Mycological profile of Bronchoalveolar Lavage (BAL) samples in Respiratory infections in a tertiary care centre

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Abstract

Introduction: Lower respiratory tract Infections (LRTIs) remains the most common infection seen in the community and among hospitalized patients. Despite treatment, most invasive pulmonary fungal infections are associated with high mortality rates of > 50%. As Bronchoalveolar lavage (BAL) fluid samples are generally useful specimens in the diagnosis of invasive pulmonary infections, this study was designed to evaluate the incidence of fungal elements in at-risk patients by direct microscopy and culture of BAL samples.

Materials and Methods: Total 213 BAL samples received in the Department of Microbiology subjected to microscopy and culture. Samples were processed and isolates identified by standard techniques.

Results: Out of 213 samples of BAL, 34(15.96%) samples showed fungal growth. Of these, *Candida species* were isolated in 19(55.88%) samples, *Aspergillus species* in 12 (35.39%) samples, *Fusarium species* in 2(5.88%), and *Mucor species* in 1(2.94%) sample .

Conclusion: Adequate measures need to be taken for the early identification and treatment of respiratory fungal infections which are associated with high rates of morbidity and mortality. These infections if diagnosed early can be treated effectively to prevent the progression of disease.

Keywords: Bronchoalveolar lavage, Candida, Aspergillus

Introduction

Lower respiratory tract Infections (LRTIs) caused by fungi remains the most common infections seen among hospitalized patients. Despite treatment, most invasive pulmonary fungal infections are associated with high mortality rates of >50%[1,2]. Bronchoalveolar lavage (BAL) fluid samples are generally useful specimens in the diagnosis of invasive pulmonary infections. Fungal lung infections are frequently encountered by pulmonary and critical care practitioners. The increased prevalence of fungal lung infections is largely related to increased numbers of immunocompromised and susceptible patients, heightened awareness of these infections, and improved laboratory methods for the diagnosis of fungal infection[3].

Diagnosis of deep seated fungal infections in lung parenchyma can be made easier by bronchoscopy and collection of bronchial wash specimens for better isolation of fungal pathogens. Invasive fungal infections are a growing problem in critically ill patients and are associated with increased morbidity and mortality. Most of them are due to *Candida* species(*spp).* Invasive candidiasis includes candidemia, disseminated candidiasis with deep organ involvement and chronic disseminated candidiasis[4].

Other fungal respiratory infections are caused by *Aspergillus fumigatus*, followed by *A. flavus*, *A. niger* and *A. nidulans*[5]. During the last decades, rare pathogenic fungi, such as *Fusarium spp., Penicillium spp., Zygomycetes, Scedosporium, Trichoderma spp.* have also emerged.[6] Isolation of fungus from clinical specimen helps the physician to make empirical choice of antifungal drugs for treatment.

Recent years have seen an increase in opportunistic fungal infections in immuno-compromised patients[7]. Respiratory fungal infections are important cause for mortality and morbidity in these patients[8] .Hence, early diagnosis, proper understanding about agents and host factors involved will help the clinician to improve the outcome of these patients.

Thus, this study was planned to study the mycological profile of respiratory samples in a tertiary care centre.

Material and methods

The present study is a retrospective study that was performed during 5 year period (from 2017 to 2021) . Out of 414 samples obtained from several groups of different patients with pulmonary and respiratory disorders, a total of 213 BAL samples were taken in the study. Samples were obtained by a specialist physician, collected in sterile tubes and transferred to department of Microbiology GMCH, Nagpur. Homogenized BAL specimens were subjected to mycological study by direct microscopy by making 10% KOH mount and Gram stain. BAL specimens were inoculated on two sets of Sabourouds Dextrose Agar (SDA) after adding Chloramphenicol (0.05 mg/ml) and were incubated at 25°C and 37°C. Identification of yeast and molds was 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 into albicans and non albicans, germ tube test was done[9,10].

Results

A total number of 414 respiratory samples were received in department of Microbiology GMCH, Nagpur. Out of 414 respiratory samples, 213(51.44%) BAL was most common followed by 191(46.13%) sputum, 6(1.44%) Endo tracheal secretions and 2(0.48%) pleural fluid (Fig.1) .

Out of 213 BAL samples 34 (15.96%) showed fungal growth (Fig.2).Highest number of samples positive for fungal growth was 12 (35.29%) found in the year 2021 followed by 9 (26.47%) in 2020, 6 (17.64%) in 2017, 5 (14.70%) in 2018 and 2 (5.88%) in 2019.

Maximum number of positive samples were found in age group of 41 to 60 years (Fig 3). Males 19(55.88%) were more commonly affected than females 15(44.11%) (Fig 4).
Fungal infections caused by yeast 19 (55.88%) were found to be more as compared to moulds 15 (44.11%). Direct microscopic examination of BAL revealed the presence of budding yeast cells and pseudo hyphaein 15(71.42%) samples and septate hyphae (Fig.5) with dichotomous branching in 6(28.57%) samples.
Thus in microscopy 21(9.85%)were positive whereas culture reveal 34/213 (15.96%) positivity. Thus adding culture in diagnostic method we were able to detect 13(6.10%) more cases. (Table 1)
Fungal culture on SDA yielded *Candida sp.*(fig 6) in 19 (55.88%) BAL samples, which includes *C. albicans* in 16 (84.21%) and *Candida non albicans* in 3(15.78%). *Aspergillus sp*. (Fig.7) were isolated in 12 (35.29%) samples which includes *A. flavus* in 4 (33.33%)samples (Fig.8), *A. fumigatus* in 3(25%), *A. nidulans* in 3(25%) and *A. niger* in 2(16.66%) samples(Fig.8) *Fusarium spp.* were isolated from 2 (5.88%) (figure.9) and *Mucor spp.* from 1 (2.94%) sample (Fig.10)

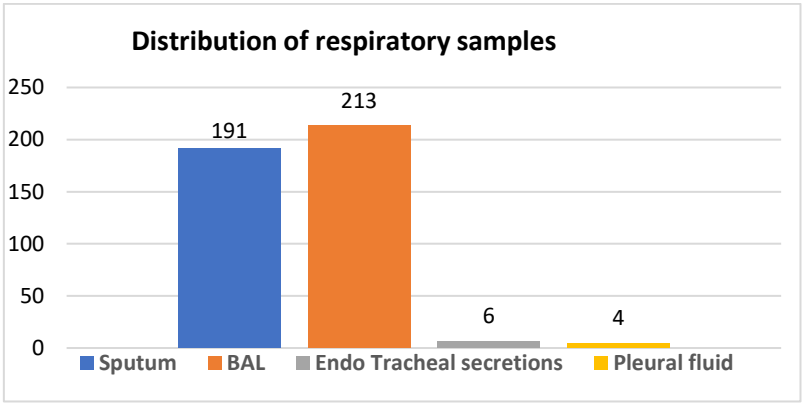

Fig 1: Distribution of respiratory samples (n=414)

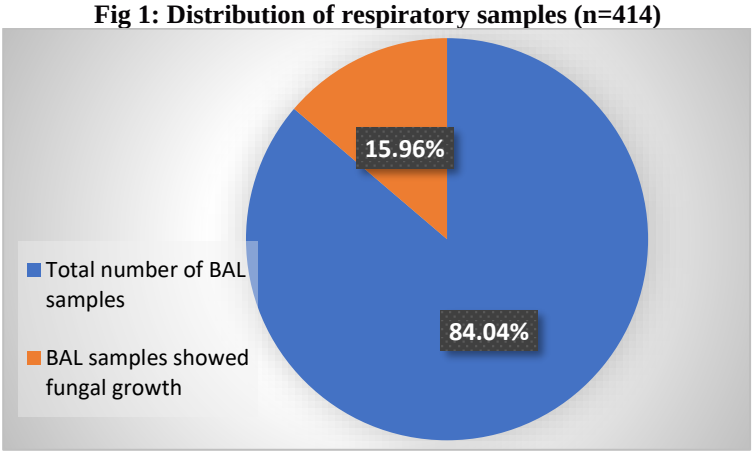

Fig 2 : Distribution of BAL samples positive for fungal

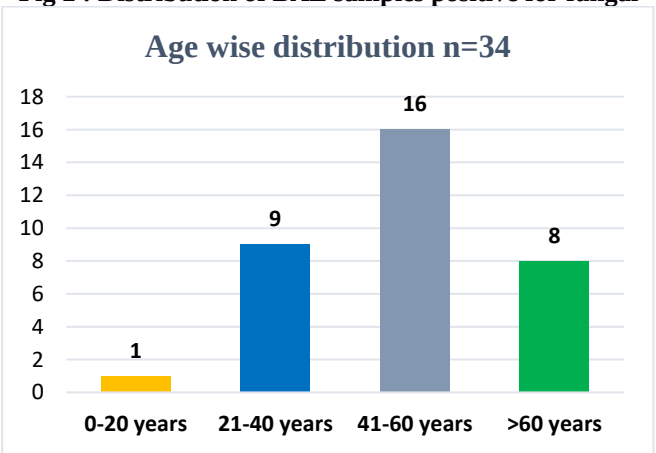

Fig 3 : Age wise distribution of BAL samples positive for fungal

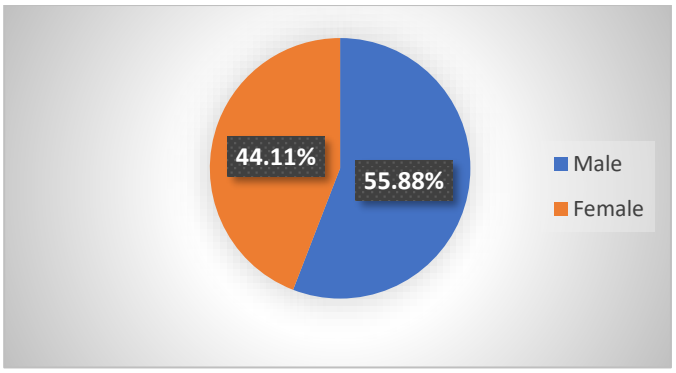

Fig 4: Gender wise distribution of positive BAL samples

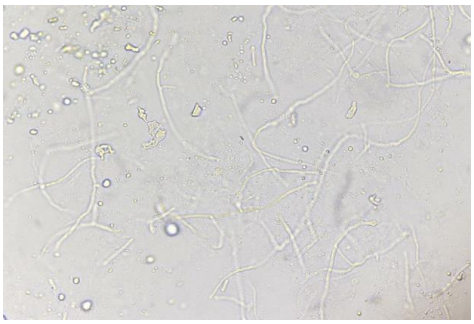

Fig 5: Direct microscopy showing fungal hyphae.

Table 1.Comparison of Direct Microscopy and Culture

Morphology	**Direct Microscopy positive & Culture positive**	**Direct Microscopy negative & Culture positive**	**Total Culture positive (n=34)**
Budding yeast & pseudohyphae	15	4	19
Septate hyphae	6	9	15
Total= 213	**21(9.85%)**	**13(6.10%)**	**34(15.96%)**

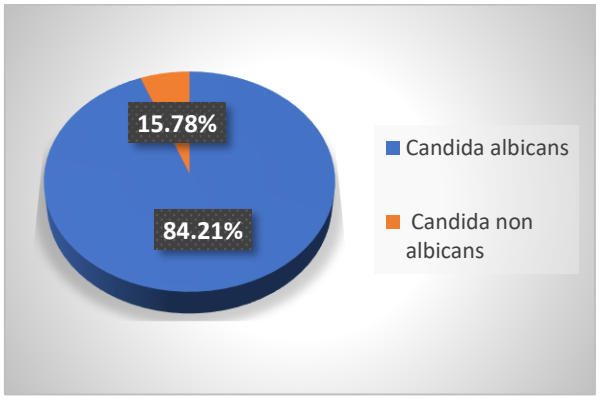

Fig 6: Distribution of yeast isolates (%)

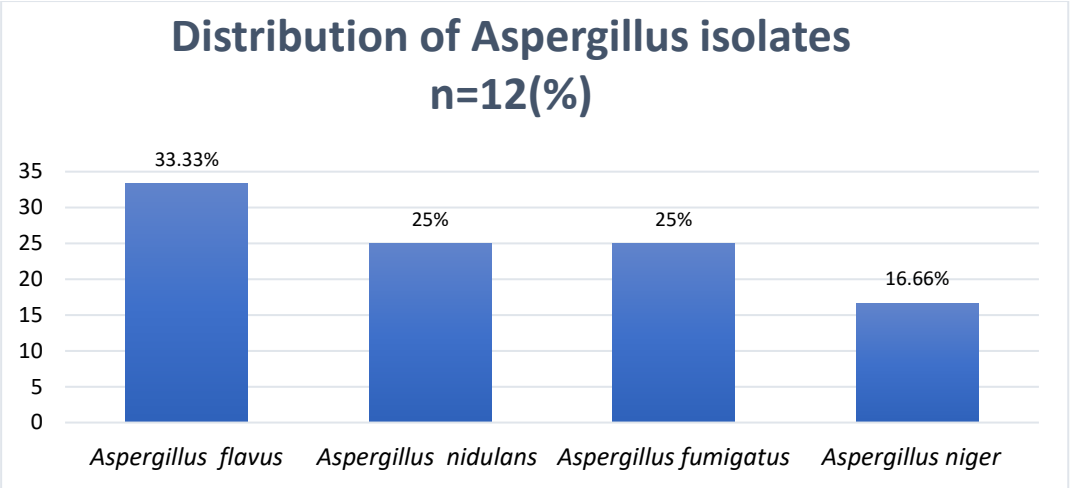

Fig 7: Distribution of Aspergillus isolates

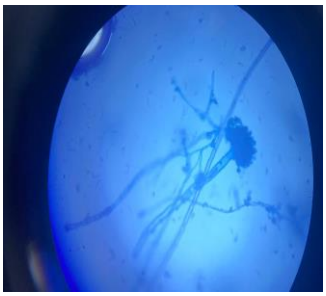

Fig 8: LPCB mount showing *A. flavus*

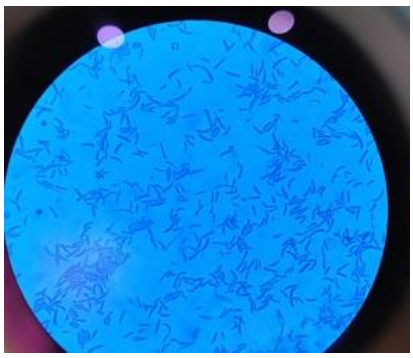

Fig 9: LPCB mount of *Fusarium spp.*

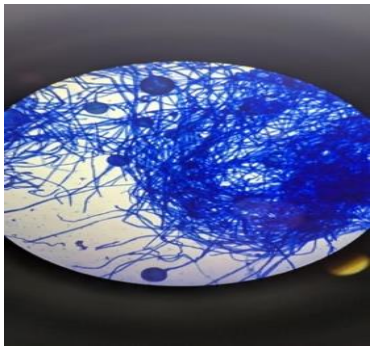

Fig 10: LPCB mount showing *Mucor spp.*

Discussion

This study comprised of 213 BAL samples out of which 34(15.96%) showed fungal growth over a period of 5 years. In retrospective study conducted by Jayaram A et al [11] over the period of 2 years showed 37% of fungal growth .

Maximum number of isolates were 12(35.29%) found in the year 2021. Maximum number of fungal positive samples were 16(47.05%) found in age group of 41 to 60 years. However, study done by Vivek KU et al [12] showed 22% cases in 51-60 years Male preponderance were seen in our study which is in accordance with studies conducted by Farooq S et al et al related to mycological profile of LRTI[13].

In this study, 21(61.76%) samples were positive on both direct microscopy and culture. Whereas 13 (38.23%) samples were positive only on culture. This is similar to study conducted by Farooq S et al [13] that showed 40.6% positive on both direct microscopy and culture and 39.5% positive on culture.

Fungal culture on SDA yielded *Candida spp.* in 19(55.88%) samples. Which included *C. albicans* in 16(84.21%) samples and *Candida nonalbicans* in 3(15.78%) samples. These findings are similar to study conducted by Sripriya CS et al [14]who showed 48.69% *Candida spp.*

In this study, *Aspergillus spp*. were isolated in 12(35.29%) samples, out of which *A. flavus* in 4(33.33%)samples were the most common which was in accordance with study conducted by Rafat et al[15] and Zarrinfar et al[16]. *A. nidulans* was found in 3 (25%)samples, *A.fumigatus* in 3(25) samples and *A. niger* in 2 (16.66%)samples.

Fusarium spp. were isolated from 2 (5.88%) samples which is in accordance to Walsh et al[6]. *Mucor spp.* 1(2.94%) was isolated. Most common fungal isolate in BAL samples was found to be *Candida spp.* by large number of workers followed by *Aspergillus spp*. Similar findings were noted in our study also. Some workers reported *A. fumigatus as the most* common species while others

found *A. flavus spp.* to be common. This difference in fungal isolates may be due to the epidemiological variations and climatic conditions.

The present study indicates that fungal etiology should be thought of in all Bronchioalveolar lavage. These fungal infections are associated with persistence of lung symptoms in spite of successful completion of drug therapy and if diagnosed early can be treated effectively to prevent the progression of disease

Hence adequate measures need to be taken for the early identification of fungal pathogens in BAL samples and treatment of respiratory infections associated with them.

Literature search suggests that there are only a very few studies related to mycological aspects of Respiratory samples. Fungal respiratory infections are increasing daily, hence more researches related to this has to be done for developing faster diagnostic techniques for better management of patients.

Conclusion

Respiratory fungal infections remain a significant cause for morbidity and mortality in the World. This study provides a basis for early detection of fungal etiology in respiratory tract infections. Early diagnosis, proper understanding of agent is important for appropriate antifungal therapy and it plays an important role to improve the outcome of these patients.

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