

Current Trends of Various *Candida* Coinfection using KB006Hi Candida Kit and CHROM Agar in Pulmonary Tuberculosis Patients at a Tertiary Health Care Center in Patna

Sweta Muni¹, Rakesh Kumar², Shailesh Kumar³, Vidyut Prakash⁴, Namrata Kumari⁵, S K Shahi⁶, Deepak Pankaj⁷

¹Assistant Professor, Department of Microbiology, Indira Gandhi Institute of Medical Sciences, Patna, Bihar, India, ²Associate Professor, Department of Microbiology, Indira Gandhi Institute of Medical Sciences, Patna, Bihar, India, ³Additional Professor, Department of Microbiology, Indira Gandhi Institute of Medical Sciences, Patna, Bihar, India, ⁴Senior Resident, Department of Microbiology, Indira Gandhi Institute of Medical Sciences, Patna, Bihar, India, ⁵Professor, Department of Microbiology, Indira Gandhi Institute of Medical Sciences, Patna, Bihar, India, ⁶Professor and Head, Department of Microbiology, Indira Gandhi Institute of Medical Sciences, Patna, Bihar, India, ⁷Assistant Professor, Department of General Surgery, Indira Gandhi Institute of Medical Sciences, Patna, Bihar, India

Abstract

Introduction: The various *Candida* species have long been associated with pulmonary tuberculosis (TB). These *Candida* have assumed significance as emerging pathogen in these TB patients with some degree resistance to antifungal therapy thus complicating the disease and its treatment. Early identification of *Candida* species and instituting appropriate treatment therapy is important in reducing the morbidity and mortality in patients with TB.

Aim: The aim is to study trends and prevalence of various *Candida* coinfection using KB006Hi *Candida* kit and CHROM agar in pulmonary TB patients and evaluating usefulness of these *Candida* identification kit.

Material and Methods: This study was done in the Department of Microbiology at Indira Gandhi Institute of Medical Sciences, Patna, Bihar, over a period of 1 year in seventy patients of pulmonary TB. Prior Institutional Ethical Committee approval was also obtained for this study.

Results: Out of a total of 200 patients, 70 patients (35%) whose sputum samples were positive for acid-fast bacilli were included in the study. *Candida* coinfection was observed in 26 (37.14%) patients which included 16 male and 10 female patients. The ratio of male to female in *Candida* infection group was 1.6:1. *Candida albicans* was the most common isolate among *Candida* species in pulmonary TB patients which was isolated in 50% of the patients.

Conclusion: *Candida* coinfection in pulmonary TB patient is common, and synergistic growth between *Candida* species and TB exists. Along with *C. albicans*, the prevalence of non-*albicans* species is also increasing with variable degree of resistance. Prompt measures need to be taken for routine identification and treatment of these opportunistic *Candida* infections in TB patients.

Key words: *Candida albicans*, CHROM agar, Non-*albicans* *Candida*, Prevalence, Pulmonary tuberculosis

INTRODUCTION

Close to 200 species of genus *Candida* exist out of which almost two dozen has been associated with pathology

in human and animals.^[1] Respiratory *Candida* infection involving the lungs or bronchial system is associated with secondary infections in tuberculosis (TB) patients. *Candida* species are one of the most potentially pathogenic fungal agents in patients with bronchopulmonary disease. Whenever the host resistance is lowered as in immunocompromised patients, cancer patients on chemotherapy, long-term steroid use, and the unrecognized opportunistic fungi affect the progression of disease and may even prove fatal. The synergistic growth promoting the association of *Candida* and *Mycobacterium tuberculosis* is

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Corresponding Author: Dr. Shailesh Kumar, Department of Microbiology, Indira Gandhi Institute of Medical Sciences, Patna - 800 014, Bihar, India

well documented by various authors all over the world, but still, the sputum isolates of pulmonary TB patients are ignored for fungal studies which might prevent morbidity and mortality resulting from the disease as *Candida* species are innocuous throat commensals.^[2,3] The effect of polysaccharide fraction of *Candida albicans* for enhancement of the growth as well as reduction of the generation time of tubercle bacilli is confirmed by different studies.^[4] Most of the individuals affected with mycoses have a history of receiving long-term antibiotics and corticosteroids therapy, other immunodeficiency such as AIDS, and majority of them have severe pulmonary diseases such as TB. Concerning immunocompromised patients, *C. albicans* is the most frequently isolated pathogen, but the increasing incidence of infections due to non-albicans *Candida* such as *Candida tropicalis*, *Candida glabrata*, *Candida krusei*, and *Candida parapsilosis* is a cause of perturb.^[5,6] Out of various isolation media available commercially for identification of fungal pathogens such as candida, CHROM agar serves as a medium for detection and identification of major *Candida* species with accuracy and reduces the time of identification and its characterization from poly fungal specimens.^[7] CHROM agar Candida is a selective and differential medium which allows simultaneous isolation and identification of yeast based on the color and colony morphology.^[8] Hence, these chromogenic media for *Candida* detection are an alternative to the older conventional techniques which used to take more time and were unwieldy. Species identification is pivotal when treating candidiasis as prolonged use of azoles in treatment has led to the emergence of drug resistance in *C. albicans* and other species.^[9] This type of study will not only be helpful in screening patients for coinfection with *Candida* in pulmonary TB patients but can also be worthwhile in treating patients with improper response to antitubercular medications.

Objectives of the Study

- To determine the prevalence of *Candida* coinfections among TB patients.
- To differentiate *Candida* to the species level due to pathogenicity variation.
- Evaluating the usefulness of CHROM agar and KB006 identification kit.
- Early identification of *Candida* species and instituting appropriate treatment therapy thus reducing the morbidity and mortality in patients with TB.

MATERIALS AND METHODS

This prospective analytical study was done at Indira Gandhi Institute of Medical Sciences, Patna, Bihar, over a period of 1 year in seventy patients. Samples were collected from in and outpatients from the Department of Pulmonary Medicine

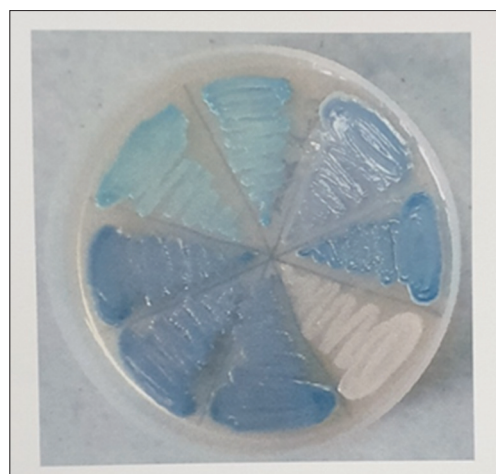


Figure 1: Growth of *Candida* species on CHROM agar

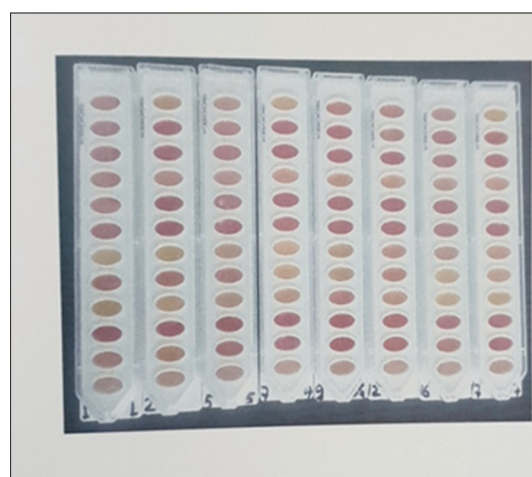


Figure 2: KB006 HI identification kit (sugar assimilation reaction)

and Revised National TB Control Program (RNTCP) designated microscopic center for acid-fast bacilli (AFB) staining of sputum. Prior Institutional Ethical Committee approval was also obtained for this study. Clinically diagnosed cases of pulmonary TB of all age groups and AFB smear positive or negative in Ziehl–Neelsen staining were included in the study. Repeat samples from the same patient, non-cooperative patients, and patients on antifungal therapy were excluded from the study.

Clinical specimens such as sputum and pleural effusion fluid were collected from pulmonary medicine department, and spot samples or morning two samples were collected from DMC, RNTCP after proper instructions to the patients. All the clinical specimens were Gram stained and observed under the microscope for yeast cells. In doing culture for primary isolation, Sabouraud dextrose agar was used for primary isolation of *Candida* species. The culture plates were incubated at 37°C for 48 h. *Candida* isolates were speciated by the following features -

- Morphology and culture characteristic (less informative)
- Germ tube test
- Cornmeal agar for chlamydospore formation
- Color of the colonies on CHROM agar [Figure 1]
- KB006 HiCandida Identification kit (sugar fermentation) [Figure 2].

Characterization of organisms - All pure cultures were characterized to species level using different tests confirming with required standard diagnostic criteria.^[10,11] The criteria included will be morphological and cultural characteristics and small and large surface pellicle formation.

Study Methods

- KB006 HiCandida Identification kit - It is a standard test system that can be used for identification and differentiation of *Candida* species. It can also be used for validating known laboratory strains. Principle of test - Each KB006 kit is a standardized colorimetric identification system utilizing 12 conventional biochemical tests. The tests are based on the principle of pH change and substrate utilization. On incubation, organisms undergo metabolic changes which are indicated by spontaneous color change in the media.
- Germ tube test - A rapid method of identifying *C. albicans* based on its ability to form germ tubes within 2 h when incubated in human serum at 37°C (Reynolds–Braude phenomenon).
- CHROM agar - CHROM agar Candida (CHROM agar company, Himedia) - Identification of *Candida* spp. on CHROM agar culture produce different color of colonies.
- Cornmeal agar for chlamydospore formation - Culture on cornmeal agar at 20°C produce chlamydospores by *C. albicans* and *C. dubliniensis*.

RESULTS

Out of a total of 200 patients in whom pulmonary TB was suspected, sputum specimen was sent and in which 70 patients (35%) whose sputum samples were positive for AFB by Ziehl–Neelsen method were included in the study. Out of 70 patients in sputum-positive pulmonary TB, 42 were males and 28 were females. These seventy patients were screened for *Candida* coinfection. *Candida* coinfection was observed in 26 (37.14%) patients out of the 70 patients of pulmonary TB which included 16 male and 10 female patients. The ratio of male to female in *Candida* infection group was 1.6:1. Maximum number of *Candida* coinfection was observed in age group 61–80 years (57.7%) which was followed by age group 41–60 years (23.07%) and age group 21–40 years (19.23%), respectively [Table 1]. In various cases of pulmonary TB, identification of *Candida* species was done by CHROM agar and *Candida* identification kit. In this

study, it was found that *C. albicans* was the most common isolate among *Candida* species in pulmonary TB patients which was isolated in 50% of the patients (13/26). Next common *Candida* species detected was *C. tropicalis* which was present in 23.08% patients (6/26) followed by *C. glabrata* present in 15.38% of patients (4/26). One patient each of *C. parapsilosis*, *C. krusei*, and *Candida haemulonii* was isolated in sputum samples of pulmonary TB patients [Table 2].

DISCUSSION

Respiratory fungal infections are the emerging pathogen complicating pulmonary TB, and *Candida* infection is one of the most common pathogenic fungi implicated in these patients. The diseases which are immunosuppressive in nature have led to increase in the incidence of opportunistic fungal infections. In the present study, the number of patients suffering with *Candida* coinfection in pulmonary TB patients was 37.14% which is quite similar to various studies done by other researchers [Table 3].^[2,12-15]

Considering gender, in our study, there is not much difference, and male preponderance was slightly more than females with the ratio being 1.6:1. Even other studies suggest

Table 1: Age- and sex-wise distribution of *Candida* speciation among TB patients

Age group (years)	Male	Female	Total <i>Candida</i> species (n=26) (%)
21–40	3	2	5 (19.23)
41–60	3	3	6 (23.07)
61–80	10	5	15 (57.70)

TB: Tuberculosis

Table 2: Distribution of various *Candida* spp. (n=26) from patients with TB

<i>Candida</i> species	Number isolated (%)
<i>Candida albicans</i>	13 (50)
<i>Candida tropicalis</i>	6 (23.07)
<i>Candida glabrata</i>	4 (15.38)
<i>Candida parapsilosis</i>	1 (3.85)
<i>Candida krusei</i>	1 (3.85)
<i>Candida haemulonii</i>	1 (3.85)

TB: Tuberculosis

Table 3: Showing incidence of *Candida* coinfection in pulmonary TB patients in various study

Authors	<i>Candida</i> species isolated (%)
Baradkar <i>et al.</i> (2009)	26/100 (26%)
Kali <i>et al.</i> (2013)	30/75 (40%)
Mathavi <i>et al.</i> (2014)	19/107 (17.7%)
Kavitha <i>et al.</i> (2017)	41/121 (33.89%)
Nandihal <i>et al.</i> (2018)	32/100 (32%)
Present study (2019)	26/70 (37.14%)

that there is variability with regard to males and females as more males are having *Candida* coinfection in pulmonary TB patients, but some studies have also reported more female preponderance.^[2,15] Less number of females in our study could only be due to the fact that most women are homemaker prefer to stay indoor and less literacy rate among females which emphasize the need of health education of the society. Patients of old age more than 60 years of age were affected maximally (more than 50%) with the *Candida* infection. People in the old age group are more vulnerable to fungal infections as the aging process leads to decline and variable changes in the physiologic and morphological functions in these people.^[16] Another important factor could be less saliva production which limits production of peptide and presence of protein in the oral cavity, and the lack of substances with broad antimicrobial activity, such as lysozyme, contributes to oral candidiasis.^[17] *C. albicans* is the most common fungal pathogen in patients of pulmonary TB and this is also evident from the present study where the common fungal pathogen was *C. albicans* in half of the cases. The various non-albicans *Candida* comprised other half of cases which were *C. tropicalis* (most common non-albicans *Candida*) followed by *C. glabrata*. One patient each of *C. parapsilosis*, *C. krusei*, and *C. haemulonii* were found in this study. Past studies done by various researchers showed *C. albicans* to be the principal isolate followed by *C. tropicalis* which correlates also with the present study.^[2,12,18,19] *C. albicans* is a commensal organism, but it can be infectious and proves to be menacing in immunocompromised individuals under a variety of conditions as well as TB.^[20]

This percentage of pulmonary TB patients coinfecting with *Candida* is perturbing and might interfere and complicate the treatment of TB.^[21] There should be adequate guidelines, and steps should be taken for the treatment and prevention of the opportunistic candidiasis in pulmonary TB patients.

CONCLUSION

Candida from being a normal commensal in human can attain pathogenic forms and can even prove to be lethal in patients where it coexists with pulmonary TB. Detection of the various *Candida* species will aid in instituting prompt antifungal treatment for candidiasis along with the treatment of TB. There had been emerging resistance to antifungal therapy mostly in non-albicans *Candida* in recent times, so this type of study where pulmonary TB patients are coinfecting with *Candida* species will be worthwhile to the patients.

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