Original Article

Prevalence and Antifungal Susceptibility of *Candida* Infections in Patients with Pulmonary Tuberculosis: A Cross-sectional Study from the Union Territory of Puducherry, India

MAHENDRAN MURUGAVEL[1], ANUSHEELA HOWLADER[2], PRITHIVIRAJ NAGARAJAN[3], SETHUMADHAVAN KUTHALARAMALINGAM[4]

ABSTRACT

Introduction: Pulmonary Tuberculosis (TB) is a bacterial infectious disease caused by *Mycobacterium tuberculosis*. *Candida albicans* is the most commonly isolated fungal pathogen in bronchopulmonary patients. Invasive candidiasis due to *Candida* species has been on the rise in recent years. However, drug sensitivity studies of *C. albicans* isolated from TB patients are not routinely conducted in India and other developing countries.

Aim: To isolate and identify *Candida albicans* from patients with TB and to determine its antifungal susceptibility.

Materials and Methods: This cross-sectional study was conducted on 129 patients suspected of having pulmonary TB, analysed from August 2016 to January 2018 at the Department of Chest Medicine, Aarupadai Veedu Medical College and Hospital, Puducherry, India. Sputum samples were collected and processed to identify *Candida* species through Gram staining, Ziehl-Neelsen staining, germ tube formation, and sugar fermentation. Antifungal resistance testing was performed using the disk diffusion method recommended by the Clinical and Laboratory Standards Institute (CLSI). The collected data were subsequently analysed using descriptive statistics and the Chi-square test to evaluate the results.

Results: *Candida* infection was observed in 10 (7.75%) out of 129 patients with pulmonary TB. Among patients with co-infection, *C. albicans* was the most common, accounting for 4 (40%) cases, followed by *C. krusei* with 2 (20%) cases, *C. tropicalis* with 1 (10%) case, *C. kefyr* with 1 (10%) case, *C. glabrata* with 1 (10%) case, and *C. parapsilosis* with 1 (10%) case. Among the azole category, itraconazole and amphotericin B exhibited the highest sensitivity percentages against *Candida*.

Conclusion: The current study revealed a high prevalence of co-infection between pulmonary TB and *Candida* spp., which may contribute to the symptoms of pulmonary lung disorders. The prevalence of *Candida* species could also be associated with an inadequate response to antitubercular drugs. Amphotericin B and itraconazole, two commonly used drugs in the community, are often employed as the preferred treatments for fungal infections. Early detection and treatment of this opportunistic candidiasis in patients with pulmonary TB will likely enhance outcomes for such individuals.

INTRODUCTION

In recent decades, the global healthcare landscape has witnessed a concerning rise in fungal infections, especially among patients receiving medical care in hospitals [1]. These infections not only prolong hospital stays but also create significant financial burdens [2]. *Candida*, a genus of yeast, is a key contributor to approximately 70% of fungal infections within hospital settings [3]. Candidiasis, the disease caused by *Candida* species, has emerged as a major public health concern due to its high rates of morbidity and mortality [4]. Traditionally, *Candida albicans* was the primary culprit, responsible for 60-80% of fungal infections. However, Non *albicans Candida* (NAC) species are now taking the lead as predominant pathogens. This shift is partly attributed to the widespread use of antibiotics and steroids, which have contributed to the global surge in fungal infections [5].

Simultaneously, TB remains a significant global public health challenge, afflicting approximately 10.6 million individuals annually and resulting in 1.6 million deaths [6]. *Mycobacterium tuberculosis*, the causative agent of TB, imposes a substantial burden on both health and economies, particularly in developing nations, such as those in Asia and Africa [7,8]. India, notably, bears the highest burden of TB globally, with an estimated 2.64 million cases in 2019 [9]. Despite the Indian government's provision of free diagnostic and medical services for registered TB patients, research highlights the substantial financial strain and potential catastrophic costs associated with TB [10].

Furthermore, individuals with TB, especially those with pulmonary cases, have been observed to have associations with various *Candida* species [11]. Patients who are on immunosuppressive medications or have a history of such treatments are particularly vulnerable to *Candida* infections. *Candida* consists of approximately 200 species, with the most frequently implicated in clinical infections being *C. albicans, glabrata, tropicalis, stellatoidea, parapsilosis, ciferri, guilliermondii, haemulonii, kefyr*, and *krusei* [12]. The primary culprits in candidiasis are typically five prevalent species: *Candida albicans, Candida glabrata, Candida parapsilosis, Candida tropicalis*, and *Candida krusei* [13].

The treatment of candidiasis commonly involves pharmaceuticals such as amphotericin B and fluconazole, which are classified as polyenes and azoles, respectively [14]. *Candida* species exhibit varying susceptibilities to antifungal agents, with some, like *C. krusei* and *C. glabrata*, displaying resistance or reduced sensitivity to fluconazole [15]. This underscores the importance of accurately identifying and testing susceptibility to select the most effective antifungal treatment [16]. Moreover, it is worth noting that even individuals with robust immune systems can experience invasive candidiasis.

This is especially relevant in the context of TB, as it compromises the immune response, heightening susceptibility to fungal infections [17]. Consequently, studying the types of *Candida* species and their antifungal susceptibility in clinical isolates has become crucial for managing *Candida* infections. While numerous studies worldwide, including various regions of India, have investigated the speciation and antifungal susceptibility of *Candida* isolates [18,19], there is a notable gap in research examining the prevalence of *Candida* species across all hospital wards and specimen types in a tertiary care center. Additionally, more rigorous and systematic investigations are needed to assess the prevalence of pulmonary TB among patients in the Union Territory of Puducherry. The aim of this study is to isolate and identify *Candida albicans* from TB patients and determine its antifungal susceptibility.

MATERIALS AND METHODS

The present cross-sectional study was conducted at Aarupadai Veedu Medical College and Hospital in Puducherry, India, specifically in the Department of Microbiology and the Department of Chest Medicine. Permission from the Institutional Human Ethical Committee (IEC) was obtained. Data collection took place between August 2016 and January 2018. Informed written consent was obtained from the subjects who participated in the study, with individuals under the age of majority requiring parental or legal guardian authorisation after receiving appropriate information.

Inclusion criteria: Patients aged 21 to 60 years, all genders, including transgender individuals, known and previous TB patients were included in the study.

Exclusion criteria: Patients under antifungal therapy, patients undergoing chemotherapy for malignancy, patients who refused to participate in the study were excluded from the study.

Sample size estimation: The sample size for the study was determined to be 129 based on a 95% confidence interval, a margin of error of 4%, a Z-score of 1.96, and a prevalence rate of 4.03% [20], using the following formula:

The formula

$$n \geq \frac{Z^2_{1-\alpha/2}P(1-P)}{d^2}$$

can be utilised to derive the sample size (n). In this context, the variables are defined as follows: Z represents the Z-score, P represents the prevalence rate, Q corresponds to one minus the prevalence rate, and d represents the margin of error.

Study Procedure

The focus of the research was on patients hospitalised in the chest medicine ward who exhibited symptoms such as cough, fever, dyspnoea, and expectoration. Before collecting sputum samples, complete information about the study was provided to the patients at AVMC&H, and specific consent was obtained from each patient. A total of 129 patients suspected of having pulmonary TB were analysed (101 males and 28 females) in age groups ranging from 21 to 60 years during the study period.

Sample collection: The collected sputum samples were processed using Gram stain, Ziehl-Neelsen stain, KOH mount, and cultured on blood agar and MacConkey agar. To confirm TB in patients, sputum samples were examined for acid-fast tubercle bacilli. Further investigations were conducted on the 129 suspected TB patients who were also suspected of having fungal infections. Positive samples underwent Gram staining and microscopic examination to confirm these suspected fungal infections. Under the microscope, the presence of yeast cells and filaments consistent with fungal morphology provided evidence of co-existing fungal infections in these TB patients, characteristic of fungal morphology.

Isolation and identification of *C. albicans*: Clinical samples underwent cultured on Sabouraud Dextrose Agar (SDA) and incubated at both 25°C and 37°C. These cultures were examined daily for microbial growth and subsequently subcultured on SDA. The subcultures were incubated at 37°C for 24 hours. On SDA, *Candida* species produced creamy, smooth, pasty, and convex colonies within 24 to 72 hours. Some species required more than three days to become visible on the culture medium. The isolated colonies obtained on SDA were further subjected to Gram staining to identify budding yeast cells and pseudohyphae. Conventional methods, including the Germ tube test, demonstration of chlamydospore formation on Cornmeal agar with Tween 80, sugar fermentation tests, and sugar assimilation tests, were employed for speciation [21].

Candida isolates were subcultured on HI Chrome agar plates for further identification and incubated at 37°C for 48 hours. The colonies were identified based on the colour produced according to the manufacturer's instructions [22], resulting in the following identifications:

- *Candida albicans*: Light green-coloured smooth colonies.
- *Candida tropicalis*: Blue to metallic blue-coloured raised colonies.
- *Candida glabrata*: Cream to white smooth colonies.
- *Candida krusei*: Purple fuzzy colonies.
- *Candida parapsilosis*: Light pink colonies.

Antifungal susceptibility testing: Antifungal susceptibility testing was performed using the disk diffusion method according to the CLSI (formerly NCCLS) 2009 M44-A2 guidelines. Commercially available 6 mm antifungal discs (Himedia, Mumbai, India), including fluconazole (25 μg), voriconazole (1 μg), amphotericin B (20 μg), itraconazole (10 μg), and ketoconazole (30 μg), were used [23]. Mueller-Hinton agar supplemented with 2% glucose and 0.5 mg/L methylene blue was utilised as the medium. The inoculum was taken from the SDA tubes using a sterile swab and standardised to a 0.5 McFarland turbidity. Susceptibility testing was performed on Mueller-Hinton agar plates with 2% glucose and 0.5% methylene blue. Antifungal drug discs, including itraconazole, voriconazole, fluconazole, ketoconazole, and amphotericin B, were applied and then incubated at 37°C for 24 to 48 hours [24].

STATISTICAL ANALYSIS

The data collected was entered into a Microsoft Excel sheet. Descriptive statistics were used to analyse the data. The Chi-square test was applied, and a p-value of <0.05 was considered significant for the analytical statistics.

RESULTS

Out of the 129 patients in the study, 101 (78.29%) were males, and 28 (21.71%) were females. A higher incidence of 45 (34.88%) was observed in the age group of >60, with the highest number of patients in the age group of 41-60 years, totaling 60 (46.51%). The least number of patients was found in the age group of 21-40 years, with 24 (18.61%) patients [Table/Fig-1].

Age group (years)	Male	Female	
	n (%)	n (%)	n (%)
21-40	17 (13.18)	7 (5.43)	24 (18.61)
41-60	47 (36.43)	13 (10.08)	60 (46.51)
>60	37 (28.68)	8 (6.2)	45 (34.88)
Total	101 (78.29)	28 (21.71)	129 (100)

[Table/Fig-1]: Age-wise prevalence of pulmonary Tuberculosis (TB).

Distribution of *Candida* spp.: Out of the 129 patients, 10 (7.75%) had *Candida* species distribution is shown in [Table/Fig-2].

Positivity among the sputum samples was determined based on AFB (Acid-Fast Bacilli) testing. Out of the 129 patients from whom
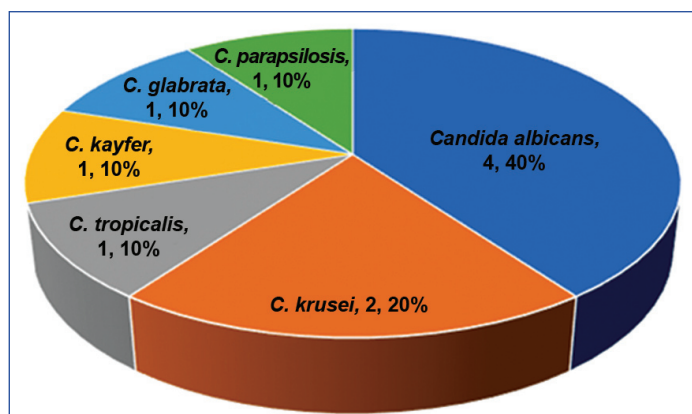
[Table/Fig-2]: Distribution percentage of *Candida* in pulmonary Tuberculosis (TB) patients.

sputum samples were obtained, 71 (55.04%) purulent samples tested positive for AFB, while 25 (19.38%) tested negative. Among the mucopurulent samples, 19 (14.73%) were AFB-positive, with 8 (6.20%) testing negative. For the mucoid sputum samples, 2 (1.55%) were AFB-positive, while 4 (3.10%) were AFB-negative. The nature and classification of these sputum samples are shown in [Table/Fig-3]. The result was not significant (p-value <0.05).

Nature of sputum	n (%)	Positive n (%)	Negative n (%)	Chi-square test	p-value
Purulent	96 (74.42)	71 (55.04)	25 (19.38)		
Muco purulent	27 (20.93)	19 (14.73)	8 (6.20)	4.571	0.107
Mucoid	6 (4.65)	2 (1.55)	4 (3.10)		

[Table/Fig-3]: Nature of clinical sputum samples.
Chi-square test applied; Significant (p<0.05)

Out of 129 patients, 5 (3.87%) had *Aspergillus* species. *A. flavus* was isolated in 1 (0.78%) of the patients whose sputum was AFB positive. *A. fumigatus* was isolated in 2 (1.56%) of the patients, 1 (0.78%) with sputum positive and the other one (0.78%) with sputum negative. *A. niger* was isolated in 2 (1.56%) of the patients, with 1 (0.78%) being sputum positive and the other sputum negative [Table/Fig-4]. *Aspergillus* species were not the primary focus of the study, but this additional observation was made and documented here.

Species	Identified n (%)	Positive n (%)	Negative n (%)
A.flavus	1 (0.78)	1 (0.78)	0
A.fumigatus	2 (1.56)	1 (0.78)	1 (0.78)
A.niger	2 (1.56)	1 (0.78)	1 (0.78)

[Table/Fig-4]: Detection of *Aspergillus spp* in pulmonary Tuberculosis (TB) patients.

Antifungal efficacy: The antifungal efficacy was tested against various species and antifungals, including fluconazole, voriconazole, itraconazole, ketoconazole, and amphotericin B [Table/Fig-5]. The maximum sensitivity of 4 (100%) was observed in *C. albicans* when treated with itraconazole and amphotericin B, followed by 3 (75%) for ketoconazole, and 2 (50%) each for fluconazole and voriconazole. The highest percentage of sensitivity to *Candida* was seen with

itraconazole and amphotericin B. Notably, *C. tropicalis* and *C. kyfer* showed 0 sensitivity to fluconazole and voriconazole. The maximum sensitivity was observed against *C. krusei*, followed by *C. glabrata* and *C. parapsilosis*.

DISCUSSION

As a global issue with a changing prevalence and incidence rate, TB is a serious health problem [25]. In recent times, there has been an increase in the prevalence of opportunistic fungal infections, which do not typically become pathogenic in normal, healthy individuals [26]. These infections can become pathogenic in people with compromised immunity due to underlying diseases, increased consumption of broad-spectrum antibiotics, or those who have pulmonary TB. When pulmonary TB is co-infected with a fungal infection, morbidity is increased [27].

Males were more likely to have pulmonary TB, and their rates of co-infection with fungi were higher than those of females in the present study. This may be because of the increased exposure to the external environment among males, as they often work outside. In addition, smoking is more prevalent in men, and in developing countries, men generally have greater access to healthcare facilities [28].

The most common age group for pulmonary fungal infection with *Aspergillus* is middle-aged, as opposed to the elderly, and males are more likely to be infected [29]. Although the detection of *Aspergillus* species was not the primary aim of the study, it was an additional observation made during the research process. Including the results related to *Aspergillus* might be relevant for providing a comprehensive overview of fungal infections in the studied population, even though the primary focus was on *Candida*.

The current study's outcomes align with those of Kohno S et al., and other researchers, affirming that individuals in their third and fourth decades have a higher prevalence of fungal mycosis compared to younger people [30]. Men within these age brackets exhibited more cases than women. Previous studies have also linked fungal infections mostly to individuals in their 30's and 40's [31,32].

According to these findings, age seems to influence the occurrence of fungal infections. Children under 10 years old did not exhibit any systemic infections, while those over 60 had a low incidence rate. Conversely, patients in middle age presented a high prevalence, possibly due to greater environmental exposure and secondary infections. The most frequently diagnosed *Aspergillus* species, such as *A. fumigatus, A. flavus*, and *A. niger*, were identified, along with *Candida albicans*, from sputum samples collected from TB cases in the current study, affirming Osman NM et al.'s results, which showed *A. fumigatus* as the most commonly observed isolate among *Aspergillus* species in their analysis of 50 TB cases [33]. Additionally, Ekenna O et al., confirmed TB [34]; however, the present research demonstrated some variation, as the predominant isolates were *A. fumigatus*, compared to Razmpa et al.'s findings, which reported a higher presence of *A. flavus* during sample collection [35].

Antifungal drugs					
Species	Fluconazole n (%)	Voriconazole n (%)	Itraconazole n (%)	Ketoconazole n (%)	Amphotercin B n (%)
C. albicans	2 (50%)	2 (50%)	4 (100%)	3 (75%)	4 (100%)
C. tropicalis	0	0	1 (100%)	1 (100%)	1 (100%)
C. krusei	2 (100%)	2 (100%)	2 (100%)	2 (100%)	2 (100%)
C. kyfer	0	0	1 (100%)	0	1 (100%)
C. glabrata	1 (100%)	1 (100%)	1 (100%)	1 (100%)	1 (100%)
C. parapsilosis	1 (100%)	0	1 (100%)	0	1 (100%)

[Table/Fig-5]: Results of Antifungal Susceptibility Testing (AST) conducted on isolated fungi.

Kashid RA et al., reported a prevalence of 13.33% for *C. albicans* [36]. In the present study, *Candida albicans* was the most common fungal species, causing co-infection in four cases (40%). This was followed by *C. krusei* in two cases (20%), *C. tropicalis* in one case (10%), *C. kefyr* in one case (10%), *C. glabrata* in one case (10%), and *C. parapsilosis* in one case (10%). The prevalence of *Candida* was higher in men compared to women, which was consistent with the findings of Roy RC et al., who reported *C. tropicalis* as the predominant species in HIV-positive patients [37]. Similar studies conducted by Bhavana C and Singh R et al., have reported *Candida tropicalis* as the major isolate, ranging between 26-59% [38,39]. A study carried out by Khadka S et al., found that *Candida tropicalis* is the major isolate among NAC species, followed by *Candida glabrata*, which was comparable to the present study [40]. These findings suggest that NAC species are becoming increasingly important pathogens, gradually replacing *Candida albicans* over the past 2-3 decades [41]. In contrast to these findings, the present study showed *Aspergillus* species as the most important fungi. The observations of the present study were consistent with previous studies by other authors, who also found a significant predominance of *Candida albicans* [Table/Fig-6] [17,19,42-48].

higher sensitivity to itraconazole and amphotericin B, followed by ketoconazole, fluconazole, and voriconazole. *C. krusei* and *C. glabrata* exhibited 100% sensitivity to all antifungal drugs used. Therefore, the antifungals itraconazole and amphotericin B, which are typically 100% sensitive to most *Candida* species, are listed and correlated with similar sensitivity patterns observed in other studies, as depicted in [Table/Fig-6] [17,19,42-48].

Males are more likely to develop the condition due to their increased exposure to the outside environment and propensity for utilising various addictive substances. However, it is important to note that the findings of this study might not be representative due to the limited study group; therefore, it must be verified through further research. From a clinical standpoint, these findings underscore the importance of personalised treatment for *Candida* infections, especially for *C. albicans*. Itraconazole and amphotericin B are potential first-line options, considering the potential resistance to other antifungals. Future research should aim to expand sample sizes, delve into genetic factors impacting drug sensitivity, and consider regional variations to enhance tailored antifungal therapies. These efforts will form a foundation for ongoing optimisation of *Candida* infection treatment.

Authors with places and years of publication / *Candida* spp. with antifungals		Jaycharan AL et al., Chennai, 2018 (%) [17]	Bhaskaran R et al., Thrissur, 2020 (%) [19]	Talukdar A et al., Guwahati, 2020 (%) [42]	Shukla R et al., Telangana, 2020 (%) [43]	Deepthi KN et al., Kerala, 2020 (%) [44]	Shwetha DC and Venkatesha DC, Karnataka, 2021 (%) [45]	Patil and Nerurkar E, Mumbai, 2021 (%) [46]	Chen J et al., China, 2021 (%) [47]	Chongtham U Manipur, 2022 (%) [48]	Present study, Puducherry 2024 (%)
C. albicans	F	88.2	100	85	80	96.7	85.1	77	92.5	79.5	50
	V	-	100	100	88	100	85.1	78	92.5	84.1	50
	It	89.2	-	-	-	-	-	94	-	79.5	100
	Kt	-	-	-	-	-	-	-	-	63.6	75
	AmpB	98.9	96	100	84	-	100	97.3	-	93.2	100
C. tropicalis	F	83.3	100	28.5	65.2	91.5	93.8	79	80.9	31.2	0
	V	-	100	85.7	82.6	100	96.9	79	61.9	81.3	0
	It	76.6	-	-	-	-	-	93.5	-	56.2	100
	Kt	-	-	-	-	-	-	-	-	50	100
	AmpB	96.6	100	71.4	89.1	-	100	94.5	-	65.6	100
C. krusei	F	0	0	28	0	0	0	-	-	0	100
	V	-	100	28	100	83.3	66.7	100	-	100	100
	It	72.7	-	-	-	-	-	-	-	40	100
	Kt	-	-	-	-	-	-	-	-	40	100
	AmpB	100	0	64	100	-	100	66	-	60	100
C. kyfer	F	-	-	-	-	-	-	-	-	-	0
	V	-	-	-	-	-	-	-	-	-	0
	It	-	-	-	-	-	-	-	-	-	100
	Kt	-	-	-	-	-	-	-	-	-	0
	AmpB	-	-	-	-	-	-	-	-	-	100
C. parapsilosis	F	100	100	-	100	87.5	-	60	91.9	50	100
	V	-	92.3	-	100	100	-	80	94.6	100	0
	It	100	-	-	-	-	-	-	-	25	100
	Kt	-	-	-	-	-	-	-	-	0	0
	AmpB	100	100	-	50	-	-	95	-	50	100

[Table/Fig-6]: Comparison of sensitivity patterns of *Candida* species to antifungals among various studies [17,19,42-48].
F: Fluconazole, V: Voriconazole, It: Itraconazole, Kt: Ketoconazole, AmpB: Amphotericin B

Antifungal susceptibility testing was performed in the study. The antifungal drugs used were fluconazole, voriconazole, itraconazole, ketoconazole, and amphotericin B. *Candida* species were identified using chrome agar for all *Candida* species. The results of the present study showed a total of 10 (7.75%) *Candida* isolates, including *C. albicans (4), C. tropicalis (1), C. krusei (2), C. kefyr (1), C. glabrata (1),* and *C. parapsilosis* (1). *C. albicans* showed

Limitation(s)

Sputum samples were used for testing, which may not be the most reliable method for determining the specific type of pulmonary TB infection a person has. Additionally, there is a need to study the increase in Minimum Inhibitory Concentration (MIC) values over time among different *Candida* isolates in specific geographic locations.

CONCLUSION(S)

Candida albicans has been found to be the most common co-infection in TB patients. Present research highlights the increasing threat posed by NAC, some of which are resistant to azole antifungals. The prevalence of *Candida* species is on the rise in tertiary care hospitals, particularly among patients with pulmonary TB who also have *Candida albicans* infections. Despite receiving antitubercular therapy, a majority of male patients continued to experience pulmonary symptoms.

It is recommended to incorporate epidemiological data on fungi and routine antifungal susceptibility testing for *Candida* in the management of pulmonary TB patients. The findings led us to conclude that drug-resistant *Candida* strains are frequently present in the sputum and respiratory tracts of individuals with respiratory tract infections. Therefore, regular screening for *Candida* infection should be conducted in pulmonary TB patients, along with antifungal sensitivity testing for NAC. Additionally, prompt therapy, effective treatment, and species identification are crucial for reducing patient morbidity and mortality.

REFERENCES

[1] Jain A, Jain S, Rawat S. Emerging fungal infections among children: A review on its clinical manifestations, diagnosis, and prevention. J Pharm Bioallied Sci. 2010;2(4):314. Available from: https://dx.doi.org/10.4103/0975-7406.72131.

[2] Schelenz S. Management of candidiasis in the intensive care unit. J Antimicrob Chemother. 2008;61(Supplement 1):i31-34. Available from: https://dx.doi.org/10.1093/jac/dkm430.

[3] Azie N, Neofytos D, Pfaller M, Meier-Kriesche HU, Quan SP, Horn D. The PATH (Prospective Antifungal Therapy) Alliance® registry and invasive fungal infections: Update 2012. Diagn Microbiol Infect Dis. 2012;73(4):293-300. Available from: https://dx.doi.org/10.1016/j.diagmicrobio.2012.06.012.

[4] de Almeida AA, Mesquita CSS, Svidzinski TIE, de Oliveira KMP. Antifungal susceptibility and distribution of Candida spp. isolates from the University Hospital in the municipality of Dourados, State of Mato Grosso do Sul, Brazil. Rev Soc Bras Med Trop. 2013;46(3):335-39. Available from: https://dx.doi.org/10.1590/0037-8682-0074-2012.

[5] Manjunath V, Vidya GS, Sharma A, Prakash MR. Speciation of *Candida* by Hicrome agar and sugar assimilation test in both HIV infected and non infected patients. Int J Biol Med Res. 2012;3(2):1778-82.

[6] Global tuberculosis reports [Internet]. Who.int. [cited 2023 Oct 19]. Available from: https://www.who.int/teams/global-tuberculosis-programme/tb-reports.

[7] Bansod S, Rai M. Emerging of mycotic infection in patients infected with mycobacterium tuberculosis. World J Med Sci. 2008;3(2):74-80.

[8] Vannberg FO, Chapman SJ, Khor CC, Tosh K, Floyd S, Jackson-Sillah D, et al. CD209 genetic polymorphism and tuberculosis disease. PLoS One. 2008;3(1):e1388. Available from: https://dx.doi.org/10.1371/journal.pone.0001388.

[9] TB statistics India [Internet]. TBFacts. 2018 [Internet] [cited 2023 Oct 19]. Available from: https://tbfacts.org/tb-statistics-india/.

[10] Chandra A, Kumar R, Kant S, Krishnan A. Costs of TB care incurred by adult patients with newly diagnosed drug-sensitive TB in Ballabgarh block in northern India. Trans R Soc Trop Med Hyg [Internet]. 2022;116(1):63-69. Available from: https://dx.doi.org/10.1093/trstmh/trab060.

[11] Amala SE, Hanson A, Wokem GN, editors. Candida Co-Infection with Mycobacterium tuberculosis in Tuberculosis Patients and Antifungal Susceptibility of the Isolates. Tuberculosis Research. 2020;8(2). Available from: https://dx.doi.org.10.4236/jtr.2020.82006.

[12] Pam VK, Akpan JU, Oduyebo OO, Nwaokorie FO, Fowora MA, Oladele RO, et al. Fluconazole susceptibility and ERG11 gene expression in vaginal candida species isolated from Lagos Nigeria. Int J Mol Epidemiol Genet. 2012;3(1):84-90.

[13] Pfaller MA, Castanheira M, Messer SA, Moet GJ, Jones RN. Variation in Candida spp. distribution and antifungal resistance rates among bloodstream infection isolates by patient age: Report from the SENTRY Antimicrobial Surveillance Program (2008-2009). Diagn Microbiol Infect Dis. 2010;68(3):278-83. Available from: https://dx.doi.org/10.1016/j.diagmicrobio.2010.06.015.

[14] Ashley ESD, Lewis R, Lewis JS, Martin C, Andes D. Pharmacology of systemic antifungal agents. Clin Infect Dis. 2006;43(Supplement_1):S28-39. Available from: https://dx.doi.org/10.1086/504492.

[15] Chander J. A text book of medical mycology, Candidiasis. New Delhi: Mehta Publishers; 2009.

[16] Chen XH, Gao YC, Zhang Y, Tang ZH, Yu YS, Zang GQ. Tuberculosis infection might increase the risk of invasive candidiasis in an immunocompetent patient. Rev Inst Med Trop Sao Paulo. 2015;57(3):273-75. Available from: https://dx.doi.org/10.1590/s0036-46652015000300016.

[17] Jayacharan AL, Katragadda R, Ravinder T, Vajravelu L, Manoranjan L, Hemalatha S. Antifungal susceptibility pattern among *Candida* species: An evaluation of disc diffusion and micro broth dilution method. J Microbiol Infect Dis. 2018;8(3):97-101.

[18] Pandita I, Wyawahare AS, Mulay MV, Hussain T, Kulkarni S. Speciation and antifungal susceptibility pattern of candida isolates from various clinical specimens at a tertiary care hospital, Maharashtra. Int J Adv Res (Indore). 2019;7(10):244-50. Available from: https://dx.doi.org/10.21474/ijar01/9827.

[19] Bhaskaran R, Valsan C, Sathiavathy KA. Molecular speciation and antifungal susceptibility profile of candida species in a tertiary care centre in central Kerala. J Evol Med Dent Sci. 2020;9(06):357-62. Available from: https://dx.doi.org/10.14260/jemds/2020/81.

[20] Bhattacharjee P. Epidemiology and antifungal susceptibility of *Candida* species in a tertiary care hospital, Kolkata, India. Curr Med Mycol. 2016;2(2):20-27. Available from: https://dx.doi.org/10.18869/acadpub.cmm.2.2.5.

[21] Standard Operating Procedures for Fungal Identification and Detection of Antifungal Resistance- National Antifungal Resistance Surveillance. New Delhi: Indian Council of Medical Research; 2019.

[22] Mehta R, Wyawahare AS. Evaluation of Hicrome *Candida* differential agar for species identification of Candida isolates from various clinical samples. Int J Cont Med Res. 2016;3(4):1219-22.

[23] M44-A2 Method for antifungal disk diffusion susceptibility testing of yeasts; Approved Guideline. CLSI document. 2009;29(17):44-A46.

[24] Alastruey-Izquierdo A, Melhem MSC, Bonfietti LX, Rodriguez-Tudela JL. Susceptibility test for fungi: Clinical and laboratorial correlations in medical mycology. Rev Inst Med Trop Sao Paulo. 2015;57(suppl 19):57-64. Available from: https://dx.doi.org/10.1590/s0036-46652015000700011.

[25] Millet JP, Moreno A, Fina L, del Baño L, Orcau A, de Olalla PG, et al. Factors that influence current tuberculosis epidemiology. Eur Spine J. 2013;22(S4):539-48. Available from: https://dx.doi.org/10.1007/s00586-012-2334-8.

[26] Pfaller MA, Diekema DJ. Rare and emerging opportunistic fungal pathogens: Concern for resistance beyond *Candida albicans* and *Aspergillus fumigatus*. J Clin Microbiol. 2004;42(10):4419-31. Available from: https://dx.doi.org/10.1128/jcm.42.10.4419-4431.2004.

[27] Muni S, Rajpal K, Kumar R, Kumari R, Sinha R, Kumar S, et al. Identification of fungal isolates in patients with pulmonary tuberculosis treated at a tertiary care hospital. Cureus. 2023;15(4):e37664. Doi: 10.7759/cureus.37664.

[28] Miller PB, Zalwango S, Galiwango R, Kakaire R, Sekandi J, Steinbaum L, et al. Association between tuberculosis in men and social network structure in Kampala, Uganda. BMC Infect Dis. 2021;21(1):1023. Available from: https://dx.doi.org/10.1186/s12879-021-06475-z.

[29] Kosmidis C, Denning DW. The clinical spectrum of pulmonary aspergillosis. Thorax. 2015;70(3):270-77. Available from: https://dx.doi.org/10.1136/thoraxjnl-2014-206291.

[30] Kohno S, Izumikawa K, Ogawa K, Kurashima A, Okimoto N, Amitani R, et al. Intravenous micafungin versus voriconazole for chronic pulmonary aspergillosis: A multicenter trial in Japan. J Infect. 2010;61(5):410-18. Available from: https://dx.doi.org/10.1016/j.jinf.2010.08.005.

[31] Ogba OM, Abia-Bassey LN, Epoke J. The relationship between opportunistic pulmonary fungal infections and CD4 count levels among HIV-seropositive patients in Calabar, Nigeria. Trans R Soc Trop Med Hyg. 2013;107(3):170-75. Available from: https://dx.doi.org/10.1093/trstmh/trs025.

[32] Ogba OM, Abia-Bassey LN, Epoke J, Mandor BI, Iwatt GD. Characterization of candida species isolated from cases of lower respiratory tract infection among HIV/AIDS patients in calabar, Nigeria. World J AIDS. 2013;03(03):201-06. Available from: https://dx.doi.org/10.4236/wja.2013.33027.

[33] Osman NM, Gomaa AA, Sayed NM. Microarray detection of fungal infection in pulmonary tuberculosis. Egypt J Chest Dis. 2013;62(1):151-57.

[34] Ekenna O, Uba A, Chikwem JO, Mambula S, Aliyu MB, Mohammed I. Relevance of moldy fungi as agents of chronic lower respiratory tract infection in patients seen in Maiduguri, Nigeria. West Afr J Med. 2007;26(2):117-20.

[35] Razmpa E, Khajavi M, Hadipour-Jahromi M, Kordbacheh P. The prevalence of fungal infections in nasal polyposis. Acta Med Iran. 2007;1:45-50.

[36] Kashid RA, Belawadi S, Devi G, Indumati I. Incidence of non-candida albicans in patients with urinary tract infection with special reference to speciation and antifungal susceptibility. J Evol Med Dent Sci. 2012;1(4):572-77. Available from: https://dx.doi.org/10.14260/jemds/89.

[37] Roy RC, Sharma GD, Barman SR. Trend of *Candida* infection and antifungal resistance in a tertiary care hospital of northeast India. Afr J Microbiol Res. 2013;7:3112-16.

[38] Bhavana C. Speciation of candida and antifungal susceptibility testing from clinical specimens in a tertiary care hospital, Bangalore. Bangalore Int J Curr Res. 2017;9(01):45832-36.

[39] Singh R, Verma RK, Kumari S, Singh A, Singh DP. Rapid identification and susceptibility pattern of various *Candida* isolates from different clinical specimens in a tertiary care hospital in Western Uttar Pradesh. Int J Res Med Sci. 2017;5(8):3466. Available from: https://dx.doi.org/10.18203/2320-6012.ijrms20173542.

[40] Khadka S, Sherchand JB, Pokhrel BM, Parajuli K, Mishra SK, Sharma S, et al. Isolation, speciation and antifungal susceptibility testing of *Candida* isolates from various clinical specimens at a tertiary care hospital, Nepal. BMC Res Notes. 2017;10(1):218. Available from: https://dx.doi.org/10.1186/s13104-017-2547-3.

[41] Meurman JH, Siikala E, Richardson M, Rautemaa R, Méndez-Vilas A, (ed.). Non-Candida albicans Candida yeasts of the oral cavity. In Communicating Current Research and Educational Topics and Trends in Applied Microbiology. Vol. 2. Badajoz: FORMATEX. 2007. p. 719-731.

[42] Talukdar A, Barman R, Sarma A, Krishnatreya M, Sharma JD, Kataki AC. Fungal profile and antifungal susceptibility pattern of candidiasis in esophageal cancer patients. J Cancer Res Ther. 2020;16(Supplement):S209-12. Available from: https://dx.doi.org/10.4103/jcrt.JCRT_581_18.

[43] Shukla R, Sg R, Ak B. A study of Candida albicans and non-albicans *Candida* species isolated from various clinical samples and their antifungal susceptibility pattern. J Med Sci Res. 2020;8(1):01-11. Available from: https://dx.doi.org/10.17727/jmsr.2020/8-1.

[44] Deepthi KN, Menon AR, Nair P. Identification of *Candida* species from clinical isolates and their antifungal susceptibility pattern. J Evol Med Dent Sci. 2020;9(24):1813-17. Available from: https://dx.doi.org/10.14260/jemds/2020/396.

[45] Shwetha DC, Venkatesha DC. Identification and antifungal susceptibility of *Candida* species isolated from various clinical samples at a tertiary care hospital. Int J Curr Microbiol App Sci. 2021;10(03):527-32.

[46] Patil S, Nerurkar E. Speciation and antifungal susceptiblity profile of *Candida* species- A study from a private diagnostic reference lab in Mumbai. IAR J Med Sci. 2021;2(4):48-55.

[47] Chen J, Hu N, Xu H, Liu Q, Yu X, Zhang Y, et al. Molecular epidemiology, antifungal susceptibility, and virulence evaluation of *Candida* isolates causing invasive infection in a tertiary care teaching hospital. Front Cell Infect Microbiol. 2021;11:721439. Available from: https://dx.doi.org/10.3389/fcimb.2021.721439.

[48] Chongtham U, Athokpam DC, Singh RM. Isolation, identification and antifungal susceptibility testing of *Candida* species: A cross-sectional study from Manipur, India. J Clin Diag Res. 2022;16(4):DC09-DC14. Available from: https://dx.doi.org/10.7860/jcdr/2022/55695.16248.

PARTICULARS OF CONTRIBUTORS:
1. Assistant Professor, Department of Microbiology, Trichy SRM Medical College Hospital and Research Centre (Affiliated to The Tamil Nadu Dr. M.G.R. Medical University, Chennai), Irungalur, Tiruchirapalli, India; Former Assistant Professor, Department of Microbiology, Aarupadai Veedu Medical College and Hospital, Vinayaka Mission's Research Foundation (Deemed to be University), Kirumampakkam, Puducherry, India.
2. Associate Professor, Department of Microbiology, Aarupadai Veedu Medical College and Hospital, Vinayaka Mission's Research Foundation (Deemed to be University), Kirumampakkam, Puducherry, India.
3. Assistant Professor, Department of Medical Biotechnology, Aarupadai Veedu Medical College and Hospital, Vinayaka Mission's Research Foundation (Deemed to be University), Kirumampakkam, Puducherry, India.
4. Professor, Department of Microbiology, Aarupadai Veedu Medical College and Hospital, Vinayaka Mission's Research Foundation (Deemed to be University), Kirumampakkam, Puducherry, India.

NAME, ADDRESS, E-MAIL ID OF THE CORRESPONDING AUTHOR:
Dr. Prithiviraj Nagarajan,
Assistant Professor, Department of Medical Biotechnology, Aarupadai Veedu Medical College and Hospital, Vinayaka Mission's Research Foundation (Deemed to be University), Kirumampakkam, Puducherry-607403, India.
E-mail: prithivinaga@gmail.com

AUTHOR DECLARATION:
- Financial or Other Competing Interests: None
- Was Ethics Committee Approval obtained for this study?   Yes
- Was informed consent obtained from the subjects involved in the study?   Yes
- For any images presented appropriate consent has been obtained from the subjects.   Yes