

Original Research Article

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Mycological Profile of Bronchial Wash Specimens in Patients with Lower Respiratory Tract Infections

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ABSTRACT

Lower respiratory tract Infections (LRTIs) remains the most common infections seen in the community and among hospitalized patients. The increased prevalence of fungal lung infections is largely seen both in immunocompromised and immunocompetent patients. Most of the fungal infections are due to *Candida* species, especially *Candida nonalbicans* and among filamentous fungi, *Aspergillus* spp is most common, caused by *Aspergillus fumigatus*, *A. flavus*, *A. niger*, *A. clavatus*, and *A. nidulans*, followed by other fungal organisms. The aim of the study is to isolate and speciate the different fungal pathogens from bronchial wash specimens from immunocompetent and immunocompromised patients with lower respiratory tract infections by conventional methods. This is a prospective study conducted for a period of 18 months from January 2015 to June 2016 at Institute of Microbiology, Madras Medical College. 488 bronchial wash specimens were collected from patients with lower respiratory tract infections. Age of patients ranged from 12 years to 80 years; 234 were males and 154 were females. Fungal growth was observed in 146 patient samples (29.9%). Yeasts were isolated in 71 samples (48.6%) and filamentous fungi were isolated in 75 samples (51.3%). Diagnosis of deep seated fungi from lower respiratory tract will be more appropriate from Bronchial wash specimens than sputum specimens as the rate of contamination with normal throat commensals is low and isolation of true pathogens is more. Early diagnosis of deep seated fungal infections leads to better control of underlying diseases and results in prolonged survival.

Keywords

Lower respiratory tract Infections (LRTIs), Bronchial wash, *Candida* species, *Aspergillus* spp.

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Introduction

Lower respiratory tract Infections (LRTIs) remains the most common infections seen in the community and among hospitalized patients. The common LRTIs include Bronchitis, Bronchiolitis, and Pneumonia and Pleural infections. Though bacterial infections of the lower respiratory tract are predominant, fungal infections occur in immunocompetent and many immunocompromised states, which predisposes to them (Velez *et al.*, 2007). 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 (Singh, 2001). 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. Bronchial wash specimens are collected at

bronchoscopy and submitted for evaluation of bronchial fluids, often as an adjunct to bronchial brushing and biopsy. Whereas the brush and biopsy specimens are directed at a specific target, the wash can provide a broader sample of the bronchial pathology, including organisms.

Bronchial washings are the secretions aspirated back through the bronchoscope channel after instillation of saline into a major airway. The secretions obtained by this method do not represent material from the bronchiolar or alveolar level. In intubated patients, bronchial washings are no different than an ETA obtained with a suction catheter (Abdolmajid *et al.*, 2006)

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* spp, especially *Candida albicans*. Invasive candidiasis includes candidaemia, disseminated candidiasis with deep organ involvement and chronic disseminated candidiasis (Paramythiotou *et al.*, 2014).

Aspergillus spores are ubiquitous in the environment and can be cultured from grain, moist soil, and vegetation, or the organism can be a laboratory contaminant. While there are more than 300 species of the organism, most human infections are caused by *Aspergillus fumigatus*, followed by *A. flavus*, *A. niger*, *A. clavatus*, and *A. nidulans* (Levy *et al.*, 1992).

During the last decades, rare pathogenic fungi, such as *Penicillium* spp, Zygomycetes, *Fusarium* species and *Scedosporium*, *Trichoderma* spp have also emerged (Walsh *et al.*, 2004).

Isolation of fungus from clinical specimen helps the physician to make empirical choice

of antifungal drugs for treatment. Factors related to patient's clinical situation and present co-morbidities, local epidemiology data and purpose of treatment (prophylactic, pre-emptive, empiric or definitive) should be taken into account for the appropriate choice of antifungal agent (Paramythiotou *et al.*, 2014).

Aims and objectives

The aim of the study is to isolate and speciate the different fungal pathogens from bronchial wash specimens from immunocompetent and immunocompromised patients with lower respiratory tract infections.

Materials and Methods

This is a prospective study conducted for a period of 18 months from January 2015 to June 2016 at Institute of Microbiology, Madras Medical College. 488 bronchial wash specimens were collected from patients with lower respiratory tract infections.

Fungal cultures and identification were performed on fresh specimens by standard procedures in microbiology laboratory. The specimens were subjected to KOH mount for initial identification and cultured on Sabouraud Dextrose Agar with antibiotics. The inoculated specimens were incubated at 37°C and 25°C.

Yeast-form fungi were identified according to standard clinical laboratory methods, including the grams staining, germ tube test and Chromagar identification. Mould-form fungi were identified using colony morphology and microscopic findings.

Results and Discussion

A total of 488 bronchial wash specimens were collected from patients with lower respiratory

tract infections. Age of patients ranged from 12 years to 80 years; 234 were males and 154 were females (Fig. 1).

Fungal growth was observed in 146 patient samples (29.9%). Yeasts were isolated in 71 samples (48.6%) and filamentous fungi were isolated in 75 samples (51.3%) (Fig. 2).

Candida non-albicans predominated yeasts, with 23 isolates of *Candida tropicalis*, 14 isolates of *Candida glabrata* and 8 isolates of *Candida krusei*. *Candida albicans* was isolated in 9 samples (Fig. 3).

Filamentous fungi were isolated in 75 (51.3%) samples. Identification was done based on macroscopic colony morphology and microscopic identification. *Aspergillus* species was the predominant fungi isolated in bronchial wash specimens, *Penicillium* spp

was isolated in 4 specimens and *Paecilomyces* spp in 4 specimens. *Rhizopus* spp, *Rhizomucor* spp, *Pseudallescheria boydii*, were isolated from one specimen each (Fig. 4).

Aspergillus fumigates was the predominant isolate among *Aspergillus* spp, with 25 *Aspergillus fumigates* isolated from bronchial wash samples. *Aspergillus flavus* was isolated from 12 samples, *Aspergillus nidulans* from 3 samples, *Aspergillus niger* from 16 samples, *Aspergillus terreus* from 6 samples and *Aspergillus versicolor* from 2 samples (Fig. 5).

Respiratory tract infections are common cause of hospitalisation. In this study we evaluated the overall prevalence of fungal infections in lower respiratory tract (Yazicioglu Mocin *et al.*, 2013)

Fig.1 Male vs. female ratio

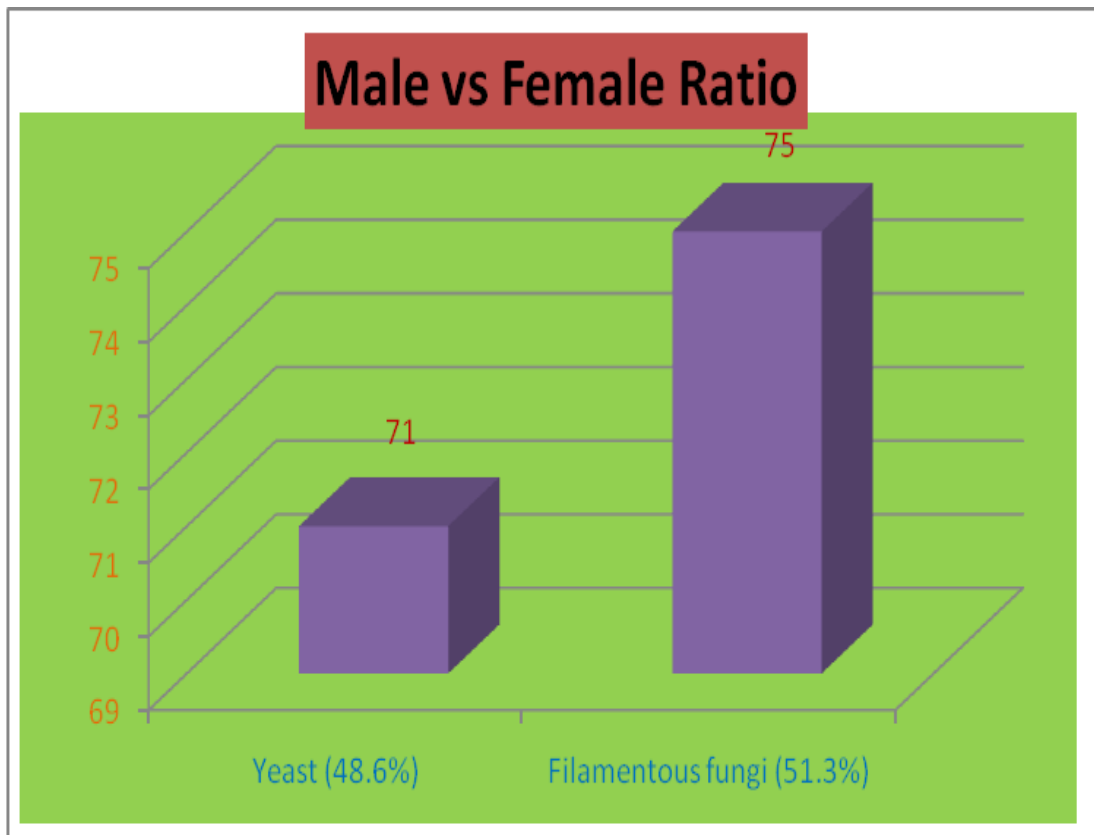


Fig.2 Fungal growth pattern in bronchial wash specimens

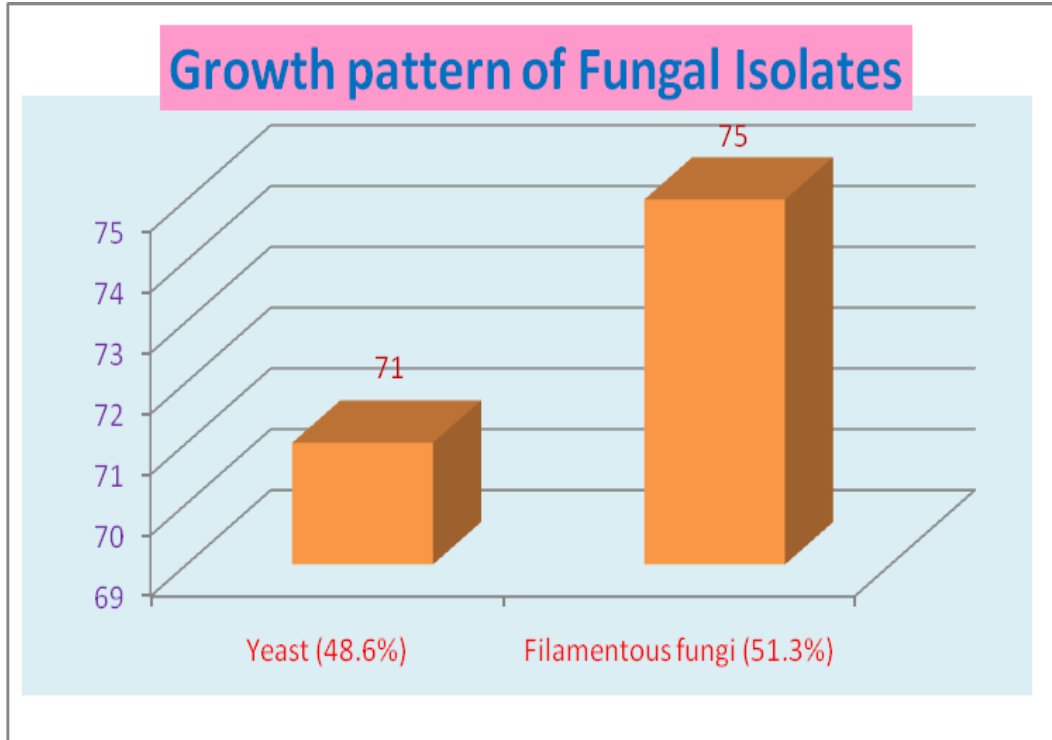


Fig.3 Yeasts identified in bronchial wash samples

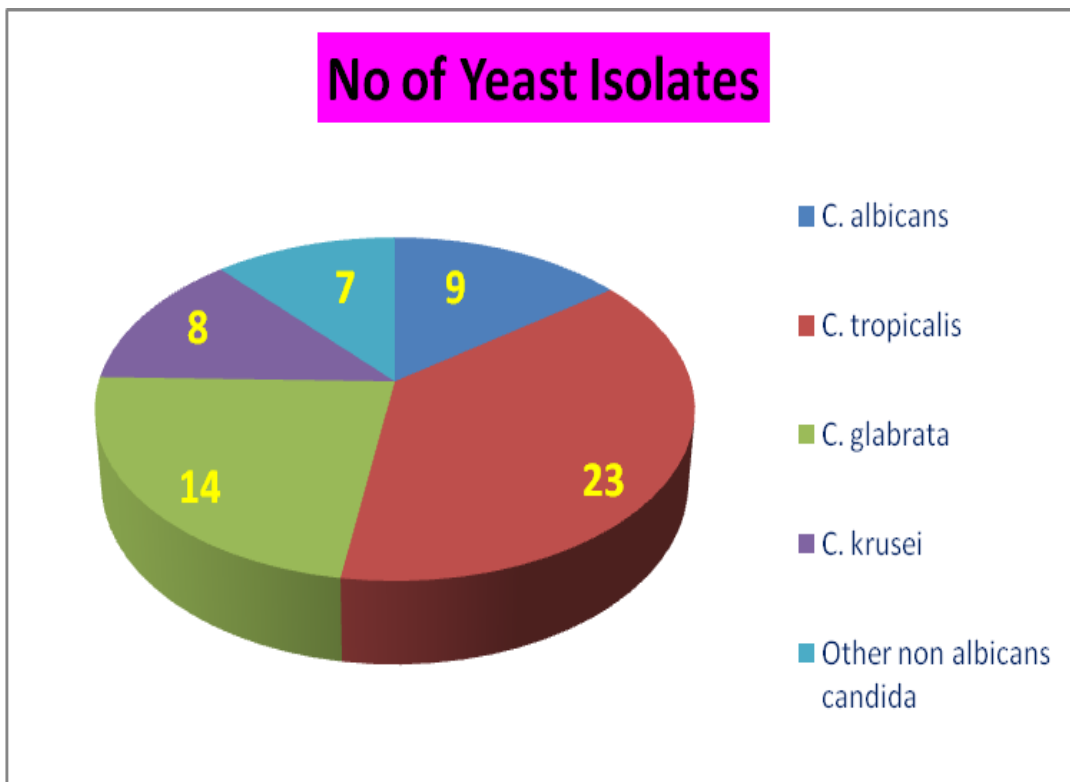


Fig.4 Distribution of other fungal isolates

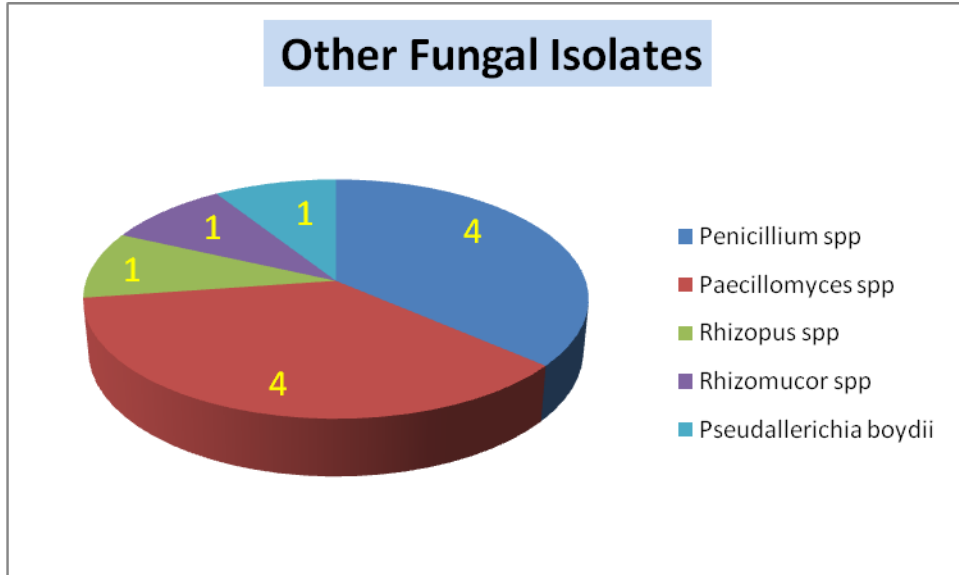
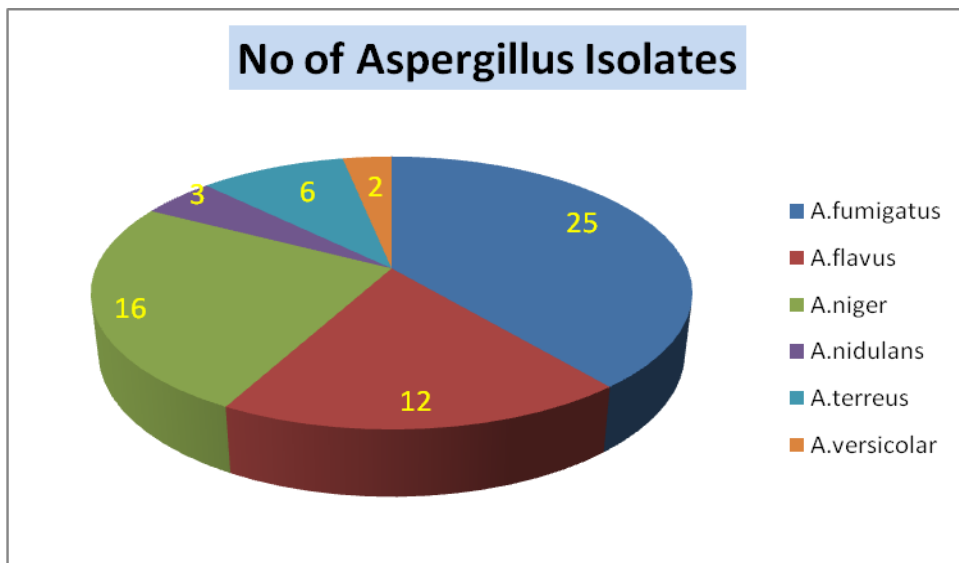


Fig.5 Distribution of *Aspergillus* species in Bronchial wash specimens



Though bacterial etiology of lower respiratory tract is more common, fungal infections are increasingly reported in both immunocompetent and immunocompromised patients.

In this study, Males were found to be more affected (47.9%) than females (31.5%). An increased incidence of yeasts was found than filamentous fungi. The increased proportion

of non-albicans species has been also observed in critically ill patients with considerable differences in the percentage of albicans vs. non-albicans spp (Falagas *et al.*, 2010). In an Italian study 40% of the fungaemia episodes were due to *C. albicans*, followed by *C. parapsilosis* (23%), *C. glabrata* (15%), *C. tropicalis* (9%) and other species (13%) (Bassetti *et al.*, 2006). Non-albicans *Candida* was found to be more in

lower respiratory tract infections than *Candida albicans* in our study.

Among the hundreds of *Aspergillus* species few are able to cause disease to humans. The most commonly encountered include *A. fumigatus* followed by *A. flavus* and *A. terreus*. The epidemiology of Aspergillosis in the ICU is difficult to establish due to the inhomogeneity of hospitalised patients, the diagnostic difficulties necessitating a biopsy and the difficulty in discriminating between colonisation and disease (Meersseman *et al.*, 2007), which was also seen from our study.

Invasive fungal infections are increasingly common among the lower respiratory tract infections in the community and nosocomial setting.

Isolating deep seated fungal infections from distal part of the lung will be of immense use in diagnosis and treatment of the same.

Diagnosis of deep seated fungi from lower respiratory tract will be more appropriate from Bronchial wash specimens than sputum specimens as the rate of contamination with normal throat commensals is low and isolation of true pathogens is more.

Furthermore, because risk-factors associated with these infections continue to increase in frequency, it is likely that the incidence of fungal infections will continue to increase in the coming decades. This expansion is based on an increase in the number of immunocompromised patients, including cancer patients with chemotherapy-induced neutropenia, transplant recipients receiving immunosuppressive therapy, and human immunodeficiency virus infected patients.

Early diagnosis of deep seated fungal infections leads to better control of underlying diseases and results in prolonged survival.

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