

Original Research Article

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Mycological Profile of Broncho Alveolar Lavage (BAL) Samples in Pulmonary Tuberculosis Patients from Central India

Bhawana Bajare, S. S. Tankhiwale* and S. S. Zodpey (Shrikhande)

Department of Microbiology Government Medical College, Nagpur-440003, India

*Corresponding author

ABSTRACT

Keywords

Fungal infections, pulmonary tuberculosis, Bronchoalveolar lavage, Aspergillus species, Candida species, Central India

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The frequency of fungal infections in tuberculosis patients has increased in recent years. A study published in the Bulletin of the World Health Organization estimates that more than a million people develop chronic pulmonary aspergillosis after being treated for TB every year. As bronchoalveolar lavage (BAL) fluid samples are generally useful specimens in the diagnosis of invasive pulmonary aspergillosis (IPA), this study was designed to evaluate the incidence of fungal infections in pulmonary tuberculosis patients by direct microscopy and culture of BAL samples. Material and Methods: Total 171 BAL samples from patients admitted in department of Respiratory Medicine were received in the Department of Microbiology subjected to microscopy and culture. Samples were processed and identified by conventional techniques. Result: Out of 171 samples, 21 (12.28%) samples were positive in direct examination and 33(19.29%) samples were culture positive. *Candida* sp were isolated in 16 (48.48%) samples, *Aspergillus* sp from 14 (42.42%) samples, *Fusarium* sp from 2(6%) samples, *Penicillium* sp from 1 (3%) sample.

Introduction

Tuberculosis in India, as in other developing countries, is a major public health problem. Nearly 40% of the Indian population is infected with Mycobacterium tuberculosis and 1.5-2.0% of this population is suffering from radiological active disease¹. Each year, more than one million people who have been treated successfully for tuberculosis (TB) go on to

develop a deadly fungal infection that is often left untreated because it is mistaken for a recurrence of TB. A study published in the *Bulletin of the World Health Organization* estimates that more than a million people develop chronic pulmonary aspergillosis after being treated for TB every year². *Candida* species are also emerging as a potentially pathogenic fungal agent in patients with broncho-pulmonary diseases³. A large

proportion of population is suffering from tuberculosis and around one third of tuberculosis patients develop cavities in their lungs, making them vulnerable to infection with molds. Most of the time these fungal infections are not diagnosed and often mistaken for recurrence of tuberculosis. These opportunistic infections if diagnosed early can be treated effectively to prevent the progression of disease⁴. So this study was done to know the prevalence of fungal infections among pulmonary tuberculosis patients in this region and to identify the common fungi causing infection in these patients.

Materials and Methods

The present study was conducted on patients infected with Mycobacterium tuberculosis on whom the bronchoscopy was performed for some reasons, admitted in the department of Respiratory Medicine from July 2016 to December 2017. Homogenized BAL specimens were subjected to mycological study by direct microscopy by making 10% KOH mount and Gram stain. BALs were streaked on two sets of Sabourouds Dextrose Agar (SDA) after adding chloramphenicol (0.05 mg/ml) and were incubated at 25 and 37°C. Species identification for yeast and molds were done according to standard methods of identification. Any fungal growth was identified based on colony morphology, pigmentation, growth rate, lactophenol cotton blue mount (LPCB), slide culture on corn meal agar, urease test, etc. as per conventional techniques. For candida speciation, germ tube test, slide culture and hi-chrome candida chromogenic agar were used.^{5,6}

Results and Discussion

Study group comprised of a total of 171 patients having tubercular squeals like persisting cavity, collapse, fibrosis, etc.

Hundred and ten were males (64%) and 61 (36%) were females with ages ranging from 40 to 85 years (median age being 55 years) for both. Male preponderance were seen. Direct microscopic examination of BAL revealed the presence of budding yeast cells (Figure 1) and pseudo hyphae in 11 (52.38%) patients and septate hyphae with dichotomous branching in 10 (47.61) patients.

Thus in microscopy 21/171 (12.28%) were positive whereas culture reveal 33/171 (19.29%) positivity. Thus adding culture in diagnostic method we were able to detect 12 (7%) more cases. (Table 1)

Fungal culture on SDA yielded *Candida sp.* in 16 (48.48%) patients, which includes *C. albicans* in 7 (44%) patients, *C. tropicalis* in 5 (31%) patients (Figure 2), *C. glabrata* in 2 (13%) and *C. parapsilosis* in 2 (13%) patients. *Aspergillus sp.* were isolated in 14 (42.42%) patients (Figure 3), which includes *A. fumigatus* in 6 (43%) patients, *A. flavus* in 4 (29%) patients, *A. nidulance* in 2 (14%) patients and *A. niger* in 2 (14%) patients. *Fusarium sp* were isolated from 2 (6%) patients (figure.4) and *Penicillium sp* from 1 (3%) patient. (Table 2)

Though several authors have examined the incidence of infection with specific fungal agents in the context of chronic lung pathology, studies on pulmonary mycoses, as a group, have been relatively limited. In this study we report isolation of fungi in patients presenting with pulmonary tuberculosis in a tertiary care teaching hospital located in the central India.

In the present study, about 19.30% tuberculosis patients were co infected with fungal elements. This is in accordance with studies conducted by Shome *et al.*,⁷ and Babita *et al.*,⁴ who reported se 18% and 24% cases respectively. Whereas some studies

reported higher incidence of co-infection ranging from 36 to 39%^{3,8,9}. This may be due to the fact that they used sputum sample for study of fungal infections whereas we done our study on bronchoalveolar lavage which is more specific sample and less chances of contamination with oral flora.

most frequent isolate, being recovered from 44% of patients, followed by *Candida tropicalis* (31%) *Candida glabrata* (13%) and *Candida parapsilosis* (13%) which is in accordance with the studies done by Babita *et al.*,⁴ who reported isolation of *Candida albicans* in 44%. But Kali *et al.*,¹⁰ and Khanna *et al.*,⁸ reported higher percentage of 50 to 62% respectively.

In present study *Candida albicans* was the

Table 1. Comparison of Direct microscopy and culture

Morphology	Direct microscopy positive & culture positive	Direct microscopy negative & culture positive	Total Culture positive (n=33)
Budding yeast & pseudohyphae	11	5	16
Septate hyphae	10	7	17
Total= 171	21 (12.28%)	12(7%)	33(19.29%)

Fig.1

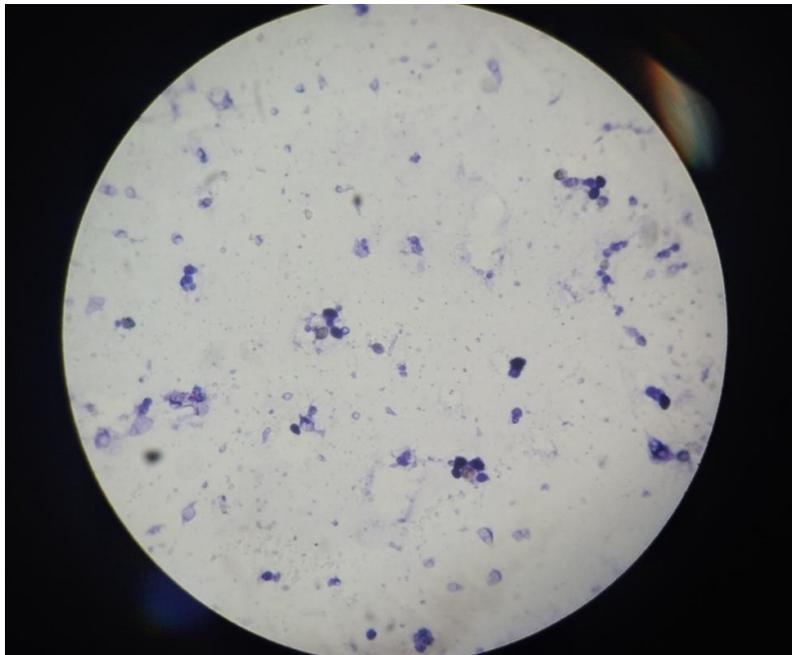


Fig.2



Fig.3

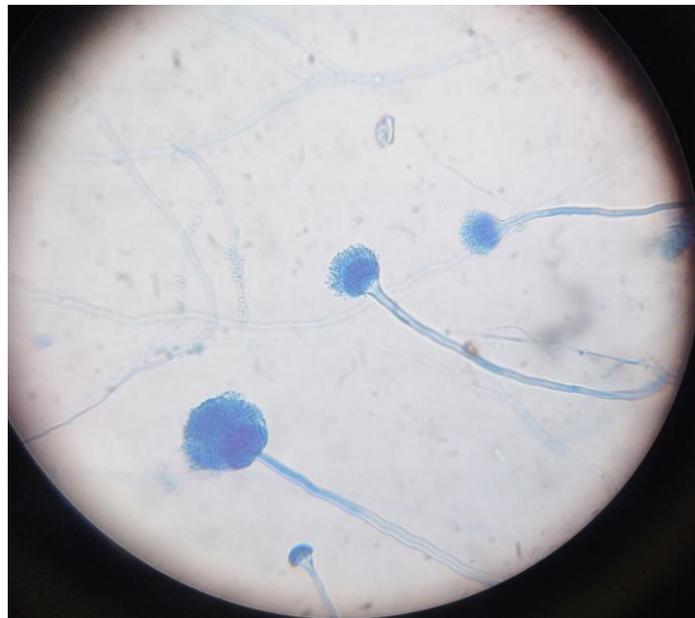


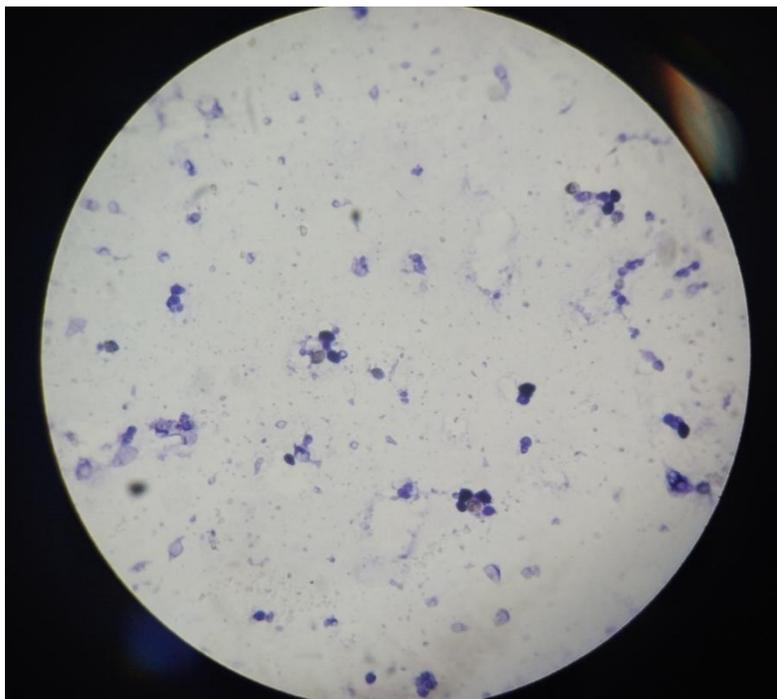
Fig.4



Fig.5



Fig.6



Among *Aspergillus* spp., *Aspergillus fumigatus* was isolated in 43% samples, followed by *Aspergillus flavus* 29% and *Aspergillus niger* 14% samples. This is in accordance with the studies done by Shahid *et al.*¹, Mathavi *et al.*,³ and Babita *et al.*,⁴ who also reported higher prevalence of *Aspergillus fumigatus* followed by *Aspergillus flavus* and *Aspergillus niger*.

We also isolated *Fusarium* spp in 2 (6%) samples. Study by Yadu, *et al.*,¹² reported *Fusarium solani* in 6.12% patients of pulmonary tuberculosis. One sample in present study also yielded growth of *Penicillium* sp. Similarly Wang, *et al.*,¹³ and Hatakayama. S. *et al.*,¹⁴ reported single case of disseminated penicilliosis in immunocompetent tubercular person and immunocompromised tubercular patient respectively.

Most common fungal infection is found to be *Candida* species by large number of workers followed by *Aspergillus* species. In

Aspergillus some reported common species as *A. fumigatus* while others found *A. flavus* species to be common. This difference in fungal isolates may be due to the epidemiological variations and climatic conditions.

As more than 19% cases of serious fungal infections are seen in treated cases of pulmonary tuberculosis patients which may lead to severe morbidity and mortality, a thorough checkup of these patients for mycosis is needed. An early suspicion and prompt treatment will save the patients.

The present study indicates that fungal etiology should also be sought in all the clinically suspected pulmonary tuberculosis patients. These secondary fungal infections are associated with persistence of lung symptoms in spite of successful completion of antituberculous drug therapy. Hence adequate measures need to be taken for the early identification and treatment of these secondary

infections which are associated with high rates of morbidity and mortality.

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