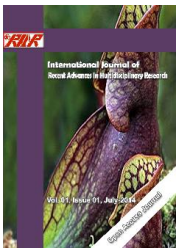

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Full Length Research Article

THE INCIDENCE OF ASPERGILLOSIS AND CANDIDIASIS AMONG PATIENTS WITH PULMONARY DISORDERS ATTENDING INFECTIOUS DISEASES HOSPITAL, KANO

[1]Taura, D. W., [*2]Adamu, S., [3]Koki, Y. A., [4]Musa, M. A., [5]Ismail, T. A. and [6]Halliru, H. A.

[1] Department of Microbiology, Bayero University, Kano
[2]Department of Pharmacy, Infectious Diseases Hospital, Kano
[3, 4, 5,] Pathology Department, Murtala Muhammad Specialist Hospital, Kano
[6] Medical Laboratory Science Department, Bayero University, Kano

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ABSTRACT

Fungal infections such as Aspergillosis and Candidiasis have been given less priority in the diagnosis of respiratory disorders compared to other respiratory tract infections. The research is therefore aimed at screening for *Aspergillus* and *Candida* infections among patients with pulmonary symptoms that attend Infectious Diseases Hospital, Kano between April and October, 2011, in order to ascertain the endemicity of the infection and the risk factors and groups associated with the diseases. Two hundred sputum samples were investigated by cultivation on Sabouraud's Dextrose Agar containing antibiotic and examination for the fungal elements. Fungal identification was carried out by direct microscopy and colony morphology. Dalamau plate technique and Germ-tube tests were employed to differentiate yeasts. Of the 200 samples examined, eighty subjects (40.00%) were found to be positive of which 49 (61.25%) were males and 31 (38.75%) were females; 41 (51.25%) were positive with *Aspergillus* spp where as 39 (48.75%) were found to be positive with *Candida* spp. Among the *Aspergillii, A. niger* was the most prevalent (21.25%) while among the candida; *C. albicans* was the most prevalent (35.00%). Significant association was found between the fungal vulnerability with age and prolonged antibiotic usage. *Aspergillus* and *Candida* species were found to preferentially result to or rather complicate bronchopulmonary diseases, hence fungal infections need to be explored in patients with the disorders.

INTRODUCTION

Fungal infections have emerged as world-wide healthcare problems in recent years, owing to the excessive use of broad spectrum antibiotics, long- term use of immunosuppressive agents and the increasing population of terminally ill, debilitated and immunocompromised patients (Chen *et al.,* 2003). In tune with the general trend, there has been a phenomenal rise in the occurrence of fungal lungs infections over the last two decades, a significant fraction which is community acquired and very few are capable of infecting a normal host (Chen *et al.,* 2003). Important progress has been achieved in understanding fungal pathogenicity; infection by opportunistic and pathogenic fungi in immunocompromised patients, caused by *Aspergillus* and *Candida* spp and other agents that had previously been considered to be non-pathogenic are now being encountered as a cause of human infections (David *et al.,* 2007).

Corresponding author: Adamu, S.
Department of Pharmacy, Infectious Diseases Hospital, Kano

The advent of these unusual infections has lead to reappraisal of the diagnostic tests involved in the investigation of fungal infections by isolation and identification to ensure that the most appropriate treatment is ascertained (David *et al.,* 2007). The genus *Aspergillus* has a great impact in various fields of research and many species are important as human and animal pathogens. Although the genus with more than 260 species has been studied for several centuries, the systematic is still in a state of flux (Varga and Samson, 2008). *Candida;* genus of yeasts, may be harmless commensals or endosymbionts of animal hosts including humans, but some species in wrong location can cause diseases. *C. albicans* can cause infection in humans and other animals especially immunocompromised patients (Ryan and Ray, 2004). Many species are found in gut flora, in mammalian hosts, others live as endosymbionts in insect hosts (Nguyen *et al.,* 2007). There are limited national and international studies into the burden of the diseases; made more difficult by non-standardized diagnostic criteria (Stevens, *et al*., 2003; Rita, *et al*., 2009). Even though there was found to be very few epidemiological data on serious fungal infection in Nigeria, it was estimated that over 11.8% of the population

suffer from the infection each year and over 800,000 are estimated to be affected with substantial mortality of which 144,195 are from *Candida* spp and 93,649 from Allergic Bronchopulmonary Aspergillosis (Rita *et al*., 2009). About 143 reported cases are due to fungi other than the aspergilli; a great many occur due to *Aspergillus* spp (Anuradha *et al*., 2013).
Candida spp were the commonest cause (19%) of all systemic infections; *C. albicans* remained the principle etiological agent in systemic Candidasis, followed by C. *tropicalis, C.glabrata, C. krusie and C. psuedotropicalis* respectively (Wilson et al., 1993). About 60% of cases worldwide were found to be due to *C. albicans* (Anuradha *et al*., 2013). Infections with *Aspergillus* and *Candida* spp have been among the hallmarks of the pulmonary diseases, few researches and literatures have been dedicated to these fungi, thereby indicating them in complicating the disease conditions (Gupta *et al.,* 2012); in spite of scanty researches regarding fungal pulmonary diseases generally in apparently healthy individuals, some researchers such as Anna *et al.,* (2012), Aluyi *et al.,* (2010), Dabo and Yusha'u, (2007), Mustapha *et al.,* (1997) and Rolston, (2001), among others, have given enormous contributions.

MATERIALS AND METHODS

Study Area

The study was carried out at Infectious Diseases Hospital Kano, being the reference center for pulmonary disorders such as Tuberculosis as well as HIV and AIDS, from the month of April to October 2011.

Sample Size

Two hundred (200) sputum samples were collected at random from patients 12 years and above with bronchopulmonary disorders attending the hospital's chest clinic from April to October, 2011.

Sample Collection

Subjects were given clean, dry, wide-naked and leak proof containers and requested to expectorate early morning before mouthwash as done by Baker *et al.,* (2001). The samples were then labelled and kept at 40^{o}C prior to laboratory procession as employed by Ochie and Kochatkhar, (2005). Demographic data of the recruited patients were collected through a questionnaire; consent form was administered to each patient prior to sample collection.

Microscopy

Direct microscopy of sputum samples was done to observe hypae, yeast or spore forms. Standard staining techniques and methods such as those employed by WHO, (2009) and Ochie and Kolhatchar, (2005); potassium hydroxide mounts, Indian ink and lactophenol cotton blue preparation were used.

Media Preparation

The Sabouraud's Deextrose Ager (SDA) was prepared by dissolving 47g in 1000mls of distilled water and 4 microgram per milliliter of gentamycin was added and autoclaved at 121^{o}C for 15 minutes as done by Bakare *et al.,* (2003). Corn meal agar was prepared by mixing 17g of the corn meal in 500mls of sterile water with addition of 10ml of tween 80 to add more contrast during microscopy, warmed and made up to 1000mls and then autoclaved at 121^{o}C for 15 minutes as employed by Ochie and Kolhatkhar, (2005).

Cultivation

Cultivation was carried out on SDA containing Gentamycin, it has low pH that inhibit the growth of most bacteria especially gram negative types (Madigan and Martinko, 2005). The sample was inoculated on the SDA and incubated at room temperature and at 37^{o}C for 7 days as affirmed by Baker *et al.* (2001).Isolated Yeats colonies were further inoculated on corn meal agar to differentiate their types as approved by WHO, (2009) and Ochie and Kolhatkhar (2005).

Germ Tube Test

A portion of the isolated yeast colony was picked up with the tip of sterile wire loop. And inoculated into 0.5mls of blood serum in a test tube and incubated for 2 hours at 37^{o}C. The serum yeast culture was examined microscopically at X10 and X40 objectives respectively for sprouting yeast cells which indicate the presence of *C. albicans* (Cheesbrough, 2000).

Dalamau Plate Technique

This involves the use of corn meal agar with tween 80 to differentiate yeasts by their structural differences in terms of production of pseudohypae, blastochonidia and clamydoconidia on microscopic examination (WHO, 2009). One quarter of Petri dish was streaked at 3mm intervals with sterile wireloop containing yeast colony holding the loop at 45^{o}, then covered with 22 x 22mm sterile coverslip. The setup was incubated at room temperature for 2 days. It was then examined by placing the plate without lid on the microscope stage and viewed using X10 and then X40 objectives as approved by WHO, 2009.

RESULTS

In this study, two hundred (200) sputum samples was collected from patients with pulmonary diseases and observed for *Aspergillus* and *Candida* species. 80 subjects were positive of which 49 (61.25%) were males and 31 (38.75%) were females. Forty one (41) (51.25%) subjects were found to be positive for *Aspergillus* spp out of which 23 (28.75%) were males and 18 (22.50%) were females. Thifrty nine (39) (48.75%) of the total positive subjects had candida infection of which 26 (32.50%) were males and 13 (16.25%) were females (Table 1). Table 2 indicates the distribution of *Aspergillus* and *Candida* species in positive cases. Among the *Aspergilli, A. niger* had the highest (21.25%) whereas *A. terreus* had the lowest occurrence (2.5%). In case of candida, the highest incidence was found with *C. albicans* (35.00%) where as *C. glabrata, C. pseudotropicalis* and *C. tropicalis* were 1.25% respectively, being the lowest incidences. Table 3 shows age related distribution of Aspergillosis and candidiasis in positive subjects. Age groups 21 – 30 had the highest incidence of *Aspergillus* (13.75%) and 31-40 for candidiasis (16.25%). Lower incidence of both was found in age groups 71 – 80 where *Aspergillosis* had 1.25% and Candidiasis accounts for (0.00%). The vulnerability of *Aspergillus* and *Candida* species on the basis of some risk

factors of pulmonary diseases has been illustrated (table 4). Prolonged antibiotic users were found to be more vulnerable to both *Aspergillosis* (50.00%) and *Candidiasis* (37.50%). Lower vulnerability was found with cigarette smokers in both cases; *Aspergillosis* (3.75%), *Candidiasis* (50.00%).

Table 1. **Distribution of *Aspergillus* and *Candida* Species based on sex**

Agent	Sex		Total (%)
	Males (%)	Females (%)	
Apsergillus spp	23 (28.75%)	18 (22.50)	41 (51.25)
Candida spp	26 (32.50)	13 (16.25)	39 (48.75)
Total	49 (61.25)	31 (38.75)	80 (100.00)

Table 2. Distribution of *Apsergillus* and *Candida* species among positive subjects (n=80)

Isolate	Number of Positive Cases	% Incidence
A. flavus	6	7.50
A. fumigates	7	8.75
A. nidulans	5	6.25
A. niger	17	21.25
A. terreus	2	2.50
A. versicolor	4	5.00
C. albicans	28	35.00
C. globrata	1	1.25
C. pseudotropicalis	1	1.25
C. krusei	8	10.00
C. tropicalis	1	1.25
Total	80	100

n=Total number of positive subjects

Table 3. Age related distribution of *Aspergillosis* and *Candidiasis* among positive subjects n=80

Age Group (Years)	*Aspergillus* spp	*Candida* spp	Total (%)
11 – 20	6 (7.50%)	8 (10.00%)	14 (17.50)
21 – 30	11 (13.75%)	9 (11.25%)	20 (25.00)
31 – 40	9 (11.25%)	13 (16.25%)	22 (28.00)
41 – 50	7 (8.75%)	7 (8.75%)	14 (17.50)
51 – 60	3 (3.75%)	1 (1.25%)	4 (5.00)
71 – 80	1 (1.25%)	0 (0.00%)	1 (1.25)
Total	41 (51.25%)	39 (48.75%)	80 (100.00)

n=Total number of positive subjects

Table 4. Vulnerability of *Aspergillus* and *Candida* species on the basis of some risk factors of pulmonary diseases

Risk Factor	Responses	Number of Respondents	Aspergillus spp (%)	Candida spp (%)
Tuberculosis	Y	44	14 (34.15)	15 (38.46)
	N	156	27 (65.85)	24 (61.54)
HIV	Y	29	9 (21.95)	17 (43.59)
	N	171	32 (78.05)	22 (56.41)
Cigarette Smoking	Y	17	3 (7.32)	4 (10.26)
	N	183	38 (92.68)	35 (89.74)
Antibiotic users	Y	164	40 (97.56)	30 (76.92)
	N	36	1 (2.44)	9 (23.08)
Pets ownership	Y	34	9 (21.95)	7 (17.95)
	N	166	32 (78.05)	32 (82.05)

Total number of respondents for each category = 200
Y = positive response
N = negative response

DISCUSSION

The diagnosis of pulmonary aspergillosis and candidiasis is difficult not only in terms of demonstrating the organism in clinical samples but because their isolation in sputum does not necessarily mean that a person is suffering from pulmonary disease. A good clinical correlation is therefore necessary to ascertain the clinical significance of the isolates (Ofonime *et al*., 2013). Previous studies have shown that more than 500 patients from 55 chest clinic after sputum became negative for AFB had remarkably 25% Aspergillus, also Pulmonary TB and Chronic Pulmonary Aspergillosis had similar symptoms (WHO, 2011). Of the two hundred (200) samples, 80 (40%) were found positive of which 41 (51.25%) had *Aspergillosis* and 39 (48.75%) had Candidiasis. Among the positive subjects, *Aspergillus* spp was found to be 23 (28.75%) in males and 18 (22.50%) in females. In the case of *Candida* spp, 26 (32.50%) subjects were males and 13 (16.25%) were females. Although from the overall result the number of males 41(51.25%) is greater than the number of females 39 (48.75%), sex was found to have no association with fungal infection as p-value is 0.03. This agrees with the finding of Mustapha *et al.,* (1997) that gender did not show any independent risk of developing pulmonary infections. Anna *et al.* (2012) also approved that fungal infection has no relationship with sex. *Aspergillus* and *Candida* spp had overall incidence of 51.25% and 48.75% respectively. Among the Aspergilli*; A. niger* had the highest incidence (21.25%) where as in the case of Candida*, C. albicans* was found to be the highest (35.00%). This result is higher than that found by Anna *et al.,* (2012) where *Aspergillus* spp had 15.00% and *C. albicans* 60.00% which is lower compared to the finding of Ofonime *et al*., (2013) and Ogba, *et al*., (2013) (80.00%); they supported that incidences of pulmonary mycoses may vary as a result of differences in Geographical location and weather condition of the research area.

With respect to age group, higher incidence was found in age range of 21 – 30 (13.75%) for *Aspergillus* spp and 31-40 (16.25%) for *Candida* spp respectively. Statistical analysis indicates positive correlation between the infections and age groups as $P<0.05$. This agrees with the finding of Aluyi *et al.,* (2010) and that of Ofonime *et al*., (2013) that age group 20 – 30 yielded more isolates among subjects and also in accordance with the finding of Ogba, *et al.,* (2013) who find out that age brackets 24-34 shows higher prevalence, which may be due to the fact that the age groups were at the most active stages of being infected. The vulnerability of *Aspergillus* and *Candida* species on the basis of some risk factors of pulmonary diseases was ascertained. Subjects having prolonged antibiotic usage were found to be more vulnerable to *Aspergillus* spp with overall incidence of 51.25% and *Candida* spp with incidence of (48.75%), positive relationship was found statistically between antibiotic use and fungal vulnerability ($P<0.05$) which can be supported with the theses of Rolston, (2001) and Spader and Catherine, (2010) that fungal infections to some degree appear to be related to medical treatments such as broad spectrum antibiotics.

Conclusion

High incidence (40.00%) was found in the study showing that *Aspergillosis* and *Candidiasis* are common across all age groups especially young adults; they were also found to be common among all sexes. Antibiotic usage frequently predisposes an individual to pulmonary mycosis.

Recommendation

Aspergillosis and *Candidiasis* need to be explored actively in patients with bronchopulmonary disorders as they may be the cause of or complicate the situation.

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