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Original Research Article

Use of various substrate/ media for germ tube test (GTT) detection in *Candida albicans* and *Candida dubliniensis* from a tertiary care hospital

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ABSTRACT

Introduction ans Objective: In recent years, non-judicious use of broad spectrum antibiotics, corticosteroids, antineoplastic agents, immune suppressive drugs, widespread increased use of bone marrow and organ transplant, and unreasonable use of indwelling catheters have led to an increased incidence invasive candidiasis (IC). The germ tube test (GTT) is a well established, inexpensive, rapid and easy to dispense test used to identify *candida dubliniensis*. So the present study was conducted to assess the reliableness of different media for GTT production in *Candida albicans* and *Candida dubliniensis*.
Materials and Methods: A total of 102 *C.albicans* and 09 *C. dubliniensis* strains were obtained from various clinical samples received at Department of Microbiology from a teaching institute and hospital located at west- central part of Maharashtra for a period of nine months. Medium that were employed for production of germ tube in *C.albicans* and C.dubliniensis in the present study were trypticase soy broth, YEPD (Yeast Extract Peptone Dextrose) broth, Muller-Hinton agar/broth and peptone water which were assessed and compared with pooled human sera routinely used for detection of germ tube.
Observation: For both the *Candida* isolates human pooled serum showed 96.39% positivity followed by YEPD (yeast extract peptone dextrose) broth with 92.79% positivity, Trypticase soy broth showed 80.18% positivity, whereas Muller Hinton broth produced 67.56% positivity and least positivity was seen with Peptone water where only 45.04% of germ tube induction was observed.
Conclusion: So our study clearly highlights that YEPD broth is better serum free media for germ tube production. Moreover, this medium is available commercially, is more stable, reliable, effective, is non biohazardous, convenient and cost effective alternative.

1. Introduction

Candida species are the commonest fungal disease found in humans affecting mucosa, skin, nails, and internal organs of the body.[1] *Candida* species are commensals of the oral cavity, intestinal tract and vagina, with newborns being colonized soon after birth. In recent years, use of non-judicious antibiotics, corticosteroids, antineoplastic agents, immune suppressive drugs, widespread increased use of bone marrow and organ transplant, and unreasonable use of indwelling catheters have led to an increased incidence of the infections caused by *Candida* species.[1,2]

Candida albicans is widely identified worldwide as being the most virulent yeast like fungi and in the majority of hospital based studies it has been found to be the most frequent cause of superficial and systemic mycosis.[1,2] In 1955, Dublin ,Ireland a new species of *Candida* was isolated in a HIV infected individuals with oropharyngeal candidiasis which was named *Candida dubliniensis*, which was found to be closely related to C. albicans, as it shares

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many phenotypic properties, production of germ tube and chlamydospores formation.[1]

The gamut of candidiasis ranges from superficial infections of the vaginal and oral mucosae, to life-threatening invasive candidiasis with systemic involvement that proliferate via the bloodstream to internal organs of the body.[2,3] The risk includes individuals admitted to intensive care units (ICUs), on dialysis, postsurgical patients; human immunodeficiency virus (HIV) infected hosts, elderly patients, premature infants and patients with hematological and terminal stage malignancies.[4]

Although various diagnostic methods are available for identification of *Candida* isolates such as morphological, molecular and biochemical modalities, but for rapid identification of *Candida* species in all mycology laboratories begins with the germ tube test.[1,4]

The presumptive clinical identification of *C.albicans* and *C. dubliniensis* is usually based on its ability to produce germ tube when incubated at 37^0 c for 2 hours in pooled human serum.[5,6]Germ tube formation was first reported and demonstrated by Reynold and Braude in 1956 and hence, the germ tube test is also known as a "Reynolds Braude Phenomenon".

Infection occurs when there is hyphal growth and biofilm formation in the tissue. These mechanisms also allow resistance of *C.albicans* to traditional antifungal agents.[7]

Timely diagnosis is required in order to reduce mortality. Currently, Invasive Candidiasis (IC) is diagnosed through the findings of hyphae on microscopic examination or through a time- consuming culture.[8] Serological tests provide relatively faster and easier way to diagnose IC. β-d glucan (BDG) test is one widely used *Candida* serological test. However, it lacks specificity due to cross reaction with other fungi.[8] Other rapid assays have been developed to identify yeasts, and most of these methods are again extremely expensive and labor intense and may not be available routinely in all laboratories.[9]

The germ tube test (GTT) is a well-established, inexpensive, and easy to administer test used to identify *C.albicans* from other species (except *Candida dubliniensis* and *Candida africana*).[10] Various media can be used for induction of germ tube, each with unique compositions and function. Human serum is the most used medium for germ tube test.[2,8]Its main limitation is the requirement of fresh human serum on a regular basis and potential biohazard.[2,6,8]

So the present study was conducted to assess the reliability of different media for germ tube test (GTT) production in *Candida albicans* and *Candida dubliniensis* isolated from various clinical samples.

2. Materials and Methods

2.1. Study design

Prospective cross-section study, conducted at Department of Microbiology, Indian Institute of Medical Science and Research Medical college from west-central part of Maharashtra

2.2. Study period

January 2022 to September 2022.

2.3. Identification and speciation

During nine months of present study, a total of 102 *C.albicans* and 09 *C. dubliniensis* strains were isolated from various clinical samples received at Department of Microbiology. All the isolate of *Candida* were identified and further speciated by conventional mycological methods such as Gram's staining, culture on Sabouraud's dextrose agar (SDA), germ tube test, chlamydospore formation on corn meal agar, sugar fermentation test, sugar assimilation test, and growth on Hi-chrome *candida* agar.[5,11]

2.4. Different medium/substrate

Pooled human sera routinely used for detection of germ tube in clinical isolates of *C.albicans* and C.dubliniensis were compared with serum free medium such as Trypticase Soy Broth, YEPD (Yeast Extract Peptone Dextrose) broth, Muller-Hinton agar/broth and Peptone Water

2.5. Germ tube test (GTT) induction

Sabouraud's dextrose agar was used to subculture all *C.albicans* and C.dubliniensis isolates and were incubated at 37°C for 24-48 hours. For Germ tube test detection, 2-3 colonies were picked up from fresh culture and light inoculum were made in 0.5ml of all serum free media mentioned above which were then transferred in 12×75 mm test tube. *C.albicans* ATCC 10231 and C. krusei were used as positive control and negative control with each batch of yeasts tested.[11,12]

The serum free medium containing test tubes inoculated with *C.albicans* and C.dubliniensis were further incubated at 37°C in a water bath for 3 hours. Post that a drop of incubated suspension was placed on a glass slide and covered with coverslip. Evaluation of germ tube formation was done by Microscopic examination under 40X magnification for formation of germ tube.[5,11,12]

Typically, C. albicans reveals thin germ tubes, 3 to 4 mm in diameter and up to 20 mm long; unlike pseudohyphae that are not constricted at their point of origin. Observation of minimum five germ tubes in entire wet mount preparation was used as criterion for germ tube positivity. Negative results were confirmed by examining atleast 10X high

power fields for the presence of germ tubes.[5,11,12]

2.6. Statistical analysis

Microsoft office 2016 was used for the analysis. Descriptive statistics like mean and percentages were used for the analysis.

3. Results and Observation

In the present study, the germ tube production for 102 *C.albicans* and 09 C dubliniensis isolates were evaluated by using five different media.

Details of the study can be seen in Table 1

3.1. GTT- Germ tube test, YEPD- yeast extract peptone dextrose

For both the *candida* isolates human pooled serum showed 96.39% positivity followed by YEPD (yeast extract peptone dextrose) broth with 92.79% positivity, Trypticase soy broth showed 80.18% positivity , whereas Muller Hinton broth produced 67.56% positivity and least positivity was seen with Peptone water where only 45.04% of germ tube induction was observed.

4. Discussion

Many diagnostic mycology laboratories uses pooled human serum for germ tube detection. Germ tube test is widely considered, accepted, reliable and easy technique for quick presumptive identification of *C.albicans* and *C. dubliniensis*. Germ tube production by yeast cells indicates their morphological adaptation to filamentous forms under unfavourable conditions.[12,13]

However, the use of pooled human serum has some disadvantages. Serum sample must be fresh or stored frozen and the inoculum size needs to be minimal (< 10^7 cells/ml) because heavy inoculum is known to hamper germ tube production.[14] The another major drawback in handling pooled human serum is the risk of transmission of infection with HIV or Hepatitis virus. Variableness in the performance noticed with different batches of serum and presence of biological inhibitors in pooled human sera might increase the chances of false negative results.[11–14]

While study conducted by Mackenzie DWR[15] highlights that pooled human serum stored at 4^0C for 15 days reduces its ability to produce germ tubes by 50%.

Studies[9–12] conducted with pooled human serum reported a 91-100% sensitivity 95–100% specificity for germ tube test, and the few other studies[5,14] reported a sensitivity of 92.3%, 90%, and 35%, by using fetal bovine serum, rabbit serum, and horse serum respectively.

Hilmioglu et al.[16] conducted a study where they compared 12 fluids for GTT production, best results for GTT were obtained with fresh human serum, similarly human serum was found to be better in the study conducted Arora et al.[17] So in our present study also maximum number (96.39%) of *C.albicans* and *C. dubliniensis* strains produced germ tubes on pooled human serum and the possible reason may be due to the inhibitors present in the human serum, yeast cell concentration and storage condition of serum.

Our study highlights that around 92.79% of positivity were seen with YEPD (Yeast Extract Peptone Dextrose) which correlates with a study conducted by KIM et al.,[18] where demonstration of Germ tubes were seen within 30 minutes in YEPD at 39^0C but after 60 min in serum at 37^0C. Whereas study conducted by Bhumla et al.,[19] showed 81.25% production of germ tube production with YEPD and another study carried out by Abiroo Jan et al.,[20] showed 89.30% germ tube production but only in *Candida albicans* and no germ tube formation in *candida dubliniensis*.

Our study showed that with Trypticase Soya Broth both *Candida* isolates could produce germ tube in 80.18% strains which is similar to study carried out by of Arora DR et al.,[17] and Makwana GE et al[21] where they reported trypticase soy broth to be less efficient than human serum in GTT production. In contrast to such findings, a study carried out by Joshi et al[11] and Deorukhkar et al.,[12] recommended trypticase soy broth to be more effective option compared to pooled human serum for germ tube test for *C.albicans* and *C. dubliniensis*. So our study finding clearly showed that YEPD is best available media for germ tube production.

Muller-Hinton broth and Peptone Water revealed positive results in 67.56 % and 45.04% of *C.albicans* and C.dubliniensis isolates respectively in present study. Mattei AS et al.,[22] recommended Mueller Hinton broth or agar as a preferred media to human serum in germ tube production test. MA Atalay et al.,[23] reported 91.5% sensitivity using commercially available Muller Hinton Agar in the identification of *C.albicans* and *C. dubliniensis*, respectively. However, interestingly both the isolates of *Candida* showed low number of germ tube in Muller-Hinton Broth in our setting. An exact explanation of this phenomenon is unknown. In regards to peptone water many researchers[15–17] found out that, role of other media like RPMI-1640 broth, Sabouraud's broth, animal serum and peptone water in GT formation was evaluated and found to be less productive.

In Peptone water only 45.04% strains of *C.albicans* and *C. dubliniensis* respectively could produce germ tubes, possible reason for low sensitivity may be due to the lower nutritive value of peptone water commensurate to other media. Similarly, Deorukhkar et al[12] also reported sensitivity of 69% in peptone water. So amongst all the non-serum based media our study suggest the less suited medium for germ tube production is peptone water

Table 1: *Candida* isolates with positive GTT on different media/substrates

S.No.	Media/ Substrate	Isolates with positive GTT		Total (n=111)	Percentage (%)
		Candida albicans (n=102)	*Candida dubliniensis* (n=09)		
01	Human pooled serum	99	08	107	96.39
02	YEPD	96	07	103	92.79
03	Trypticase soy broth	85	04	89	80.18
04	Muller-Hinton broth	72	03	75	67.56
05	Peptone water	48	02	50	45.04

5. Conclusion

So our study clearly highlights that YPED broth is superior serum free media for germ tube production and ensuing rapid identification & presumptive differentiation of *C.albicans* and *C. dubliniensis* from other clinically significant *Candida* species without considerable time required for the preparation and testing of pooled human serum. Moreover, this medium is commercially available, is more stable, effective, is non biohazardous, convenient and cost effective alternative.

6. Source of Funding

None.

7. Conflict of Interest

None.

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