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

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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, Chlamydomspore, 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 tests 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).\(^6\)

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 dispersed in 12x75 mm test tube. A positive control (C. albicans ATCC 10231) and a negative control (C. krusei) were used with each batch of yeasttested.\(^7\) 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.

| 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 tube under unfavourable conditions.\(^4\) The formation of unconstricted filaments in response to serum is the basis of the ‘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 39°C upon comparing germ tube induction in rabbit serum at 37°C. The possible cause of variability in germ tube positivity rate may be attributed to the incubation temperature and time.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 Deorukhkar et al had reported a higher sensitivity rates of 100% and 94% respectively in trypticase soy 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-tube formation.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.

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