Modified Germ Tube Test: A Rapid Test for Differentiation of *Candida Albicans* from *Candida Dubliniensis*

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ABSTRACT

Background: *C. albicans*, the most prevalent yeast isolated from clinical samples, whose presumptive identification is usually based on germ tube test using human serum at 37°C. But *C. dubliniensis* also produces true germ tubes in same conditions. So a simple and fast method is needed to differentiate *C. albicans* from *C. dubliniensis*. In the present study we evaluated germ tube formation on YEPD medium at 39°C for rapid differentiation of the two species.

Materials and Methods: A total of 200 strains were tested for germ tube formation on YEPD medium at 39°C which included 186 strains of *C. albicans* and 14 strains of *C. dubliniensis.* Standard strains of both species were included in the study.

Results: Out of 186 test strains of *C. albicans* 166 (89.3%) formed true germ tubes after 1 h at 39°C while the remaining grew as a yeast form. All the 20 test strains of *C. dubliniensis* grew as a yeast form.

Conclusion: Positive modified germ tube test can be used for presumptive identification of *C. albicans* and ruling out *C. dubliniensis* however negative test cannot be used to differentiate between the two species.

Keywords: *C. albicans, C. dubliniensis,* Germ Tube Test, Modified Germ Tube Test, PCR-RFLP, YEPD Medium.

INTRODUCTION

With the increasing number of immunocompromised individuals, the incidence of fungal infections is also rising.[1] Also with the frequent use of broad-spectrum antibiotics and immunosuppressant's, many species previously unassociated with human diseases have become important pathogens, some examples being *Penicillium marneffei*, *Emmonsia pasteurina* and *Candida dubliniensis*.[2]

Candida dubliniensis is a recently identified species, mainly associated with oral candidiasis in immunocompromised patients.[3,4] The increasing number of infections caused by *C. dubliniensis* necessitates its proper identification to facilitate the appropriate therapeutic regimens. However, the identification of *C. dubliniensis* in clinical samples is difficult due to the frequent presence of *C. albicans* with which it is closely related, sharing most of its features for identification.[5]

C. albicans is the most prevalent yeast isolated from clinical samples.[6] However an increase in the emergence of non-albicans Candida *spp.* has been reported in last decade. The presumptive identification of *C. albicans* is usually based on its ability to produce germ tube when incubated at 37°C for 2 hours in pooled human serum. But *C. dubliniensis* also produces true germ tubes and *C. tropicalis* may produce false germ tubes which basically are pseudohyphae with constriction at its origin in human serum in similar conditions.[7] So a simple, fast, safe and practical method, which can be routinely performed in average mycology laboratory, is needed to differentiate *C. albicans* from other species of candida more accurately.

In addition to human serum, a number of other media induce germ tube formation, including plasma, saliva, sheep serum, fetal bovine serum, rabbit serum, and horse serum. Newer techniques using serum-free media like egg white, YEPD medium, tissue culture medium 199 (Difco laboratories, Detriot, Mich.), trypticase soy broth, rice cream agar, 2% oxgall broth, rice infusion-oxgall-Tween 80, Mueller-Hinton agar and various other peptone media, have been evaluated for germ tube production which are safer than the conventional method where human serum is used that might be contaminated with HIV and hepatitis viruses and are easy to perform as time required to prepare human serum is saved.[6,7,8]

Identification of *C. albicans*, based upon the ability to form germ tubes at 39◦C in serum-free YEPD media, yielded more reliable results than those by conventional methods, such as the serum-induced germ tube test, chlamydospore test and colony colour test on chromogenic media. Also this method has also shown to differentiate *C. albicans* and C. *dubliniensis*.[9] So in the present study we evaluated germ tube formation on YEPD medium at 39°C to differentiate *C. dubliniensis* from *C. albicans* with Polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) as gold standard.[4]

MATERIAL AND METHODS

This study was conducted in the Mycology Division of Department of Microbiology of a tertiary care hospital in Kashmir, India. The study was approved by the Institute's

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Ethics Committee.

Standard strains of *C. albicans* 90028 obtained from National Culture Collection of Pathogenic Fungi, Department of Medical Microbiology, PGIMER, Chandigarh and *C. dubliniensis* (type strain CD36) and *C. dubliniensis* (CBS 7987) which were kindly provided by Dr. Ziauddin Khan (Professor and Chairman Department of Microbiology, Kuwait University) were included in the study. Test strains included 186 stock strains of *C. albicans* isolated from cancer patients with oral candidiasis/colonization and 14 isolates were strains of *C. dubliniensis* which were kindly provided by Dr. Ziauddin Khan. Identification of all these strains was confirmed by PCR-RFLP using *AvrII* enzyme.[4]

Methods

YEPD (yeast extract peptone dextrose) broth was prepared in the same as described by Kim D et al.[9] The media (0.5ml) was poured into a sterile small glass tube. The isolates to be tested were diluted to 0.5 McFarland with YEPD liquid and incubated at 39°C for 1-2 hours. A drop of the suspension was transferred on to a microscopic slide for examination. A clean coverslip was placed over the drop and examined under low magnification (100x) for the presence of germ tubes.

STATISTICAL ANALYSIS

Microsoft office 2007 was used for the analysis. Descriptive statistics like mean and percentages were used for the analysis.

RESULTS

Elongated daughter cells from the round mother cell without constriction at their origin are referred to as true germ tubes while constricted hyphae as pseudohyphae. In YEPD, the standard strain of *C. albicans* formed true germ tubes after 1 h at 39◦C while those of *C. dubliniensis* grew as a yeast form. Out of 186 test strains of *C. albicans,* 166 formed true germ tubes after 1 h at 39◦C while the remaining grew as a yeast form. All the 20 test strains of *C. dubliniensis* grew as a yeast form (Figure 1,2).

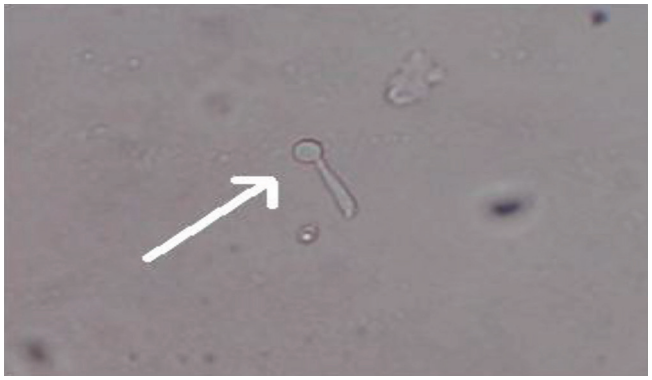

Figure-1: Germ tube formation by ***C. albicans*** at 39◦C in YEPD medium.

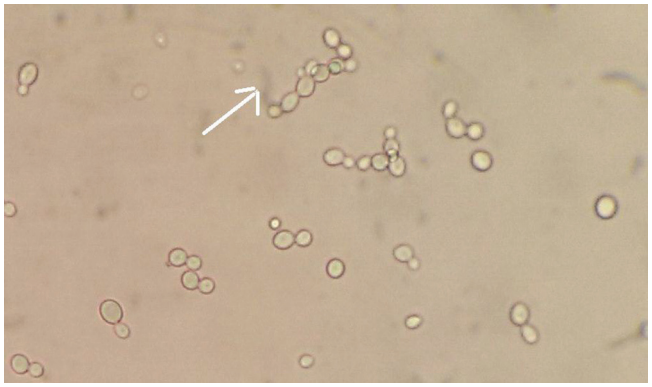

Figure-2: Yeast forms of ***C. dubliniensis*** at 39◦C in YEPD medium.

Germ tube test is very cost effective as compared to phenotypic methods and PCR-RFLP. The cost per isolate of various phenotypic methods range from 2-6 INR per isolate and for PCR-RFLP it is approximately 500 INR per isolate while for germ tube test it is only 0.1 INR per isolate. The turnaround time for other phenotypic methods is 2-4 working days and for PCR-RFLP it is 2 working days while as for germ tube test it is only 1-2 hours.

DISCUSSION

C. albicans exhibits the ability to grow as either a yeast or a mycelial form in response to different environmental factors. In vivo, the mycelial forms found in infected tissues may be important as a virulence factor in the adherence of the organisms to host epithelial tissues. In vitro, a germ tube can be induced from the yeast cell by changes in a variety of environmental factors, including ambient pH, nutritional status and temperature. However, the mechanism whereby these factors induce germ tube formation in *C. albicans* is virtually unknown.[9]

Conventionally germ tube test in human serum at 37°C for 2–3 h is used to differentiate between *C. albicans* from other non- albicans species but both *C. albicans* and *C. dubliniensis* produce germ tube by this method.[6] Also despite of the fact that germ tube test using human serum is easy to perform and has low cost this test has several disadvantages like, the serum has to be fresh or frozen; the yeast inoculum has to contain < 10^7cells mL^{-1}, otherwise, the germ tube production is inhibited. There is possible risk of infection with HIV or hepatitis virus while handling the serum and also different batches of serum may produce different results.[7]

In our study we evaluated germ tube production at 39°C after 1h of incubation in serum-free YEPD medium as done by Kim D et al. (2002) who reported that no *Candida* species other than *C. albicans* is able to form germ tubes in this medium.[9] In our study, 89.3% of *C. albicans* formed germ tubes while rest of *C. albicans* and *C. dubliniensis* strains grew as a yeast form. These results are not in agreement with that reported by Kim D et al. (2002)[9] who found that 100% of *C. albicans* form germ tubes after 1 h at 39°C. This method although very rapid and cheap (0.1 INR/isolate) if used as a sole test may lead to misidentification of about 11% of *C. albicans* as *C. dubliniensis.*

CONCLUSION

In conclusion, positive modified germ tube test can be used for presumptive identification of *C. albicans* and ruling out *C. dubliniensis* however negative test cannot be used to differentiate between the two species.

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