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Comparison of Different Media for Germ Tube Production by *Candida albicans*: A Retrospective Study

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ABSTRACT

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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 is to assess the reliability of different media for germ tube production in *Candida albicans* isolated from various clinical samples. All *Candida* isolates were identified and speciated by conventional methods such as Gram's staining, germ tube test, chlamyospore formation on corn meal agar, sugar fermentation test, sugar assimilation test, and growth on Hi-chrome candida agar. Out of 128 clinical isolates of *Candida albicans*, 5 different methods were used for germ tube production. Pooled human sera showed 121/128 (94.53%) was the most sensitive method wherein YEPD (Yeast Extract Peptone Dextrose) Broth 104/128 (81.25%) was the reliable and easy method for detection of germ tube, followed by Trypticase soy broth 93/128 (72.65%); Peptone water 79/128 (61.71%) and 2% Sucrose 76/128 (59.37%).

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 human's superficial infections, such as oral or vaginal candidiasis, and life-threatening systemic infections. Indeed, HIV is a major risk factor for developing oral

candidiasis (Ruhnke, 2006). 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 allow 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 (Sachin *et al.*, 2012).

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 (Sullivan *et al.*, 1998), *C. albicans* relies on a battery of virulence factors and fitness attributes. One such suggested contributory virulence factor in the pathogenesis of *C. albicans* is germ tube. 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 h in pooled human serum (Taschdjian *et al.*, 1960). 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 test is 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*, in various clinical samples.

Materials and Methods

During the study period of two years, a total of 128 *C. albicans* strains were isolated from various clinical samples received in Microbiology department, of a tertiary care teaching hospital. 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) (Sachin C. Deorukhkar *et al.*, 2012)

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 (Arora *et al.*, 2003). 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 done, of 2-3 colonies of each isolate from fresh culture in 0.5 ml of all the above media which were dispensed in 12x75 mm test tube. A positive control (*C. albicans* ATCC 10231) and a negative control (*C. krusei*) were used with each batch of yeast tested (Sachin C. Deorukhkar *et al.*, 2012). 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 (Kim *et al.*, 2002). 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 germtube positivity was observation of minimum five germ tubes in entire wet mount preparation. Negative results were confirmed by examining at least 10 high power fields for the presence of germ tubes (Sheppard *et al.*, 2002).

Results and Discussion

In the present study, the germ tube production for 128 *C. albicans* isolates were seen by using five different substrates. Pooled human sera showed 121/128 (94.53%), YEPD (yeast extract peptone dextrose) Broth 104/128

(81.25%); Trypticase soy broth 93/128 (72.65%); Peptone water 79/128 (61.71%) and 2% Sucrose 76/128 (59.37%). YEPD broth and pooled human serum were performed almost similar for the induction of germ tube in *C. albicans*.

The incidence of candidiasis continues to rise in proportion to the growing number of patients at risk (Gopee E. Makwana *et al.*, 2012). 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 those are not commonly available in routine microbiology laboratory services (Sachin *et al.*, 2012).

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` (Maria-Jesus Sevilla and Odds, 1986). *C. albicans* cells reproduce normally by budding, and they frequently produce germ tubes under unfavourable conditions (Sachin C. Deorukhkar *et al.*, 2012). The formation of unstricted 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 unstricted hyphae in this test. *C. tropicalis* after an extended incubation period of three hours may also produce germ tube-like structures (Sheppard *et al.*, 2002).

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 < 10⁷ cells mL⁻¹, different batches of serum may produce different results and most importantly the possible risk of biohazard (Pendru Raghunath and Seshu Kumari, 2014).

In an attempt to overcome these drawbacks, several investigators have proposed other media such as animal serum, plasma, peptone water, trypticase soya broth (TSB), Sabouraud broth, brain-heart infusion broth (BHI) (Antonella Souza *et al.*, 2014), RPMI-1640 broth, egg white and saliva. These media, however, have low sensitivity. As compared to other media; serum is more sensitive for germ tube production.

Table.1 Sensitivity of different substrate in comparison to CHROMagar

Substrate	Sensitivity (%)
Pooled human sera	94.53%
YEPD Broth	81.25%
Trypticase soy broth	72.65%
Peptone water	61.71%
2% Sucrose	59.37%

In this study, pooled human serum had highest 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.*, (2002) reported 100% positivity at 39°, 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. (Sachin C. Deorukhkar *et al.*, 2012; Kim *et al.*, 2002; Atalay *et al.*, 2017)

Trypticase soya broth had a sensitivity rate of 72.65% in our study; which is similar to the findings of (Arora *et al.*, 2003) and (Gopee E. Makwana *et al.*, 2012). In contrast, Joshi KR *et al.*, and (Sachin C. Deorukhkar *et al.*, 2012) had reported a higher sensitivity rates of 100% and 94% respectively in Trypticase soya broth. In this study, 61.71% of *C. albicans* isolates showed germ tube test positive in peptone water. Similarly, (Sachin C. Deorukhkar *et al.*, 2012) also reported sensitivity of 69% in peptone water. Among the less suited medium Germ tube production in 2% Sucrose solution was only 59.37%; but (Pendru Raghunath and Seshu Kumari, 2014) reported a higher sensitivity rate of 80%. 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 (Pollack and Hashimoto, 1987) (Table 1).

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.

YPED broth is found to be a better serum free substrate for germ tube production and subsequently for the presumptive differentiation of *C. albicans* from non-albicans candida, without the extensive time

required for the preparation and testing of pooled human serum. Furthermore, this medium is commercially available and is more stable, effective, and is not bio hazardous.

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