Medicine and Medical Sciences 2(3): 038-043, November 2014
DOI: http://dx.doi.org/10.15413/mms.2014.0104

Academia Publishing

Research Paper

Mycotic infections associated with pulmonary symptoms in patients attending infectious diseases hospital, Kano.

Accepted 14th March, 2014

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ABSTRACT

Pathogenic fungi have been given lesser consideration in the diagnosis of respiratory tract infections compared to Bacteria. This research was therefore aimed at screening for mycotic agents associated with pulmonary symptoms in patients attending Infectious Diseases Hospital, Kano, Nigeria. Two hundred sputum samples from two hundred patients were collected and investigated for mycotic infections. The samples were cultivated on sabouraud's dextrose agar (SDA) containing Gentamycin and incubated at room temperature and at 37°C for seven to fourteen days. Dalamau plate technique was employed to differentiate yeast types. Germ-tube test was used to confirm the presence of *Candida albicans.* Out of one hundred and eleven positive samples, 63.06% were males and 36.94% were females. Forty One (36.94%) were positive with *Aspergillus* species, fouty (36.04%) with *Candida* species, fifteen (13.51%) with *Geotrichum* species, ten (9.01%) with *Penicillium* species, two (1.80%) with *Alternaria* species; also *Curvularia* species (1.80%) and one subject (0.90%) was found to be positive with *Sepedonium* species. Significant association was found between fungal colonization with age and with antibiotic usage ($P<0.05$). Since the overall incidence was found to be 55.50%, it implies that fungal culture had helped in the diagnosis of fungal pulmonary disease, however, *Aspergillus and Candida* species were found more vulnerable in compounding bronchopulmonary disorders.

Key words: Infection, mycosis, pulmonary symptoms, antibiotics.

INTRODUCTION

Fungi may cause diseases of the lungs through direct infection of the pulmonary tissues, by invading pulmonary air spaces/lung cavities, or through their ability to trigger an immunological reaction when fungal material is inhaled. With the exception of *Aspergillosis,* these infections are not usually present to any significant degree in immunocompetent individuals. They are more likely to affect those who have traveled to endemic areas or as a result of opportunistic infections in patients that are immunocompromised, as a result of oncological treatments, due to immunomodulation following solid organ transplants, or HIV infection (Mandanas, 2007).

Fungal infection have emerged as a world-wide health care problem in recent years, owing to the extensive use of broad-spectrum antibiotics, long-term use of immunosuppressive agents, and the increasing population of terminally ill, debilitated and immunocompromised patients. In tune with this general trend, there has been phenomenal rise in occurrence of fungal lung infections over the last two decades, a significant fraction of which is community acquired and very few are capable of infecting a normal host (Chen et al., 2001).

Many species of fungi causes allergic reaction in humans. The most common and best described mold allergen sources belong to the taxonomic fungi in perfecti, which includes *Alternaria, Cladosporium* and *Aspergillus* species. Species of Basidiomycetes and yeasts such as *Candida albicans* are also important allergen sources (Kumar and

Saunders, 1998).

Fungal infections have been one of the notable opportunistic pathogens in Bronchopulmonary disorders. Several researches have been made with abundant literature concerning fungal respiratory diseases as cited by Gupta et al. (2012) in India; in Taiwan (Yang et al., 2006), in Fako-Cameroon Republic by Anna et al. (2012); Usman and Uba (2011); Dabo and Usha'u (2007) in Kano, Nigeria and Enwuru et al. (2008), in Enugu – Nigeria, among others.

MATERIALS AND METHODS

Study area

Two hundred sputum samples were collected from patients with pulmonary symptoms that visit the chest clinic of the Infectious Disease Hospital, Kano, Nigeria, whose ages were 12 years and above from April to October 2011 and examined for fungal pathogens.

Sample collection

Subjects were given clean, dry, wide-naked, leak proof containers and requested to cough deeply to expectorate sputum first time in the morning before mouth-washing (Baker et al., 2001). The subjects were already introduced to a questionnaire for other demography to ensure their full consent. The samples were then labeled and kept at 4°C prior to laboratory procession (Ochie and Kolhatkhar, 2005).

Cultivation

The samples were inoculated on Sabouraud's Dextrose Agar (SDA) containing Gentamycin as employed by Patrick et al. (2010) and incubated at room temperature and at 37°C for 7 to 14 days as used by Baker et al. (2001). A volume of 0.1 ml of sputum was stroken on the surface of sterile medium using a sterile wire loop as employed by John (2002); Grown colonies were subcultured on the SDA; corn meal agar was used to differentiate yeast types using Dalamau Plate Technique as affirmed by WHO (2009) and Ochie and Kolhatkhar (2005).

Germ tube test

The technique was employed for rapid identification of *C. albicans;* 0.5 ml of human serum was pipette and transferred into sterile glass test-tube. With a sterile wire loop, a portion of yeast colony from the culture was inoculated into the serum and incubated at 37°C for 2 h. Using a Pasteur pipette, a drop of the serum-yeast culture was placed on a clean glass slide and cover-sliped. The set up was examined under ×10 and ×40 objectives with the condenser closed sufficiently to give a good contrast. Sprouting yeast cells indicate the presence of *C. albians* (Cheesbrough, 2000).

Dalamau plate technique

This involves the use of corn meal agar with 1% Tween 80 to differentiate yeasts through their structural differences in terms of the nature of pseudohypae, blastoconidia, arthrospore formation and clamydospores formation microscopically. One quarter of Petri dish was used for each colony, and 3 streaks were made 3 mm apart with sterile wire loop, holding the loop at an angle of 45° and then covered with a sterile cover slip which was passed through a Bunsen flame. The set up was incubated at room temperature for 2 days. It was then examined by placing the plate without its lid on the microscope stage and using low power (×10) and high power (×40) objectives. The most characteristic morphology was found near the edge of the coverslip especially the terminal clamydoconidia of *C. albicans* (WHO, 2009).

Microscopy

Direct microscopy of sputum samples was done to observe hypae, yeast or spore forms. This provides valuable information about the fungal etiology. Various methods and staining techniques employed by WHO (2009) and Ochie and Kolhatkhar (2005) that is potassium hydroxide (KOH) mount preparation, India ink and Lactophenol cotton blue or methylene blue preparations were used.

KOH wet mount

A clean grease – free glass slide was taken and a large drop of KOH solution was placed on the slide with a Pasteur pipette. Small quantity of the specimen was transferred with a loop into the KOH drop. A clean covership was then placed over the preparation gently to avoid air bubble. The slide was kept in a moist chamber at room temperature for 15 min and then observed under ×10 and ×40 objectives (Ochie and Kolhatkhar, 2005).

Methylene blue preparation

A large drop of methylene blue was placed on a clean grease free glass slide with the help of Pasteur pipette. A small quantity of the sample was picked up with the tip of wire loop and stirred gently. In case of a colony, small portion was teased with sterile teasing needles and spread

uniformly onto the glass slide. The set up was coversliped gently in such a way that air bubble is avoided, and microscopy follows. Methylene blue used long with KOH imparts coloured background; and the fungal elements when present shows prominent refractive objects (WHO, 2009).

Indian ink preparation

The preparation was made in the center of a clean, grease free glass slide. A drop of the ink was placed on the slide and a loopful of the specimen was placed close to the drop and mixed well. A coverslip was held vertically such that one edge just touches the fluid on the slide. Keeping that edge in contact with the fluid, surface, the coverslip was gently dropped on the fluid so that air bubble was not trapped. The immediate microscopic examination follows (WHO, 2009).

RESULTS

A total of 200 sputum samples was collected from 200 patients with pulmonary disorders and examined for fungal infection. A number of 116 patients (58%) were males and 84 (42%) were females. One hundred and eleven (55.50%) of the total number of patients were found positive out of which seventy (63.06%) were males and forty- one (36.94) were females.

Table 1 shows the percentage distribution of fungal infection based on sex of the overall total positive patients, 63.06% were males and 36.94% were females. Fungal species with the highest incidence was found to be *Aspergillus* species (36.94%) and the lowest being *sepedonium* species (0.90%).

Table 2 indicates age related distribution of fungal infection among positive subjects. The age group 31-40 was found to have the highest incidence (27.92%) where as age groups 61 – 80 having the lowest (2.70%).

The percentage distribution of fungal infection in relation to marital status was also ascertained (Table 2). Higher incidence was found with married subjects (50.45%) and lowest with the separated (4.50%).

Table 4 shows the distribution of fungal infection in relation to risk factors of pulmonary diseases. Antibiotic users had the highest incidence (90.99%) where as cigarette smokers having the lowest (8.11%).

DISCUSSION

From the total of two hundred patients recruited, one hundred and sixteen (58%) were males and eighty- four (42%) were females. One hundred and eleven (55.50%) were found to be positive out of which seventy (63.06%) were males and forty one (36.94%) were females; the fungal species with highest incidence was found to be *Aspergillus* species (36.94%) followed by *Candida* spp. (36.04%) and the lowest being *Sepedonium* spp (0.90%). Gupta et al. (2012) conducted similar research with one hundred and sixty sputum samples from two hundred patients with Bronchopulmonary disorders. In their research, one hundred and nine (54.5%) were found positive of fungi with 80% *Candida* spp; *C. albicans* being (29.50%) and *Aspergillus* spp having (35.00%) incidence. The decrease in prevalences of *Aspergillus* and *Candida* species and the slight increase in the overall prevalence in this research may be due to geographical location and weather condition as affirmed by Gupta et al. (2012). In a study carried out by Anna et al. (2012), out of 200 patients with respiratory symptoms examined, *Aspergillus* spp was found in 15.00% of the patients; *A. fumigates* being 5.00%, *A. niger* and *A. flavus* being (3.00%) each and *C. albicans* was found to be 60.00%. In this research we were able to find higher incidences; with *Aspergillus* spp 36.94% among which *A. niger* had 8.50%, *A. fumigates* 3.50%, *A. flavus (*3.00%) and *C. albicans* 14.00 prevalence.

With respect to sex (Table 1), positive males were found to be seventy (63.06%) and positive females were forty-one (36.94). When analysed, the association was found to be statistically non-significant which means that fungal infection is irrespective of sex. This correlates with the finding of Mustapha et al. (1997) that gender did not show any independent risk for developing pulmonary biopsies with regards to fungal infection. Also Anna et al. (2012) found out that there is no difference in fungal infection between males and females (p=0.9048) in a study conducted on patients with Tuberculosis.

Pulmonary mycoses occur in all age groups as shown in the study (Table 2); however, age group (31- 40) was found to have higher incidence (27.9%) whereas age group (71 – 80) had the lowest (2.7%). When analysed, age distribution was found to be statistically significant ($p \leq 0.05$) which indicate that it is age related. This agrees with the findings of Aluyi et al. (2010) in the analysis of pulmonary mycoses in patients with Acquired Immunodeficiency Syndrome (AIDS) according to age groups in which they found out that nine (9) species of fungi were isolated within ages 21-30 and 8 organisms from 40-50 age groups and 3 organisms isolated from ≤20 and ≥50 age brackets (21- 45 years). Comparison can also be done with the finding of Anna et al. (2012), they found out that age groups (60-70) years have 41% incidence of *Aspergillus* infection in patients with TB infection. Possible reason given was that high incidence could be because of the depressed immunity of the elderly. The lower incidence in this study could possibly be because patients of 71-80 years were very few in number. In a study carried out in China, patients with positive cultures for *A. fumigatus* were older than those with whom no positive cultures for fungus were obtained (Bai-Ling et al., 2011).

Table 3 indicates the distribution of fungal infection on

Table 1. Percentage distribution of fungal infection based on sex.

Isolates	Sex		Total (%)
	Males (%)	Females (%)	
Aspergillus spp	23 (20.72)	18 (16.22)	41 (36.94)
Alternaria spp	1 (0.90)	1 (0.90)	2 (1.80)
Curvularis spp	2 (1.80)	0 (0.00)	2 (1.80)
Geotrichum spp	9 (8.11)	6(5.41)	15 (13.51)
Penicillium spp	7 (6.31)	3 (2.70)	10 (9.01)
Sepedonium spp	1 (0.90)	0 (0.00)	1 (0.90)
Yeasts	27 (24.32)	3 (11.71)	40 (36.04)
Total	70 (63.06)	41 (36.94)	111 (100)

Table 2. Age related distribution of fungal infection among positive subjects (n=111).

Isolates	Age Groups (Years)							Total
	11 – 20	21-30	31-40	41-50	51-60	61-70	71-80	
Aspergillus spp.	6 (5.41)	11 (9.91)	9 (8.12)	7 (6.31)	3 (2.70)	4 (3.60)	1 (0.90)	41 (36.94)
Alternaria spp.	0 (0.00)	0 (0.00)	0 (0.00)	1 (0.90)	0 (0.00)	1 (0.90)	0 (0.00)	2 (1.80)
Curvularia	1 (0.90)	0 (0.00)	0 (0.00)	1 (0.90)	0 (0.00)	0 (0.00)	0 (0.00)	2 (1.80)
Geotrichum spp	6 (5.41)	2 (1.80)	6 (5.41)	0 (0.00)	0 (0.00)	0 (0.00)	1 (0.90)	15 (13.51)
Penicillium spp	2 (1.80)	1 (0.90)	3 (2.70)	3 (2.70)	1 (0.90)	0 (0.00)	0 (0.00)	10 (9.01)
Sepedonium spp.	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	1 (0.90)	1 (0.90)
Yeasts	8 (7.21)	9 (8.12)	13 (11.71)	7 (6.31)	1 (0.90)	2 (1.80)	0 (0.00)	40 (36.04)
Total (%)	23 (20.72)	23 (20.72)	31 (27.92)	19 (17.11)	5 (4.50)	7 (6.31)	3 (2.70)	111 (100)

Table 3. Percentage distribution of fungal infection among positive subjects based on marital status (n=111).

Isolates	Marital status				Total (%)
	Married	Single	Separated	Widowed	
Aspergillus spp	13 (11.71)	19 (17.12)	3 (2.70)	6 (5.41)	41 (36.44)
Alternaria spp	1 (0.90)	0 (0.00)	0 (0.00)	1 (0.90)	2 (1.80)
Curvularia spp.	1 (0.90)	1 (0.90)	0 (0.00)	0 (0.00)	2 (1.80)
Geotrichum spp	6 (5.41)	7 (6.31)	1 (0.90)	1 (0.90)	15 (13.51)
Penicillium spp	8 (7.21)	2 (1.80)	0 (0.00)	0 (0.00)	10 (0.91)
Spedemum spp	1 (0.90)	0 (0.00)	0 (0.00)	0 (0.00)	1 (0.90)
Yeasts	26 (23.42)	8 (7.21)	1 (0.90)	5 (4.50)	40 (36.04)
Total (%)	56 (50.45)	37 (33.33)	5 (4.50)	13 (11.71)	111 (100)

the basis of marital status; married subjects had higher incidence of fungal infection (50.45%), and the separated being the lowest (4.5%). However, the relationship was found to be statistically non-significant (p=0.483). This can be because exogenous fungi are not known to be transmissible from person to person as confirmed by Riddel and Yvenne (2012).

Taking the risk factors of pulmonary diseases into consideration (Table 4), subjects having prolonged usage of antibiotics were found to have higher incidence (90.99%) and cigarette smokers had the lowest (8.11%). Statistical analysis indicates a signified relation between fungal infection and antibiotic usage ($P<0.05$) and non-significant association was found with the rest of the risk factors in the study ($P>0.05$). This could be because fungal infection to some degree appears to be related to medical treatments

Table 4. Distribution of fungal infection in relation to risk factors of pulmonary diseases (n=111).

Parameter	Response	Number of respondents	Isolates							Total (%)
			Alternaria spp.	*Aspergillus* spp.	Curvularia	*Geotrichum* spp.	*Penicillium* spp	*Sepedonium*	Yeasts	
TB	Y	44	1	14	2	5	2	0	15	39 (35.14)
	N	156	1	27	0	10	8	1	25	72 64.86)
HIV	Y	29	1	9	0	3	2	0	17	32 (28.83)
	N	171	1	32	2	12	8	1	23	79 (71.17)
Cigarette smoking	Y	17	0	3	0	0	2	0	4	9 (8.11)
	N	183	2	38	2	15	8	1	36	102 (91.89)
Antibiotic use	Y	164	2	40	2	15	10	1	31	101 (90.99)
	N	36	0	1	0	0	0	0	9	10 (9.01)
Pets owneship	Y	34	0	9	0	2	0	0	7	18 (16.22)
	N	166	2	32	2	13	10	1	33	93 (83.78)

Y=Yes (Positive responses), N = No (Negative responses), Antibiotic use ($P<0.05$), Cigarette smoking ($P>0.05$), Pets ownership ($P>0.05$).

such as chemotherapeutic agents, irradiation and broad- spectrum antibiotics as confirmed by Rolston (2001).

Conclusion

High incidence of fungal infection (55.50%) was obtained in patients with pulmonary diseases out of which 90.99% were from prolonged antibiotic users which were found to be statistically significant. *Aspergillus* and *Candida* were the predominant species among the positive subjects and therefore found to preferentially cause or help in complicating Bronchopulmonary disorders, hence, in the absence of specific predictions with regards to pulmonary symptoms, the possibility of fungal colonization needs to be explored.

Recommendation

Fungal screening in prolonged pulmonary disorders is hereby recommended as fungal infection has an overall incidence of 55.50% which indicates strong relationship between mycotic agents in Bronchpulmonary disorders.

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Cite this article as:

Taura DW, Adamu S, Koki YA, Bello BM, Muhammad MA (2014). Mycotic infections associated with pulmonary symptoms in patients attending infectious diseases hospital, Kano. Med. Med. Sci. 2(3): 038-043.

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