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RESEARCH ARTICLE

Identification of *Candida* and *Trichosporon* species by oxidase and coagulase test.

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Abstract

Candidiasis is one of the most important opportunistic fungal infections, caused by over 20 species of *Candida.* After the genus *Candida, Trichosporon* is the most common agent of yeast disseminated infections. In routine laboratory, yeast species are identified on the basis of culture, germ tube production, carbohydrate fermentation and chlamydospore formation. These conventional methods often lack specificity. These are time consuming methods involving microscopic examination that requires considerable skill and experience. Delayed identification leads to delay in empirical therapies of such infections. The objective of this study was, therefore, to evaluate the reliability of oxidase and coagulase tests for the rapid identification of *Candida* and *Trichosporon* species.

Introduction:-

The Human Microbiota plays an inevitable role in human metabolism and in understanding the pathogenesis and the optimized therapy for many diseases. In our body, populations of microbes such as bacteria and yeasts form part of normal healthy human flora. However, when the host's immune defenses are compromised, as in anticancer chemotherapy, or during steroid or antibacterial therapy, or long term catheterization or hemodialysis, microbe numbers grow beyond their typical ranges and cause infections [(1)]. One such opportunistic infection is Candidiasis. It is a fungal infection caused by yeasts that belong to the genus *Candida*. There are over 20 species of *Candida* yeasts that can cause infection in humans, the most common of which is *Candida albicans*. Other *Candida* species that can cause the superficial infections include *C. tropicalis, C. glabrata, C. parapsilosis, C. stellatoidea, C. dubliniensis, and C. krusei* [(2).]

Candida species are the fourth most common cause of bloodstream infections among hospital patients in the United States [(3)]. Chakrabarti *et al* from Postgraduate Institute of Medical Education and Research (PGIMER), Chandigarh, reported high rates of Candida bloodstream infection (Candidemia) in 27 Intensive Care Units (ICU) in India [(4)]. It is, therefore, important to identify the species of *Candida* for the successful treatment of the disease. The conventional methods of identifying species in the clinical microbiology laboratory include either direct microscopic examination using potassium hydroxide (KOH) or laboratory culturing. Identification by Culturing methods relies on criteria such as colony characteristics and morphology on different media and sugar assimilation or fermentation. Isolates of *C. albicans* are typically identified by their ability to form germ tubes (Germ Tube Test) or chlamydospores (Dalmau Plate Technique) under the appropriate conditions [(5)].

The germ tube test first described in 1960 is still used for rapid presumptive identification of the yeast inoculated in serum [(6)]. Although rapid, the germ tube test has many shortcomings. Over inoculation of the serum can inhibit germ tube formation and too short incubation time (less than two to four hours) can lead to false results as it may be difficult to discern a true germ tube from that of an early pseudohyphal cell. Additionally, specificity can be lacking as *C. tropicalis* has been reported to rarely form germ tubes [(7)]. The use of human serum in this test has several disadvantages. For example, the serum has to be fresh or frozen; the yeast inoculum has to contain $<10^7$ cells mL^{-1},

otherwise, the germ tube production is inhibited. In addition, the handling of pooled human serum includes the possible risk of infection with HIV or hepatitis virus and different batches of serum may produce different results [8].

Another approach for yeast identification is Dalmau Plate Technique which requires longer (one to several days) incubation of isolates on morphology agar (e.g., corn meal, corn meal/Tween 80, rice extract, rice extract/Tween 80, etc.) that is observed microscopically for the presence of yeast cells, pseudohyphae and spores [9, 10]. Since both methods above demand microscopic observation that requires considerable skill and experience, there is a need to develop alternate tests that are sensitive and specific but require less technical expertise and shorter incubation time [11].

The present study was, therefore, intended to evaluate alternative tests to rapidly and reliably identify *C. albicans* as well as other *Candida* and *Trichosporon* species in routine clinical microbiology practice.

Materials and methods:-

Type of study

This was a laboratory based observational study which was carried out in the Department of Microbiology, AIIMS, Patna, Bihar, India.

Duration of study

The study was done in 3 months from February 2016 to April 2016.

Method

Sources of isolates: Isolates were selected from clinical isolates of *Candida* and *Trichosporon* species.

Isolation and purification: The clinical isolates of yeast were examined under microscope by preparing lactophenol cotton blue (LPCB) mounts. The selected yeast isolates were purified on fresh Sabouraud Dextrose Agar (SDA) (Glucose, 2g; Peptone, 1g; Agar, 2g; Deionized water, 100ml) slants. The slants were incubated at 37^0 C for 24 hours. These purified isolates were identified by conventional methods.

Conventional methods for yeast identification:-

Germ tube test:-

The yeast isolates were inoculated into 0.5ml of human serum and incubated in a water bath at 37^0 C for 2 to 4 hours. After incubation, a drop of suspension was placed on a clean glass slide and overlaid with a cover slip and examined under microscope for germ tube formation [12].

High temperature (42^0C) tolerance test:-

Germ tube positive isolates were streaked on SDA slants and incubated in a water bath at 42^0 C for 24 hours. Growth at this elevated temperature was observed to facilitate differentiation of *C. albicans* and *C. dubliniensis*.

Morphology on rice extract agar:-

Light inoculum of yeast isolates was partially streaked into the thickness of Rice Extract Agar (REA) (Rice powder, 0.04g; Agar, 1.5g; Deionized water, 100ml) media making 4-5 parallel lines of approximately 2 to 2.5 cm long and 0.5 to 0.8 cm apart. A flame sterilized coverslip was placed over it and incubated at 22^0 C for 3 to 5 days. After incubation, the lid of the petri plate was removed and the edge of the cover slip was observed under the microscope for morphological features like yeast cells, hyphae, pseudohyphae or spores [12].

Characterization of the selected isolates:-

The isolates were characterized by evaluating their oxidase and coagulase producing ability on Sabouraud Dextrose Agar (SDA) and Mueller Hinton Agar (MHA) media.

Oxidase Test:-

The yeast isolates were grown on both SDA and Mueller Hinton Agar (MHA) (Beef infusion, 30ml; Starch, 0.15g; Casein hydrolysate, 1.75g; Agar, 1g; Deionized water, 100ml) plates at 37^oC for 24 hours. A small amount of inoculum was placed onto the oxidase disc. Production of purple color within 10 seconds was recorded as positive [13].

Coagulase Test:-
The yeast isolates were grown on both SDA and MHA plates at 37^0 C for 24 hours. Yeast emulsion in a drop of normal saline was prepared on a glass slide. A drop of plasma was added and slide was observed for clumping [14].

Results:-
A total of 35 yeast isolates were recovered from various clinical samples (blood, urine and sputum). These clinical isolates, namely *C. albicans, C. dubliniensis, C. tropicalis, C. kefyr, C. glabrata, C. parapsilosis* and *T. cutaneum* were presumptively identified to the species level by using germ tube test, high temperature (42^0C) tolerance test, carbohydrate (glucose, lactose, maltose, sucrose, with phenol red indicator) fermentation test and by observing their microscopic morphology on rice extract agar (REA) medium.

Oxidase Test:-
All yeast isolates on SDA were negative for oxidase production (Table 1). When grown on MHA, all the isolates of *C. albicans, C. dubliniensis, C. tropicalis, C. kefyr, C. parapsilosis* and *T. cutaneum* and 5 out of 6 clinical isolates of *C. glabrata* were found to be positive for oxidase production (Table 2).

Coagulase Test:-
On SDA, 56.25% *C. albicans*, 75% *C. tropicalis, 83.33% C. glabrata, 75 % C. parapsilosis,* 50% *T. cutaneum, 0% C. dubliniensis and C. kefyr* were found to be positive for coagulase production (Table 1). When grown on MHA, 68.75% *C. albicans*, 75% *C. tropicalis, 83.33% C. glabrata, 75 % C. parapsilosis, 100% T. cutaneum, 100% C. dubliniensis and 0% C. kefyr* were found to be positive for coagulase production (Table 2).

Table 1: Results of oxidase and coagulase tests on SDA medium.

Yeast Species	No. Of isolates	Oxidase test(no. of positive/out of total)	Coagulase test(no. of positive/out of total)	%positive (Oxidase test)	%positive (Coagulase test)
C. albicans	16	0/16	9/16	0	56.25
C. dubliniensis	2	0/2	0/2	0	0
C. glabrata	6	0/6	4/6	0	66.66
C. kefyr	1	0/1	0/1	0	0
C. parapsilosis	4	0/4	1/4	0	25
C. tropicalis	4	0/4	3/4	0	75
T. cutaneum	2	0/2	1/2	0	50

Table 2: Results of oxidase and coagulase tests on MHA medium.

Yeast Species	No. Of isolates	Oxidase test(no. of positive/out of total)	Coagulase test(no. of positive/out of total)	%positive (Oxidase test)	%positive (Coagulase test)
C. albicans	16	16/16	11/16	100	68.75
C. dubliniensis	2	2/2	2/2	100	100
C. glabrata	6	5/6	4/6	83.33	66.66
C. kefyr	1	0/1	0/1	0	0
C. parapsilosis	4	4/4	2/4	100	50
C. tropicalis	4	4/4	3/4	100	75
T. cutaneum	2	2/2	1/2	100	50

Discussion:-
The conventional methods for the identification of *Candida* spp., although simple and inexpensive, have some limitations. These methods are time consuming and are often unable to discriminate between different species (for e.g. germ tube test fails to differentiate between *C. albicans* and *C. dubliniensis)*. Recognition of yeast microscopic morphologies on Dalmau plates requires considerable skill and experience.

Newer techniques like real time PCR, matrix assisted laser desorption ionization time of flight mass spectrometry (MALDI TOF) and multiplex tandem PCR are now being employed for the rapid identification of yeast species [15-18]. However, such expensive techniques are not commonly available for use in routine clinical microbiology laboratories.

The present study, therefore, evaluated the simple and cost effective alternative methods for the identification of different *Candida* and *Trichosporon* species. The oxidase test was found to be negative in all the species of *Candida* and *Trichosporon* when grown on SDA. This result matched with the reports of Kumar *et al*. (2000) who tested 100 yeast isolates [19]. On MHA, however, 34 out of 35 (97.14%) yeast isolates gave positive result for oxidase test (Table 2).

Plasma coagulase, an enzyme that binds plasma fibrinogen and activates a cascade of reactions that induce plasma to clot, is an established virulent determinant in *Staphylococcus* species but its relation to Candidal species is least studied. The present study, therefore, detected the coagulase activity in different *Candida* and *Trichosporon* species using slide coagulase method. *C. tropicalis*, *C. glabrata* and *C. albicans* isolates that were grown on SDA showed high coagulase activity with human plasma while *C. dubliniensis* and *C. kefyr* showed no coagulase production. Except for *C. kefyr*, all the isolates grown on MHA gave positive coagulase test. Autoagglutination was observed in some *C. albicans*, *C. parapsilosis* and *C. glabrata* isolates which were then tested by tube coagulase method using *S. aureus* starins as positive control, and were found negative.

This information can help in devising newer rapid tests for identification of these pathogens. Slide coagulase, can therefore be a very important test for species diferentiation in *Candida* spp. This is all the more important because a delay in accurate identification can result in delay of therapy [20]. Identification by these newer methods will lead to rapid institution of timely therapy.
The present study, however, has a limitation as it was carried out on only 35 yeast isolates belonging to 7 species only. So, these alternative tests are needed to be carried out on many other clinically significant yeast species that are not studied in this research and with a larger number of yeast isolates.

Conclusion:-

Because of the lack of specificity and delay in obtaining identification by conventional methods, there is a need to evaluate alternative tests and suitable methods for rapid identification of different *Candida* and *Trichosporon* species in a routine microbiology laboratory.

Conflicts of interest: None

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