

## Evaluation of Indole production and Tellurite reduction for speciation of *Candida* species and *Trichosporon* species

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### ABSTRACT

**Background:** Candidiasis is one of the commonest infections in man, along with *Trichosporon* infection. Conventional methods for identification are often delayed, which leads to delay in empirical therapy in these infections. **Methods:** We here describe two newer methods, i.e. Indole production and Tellurite reduction for identification of these two genera. **Results:** Both these tests, combined together, were equally good as compared to conventional identification techniques. **Conclusion:** Indole production and Tellurite reduction are useful tests to identify these common yeast pathogens in the laboratory.

**Keywords:** *Candida*, Indole, Tellurite, *Trichosporon*

The human microbiota plays an inevitable role in human metabolism and in understanding the pathogenesis and the optimized therapy for many diseases [1]. In our body, populations of microbes such as bacteria and yeasts form part of normal healthy human flora. However, when the host's immune defenses are altered, as in immunosuppressive therapy, or during steroid or antibacterial therapy, or long term catheterization or hemodialysis, microbe numbers grow beyond their typical ranges and cause infections [1]. One such opportunistic infection is Candidiasis. It is a fungal infection caused by yeasts that belong to the genus *Candida*. There are over 20 species of *Candida* yeasts that can cause infection in humans, the most common of which is *Candida albicans*. Other *Candida* species that can cause superficial and deep infections in man include *C. tropicalis*, *C. glabrata*, *C. parapsilosis*, *C. stellatoidea*, *C. dubliniensis*, and *C. krusei* [2].

*Candida* species are the fourth most common cause of bloodstream infections among hospital patients in the

United States [3]. Chakrabarti *et al* from Chandigarh, reported high rates of *Candida* bloodstream infection (Candidemia) in 27 Intensive Care Units (ICU) in India [4]. It is, therefore, important to identify the species of *Candida* for the successful treatment of the disease. The conventional methods of identifying species in the clinical microbiology laboratory include either direct microscopic examination using potassium hydroxide (KOH) or laboratory culturing. Identification by culturing methods relies on criteria such as colony characteristics and morphology on different media and sugar assimilation or fermentation. Isolates of *C. albicans* are typically identified by their ability to form germ tubes (Germ Tube Test) or terminal chlamydospores (Dalmau Plate technique) under the appropriate conditions [5,6].

Although rapid, the germ tube test has many shortcomings. Over-inoculation of the serum can inhibit germ tube formation and too short incubation time (less than two to four hours) can lead to false results as it may be difficult to discern a true germ tube from that of an

early pseudohyphal cell. Additionally, specificity can be lacking as *C. tropicalis* has been reported to rarely form germ tubes [7]. In addition, the handling of pooled human serum includes the possible risk of infection with HIV or hepatitis virus and different batches of serum may produce different results [8].

Dalmau Plate Technique requires longer (two to several days) incubation of isolates on morphology agar (e.g., corn meal, corn meal/Tween 80, rice extract, rice extract/Tween 80, etc.) that is observed microscopically for the presence of yeast cells, pseudohyphae and spores [9,10]. As both of the above methods demand microscopic observation and considerable skill and experience, there is a need to develop alternate tests that are sensitive and specific but require less technical expertise and shorter incubation time [11]. The present study is, therefore, intended to evaluate alternative tests and to employ alternative media and reagents to rapidly and reliably identify *C. albicans* as well as other *Candida* and *Trichosporon* species in routine clinical microbiology practice.

## MATERIALS AND METHODS

This laboratory based observational study was carried out in the Department of Microbiology, AIIMS, Patna, Bihar, India as a part of summer training research cum

dissertation over a period of 3 months from February 2016 to April 2016. Samples had been selected from clinical isolates of *Candida* and *Trichosporon* species isolated in the laboratory.

**Isolation and purification:** Samples were inoculated on Sabouraud's dextrose Agar (SDA) slants. The slants were incubated at 37<sup>0</sup> C for up to 3 weeks. The obtained colonies were examined under microscope by preparing LPCB mounts. The selected yeast isolates were purified on fresh SDA slants. These purified isolates were then identified by germ tube test, high temperature (42<sup>0</sup>C) tolerance test, morphology on rice extract agar (Dalmau technique) and carbohydrate (glucose, maltose, lactose and sucrose) fermentation tests [12].

**Characterization of the selected isolates:** The isolates were characterized on the basis of their tellurite reduction and indole production. Indole tests were detected by growing the isolate overnight in peptone water and the adding a drop of Kovac's indole reagent in it. Red colour indicated positive reaction while yellow indicated negative reaction [12]. Tellurite reduction was assessed by growing the yeast isolates on nutrient agar with 0.04% Potassium tellurite and incubating overnight at 37 Degree C. Black colouration of colonies in this medium indicated positive reduction while no such colour indicated negative reduction [12].

**Table 1 – Yeast isolates with results of indole production and tellurite reduction**

Fungus	Number of isolates	Indole test		Tellurite reduction test	
		(positive/total)	% positive	(positive/ total)	% positive
<i>C. albicans</i>	16	3/16	18.75	9/16	56.25
<i>C. tropicalis</i>	4	2/4	50	¾	75
<i>C. kefyr</i>	1	1/1	100	0/1	0
<i>C. glabrata</i>	6	0/6	0	2/6	33.33
<i>C. parapsilosis</i>	4	0/4	0	¼	25
<i>Trichosporon cutaneum</i>	2	0/2	0	½	50
<i>C. dublinensis</i>	2	0/2		2/2	0

## RESULTS

Results are given in table 1. Out of 16 clinical isolates of *C.albicans*, 3 isolates (18.75%) were positive for indole production and 9 (56.25%) were positive for tellurite reduction. Out of 4 clinical isolates of *C.tropicalis*, 2 isolates (50%) were positive for indole production and 3 (75%) were positive for tellurite reduction. Out of 1 clinical isolate of *C.kefyr*, 1 (100%) was positive for indole production and none (0%) was positive for tellurite reduction. Out of 6 clinical isolates of *C.glabrata*, no isolate positive for indole production and 2 (33.33%) were positive for tellurite reduction.

Out of 4 clinical isolates of *C.parapsilosis*, none was positive for indole production and 1 (25%) was positive for tellurite reduction. Out of 2 clinical isolates of *Trichosporon cutaneum*, no isolates was positive for indole production and 1 (50%) was positive for tellurite reduction. Out of 2 clinical isolates of *C. dublinensis*, no isolate was positive for indole production and both (100%) were positive for tellurite reduction.

## DISCUSSION

Thus, indole production and tellurite reduction can be suitable tests to replace conventional ones, especially Dalmau technique, and need to be evaluated further in upcoming studies. *Candida tropicalis* was mostly positive for tellurite reduction and *C. glabrata* was mostly negative. Indole production was always negative in *C. glabrata*, *C. parapsilosis* and *Trichosporon* sp.. This information can help in devising newer rapid tests for identification of these pathogens. This is all the more important because a delay in accurate identification can frequently result in delay of therapy [13].

Identification by these newer methods will lead to rapid institution of timely therapy in case of infections by these pathogens. A yeast isolate that is indole positive and can reduce tellurite, is most likely to be *Candida tropicalis*, according to our finding. On the contrary, *Candida glabrata* is likely to be negative for both the tests. *Candida albicans*, on the other hand, can reduce tellurite and is mostly indole negative. As far as we know, both these tests together, have not been used or evaluated for

identification of yeasts. Bonfante had studied tellurite reduction, but at 0.02% concentration and his study did not involve *Trichosporon* spp. [14].

After thorough literature search, we could not find any such report on use of indole and tellurite reduction tests to characterize *Candida* spp. or *Trichosporon* spp. On combining these 2 tests with germ tube test and sugar fermentation, very accurate identification can be obtained within 24 hours of growing the isolate in vitro. All these need to be assessed further and more such newer, cheap tests need to be developed for yeast identification.

## CONCLUSION

Because of the delay in obtaining identification by conventional methods, there is a need to evaluate alternative tests and suitable methods for rapid identification of different *Candida* and *Trichosporon* species in a routine microbiology laboratory.

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