Prevalence Of Aspergillus Species In The Sputum Samples Of Patients With Lower Respiratory Tract Infections In A Tertiary Hospital In Enugu, Nigeria

U Maduakor, N Onyemelukwe, M Ohanu, U Okongwu, C Uchenna, I Okonkwo

Citation

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Abstract

Fungi have emerged globally as major public health concerns. These have become an important cause of respiratory tract infections. The rise in incidence has been attributed to the use of broad-spectrum antibiotics, antifungal agents, cytotoxic therapy, corticosteroid and immune-suppressants. **Objective**: To determine the mycological profile of Aspergillus species in sputum samples of patients with signs and symptoms of lower respiratory tract infection, from the University of Nigeria Teaching Hospital Ituku-Ozalla, Enugu.

Method: Sputum samples were collected from 303 patients who gave their consent. A standard questionnaire was used to record demographic variables. Samples were analyzed using a standard mycological technique.

Results: Of the 303 sputum sample, 169 fungi isolates were recovered while 134 were culture negative. Aspergillus species were 129, giving a prevalence of 42.6% and other fungi 13%. Aspergillus fumigatus ranked highest (36.1%), others were A. niger (28.4%), A. flavus and A. nidulans (3.6%), A.oryzae and A terreus (2.4%) respectively. Many of the patients were farmers and the age group with the highest number of isolates was 31-40.

Conclusion: Aspergillus spores have the tendency of colonizing damaged lung tissue and epithelia. The conidia can cause diverse clinical manifestations based on the immunity of the host. However, recovering of Aspergillus spp. from sputum does not validate it as the etiologic agent because respiratory tract colonization by Aspergillus spp. is common in several chronic lung diseases. Consistent isolation of the same Aspergillus spp is needed to confirm. Creating awareness to the public of the increasing role of fungi in human-animal diseases is necessary.

INTRODUCTION

Aspergillus species are environmental molds that are widespread in nature. They reproduce through the production of airborne spores/conidia. They are known to cause a wide range of pulmonary diseases [1]. It has been estimated that humans inhale hundreds of Aspergillus conidia daily [2]. The immunological status of the host determines the outcome of daily exposure [3]. In the normal healthy lungs, the spores are handled by the mucociliary clearance of the bronchi and are engulfed and exterminated by the macrophages and so these people may not come down with any infections or allergic symptoms [4, 5]. The polymorphonuclear neutrophils target the germinating spores and hyphae that ever succeed by degranulation and release of oxidants [6]. On the other hand, in patients with an impaired immune system, there is a risk of developing acute invasive aspergillosis characterized by hyphal invasion of the lung tissues and disseminating to other organs [1]. Approximately 10% of over two hundred species of Aspergillus are known to be pathogenic to man and interspecies variations in the antigenic response give rise to various disease spectra encountered in the clinical practice [7, 8, 9]. The most commonly isolated species is A. fumigatus [3]. In immune-competent individual, inhalation of Aspergillus spores can trigger allergic bronchopulmonary aspergillosis ABPA or hypersensitization response, while in immune-compromised status, it may lead to invasive aspergillosis (IPA) resulting in invasive life-threatening

septicemia [10, 11, 12]. IPA occurs in settings of pre-existing pulmonary disease, bronchiectasis, chronic bronchitis, asthma, tuberculosis or immune-suppression [13]. Immuno-suppression may be a risk factor for pulmonary aspergillosis due to increased use of cytotoxic drugs, irrational use of broad-spectrum antibiotics, corticosteroids and other immune-suppressants [14]. Fungal diseases are seen in many occupational groups such as physicians, laboratory workers, veterinarians, zoo employees, livestock handlers, dog owners, butchers, gardeners, agriculturists and florists [15].

Fungi have evolved as a major health problem both in developing and developed countries of the world and recent years have witnessed a sudden rise in the incidence of fungal infections because of the upsurge in immune-compromised patients [16].

Recovering of Aspergillus spp, from sputum samples does not validate it as an etiologic agent because respiratory tract colonisation by these fungi is a common occurrence in several pulmonary diseases [17]. The demonstration of fungal agents and their isolation from clinical specimens is still considered the gold standard in the diagnosis of mycotic disease [16]. Laboratory diagnosis of pulmonary aspergillosis associated diseases are not routinely done and this makes these disease undiagnosed and untreated. Diagnosis is mostly based on clinical, radiological and immunological testing [3]. There is a paucity of information on the profile of Aspergillus species in the sputum samples of patients with lower respiratory tract infection in Enugu metropolis, hence this work.

MATERIALS AND METHODS

Study Design and Population

It was a cross-sectional study on 303 patients attending the University of Nigeria Teaching Hospital Ituku- Ozalla Enugu, diagnosed with lower respiratory tract infections. They were randomly selected and seen between February and September 2018.

Inclusion Criteria

Patients with signs and symptoms suggestive of lower respiratory tract infections (cough, pleural pain, dyspnoea, etc) were included in the study

Exclusion Criteria

Patients who have taken antibiotics in the last 14 days were excluded from the study.

Ethical Clearance

The work was approved by the Ethics Committee of the University of Nigeria.

Data Collection

Data were collected from patients who gave their consent. A standard questionnaire was used to collect demographic variables and other necessary information. Early morning sputum was collected in sterile containers from all patients included in the study

Processing of Specimen

The quality of the sputum was evaluated macroscopically and microscopically as per protocol and only sputum samples that qualified were worked on [18]. KOH wet mounts were performed to check for the presence of fungal hyphae, spores and yeast cells.

Fungal culture was streaked in duplicate on Sabouraud dextrose agar, with and without chloramphenicol. One set of inoculated slants was incubated at 28oC and the other at 37oC and they were examined every other day for growth up to 21 days after which the culture was discarded and taken as negative. Any significant growth was identified based on gross appearance, colonial morphology, pigmentation, and microscopic appearance on lactophenol cotton blue preparation [19].

Statistical Analysis

All statistical analyses were performed using GraphPad Prism version 5.0. Quantitative and qualitative variables were expressed as absolute values and percentages. Chi-square or Fisher's exact test was used to test for significant association. P-value <0.05 was considered to be statistically significant.

RESULTS

In our study, sputum samples were collected from 303 patients with signs and symptoms of lower respiratory tract infections for the isolation of Aspergillus species. Data were classified based on age group, gender, and the engagements/status of patients. Table 1 shows that there was 45.5% male and 54.5% were female. The highest number of patients was from the age group 31-40 and this decreased

with increasing age and this applies to both males and females.

Table 2 shows that the distribution of the isolates, of the 169 fungal isolates, A. fumigatus (36.1%) ranked, highest followed by A. niger (28.4%), A. flavus (3.6%), A. nidulans (3.6%), A. oryzae, and A. terreus were the least (2.4%) respectively. When this was compared with the sampled population, it was found to be statistically significant p< 0.0001.

Table 3 shows the distribution of fungi based on sex, a higher number of isolates were found in females (103) when compared to the male counterparts (66) and gender was found to be a significant risk factor p< 0.0342

Table 4 shows the distribution of Aspergillus species based on the age group of patients, 31-40 years (63) ranked highest followed by 21-30years (57) and the least was 0-10 which no fungal isolate was recovered. Age was also found to be a significant risk factor p< 0.0002.

Table 5 shows the distribution of patients based on engagements, of the 169 positive cultures, 68 were found to be farmers, 32 housewives, 28 artisans and the least were the professionals.

Table 1

Age and Gender distribution of patients

AGE Group	MALE	FEMALE	TOTAL
0-10	0	0	0
11-20	18	15	33
21-30	30	39	69
31-40	47	53	100
41-50	22	25	47
51-60	10	17	23
61-70	8	10	18
71+	3	6	9
Total	138	165	303

Table 2

Distribution of fungal isolates in sputum Cultures

Organism isolated	Number of isolates
Aspergillus fumigatus	61
Aspergillus niger	48
Aspergillus flavus	6
Aspergillus nidulans	6
Aspergillus oryzae	4
Aspergillus terreus	4
Other fungi	40
Total	169

Table 3

Distribution of fungi based on gender of patients

Organism isolated	Male	Female	Total
Aspergillus fumigatus	24	37	61(36.1)
Aspergillus niger	20	28	48(28.4)
Aspergillus flavus	1	5	6(3.6)
Aspergillus nidulans	2	4	6(3.6)
Aspergillus oryzae	2	2	4(2.4)
Aspergillus terreus	2	2	4(2.4)
Other fungi	15	25	40(26.6)
Total	66	103	169

Table 4

Distribution of fungi based on age group of patients

Organism isolated	Age Group							
	0-10	11-20	21-30	31-40	41-50	51-60	61-70	71+
Aspergillus fumigatus	0	2	20	25	10	3	0	1
Aspergillus niger	0	6	18	15	4	3	2	0
Aspergillus flavus	0	0	1	2	3	0	0	0
Aspergillus nidulans	0	1	2	3	0	0	0	0
Aspergillus oryzae	0	1	1	2	0	0	0	0
Aspergillus terreus	0	0	2	2	0	0	0	0
Other fungi	0	2	13	14	4	3	1	3
Total	0	12	57	63	21	09	3	4

Table 5

Distribution of patients based on Engagement/Status

Engagement/Status	No positive
Artisans	28
Business/Traders	17
Students	13
Farmers	68
Housewives	32
Professionals	11
Total	169

DISCUSSION

In developing and developed countries of the world, fungi have emerged as a major health challenge. Recent years have witnessed a global spike in the incidence of fungal infections due to an increase in patients who are immunocompromised. Many opportunistic fungi can cause life-threatening systemic infections in patients with compromised immune system [16].

Aspergillus species are widespread saprophytic fungi, and indeed an average individual can inhale dozens of Aspergillus conidia daily due to their ubiquitous nature in the atmosphere. Aspergillosis is seen in humans and animals. Aspergillus conidia can colonize damaged lung tissue and epithelia. While such invasion often has no clinical outcome, Aspergillus spores may cause several clinical consequences based on the host's immune status. In humans, the disease is characterized by low-grade fever, productive cough, headache, chest-pain, hemoptysis besides keratitis, sinusitis, otitis, dermatitis, and meningitis [16]. Aspergillosis is an important fungal disease of public health and economic importance. Numerous factors predispose subjects to aspergillosis including asthma, cystic fibrosis, HIV/AIDS, cancer, neutropenia, and prolonged use of corticosteroids [20]. Clinical manifestations include allergic bronchopulmonary aspergillosis (ABPA), aspergilloma, chronic necrotizing pulmonary aspergillosis (CNPA), and invasive pulmonary aspergillosis (IPA) [17]. Patient to the patient transmission had also been reported [21].

In our study, 129 Aspergillus species isolates were recovered giving a prevalence of 42.6% and this was found to be statistically significant. Our work was consistent with the study of Tashiro et al., [17] in Japan that recovered 165 Aspergillus species from 139 patients. According to them, 62(45%) showed no clinical symptoms of aspergillosis but were colonized with Aspergillus spp., 77(55%) had a form of pulmonary aspergillosis, identified as either chronic necrotizing pulmonary aspergillosis (CNPA) (48%), aspergilloma (29%), IPA (13%), or allergic bronchopulmonary aspergillosis(ABPA) (10%). Maiz et al reported a lower prevalence of 24.2% and Shrimali et al., 29% [22, 23]. The high prevalence in our work is not unrelated to the type of climatic conditions, namely temperature, humidity and contaminated air currents favoring spore spread and sampled population. A. fumigatus had the highest isolation rate of (36.1%) followed by A. niger (28.4%). The work aligns with the work of many researchers [23, 17] but at variance with the work of Amiri et al., [24] that reported A flavus as the most prevalent fungi in the sputum of patients with Tuberculosis. The difference may then be due to the underlying disease. Aspergillus flavus ranked third (3.6%), the prevalence of this mold in the hospital environment like surgical instruments, bedrails, potted plants, air cooler and air conditioner dust had been reported and it is a serious risk to people with a compromised immune system or underlying diseases. The disease is known to occur in sporadic and epidemic form and has public health and economic implications [20]. Others were A. nidulans (3.6%), A. oryzae (2.4%) and A. terreus (2.4%). Many researchers had reported different isolation rates, the differences may be due to different diagnostic criteria used, regional climates, living conditions, and racial differences [25]. A fumigatus has been reported to be the principal cause of aspergillosis and it is found in immune-compromised and immune-competent individuals. Three million people are diagnosed annually with chronic pulmonary aspergillosis [16]. Many researchers believed that the recovery of Aspergillus spp. does not validate it as etiologic agents of the patients. The diagnostic value of Aspergillus spp. in sputum samples is not straight forward, mainly due to difficulties in distinguishing between colonization and disease [17].

Age-wise distribution showed that a large number of patients fall within the age group 21-40 and was consistent with the work of Shrimali et al., [23]. Many researchers reported increasing age as a risk factor [22]. This may have been as a result of continuous exposure to the immediate environment.

Gender distribution showed that females were more when compared to their male counterparts this was at variance

with the work of Shrimali et al., [23] that reported otherwise. The higher isolation rate may be a result of tradition and custom. Females in Nigeria tend to engage more in activities that expose them to spore inhalation. They make contact with areas where these fungi abound, namely, domestic chores like sweeping, making contact with grains during cooking, weeding, harvesting, gardening and other dusty jobs.

The engagement/status of patients showed that farmers had the highest isolation rate. This is not very surprising as they come in contact with fungi spores in their everyday endeavors. Aspergillus species are opportunistic and allergenic fungi and they may cause several diseases.

CONCLUSION

The prevalence of Aspergillus species in our setting was high. To protect human and animal health, careful monitoring of the evolving fungal pathogens is needed. It is necessary to raise awareness about the role of fungi in human and animal diseases. Since most of these fungal infections are life-threatening and fulminating, efforts must be made to develop a safe and effective vaccine to protect high-risk groups and vulnerable individuals from fungal diseases [20]. Early detection and timely treatment are necessary to avoid the terrible consequences of the disease. The simple and cheap gold standard method for diagnosing fungal infection remains the direct microscopic detection of microorganisms in clinical specimens and its isolation in pure culture. Further research is recommended concerning the increasing role of fungi in human and animal health.

References

1. Takazono, T , Sheppard, DC. Aspergillus in chronic lung disease. Modeling what goes on in the airways. Medical Mycolology, 2017; 55(1), 39-47.
2. Hospentha,l DR, Kwaon-Chung, KJ, Bennett, JE. Concentrations of airborne Aspergillus compared to the incidence of invasive aspergillosis: lack of correlation. Medical Mycology, 1998; 36, 165-168
3. Chortirmall, S.H, Al-Alawi, M., Mirkovic, B., Lavelle, G, Logan, PM, Greene, CM,
4. McElvancy, NG. Aspergillus- Associated Airway Disease, Inflammation, and the Innate Immune Response. Review Article, Hindawi Publishing Corporation BioMedical Research International, 2013; Article ID 723129, 14 pages http://dx.doi.org/10.1155/2013/723129
5. Patterson, TF, Thompson II, GR. Denning, DW, Fisherman, JA, Hadley, S, Herbrecht, R., ... Segal, BH. Practical guidelines for the diagnosis and management of aspergillosis: 2016 update by the Infections Diseases Society of America. Clinical Infectious Diseases, 2016; 63(4),e1-e60.
6. Baddley, JW. Clinical risk factors for invasive aspergillosis. Medical Mycology, 2011; 49 Suppl 1: S7-S12.
7. Dagenais, TR, Keller, NP. Pathogenesis of Aspergillus fumigatus in invasive aspergillosis. Clinical Microbiology Reviews, 2019; 22, 447-465
8. Greenberger, PA. 'Allergic bronchopulmonary aspergillosis' Journal of Allergy and Clinical Immunology, 2002; 110, 685-692
9. Greenberger, PA. 'Clinical aspects of allergic bronchopulmonary aspergillosis' Frontiers in Bioscience, 2003; 8, s119-s127
10. Kauffman,HE. Immunopathogenesis of allergic bronchopulmonary aspergillosis and airway remodelling. Frontiers in Bioscience, 2003; 8, e190- e196
11. Kauten, A.P, Slavin, RG. . 'Allergic bronchopulmonary aspergillosis in asthma and cystic fibrosis' Clinical and Developmental Immunology. 2011, Article ID 843763, 13 pages
12. Hope, WW, Kruhlak, MJ, Layman, CA, Petraitiene, R, Petraitis, V, Francesconi, A, ... Kelaher, AM. et al. Pathogenesis of Aspergillus fumigatus and the kinetics of galactomannan in an in vitro model of early invasive pulmonary aspergillosis; implications for antifungal therapy. The Journal of Infectious Diseases, 2007; 195 (3), 455-466
13. Thompson, GR, Patterson, TF. "Pulmonary aspergillosis: Recent Advances. In Seminars in respiratory and critical care medicine, 2011;32: (6): 673-681). Thieme Medical Publishers,
14. Ryan, KJ, Ray, CG. Medical Microbiology; McGraw-Hill, 2004;4, 370
15. Kusne S, Cisneros JT, Manez R, Irish, W, Martin M, Fung J, ... Starzi TE. Factors associated with invasive lung aspergillosis and the significance of positive Aspergillus culture after liver transplantation. Journal of Infectious Diseases, 1992; 166(6), 1379-1383
16. Pal M. Contribution of Prof . Dr. Mahendra Pal in Veterinary and Medical Mycology. 2007
17. Pal M. Morbidity and Mortality due to fungal infection. Journal of Applied Microbiology and Biochemistry, 2017; 1:2 DOI: 10: 21767/2576-1412.100002
18. Tashiro T, Izumikawa K, Tahiro, M , Takazono, T, Morinaga, Y, Yamamoto K.,... Yamamoto Y. Diagnostic significance of Aspergillus species isolated from respiratory samples in an adult pneumology ward. Medical Mycology, 2011: 49(6), 581-587
19. Maduakor U, Onyemelukwe N, Ogboi S, Ohanu M. (2017). Bacterial etiology of lower respiratory tract infection and their Antibiotic Susceptibility. American Journal of Medical Sciences 2017; 354 (5): 471-475.
20. Procop G.W, Robert, GD. Laboratory Methods in Basic Mycology. Chapter 65. In : Bailey and Scott's Diagnostic Microbiology, 10th edition, Betty A. Forbes Daniel F. Sahm, Alice S, Weissfied (Mosby), 1998; 871.
21. Pal M, Dave P, Manna, AK. Emerging role of Aspergillus flavus in humans and animal disorders. Journal of Mycopathological Research, 2014; 52(2), 211-216
22. Engel TGP, Erren E, Vanden Driessche KSJ, Melchers WJG, Reijers MH, Merko P, Verweij PE. Emerg Infect Dis. 2019: 25(4): 797-799
23. Maiz L, Vendrell M, Olveira C, Giron R, Nieto R, Martinez-Garcia MA. Prevalence and factors associated with isolation of Aspergillus and Candida from sputum in patients with non-cystic fibrosis bronchiectasis. Respiration 2015; 89:396-403
24. Shrimali GP, Bhatt, JK, Rajat R, Parmar RV, Nayak, S, Chandralekha D. Isolation of Aspergillus species from sputum samples: A study conducted in a Tertiary Care Hospital, Ahmeddbad. National Journal of Medical Research, 2013; 3, 239-291

25. Amiri MRJ, Aghali, SR, Shokohi T, Hedayati MT, Abastabar M, Aliyali, M, ... Hasanpour, H. Invasive forms of Candida and Aspergillus in sputum samples of pulmonary tuberculosis reference laboratory in Ghaemshahr, Northern Iran: An analysis of samples collected during the past 10 year. International Journal of Mycobacteriology, 2016; S179-S180
26. Xu H, Li L, Huang WJ, Wang LX, Li WF, Yuan WF. Invasive pulmonary aspergillosis in patients with chronic obstructive pulmonary disease: a case control study from China. Clinical Microbiology and Infection, 2012; 18, 403-408

Author Information

U.C. Maduakor
Department of Medical Laboratory Sciences, Faculty of Health Sciences and Technology, College of Medicine, University of Nigeria
Enugu Campus, Nigeria

N.F. Onyemelukwe
Department of Medical Laboratory Sciences, Faculty of Health Sciences and Technology, College of Medicine, University of Nigeria
Enugu Campus, Nigeria

M.E. Ohanu
Department of Medical Microbiology, College of Medicine, University of Nigeria Teaching Hospital
Itukku-Ozalla, Enugu.Nigeria

U.C. Okongwu
Department of Medical Laboratory Sciences, Faculty of Health Sciences and Technology, College of Medicine, University of Nigeria
Enugu Campus, Nigeria

C.A. Uchenna
Department of Medical Laboratory Sciences, Faculty of Health Sciences and Technology, College of Medicine, University of Nigeria
Enugu Campus, Nigeria

I.N. Okonkwo
Department of Medical Laboratory Sciences, Faculty of Health Sciences and Technology, College of Medicine, University of Nigeria
Enugu Campus, Nigeria