The X^2 value of 5.763 and a p-value of 0.222 indicate that the difference in the distribution of Candida isolates among the different age groups is not statistically significant. This suggests that age may not be a significant factor in the distribution of Candida isolates in this study population

The results presented in Table 4.5 reveal the anti-fungal susceptibility patterns of Candida isolates to various anti-fungal drugs. The findings indicate that the susceptibility patterns vary widely among the different anti-fungal agents. Fluconazole, a widely used anti-fungal agent, showed a relatively low sensitivity rate of 20% among the Candida isolates. Moreover, 16% of the isolates were found to be resistant to fluconazole, while 9% showed intermediate susceptibility. These findings suggest that fluconazole may not be the most effective anti-fungal agent against Candida infections in this population. In contrast, itraconazole showed a higher sensitivity rate of 34% among the Candida isolates. Only 8% of the isolates were found to be resistant to itraconazole, while 3% showed intermediate susceptibility. These findings suggest that itraconazole may be a more effective anti-fungal agent against Candida infections in this population. Nystatin showed the highest sensitivity rate of 40% among the Candida isolates. Only 4% of the isolates were found to be resistant to nystatin, while 1% showed intermediate susceptibility. These findings suggest that nystatin may be an effective anti-fungal agent against Candida infections in this population. Ketoconazole showed a moderate sensitivity rate of 37% among the Candida isolates. Only 6% of the isolates were found to be resistant to ketoconazole, while 2% showed intermediate susceptibility. These findings suggest that ketoconazole may be a moderately effective anti-fungal agent against Candida infections in this population. Clotrimazole showed a relatively low sensitivity rate of 32% among the Candida isolates. Moreover, 10% of the isolates were found to be resistant to clotrimazole, while 3% showed intermediate susceptibility. These findings suggest that clotrimazole may not be the most effective anti-fungal agent against Candida infections in this population.

The results presented in Table 4.6 reveal the anti-fungal susceptibility patterns of Aspergillus isolates to various anti-fungal agents. The findings indicate that the susceptibility patterns vary widely among the different anti-fungal agents. Fluconazole, a widely used anti-fungal agent, showed a disturbingly low efficacy against Aspergillus isolates, with 100% of the isolates found to be resistant to this agent. This suggests that fluconazole may not be an effective treatment option for Aspergillus infections in this population. In contrast, itraconazole showed a moderate efficacy against Aspergillus isolates, with 17% of the isolates found to be sensitive to this agent. However, 6% of the isolates were found to be resistant to itraconazole, highlighting

the need for careful selection of anti-fungal agents for the treatment of Aspergillus infections. Nystatin showed a relatively high efficacy against Aspergillus isolates, with 24% of the isolates found to be sensitive to this agent. Only 2% of the isolates were found to be resistant to nystatin, suggesting that this agent may be a viable treatment option for Aspergillus infections in this population. Ketoconazole showed a moderate efficacy against Aspergillus isolates, with 15% of the isolates found to be sensitive to this agent. However, 7% of the isolates were found to be resistant to ketoconazole, highlighting the need for careful selection of anti-fungal agents for the treatment of Aspergillus infections. Clotrimazole showed a relatively high efficacy against Aspergillus isolates, with 24% of the isolates found to be sensitive to this agent. Only 1% of the isolates were found to be resistant to clotrimazole, suggesting that this agent may be a viable treatment option for Aspergillus infections in this population. The findings of this study highlight the importance of anti-fungal susceptibility testing in guiding the selection of effective anti-fungal agents for the treatment of Aspergillus infections. The results also suggest that nystatin and clotrimazole may be effective anti-fungal agents against Aspergillus infections in this population, while fluconazole may not be an effective treatment option.

CONCLUSION

The results of this study provide details into the distribution of fungal isolates, their susceptibility to various antifungal agents, and the use of antifungal drugs among the study population. The study found that Candida and Aspergillus were the most common fungal isolates, accounting for 30% and 18.67% of all isolates, respectively. Other fungal isolates, including Penicillium and Mucor, were also identified. The distribution of fungal isolates was found to be similar among males and females, with no significant differences observed. However, the distribution of Candida isolates was found to vary among different age groups, with the highest frequency observed among the 30-39 age group. The study also found that the use of antifungal drugs was common among the study population, with fluconazole being the most widely used antifungal agent. However, the susceptibility of fungal isolates to various antifungal agents was found to be variable, with some isolates showing resistance to commonly used antifungal agents. The results of this study highlight the importance of antifungal susceptibility testing guiding the selection of effective antifungal agents for the treatment of fungal infections. The findings also suggest that the use of antifungal agents should be judicious and based on the results of susceptibility testing to minimize the development

REFERENCES

1. Usharani, A., Bharathi, M. and Sandhya, C. (2011). "Isolation and Characterization of Candida Species from Oropharyngeal Secretions of HIV Positive Individuals," Nasza Dermatology Online; 2 (3): 119-124.
2. Juliana, C. R. (2004). "Phenotypic and Genotypic Identification of Candida spp. Isolated from Hospitalized Patients," Revista Iberoamericana de Micología; 21: 24-26.
3. Soll, D. R.(2002). Candida and Virulence: The Evolution of Phenotypic Plasticity. Acta Tropica; 81(2): 101. doi:10.1016/S0001-706X(01)00200-5.
4. Diaz-Fuentes G, Shin C, Sy ER, Niazi M. and L. Me-non, "Pulmonary Fungal Involvement in HIV-Positive Pa- tients in an Inner City Hospital in New York," The Inter- net Journal of Pulmonary Medicine, Vol. 7, No. 2, 2007, pp. 1531-2984.
5. Pfaller, M. A., Rex, J. H. & Rinaldi, M. G. (1997). Antifungal testing: technical advances and potential clinical applications. Journal of Clinical Infectious Disease, 24, 776- 784.
6. Espinel-Ingroff, A. (1998). In vitro activities of a new triazole voriconazole (UK-109,496) against opportunistic filamentous and

dimorphic fungi and common and emerging yeast pathogens. Journal of Clinical Microbiology, 36, 198-202.
7. Rex, J.H., Pfaller, M. A., Walsh, T. J., Chaturvedi, V., Espinel-Ingroff, A., Ghannoum, M. A., Gosey, L. L., Odds, F. C., Rinaldi, M. G., Sheehan, D. J. & Warnock, D. W. (2001). Antifungal susceptibility testing; Practical aspects and current challenges. Clinical Microbiology Reviews, 14(4), 643-658.
8. Khan, S., Singhal, S., Mathur, T., Upadhyay, D. J. & Rattan, A. (2006). Antifungal susceptibility testing method for resource constrained laboratories. Indian Journal of Medical Microbiology, 24(3), 171-176.
9.Chakrabarti A, Shivaprakash MR.(2005) Microbiology of systemic fungal infections. J Postgrad Med ;51;16-20.-.
10.Akortha EE, Nwaugo VO, Chikwe (2009). Antifungal resistance among Candida species from patients with genitourinary tract infection isolated in Benin City, Edo state, Nigeria. African Journal of Microbiology Research 3(1 1).
11 Koneman FW, Allen SD, Janda WM, Wine WC, Schreckenberger PC, Procop GW Koneman's (2006)Color Atlas and Textbook of Diagnostic Microbiology. 6th ed. Philadelphia: Lippincott Williams and Wilkins;
12.Chakrabarti A, MR Shivprakash, Venugopal PV, Venugopal TV. National workshop in medical Microbiology: Medical mycology laboratory procedure. Proceedings of 29th Annual Congress of Indian Association of Medical Microbiologist; 19-20
13 Hageage GJ, Harrington BJ(1998). Use of Calcoflor white in clinical Mycology. Lab Med 15:109-12.
14.Milne LIR. Fungi. In: Colle JG, Frase AG, Marmion BP, Simmons A. Mackie and McCartney (2007).Practical Medical Microbiology. 14th ed. Edinburgh: Churchill Livingstone; :695-717.
15 Baradkar VP, Mathur M, Kumar S. Hichrom(1996). Candida agar for identification of Candida species. Indian J Clin Microbiol
16. Chander J.(2009) Textbook of Medical Mycology. Ed.3. New Delhi: Mehta; 2009:266-283.
17. Forbes BA, Sahm DF, Weissfeld AS (2007). Laboratory methods in basic Mycology. In: Bailey and Scott's Diagnostic Microbiology. 12th Ed. St. Louis: Mosby; :629-7.
18. Clinical Laboratory Standards Institute (CLSI). Method for antifungal disk diffusion Susceptibility testing of Yeasts; Approved Guideline- second edition. CLSI document M44-A2 (ISBN 1-56238-703-0). Clinical Laboratory Standard Institute, Waync: Pennsylvania; 2009.