Mycotic Infections in Bronchopulmonary Diseases

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ABSTRACT :

Background: Recently pulmonary mycotic infections have gained increasing recognition in the field of infectious diseases and pose a difficult challenge.Since clinicians now see an increasing number of immuno-compromised patients, high index of suspicion is necessary for timely diagnosis and treatment of mycotic infections.

Aim: The study was designed to document the prevalence and sensitivity pattern of fungal pathogens associated with bronchopulmonary infections.

Material & Methods: The study includes 60 clinically diagnosed cases of bronchopulmonary infections along with 20 normal healthy individuals taken as control. Samples collected from cases and controls were processed to examine the presence of fungal pathogens and their sensitivity pattern was studied.

Results: 40 fungal pathogens were isolated. Majority male patients (70%) were affected, among them(50%) belong to the age group of (41-60 years). Among the isolated fungal pathogens Candida spp predominated(40%) followed by Aspergillus spp (32.5%).One spp of Penicillium marneffei was isolated from an HIV sero positive patient. Two non albicans Candida spp were isolated from controlgroup. Among the antifungals used all the isolates were resistant to Trimethoprim (Tr),Co-trimazine(C-m),Co-trimoxazole(Co) and Polymyxin-B(Pb). All spp of Candida, Aspergillus and Penicillium were found to be sensitive to Clotrimazole(Cc).But Fusarium and Mucor spp were resistant to all the antifungal agents used.

Conclusion: Hence a great awareness is required for selective isolation and identification of fungi along with their antifungal susceptibility pattern.

Keywords: Bronchopulmonary mycoses, antifungal susceptibility testing.

INTRODUCTION:

Though respiratory mycoses are less common than bacterial infection but the diseases have recently gained increasing importance in the field of infectious disease and they pose a difficult diagnostic challenge in many parts of the world[1].The different modes of presentation of respiratory mycoses are pyrexia of unknown origin, bronchopneumonia, fibrocavitary lesions, chronic lung abscess or a mass lesion on chest[3]. The current opinion suggests that due to wide spread of immunosuppressive treatment, newer therapeutic modalities in cancer therapy, generalized immune-suppression, the frequency of pulmonary mycoses and their systemic manifestations has gone up in India. As there is lack of sophisticated medical mycology laboratory, the diagnosis of pulmonary mycoses are more often than not likely to be missed. Since clinicians now see an increasing number of immuno-compromised patients, a high index of suspicion is necessary for timely diagnosis and treatment[2].

Keeping all these things in view, the present study was undertaken to find the incidence of fungal pathogens causing lower respiratory tract infections and their sensitivity pattern to commonly used antifungal agents.

Materials and Methods:

The study was carried out in Department of Microbiology with collaboration of Department of Pulmonary Medicine, S.C.B. Medical College & Hospital, Cuttack, Odisha. Sixty patients clinically diagnosed to have lower respiratory tract infection and 20 normal

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healthy individual (control) without any lower respiratory tract infection formed the study groups.

Samples like sputum, bronchial aspirate and bronchoalveolar lavage fluid were collected in a clean screw capped wide mouth sterile container but pleural fluid and cavitary aspirate were collected with 18 gauge needle. Patients were advised to wash their mouth vigorously with plain water before collection of sputum sample. Induced sputum was collected from control group.

Samples collected were subjected for microscopy (10% KOH, India Ink and Gram Stain) and culture on SDA (Sabouraud dextrose agar) medium with and without antibiotics in duplicate and incubated at 37^0C and 25 ^{0}C respectively. Any significant growth of fungal pathogen were further identified as per standard protocol[4].Antifungal susceptibility testing was done with 10 standard antifungal disks supplied by Himedia by disk diffusion method[5].Data Analysis in terms of simple statistical tools such as percentage was used. The ethical clearance for the study was obtained from ethical committee of SCB Medical College.

Results:

Out of 60 clinically diagnosed cases of bronchopulmonary infections, fungal pathogens were isolated in 40 cases (66.6%).Among the 40 positive cases majority were males(70%),amongst which 50% belong to the age group of 41-60 years as shown in Table-1.

	Positive Cases(n)= 40			Control(n)= 20		
Age	Male	Female	Total (%)	Male	Female	Total
0-20 years	3	0	3(7.5%)	4	1	5
21-40 years	10	4	14(35%)	4	0	4
41-60 years	13	7	20(50%)	5	2	7
61-80 years	2	1	3(7.5%)	4	0	4
Total	28(70%)	12(30%)	40(100%)	17	3	20

Table-1: Age & Sex-Wise Distribution of Culture Positive Cases

Table-2 shows among the 40 fungal isolates 16(40%) were Candida spp followed by Aspergillus spp 13(32.5%), Penicillium spp7 (17.5%), Fusarium spp 3 (7.5%) and Mucor 1 (2.5%).Out of 40 culture positive cases fungal elements could be detected by microscopy in 36 cases. In control group only 2 non-albicans Candida spp were isolated. However, unlike the cases with Candida albicans which showed plenty of pseudohyphae, in these 2 cases there were no pseudohyphae visible by Gram-stain and candidal growth was mixed with normal flora of oropharynax.

Of the Candida spp 12/16(75%) were Candida albicans. Similarly amongst Aspergillus spp majority were Aspergillus niger 5/13(38.46%).Out of Penicillium spp isolated one was Penicillium marneffei from an HIV sero positive patient.

Name of fungus Isolated	Number of Fungal Isolates			Control(n=20)
	Culture Positive Cases(n)= 40			
	Male	Female	Total (%)	
Candida spp	3	0	3(7.5%)	2
Aspergillus spp	10	4	14(35%)	0
Penicillum spp.	13	7	20(50%)	0
Fusarium spp	2	1	3(7.5%)	0
Mucor	28(70%)	12(30%)	40(100%)	0

Table-2: Distribution of Fungal isolates in Cases &Controls

Table-3 shows that all the spp of Candida, Aspergillus and Penicillum were found to be sensitive to Clotrimazole (Cc) and next to it was Ketoconazole(Kt) which was only resistant to non-albicans *Candida* spp. All the isolated fungal pathogens were resistant to Trimethoprim (Tr), Co-trimazine(Cm),Co-trimxazole(-Co) and Polymyxin-B(Pb).Besides that *Fusarium* and *Mucor spp* were resistant to all the antifungal used.

Discussion:

The developing countries like India have an enlarging population of patients with various immunodeficiencies and HIV infection. Since opportunistic mycoses are serious threat to patients, it is anticipated that these infections may break out in epidemic proportions under suitable circumstances. Beside these, a growing number of multi-drug resistant opportunistic fungi posing a more serious challenge of life threatening infection of immune compromised patients.

Current knowledge on the global distribution of respiratory mycotic infection does not depict there true prevalence. It largely reflects the geographic distribution of medical mycologists or other investigators engaged in the study of fungal diseases and their research interest[6].

In the present study of the 60 clinically diagnosed cases having lower respiratory tract infections, etiologic agent was identified in 40 patients. Our study showed lower respiratory tract fungal infections

Name of the fungus	Anti fungal discs used									
	Fu	It	Ap	Pb	Kt	Ns	Cc	Tr	Co	Cm
A. niger (5)	R	R	R	R	S	M	S	R	R	R
A.glaucus(4)	R	M	R	R	S	M	S	R	R	R
A.flavus (3)	R	M	R	R	S	M	S	R	R	R
A. terreus (1)	R	M	M	R	S	M	S	R	R	R
Penicillum spp. (6)	R	M	S	R	S	M	S	R	R	R
P. marneffei (1)	R	M	S	R	S	S	S	R	R	R
Candida spp.(3)	S	M	M	R	R	S	S	R	R	R
Candida albicans (12)	S	S	S	R	S	M	S	R	R	R
Fusarium (3)	R	R	R	R	R	R	R	R	R	R
Mucor (1)	R	R	R	R	R	R	R	R	R	R

NOTE: R – Resistant , S – Sensitive, M – Moderate

Table 3: Antifungal Sensitivity Test

were more common in the age group of 41-60 years (50%)(Table-1). Lakshmi *et al* (1972) and Shome et al (1976) have also reported similar findings [7,8]. This could be due to many factors. The most important factor of which appears to be that, this is the age group in which immunity in the body appears to be in the declining phase and immune deficiency diseases are more prevalent over patients who are most exposed to stress and strain of life that might tend to reduce the host resistance. In our study fungal infections are predominantly seen in males(70%) in comparison to females (30%)(Table-1).This may be due to outdoor work and frequent exposure to outside environment [7,8].

Maximum number of Candida spp (40%) were isolated during our study period followed by *Aspergillus*(32.5%) and *Penicillium spp* (17.57%) respectively. (Table-2).

Lakshmi *et al* (1972) reported isolation of *Aspergillus* in 40% cases[11]. The variation in the percentage of isolation of fungus could easily be explained as various places will vary with environmental and soil factors. The history of previous treatment taken by the patient is another important contributory factor to the same problem. Beside *Candida, Aspergillus* and *Penicillium spp,* the other fungus isolated were *Fusarium and Mucor.* These rare fungal pathogens now taking upper hand in causing opportunistic infection[6].

Of the Candida spp isolated 12/16(75%) were *Candida albicans.*This is in conformity with reports from Jha *et al* (1974)[9].Among the isolated *Penicillium* spp, *Penicillium marneffei* was isolated from an HIV positive patient.Very few cases have been reported earlier.To the best of our knowledge this is the first case reported from Odisha. In our study we got(32.5%) Aspergillus spp out of which *Aspergillus niger* was (38.46%), *Aspergillus glaucus*(30.76%), *Aspergillus flavus*(23.08%) and *Aspergillus terreus*(7.69%)-(Table-3). Shivananda in his study in 1992 on 825 patients with pulmonary infections found (15.39%) of isolates to be *Aspergillus spp* of these Aspergillus fumigates was(11.15%),*Aspergillus niger* was (3.2%) and *Aspergillus flavus* was(0.96%)[10].Geetalakshmi from Chennai documented pulmonary aspergillosis in 36 samples. The isolates in her study were *Aspergillus fumigates, Aspergillus niger* and *Candida spp* [11].

All spp of *Candida, Aspergillus* and *Penicillium* were sensitive to Clotrimazole(Cc) and Ketoconazole(Kt) was the next drug of choice which was only resistant in case of non albican *Candida spp*.All the isolated fungus were resistant to Trimethoprim(Tr),Co-trimoxazole(Co),Co-trimazine and Polymyxin-B(Pb) whereas *Fusarium* and *Mucor spp* were found to be resistant to all antifungal agents used [12].

Conclusion:

Since respiratory and systemic mycoses have no pathognomonic clinical or radiologic syndrome and mycological diagnostic facilities are restricted to only some of the major metropolitan centres, these diseases may be frequently confused with tuberculosis or other diseases of obscure etiology in India and other developing countries. Greater awareness and a high index of clinical suspicion are important pre-requisites for their diagnosis. Also, active collaboration of internists, pathologists, mycologists and microbiologists is advocated for their expeditious diagnosis and successful management. Further studies should focus on the development of rapid techniques for selective isolation and identification of systemic pathogenic fungi. The problem of antifungal resistance is likely to become more serious in the future as more and more patients with AIDS, bone marrow transplantation and neutropenia will require chemoprophylaxis cover against systemic fungal infections. Thus, it would be

of vital importance to intensify search for more potent and less toxic antifungal drugs.

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