

Antifungal susceptibility pattern of *Candida* species isolated from suspected cases of tuberculosis

Sharadadevi Mannur Y¹, Anusuya Devi D^{2,*}, Nagaraj ER³

^{1,3}Professor, ²Assistant Professor, Sri Siddhartha Medical College, Hospital & Research Centre, Tumkur

*Corresponding Author:

Email: annu151983@gmail.com

Abstract

Background: *Candida* species are emerging as a potentially pathogenic fungus in patients with broncho-pulmonary diseases. The synergistic growth promoting association of *Candida* and *Mycobacterium tuberculosis* has raised increased concern for studying the various *Candida* spp. and its significance in tuberculosis patients during current years.

Aim: Aim of our study was isolation and identification of *Candida* spp from tuberculosis patients; as well as testing sensitivity of these clinical isolates to commonly prescribed antifungal drugs.

Method: Totally 178 sputum specimens were screened by RNTCP Unit were included in the study. In vitro susceptibility testing of *C. albicans* isolated from pulmonary tuberculosis patients to five antifungal drugs was carried by direct susceptibility method using CHROM agar.

Results: We could able to obtain 20.2% of *Candida* strains from 178 RNTCP clinic attendees and *Candida albicans*, *Candida parapsilosis*, *Candida dubliniensis* and *Candida krusei* was identified. Overall based on our study report we could able to report 33.3%, 19.4% and 8.3% antifungal resistance by *Candida* strains towards the antifungal drugs Itraconazole, Nystatin, and Amphotericin B, respectively.

Conclusion: In this study we found a shifting pattern of epidemiology of *Candida* species from commensal to emerging pathogen. Therefore, screening of tuberculosis patients for *Candida* infection should be routinely practiced along with anti-fungal sensitivity testing for non-albicans *Candida* isolates.

Keywords: Antifungal susceptibility, azoles, *Candida*, disk diffusion, tuberculosis, Non-albicans *Candida*(NAC)

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Introduction

Tuberculosis is a chronic infectious disease caused by *Mycobacterium tuberculosis* or other pathogenic mycobacteria of the *Mycobacterium tuberculosis* complex. Tuberculosis (TB) causes significant morbidity and mortality throughout the world, particularly in developing countries in Asia and Africa.^{1,2} At present about one third of the human population is infected with *Mycobacterium tuberculosis* and every year two million persons die because of it.² Progress of the disease and prolonged treatment with antibiotics or immunosuppressive agents makes tuberculosis patients immunocompromised and hence susceptible to fungal infections.³⁻⁶

Candidiasis is one of the most frequent opportunistic infection found in human beings. The incidence rate of fungal infections rose significantly on past two decades.⁴ While *Candida albicans* is considered as primary agent of these diseases. Other species like *Candida dubliniensis*, *Candida tropicalis*,

Candida parapsilosis etc. has also been shown to produce severe systemic infection.⁵

Candida spp. is present in the oral cavity and is considered commensal fungi. *C. albicans* is the predominant species, accounting for 60 to 70% of all isolations, followed by *C. tropicalis* and *C. glabrata*.^{2,6} The incidence of *Candida* spp. in the oral cavity and the reasons for the establishment of infections caused by these microorganisms have been associated to immunosuppression, endocrine disorders, mucosal lesions, poor oral hygiene, and long-term treatment with antibiotics or corticosteroids.^{3,7}

Since *Candida* involves in opportunistic infection with debilitated persons and also causes secondary infection even among immunocompetent persons and also developing drug resistance, it is necessary to give importance to the evaluation of the drug resistant *Candida* strains present in the respiratory tract of the patients suffering from the respiratory tract infections.

In our present study, in order to evaluate the occurrence of drug resistant *Candida* present in the respiratory tract of the individuals suffering from the respiratory tract infection, we selectively chosen the RNTCP clinic attendees since these cases contains both TB + ve and TB – ve individuals with clinically proven respiratory tract infections.

There is increased concern with studying altered mycotic respiratory flora and its significance in pulmonary tuberculosis patients in current years due to this change in trends. The present study aim to focus on

the isolation of different *Candida* species present in the sputum of the RNTCP clinic attendees in order to perform the in vitro antifungal susceptibility pattern of these isolates and to screen the drug resistant *Candida* strains.

Materials and Methods

A total of 178 Sputum samples were collected from patients suspected for tuberculosis (clinically and radiologically) attended the RNTCP clinics at Shri Siddhartha Medical College, Hospital & Research Centre, Tumkur were included in this study during the period of May 2015 - December 2015. HIV-positive patients, diabetics, patients with any other systemic diseases, chronic asthma, smokers, and patients on prolonged treatment of antibiotics or corticosteroids were also included in this study.

Specimen Collection

Two sputum samples, spot sample (i.e. at the time when patient was examined) and the next day early morning sample were collected from RNTCP clinic attendees in a separate sterile container. Early morning sputum sample were collected in a sterile container after gargling mouth with sterile normal saline. Patients were instructed to collect sputum samples after the deep cough in order to correlate *Candida* infection.⁸

Direct microscopic examination

Gram's Staining: Uniform smears from purulent, muco-purulent or blood stained portion of the sputum was prepared and subjected to Gram's stain and examine for the presence of gram positive budding yeast cells with or without pseudohyphae.

Auramine-Rhodamine stain: Sputum smears were stained with Auramine-Rhodamine stain and examined under fluorescent microscopy for the presence of acid fast bacilli.

Candida isolation: The sputum sample were inoculated on to two sets of SDA with antibiotic (Chloramphenicol 50mg/lit), one set incubated at 37°C, and the other set at 25-30°C(BOD) and observed for 48-72 hrs. The typical creamy, smooth, pasty colonies with yeasty odour were noted and recorded. The isolated *Candida* strains were further identified as *Candida* species by the following methods i.e., identifying pseudo mycelium, blastospore and Chlamyospore formation on corn meal agar, germ tube production and also on growth on CHROM agar (Hi-Media, Mumbai).⁹

Antifungal susceptibility test: Antifungal susceptibility test was performed by disc diffusion method with commercially available antifungal discs- Amphotericin B 100 units, Fluconazole 25 mcg, Nystatin 100 units, Voriconazole 1 mcg and Itraconazole 10 mcg all are supplied by Hi-Media pharmaceuticals, Mumbai. Inoculum was prepared by picking five distinct colonies of *Candida* from fresh 24

hour old culture grown on SDA. Colonies are suspended in 5ml sterile 0.85% normal saline and adjusted to the turbidity to yield 10⁶ cells per ml (i.e. 0.5 McFarland standards).¹⁰

Direct susceptibility testing: The isolates were inoculated directly onto CHROM agar (Hi-Media) to identify *Candida* to species level and to predict the susceptibility to various antifungal agents has been evaluated. A sterile non-toxic cotton swab dipped in the standard inoculum and streaked the entire agar plate. Then apply the discs using aseptic technique with a distance of at least 24 mm, incubate the plates at 37°C for 24-48 hrs. If it showed insufficient growth; read only after 48 hrs. The zone of inhibition around the discs were noted and recorded.¹¹ Quality control was performed using *Candida albicans* ATCC 90028(Hi-Media, Mumbai) as reference strain.

Results

Out of the total 178 patients with suspected tuberculosis, 29 patients (16.29%) who were positive for Tuberculosis, whereas 149 (83.7%) patients were negative for Tuberculosis. Among the 178 patients with suspected pulmonary tuberculosis, *Candida spp* were isolated from 36 (20.2%) patients. *Candida albicans* was the most common isolate 26 (72.2%), followed by *C. parapsilosis* 6 (16.6%) *C.dubliniensis* 2(5.5%) and *C.krusei*.2 (5.5%). (Table 1) (Fig. 3, 4)

Candida infection was found in 71.9% male patients, while it was observed in only 28.08% of the female patients (Fig. 1). There was a significant male preponderance for occurrence of *Candida* infection.

All the patients were screened for HIV status simultaneously. Out of the total 178 patients with suspected tuberculosis, 7 patients (3.93%) were HIV reactive whereas 171(96.06%) patients were HIV non-reactive. (Fig. 2)

Candida albicans were highly susceptible to Voriconazole (72.2%), Fluconazole (72.2%) (Table 2). Amphotericin B was the next effective drug showed intermediate sensitive with 63.8% susceptibility followed by Nystatin 52.7% and Itraconazole 38.8%. *Non-albicans candida* (NAC) were highly susceptible to Voriconazole (27.7%), Fluconazole (22.2%) (Table 2)(Fig. 5). Amphotericin B was the next effective drug showed 5.5% susceptibility. Nystatin showed intermediate sensitive with 18.1% followed by Itraconazole 11.1% which showed dose dependant susceptibility.

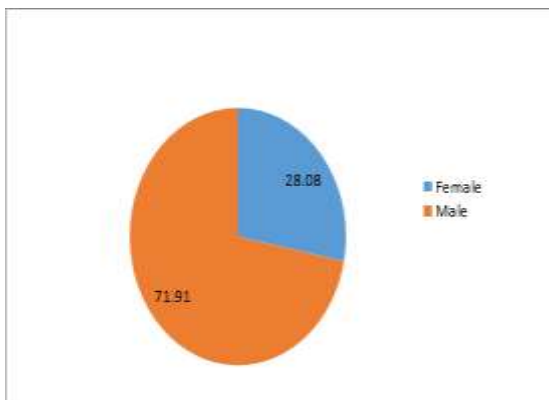


Fig. 1: Gender distribution of suspected tuberculosis patients

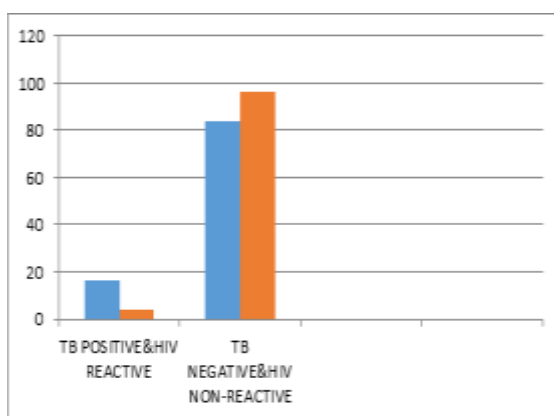


Fig. 2: Correlation between Tuberculosis & HIV status

Table1: Candida species isolated from the sputum of Tuberculosis patients

Tuberculosis status	AFB	Candida spp isolated	Candida spp not isolated
Sputum positive	AFB	4	25
Sputum negative	AFB	32	119

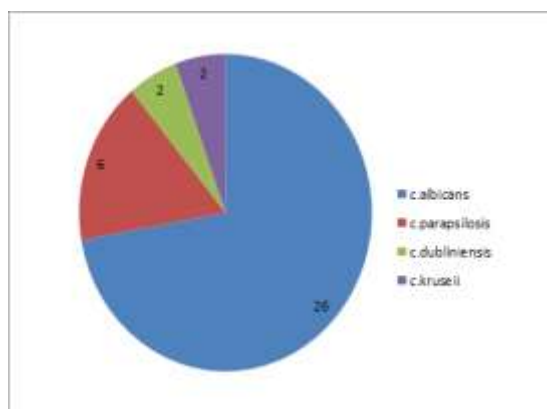


Fig. 3: Distribution of various Candida spp. from patients with tuberculosis

Table 2: Antifungal drug resistance pattern of Candida strains in percentage

Candida strains	Amphotericin B			Nystatin			Fluconazole			Itraconazole			Voriconazole		
	S	IS	R	S	IS	R	S	IS	R	S	IS	R	S	IS	R
<i>C. albicans</i> (26)	2.7	61.1	8.3	-	52.7	19.4	72.2	-	-	5.5	33.3	33.3	72.2	-	-
<i>C. parapsilosis</i> (6)	2.7	8.3	5.5	-	11.1	5.5	18.1	-	-	-	2.7	13.8	18.1	-	-
<i>C. dubliniensis</i> (2)	2.7	2.7	-	-	2.7	2.7	5.5	-	-	-	5.5	-	5.5	-	-
<i>C. kruseii</i> (2)	-	5.5	-	-	2.7	2.7	2.7	-	2.7	-	2.7	2.7	5.5	-	-



Fig. 4: Candida speciation on CHROMagar- *Candida albicans*, *C. parapsilosis*, *C. dubliniensis* and *C. kruseii*



Fig. 5: Antifungal resistance of Candida species

Discussion

Tuberculosis is well recognized for its wide range of clinical spectrum, chronicity and sequelae. Respiratory fungal infections are one of the emerging conditions complicating pulmonary tuberculosis. Weak immune status, destruction of lung tissues and lesions formed due to TB are the predisposing factors for fungal infections. Even after successful recovery from TB, prolonged treatment with antibiotics and corticosteroids makes the patients very much prone to opportunistic infections.^{12,13} Though several authors have documented *Candida* species as the most common fungal agent isolated from sputum of pulmonary tuberculosis patients, its significance has always been a matter of controversy due to the fact that up to 32.5% healthy people carry *Candida* in their throat. This can contaminate the sputum sample during collection.¹⁴ Various studies discussing prevalence of *C.albicans* in pulmonary tuberculosis patients are available.^{9,11,15} Although *Candida* infections in pulmonary tuberculosis is not well recognized, in few cases it was shown to be associated with chronic secondary infections responsible for cough, expectoration, dyspnea, anemia and fever which may prove fatal in severe cases.^{3,4,16}

Infections due to *Candida* species necessitate the use of Fluconazole, Voriconazole, or the Echinocandins because these isolates are frequently intrinsically resistant to Amphotericin B or develop resistance to Amphotericin B while the patient is on therapy and the antifungal therapy regimen needs constant vigil and requires periodical antifungal susceptibility/resistance evaluations.^{9,11}

Resistance pattern of the *Candida* species to the azoles have been discussed by Jose et al 2010¹⁵ and shown to have resistance to both azoles and Amphotericin B antifungal. So the alternative antifungal regimens are essential and the role of other combinations of antifungals to treat complicated *Candida* infections needs to be evaluated.^{18,19}

C. albicans has been reported to be the most predominant isolate from sputum of tuberculosis patients followed by *C. tropicalis*.^{2,5,16,17} Wide variation ranging from 45–92% was seen in the prevalence of *C.*

albicans in several Indian studies.^{2,5,16,17} In the present study we detected 72.2%, 16.6%, 5.5% and 5.5% prevalence of *C. albicans*, *C. parapsilosis*, *C.dubliniensis* and *C. krusei* respectively. This result is in keeping with other similar studies.^{2,5,16,20}

In this study we present evidence that *Candida* isolates inoculated directly onto CHROM agar allows the rapid identification determination of susceptibility for the majority of *Candida* isolates encountered in the clinical laboratory.

From our study we found that the significant percentage (12.3%) of the resistant *Candida* strains have been isolated from the sputum of the TB clinic attendees with both the pulmonary TB cases and patients with other respiratory tract infections without TB. Quite high level percentage (33.3%) of the resistance towards the antifungal drug Itraconazole was observed from all the *Candida* species followed by Nystatin drug (19.4%) and Amphotericin B (8.3%).

Conclusion

From our study, we could conclude that the occurrence of drug resistant *Candida* strains in the sputum/ respiratory tract of the patients with respiratory tract infections is not uncommon.

Therefore, screening of pulmonary tuberculosis patient for *Candida* infection should be routinely practiced along with anti-fungal sensitivity testing for non-albicans *Candida* isolates.

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