Mycological profile and antifungal susceptibility pattern of lower respiratory tract samples – an in vitro prospective study from a tertiary care hospital

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ABSTRACT

Introduction: Opportunistic fungal infections are increasing in recent years and the spectrum of pathogens also changing with the current condition. Identifying and isolating the deep-seated fungal infections will be useful. The samples of the deep-seated fungal infections were difficult to obtain and invasive procedures like bronchoscopy is required to collect samples from the distal part of the lungs, while samples collected from lung parenchyma is difficult. Therefore, the identification and diagnosis of these fungal infections are challenging. **Aim & Objectives:** To identify fungal pathogens from bronchial wash and bronchoalveolar lavage (BAL) specimens. To determine the antifungal susceptibility of the various isolates to amphotericin B, itraconazole,

ketoconazole, and fluconazole by disk diffusion method. **Materials and methods**: The prospective study, was conducted for a period of six months (April 2019 to September 2019) from the department of microbiology, Government Kilpauk Medical College,Chennai. A total of 100 lower respiratory tract samples were received from patients submitted for routine bronchoscopy including in-patients and out-patients, suspected of chronic non-resolving lower respiratory tract infections. All the samples were processed for the detection of fungal pathogens as per standard laboratory mycology methods. **Results:** Out of 100 samples, 10% of samples demonstrated the presence of fungi, amongst 70% was *Candida albicans* and 30% were *Candida non-albicans*. In antifungal susceptibility testing, all *Candida albicans* were susceptible to all four antifungals, while *Candida non-albicans* were susceptible to ketoconazole, itraconazole, and fluconazole while resistant to amphotericin B. **Conclusion:** Patients with deep-seated lower respiratory tract infections should be screened for the presence fungi by routine fungal identification methods in laboratories in addition to other diagnostic modalities. Bronchoscopy with BAL samples and bronchial wash is useful in diagnosing these deep-seated fungal infections using standard laboratory mycology methods, which further guides the physician in starting the early treatment with specific antifungal therapy.

Key words: Bronchoalveolar lavage (BAL)samples, Bronchoscopy, *Candida albicans* and *Candida non-albicans*.

INTRODUCTION

Globally there is a recent increase in trends of opportunistic fungal infections with a higher number of cases being reported. According to 2015 Global burden of disease, lower respiratory tract infections is one among the most common cause of death following cerebrovascular disease

and ischemic heart disease. A developing country like India, there is a variation in incidence of invasive fungal diseases. Among the opportunistic infections, the invasive fungal disease with mucormycosis, candidiasis, and aspergillosis were more common [1]. The factors contributing to the increased number of cases due to invasive fungal infections were mainly due to lower immune status such as an immunocompromised state and also, patients with lower respiratory symptoms with high clinical suspicion. These opportunistic infections requires confirmation by laboratory methods such as identification from fungal culture. Earlier, there was an underestimation of the prevalence of invasive fungal diseases worldwide [2]. Therefore the data on opportunistic fungal respiratory tract infections and its antifungal susceptibility from our demographic area were sparse, so we postulated this study to evaluate the presence of opportunistic fungi and their antifungal susceptibility in our locality [3].

One among the indications for the patient's hospitalization remains to be lower respiratory tract infections. The common infections are pneumonia, acute bronchitis, chronic obstructive pulmonary diseas (COPD), bronchiolitis,and acute bronchiectasis [4]. The bacterial infections were predominant among these infections, while fungal infections were more common in immunocompromised or immunosuppressed patients such as patients on long-term steroids, chemotherapy, splenectomy, and transplant recipients [5]. Amongst the patients admitted to the critical care units, lung infections were frequently encountered due to fungal infections. The diagnosis and identification of fungi causing fungal infections remains challenging due to the location of deep-seated fungal infections. Currently, to identify deep-seated fungal infections, lower respiratory samples such as BAL and bronchial wash were found to be useful. Identification of these fungal pathogens from such clinical specimens helps the physician to make empirical choices for the treatment [6] .

Systemic fungal infections caused by an opportunistic pathogen is associated with a more invasive disease. The clinically important opportunistic pathogens among yeasts were *Candida spp*, and molds like *Aspergillus spp*[7]. Both the above-mentioned pathogens cause invasive fungal disease in immunosuppressed patients. *Candida spp* is most commonly associated with bloodstream infections [8]. In *Candida spp* with invasive disease, therapy with amphotericin B and flucytosine was found to be useful. This therapy is particularly used in conditions such as peritonitis, meningitis, and osteomyelitis than monotherapy using fluconazole [9] .

MATERIALS AND METHODS

Study samples

In our prospective study, a total of 100 patient samples (n=100) received in the department of Microbiology, Government Kilpauk Medical College & Hospital, Chennai were collected for six months (April 2019 to September 2019). The samples included in this study were of all ages both in-patients and outpatients, irrespective of their immune status and comorbidities. The lower respiratory tract samples such as bronchoalveolar (BAL) lavage and bronchial wash of suspected fungal infections were included. Inclusion criteria was patients reported with only lower respiratory tract infections and other respiratory samples were excluded. And also patients on antifungal therapy were excluded.

Phenotypic identification

The study samples were processed in a biosafety cabinet with careful consideration and prevention to control contamination.Both the identification of fungi and culture were performed on freshly received samples by standard laboratory mycology procedures in laboratories [10]. The samples were subjected to initial identification, for the presence of fungal elements using

potassium hydroxide (KOH) mount. Followed by culturing onto SDA (Sabouraud Dextrose Agar) with antibiotic chloramphenicol, for isolation of *Candida* species and SDA without antibiotics for isolation of *Aspergillus spp*. The inoculated samples were incubated at 37ºC incubator and 25ºC incubator. The followup of yeasts includes Gram staining and germ tube test for identification [11]. The *Candida spp* were grouped into *Candida albicans* and *Candida non-albicans* based on conventional identification methods. The *Aspergillusspp* is identified based on the morphology of LPCB (lactophenol cotton blue mount).

Antifungal susceptibility testing

The fungal isolates were subjected to antifungal susceptibility testing according to document M44 of CLSI (Clinical and Laboratory Standards Institute) guidelines. *Candida spp* were tested against amphotericin B, ketoconazole, itraconazole and fluconazole by disk diffusion method. From a freshly isolated *Candidaspp* a suspension matching 0.5 McFarland for each sample, were inoculated onto the Mueller-Hinton agar(MHA) supplemented with methylene blue (0.5µg/ml), and 2% glucose. Allow the plates to air dry and using paper discs of 6.3mm diameter, made of Whatman filter paper number 2, containing amphotericin B (20 µg/µl), ketoconazole(10 µg/µl), itraconazole(10 µg/µl) and fluconazole(10 µg/µl) were used for determination of antifungal susceptibility [12].

RESULTS

Out of the 100 included samples in our study, 10 samples (10%) were found positive for fungal elements on direct KOH microscopy and Gram staining showed Gram positive budding yeasts along with pseudohyphae (Fig 1).

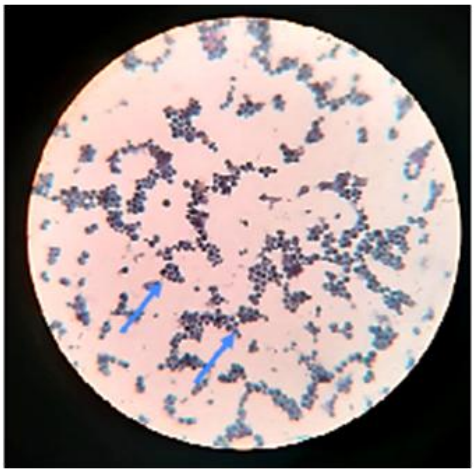

Figure 1: Gram staining shows Gram positive budding yeasts

The fungal culture of the same isolates demonstrated the growth of *Candida spp*. The colony morphology on SDA agar with chloramphenicol shows non-mucoid, cream coloured colonies (Fig 2). The predominant pathogen in our study was *Candida spp* (10%, n=10). (Fig 1 c and d). Among these *Candida albicans* and *Candida non-albicans* were 70% and 30% respectively.

Figure 2: SDA agar showing *Candida spp* colonies

The antifungal susceptibility pattern of the *Candida albicans* (n=7), all isolates were susceptible to amphotericin B (14.30 %), ketoconazole(100%), itraconazole (71.40 %) and fluconazole (85.70%). Among the *Candida non-albicans* (n=3), ketoconazole, itraconazole and fluconazole 100% susceptible while amphotericin B showed 100% resistance to all the isolates (Fig.3).

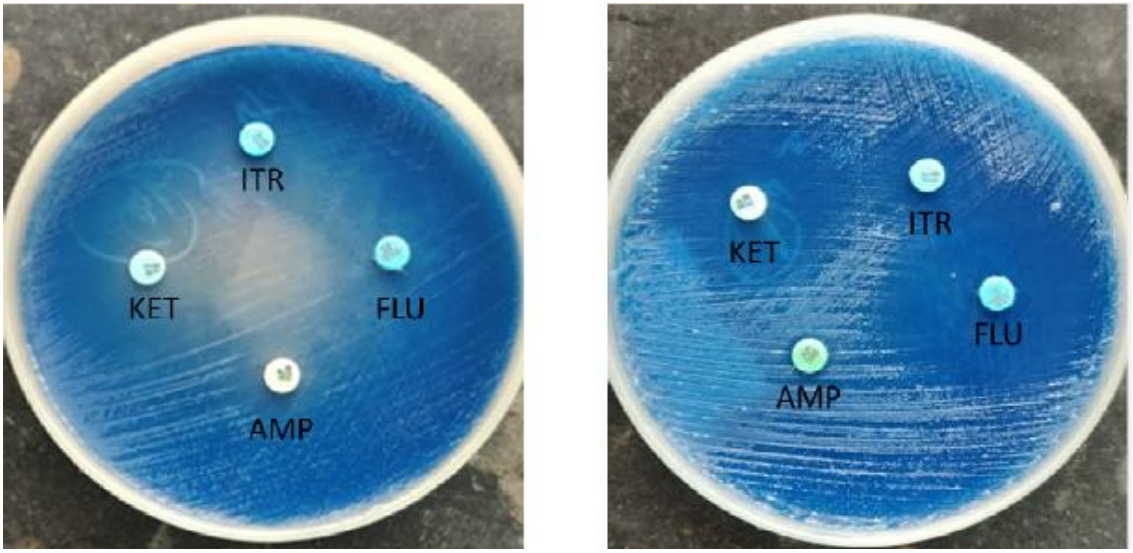

Figure 3: Antifungal susceptibility testing - *Candida albicans*&*Candida non-albicans*

DISCUSSION

Fungal infections incidence were increasing at a higher rate and with an enormous challenge to diagnose in laboratories. In a developing country like India, the common infection causing pneumonia was opportunistic fungal pathogens rather than the endemic mycoses in the geographical area. In our study, among the lower respiratory tract samples, 10% were positive for *Candida spp.* Sripriya *et al*., stated the presence of fungi (29%) in lower respiratory samples including yeasts especially *Candida spp* (48%) [13]. Identification of fungi early in the course of disease in the clinically suspected illness poses a challenge in laboratories.

The antifungal therapy has been expanded widely and allows the physician to choose old class polyenes as a treatment of choice, or the newer azoles, echinocandins as used for treatment of individual patients [14]. The right choice of choosing an antifungal treatment should be considered factors such as purpose of treatment, local and epidemiology data, patient's clinical condition, and co-morbidities.

Azole compounds were available and used for more than a decade for treatment of the invasive fungal diseases. Azoles had a profound impact on curtailing infections caused by *Candida spp* yet, long-term use of these azoles might cause a dramatic increase in azole resistance among molds and *Candidaspp* infections [15]. In our study, all *Candida spp* from the BAL and bronchial wash samples were tested for azoles sensitivity. The antifungal drugs tested were amphotericin B, ketoconazole, itraconazole and fluconazole [16].

Candidaspp is one among the common fungal pathogen of lower respiratory tract. In our study, the predominant common pathogen isolated was *Candida spp.* Kim *et al.,* stated in his study that 40% of the fungal respiratory infections were due the to *C. albicans*, followed by *C. parapsilosis* (23%), *C. glabrata* (15%), *C. tropicalis* (9%), and other *Candida* species (13%) [17].

CONCLUSION

The invasive fungal disease mainly due to *Candidaspp* was a concern in the patients admitted to critical care units and were associated with the increased morbidity & mortality. Invasive candidiasis includes invasion of blood (candidemia), and involvement of various organs (disseminated candidiasis). Fungal culture provides both speciation and identification of the pathogen. The common fungal pathogen isolated in our study was *Candida albicans* and *Candida non-albicans*. Limitation of our study was only *Candida spp* was isolated from lower respiratory infections. The identification of deep-seated fungi using BAL and bronchial wash samples rather than a sputum or induced sputum will be more appropriate as their contamination were lower with normal commensals of throat and it further helps in identifying the true pathogens. Early detection in laboratory and management of these fungal infections reduces the

burden of disease[18]. The patients samples in which fungi were not detected by laboratory methods, an empirical therapy is indicated. The patients who were diagnosed with fungal infections proven by the laboratory findings, requires a targeted or specific antifungal therapy [19].

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