International Journal of Community Medicine and Public Health
Bhumbla U et al. Int J Community Med Public Health. 2021 Dec;8(12):5968-5971
http://www.ijcmph.com
pISSN 2394-6032 | eISSN 2394-6040

DOI: https://dx.doi.org/10.18203/2394-6040.ijcmph20214598

Original Research Article

A comparative study for production of germ tube in *Candida albicans* of various pulmonary samples, by different methods in a tertiary care hospital of south west Rajasthan

Upasana Bhumbla[1]*, Amit Gupta[2]

[1]Department of Microbiology, Associate Professor, Adesh Institute of Medical Sciences, Bathinda, Punjab, India
[2]Department of Pulmonology, Assistant Professor, Geetanjali Medical and Hospital, Udaipur, Rajasthan, India

Received: 07 October 2021
Accepted: 11 November 2021

***Correspondence:**
Dr. Upasana Bhumbla,
E-mail: ucupasana124@gmail.com

ABSTRACT

Background: Systemic candidiasis is associated with a high crude mortality rate, even with first line antifungal therapy. *C. albicans* is the predominant cause of invasive fungal diseases which is a serious public health issue. The main objective was to assess the reliability of different media for germ tube production in *Candida albicans* isolated from various clinically diagnosed pulmonary samples.
Methods: All Candida isolates were identified and speciated by conventional methods such as Gram's staining, germ tube test, chlamydospore formation on corn meal agar, sugar fermentation test, sugar assimilation test, and growth on Hi-chrome candida agar.
Results: Out of 108 clinical isolates of *Candida albicans*, 5 different methods were used for germ tube production. Pooled human sera showed 93/108 (86.1%) was the most sensitive method wherein YEPD (yeast extract peptone dextrose) broth 91/108 (84.7%) was the reliable and easy method for detection of germ tube, followed by trypticase soy broth 81/108 (81.4%); peptone water 80/108 (74.7%) and 2% sucrose 71/108 (65.7%).
Conclusions: YPED broth is found to be a better serum free substrate and subsequently for the presumptive differentiation of *C. albicans* from non-albicans candida (NAC), without the extensive time required for the preparation and testing of pooled human serum. Furthermore, this medium is commercially available, more stable, effective, and is not bio hazardous.

Keywords: *Candida albicans*, Chlamydospore, Trypticase

INTRODUCTION

The incidence and prevalence of invasive fungal infections have increased in the last decade, especially due to large population of immunocompromised patients and/or those hospitalized with serious underlying diseases. Indeed, *Candida spp.* are the fourth most common cause of hospital-acquired systemic infections in India with crude mortality rates of up to 30%. In healthy individuals this colonization generally remains benign. *C. albicans* can cause two major types of infections in humans: superficial infections, such as oral or vaginal candidiasis, and life-threatening systemic infections. Indeed, HIV is a major risk factor for developing oral candidiasis.[1] Systemic candidiasis is associated with a high crude mortality rate, even with first line antifungal therapy. Both neutropenia and damage of the gastrointestinal mucosa are risk factors for the development of systemic (disseminated) candidiasis. Further risk factors include central venous catheters, which allows direct access of the fungus to the bloodstream, the application of broad-spectrum antibacterials, which enable fungal overgrowth, and trauma or gastrointestinal surgery, which disrupts mucosal barriers.[2]

C. albicans is the predominant cause of invasive fungal infections and represents a serious public health challenge with increasing medical and economic importance due to the high mortality rates and increased costs of care and duration of hospitalization. During both superficial and systemic infection, *C. albicans* relies on a battery of virulence factors and fitness attributes.[3] One such suggested contributory virulence factor in the pathogenesis of *C. albicans* is germ tube.[4] Also it is a presumptive clinical identification of *C. albicans* usually made on the basis of its ability to produce short, slender, tube-like structures called germ tubes when incubated at 35°C to 37°C for 2 to 4 hours in pooled human serum.[5] The germ tube has parallel walls and no constriction at the point of origin at the blastospore mother cell. The use of human serum routinely for culture and microscopic examination of *C. albicans* in the germ tube testis cheap but presents a hazard for transmission of diseases. This study investigated the possibility of using other four different substrates for induction of germ tube by *C. albicans* and to compare their efficacy with pooled human serum in routine laboratory.

The aim of the study was to assess the reliability of different media for germ tube production in *Candida albicans* isolated from various clinically diagnosed pulmonary samples.

METHODS

During the study period of one year, a total of 108 *C. albicans* strains were isolated from various clinical pulmonary samples received in microbiology department of a tertiary care teaching hospital in South west Rajasthan. All Candida isolates were identified and speciated by conventional methods such as Gram's staining, germ tube test, chlamydospore formation on corn meal agar, sugar fermentation test, sugar assimilation test, and growth on Hi-chrome candida agar (Himedia, Mumbai).[4]

For germ tube test substrates that were employed for induction of germ tube in clinical isolates of *C. albicans* in the present study were trypticase soy broth, YEPD (yeast extract peptone dextrose) broth, brain heart infusion, 2% sucrose, peptone water were evaluated and compared with regular substrate employing pooled human sera.[6] All *C. albicans* isolates were sub-cultured onto Sabouraud's dextrose agar and were incubated at 37°C for 24-48 hours before performing the germ tube test. For the germ tube test, a light inoculum was made, of 2-3 colonies of each isolate from fresh culture in 0.5 ml of all the above media which were dispensed in 12×75 mm test tube. A positive control (*C. albicans* ATCC 10231) and a negative control (*C. krusei*) were used with each batch of yeaststested.[7] Then the inoculated test tubes were incubated at 37°C in a water bath for 3 hours. Evaluation of germ tube formation was done by placing a drop of incubated suspension placed on a glass slide and covered with coverslip. Microscopic examination was done under magnification of 40X for the presence of germ tube.[8] Of typical *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. A criterion for germ tube positivity was observation of minimum five germ tubes in entire wet mount preparation. Negative results were confirmed by examining atleast 10X high power fields for the presence of germ tubes.[9]

RESULTS

In the present study, the germ tube production for 108 *C. albicans* isolates were seen by using five different substrates. Pooled human sera showed 93/108 (86.1%), YEPD broth (yeast extract peptone dextrose) 91/108(84.7%); trypticase soya broth 81/108 (81.4%); peptone water 80/108 (74.7%) and 2% sucrose showed 71/108 (65.7%). YEPD broth and pooled human serum were performed almost similar for the induction of germ tube in *C. albicans*.

Table 1: Sensitivity of different substrate in comparison to CHROM agar.

Substrate	Sensitivity (%)
Pooled human sera	86.1
YEPD broth	84.7
Trypticase soy broth	81.4
Peptone water	74.7
2% sucrose	65.7

DISCUSSION

The incidence of candidiasis continues to rise in proportion to the growing number of patients at risk.[10] Thus rapid identification of Candida isolates to the species level in the clinical laboratory has become important. Several methods for the identification of yeasts have been developed. However, most of these techniques are labour-intensive technologies and expensive that are not commonly available in routine microbiology laboratory services.[2]

The germ tube test has been a long well-established, rapid and highly reliable presumptive test for identification of medically important *C. albicans.* This technique is a simple, cheap method and may therefore a favoured method for laboratories trying to work economically.[10] *C. albicans* cells reproduce normally by budding, and they frequently produce germ tubesunder unfavourable conditions.[4] The formation of unconstricted filaments in response to serum is the basis ofthe 'germ tube test', to distinguish *C. albicans* from other Candida species; although *C. dubliniensis*, the nearest relative to *C. albicans*, also forms unconstricted hyphae in this test. *C. tropicalis* after an extended incubation period of three hours may also produce germ tube-like structures.[9]

The classical method of pooled human sera has been widely used by laboratories for several years and had a sensitivity range of 91-100%. The results obtained by this study are in agreement with this parameter (sensitivity 94.53%). In spite of its low cost and easiness, the use of human serum for this test may have several disadvantages for example; serum has to be fresh otherwise frozen serum at 4°C for 15 days may have 50% decrease in germ tube production, false negative result due to the effect of biological inhibitors present in it, the yeast inoculum has to contain $<10^7$ cells/ml, different batches of serum may produce different results and most importantly the possible risk of biohazard.[11]

In an attempt to overcome these drawbacks, several investigators have proposed other media such as animal serum, plasma, peptone water, tryptic soy broth (TSB), Sabouraud broth, brain-heart infusion broth (BHI), RPMI-1640 broth, egg white and saliva.[12] These media, however, have low sensitivity. As compared to other media; serum is more sensitive for germ tube production.

In this study, pooled human serum had sensitivity (94.53%) and the possible reason may be due to the inhibitors present in the human serum, yeast cell concentration and storage condition of serum. Further, YEPD medium had sensitivity (81.25%) and also Kim et al reported 100% positivity at 39ºC upon comparing germ tube induction in rabbit serum at 37ºC. The possible cause of variability in germ tube positivity rate may be attributed to the incubation temperature andtime.[4,8,13,14]

Trypticase soy broth had a sensitivity rate of 72.65% in our study; which is similar to the findings of Arora et al and Makwana et al.[6,10] In contrast, Kim et al and Deorukhar et al had reported a higher sensitivity rates of 100% and 94%respectively in trypticase soya broth.[7] In this study, 61.71% of *C. albicans* isolates showed germ tube test positive in peptone water. Similarly, Deorukhkar et al also reported sensitivity of 69% in peptone water.[7] Among the less suited medium germ tube production in 2% sucrose solution was only 59.37%; but Raghunath et al reported a higher sensitivity rate of 80%.[12] This may be due to initial pH which have allowed germ-tube formation to occur and later a drop in pH could suppress germ-tubeformation.[15]

Germ tube formation in *C. albicans* is an endotrophic process, with new factors which affect germ tube formation, and the complex interrelationships between the many environmental factors.

CONCLUSION

YPED broth is found to be a better serum free substrate and subsequently for the presumptive differentiation of *C. albicans* from non-albicans candida (NAC), without the extensive time required for the preparation and testing of pooled human serum. Furthermore, this medium is commercially available, more stable, effective, and is not bio hazardous.

Funding: No funding sources
Conflict of interest: None declared
Ethical approval: The study was approved by the Institutional Ethics Committee

REFERENCES

1. Ruhnke M. Epidemiology of *Candida albicans* infections and role non-candida albicans yeasts. Curr Drug Targets. 2006;7:495-504.
2. Sachin CD, Ruchi K, Santosh S. In vitro evaluation of proteinase, phospholipase and haemolysin activities of Candida species isolated from clinical specimens. Int J Med Biomed Res. 2012;1:153-7.
3. Sullivan D, Haynes K, Bille J, Boerlin P, Rodero L. widespread geographical distribution of oral candida dubliniensisstrains in human immunodeficiency virus-infected individuals. J Clin Microbiol. 1998;36:3007-12.
4. Deorukhkar SC, Saini S, Jadhav PA. Evaluation of different media for germ tube production of *Candida albicans* and *Candida dubliniensis*. IJBAR. 2012;3(09):704-7.
5. Taschdjian CL, Burchall JJ, Kozinn PJ. Rapid identification of *Candida albicans* by filamentation on serum and serum substitutes. Am J Clin Pathol. 1960;90:212-5.
6. Arora DR, Saini S, Aparna, Gupta N. Evaluation of germ tube test in various media. Indian J Pathol Microbiol. 2003;46(1):124-6.
7. Kim D, Shin WS, Lee K H , Park J Y, Koh CM. Rapid differentiation of Candida albicans from other Candida species using its unique germ tube formation at 39 dgeree C. Yeats 2002;19(11):957-62.
8. Kim D, Shin WS, Lee KH, Kim K, Park JY, Koch CM. Rapid differentiation of *Candida albicans* from Candida species using its unique germ tube formation at 39°C. Yeast. 2002;19:957-62.
9. Sheppard DC, Locas MC, Restieri C, Laverdiere. Utility of germ tube formation at 39°C. Yeast. 2002;19:957-62.
10. Makwana GE, Gadhavi H, Sinha M. Comparison of germ tube production by *Candida albicans* in various media. NJIRM. 2012;3:6-8.
11. Mattei AS, Alves SH, Severo CB, Guazzelli LD, Oliveira FD, Severo LC. Use of Mueller-Hinton broth and agar in the germ tube test. Revista do Inst Med Trop São Paulo. 2014;56:483-5.
12. Raghunath P, Kumari KS, Subbannayya K. SST broth, a new serum free germ tube induction medium for identification of *Candida albicans*. World J Microbiol Biotechnol. 2014;30(7):1955-8.
13. Sevilla MJ, Odds FC. Development of Candida albicans hyphae in different growth media-variations in growth rates, cell dimensions and

timing of morphogenetic events. Microbiology. 1986;132(11):3083-8.
14. Atalay MA, Koc AN, Parkan OM, Aydemir G, Elmali F, Sav H. Can serums be replaced by Mueller-Hinton agar in germ tube test? Niger J Clin Pract. 2017;20(1):61-3.
15. Pollack JH, Hashimoto T. The role of glucose in the pH regulation of germ-tube formation in *Candida albicans*. Microbiology. 1987;133(2):415-24.

Cite this article as: Bhumbla U, Gupta A. A comparative study for production of germ tube in *Candida albicans* of various pulmonary samples, by different methods in a tertiary care hospital of south west Rajasthan. Int J Community Med Public Health 2021;8:5968-71.