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Coagulase Activity: A Virulence Trait in Pathogenic Candida species

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ABSTRACT

Candida species are ubiquitous fungi. It is the most frequent human fungal pathogen and 4th leading cause of hospital acquired infections. Candida species also contain well-recognized but not well-characterized virulence factors that may contribute towards their ability to cause infection. Coagulase activity of various cultures of *C. albican* were examined using a classical tube test and a slide test. A total of 20 clinical isolates of *Candida albican* were incubated in rabbit plasma for overnight. Positive strains of *Candida albican* for coagulase test accounts for 45% and negative results were account for 55% in rabbit plasma. In sheep plasma *C. albican*s showed positive results. None of the candida isolates have positive result in human plasma. In the study, rabbit plasma is found to be most suitable medium for the screening of coagulase enzyme. Therefore, the study concluded that detection of coagulase enzyme in laboratory possibly will aid in the diagnosis of Candida related infections.

Keywords: *Candida, Fungi, Coagulase activity*

INTRODUCTION

Candida species are omnipresent fungi that characterize mainly as widespread human fungal pathogens. Women are more vulnerable to genital yeast infections as compared to men. Also, individuals with diabetes or immune compromised individuals are more likely to acquire yeast infections. Important pathogenic species possibly include Candida albicans, *Candida tropicalis*, *Candida parapsilosis*, *Candida glabrata* and *Candida krusei*. Many species are found in gut flora, including *C. albicans* in mammalian hosts (Nguyen *et al.*, 2007). These fungal infections can be treated with antifungal drugs in otherwise healthy individuals (DEnfert and Hube, 2007). In debilitated or immune compromised patients it may become a systemic disease producing pathogenic organism.

Hydrolytic enzyme production is also well-known factor to play a fundamental role in the process of virulence of bacteria (Finlay and Falkow, 1989), protozoa (McKerrow, 1993) and pathogenic yeasts (Ogrydziak, 1990). Extracellular enzymes for instance lipases, coagulase, proteases phospholipases, esterases, and phosphatases are the recognized virulence factors of Candida albicans (Calderone and Fonzi, 2001; Chen *et al,* 2002). Other virulence factors involved in the pathogenecity of Candida albicans possibly include adhesion factors, phenotypic switching and morphogenesis. *C. albicans* grows in yeast form when cultured in ambient temperature and standard conditions. On the contrary, slight changes in environmental conditions such as temperature and pH can result in yeast to pseudohyphal transition. Although pseudohyphae morphology exhibit numerous similarities with yeast cells (Berman and Sudbery, 2002), but their role during infection remains unspecified. When Candida albicans cells are cultured in a growth medium that imitate the environmental conditions of a human body, they develop as true hypha. This capability of Candida albicans to grow in hyphal form has been suggested as a pathogenic factor, as these hyphal forms are often pragmatic invading tissue, and those strains defective of producing hyphal form are also not capable to cause infection. (Davies, 1990). This miscellany of virulence characteristics of organism may

assist adjustment to distinct phases of infection and acting synergistically to augment fungal survival (Soll, 2002).

Candida albicans display mixed coagulase activity against varied plasma but exact mechanism of this activity is not clearly elucidated (Yigit *et al.*, 2008). Coagulase is a protein enzyme which enables conversion of plasma fibrinogen to fibrin. *C. albicans* can produce two forms of coagulase enzyme one is bound coagulase and other is free coagulase. Bound coagulase, otherwise known as "clumping factor", can be detected by a slide coagulase test. Free coagulase can be detected using a tube coagulase. The aim of present study was to demonstrate the coagulase activity by pathogenic *Candida spps.*

MATERIALS AND METHODS

Plasma coagulase preparation: Cardiac puncture of healthy adult rabbit was done to collect rabbit blood. Blood is treated with anticoagulant. Centrifuged for 15min at 6000rpm. Filtrates were placed in screw-capped tubes. Plasma was separated from blood and freeze until use.

Microscopy: The organism can be confirmed by a presence of a germ tube.

Tube coagulase test: Rabbit plasma was inoculated with a Candida colony. The tube is then incubated at 37°C for 1 – 1.5 hr. If negative, then tube was further incubated for 24 hours. Coagulase activity was evaluated by the clot formation which possibly will not be re suspended by gentle shaking (Dorlands, 2007).

Slide coagulase test: This test is usually performed on a glass slide. A negative control is also run to discount auto agglutination. Saline drops were put onto the slide labelled with sample number, Test (T) and control (C). By using wireloop drops were emulsified with the test organism. A drop of plasma was placed on test and mixed well; the slide was then shaken gently for 10 seconds (Bailey *et al,* 1986). If clumping observed in the plasma within 10 seconds then it is considered positive, while no clumping in the control. No clumping will be observed if test is negative.

RESULTS

A total of 20 clinical isolates of Candida albicans were incubated for overnight in rabbit plasma. 9 strains of Candida albican show Positive results for coagulase test and they accounts for up to 45%. On the other hand, 11 strains of Candida albican show negative results and they account for up to 55% in rabbit plasma.

C. albicans strains also show positive reactions for coagulase test in sheep plasma. In human plasma none of the Candida isolates have shown positive results.

DISCUSSION

There are only few published studies reporting on coagulase activity by Candida albicans while numerous research on pathogenecity have focused on enzymes such as such as proteinase, phospholipase and hemolysin. In present study we have checked the production of coagulase enzyme by various pathogenic Candida spp. using rabbit plasma. The total number of 20 *C. albicans* isolates have been taken out of which only 9 strains show positive results and 11 showed negative i.e 45% were positive and 55% were negative. In a similar study, strains of *C. albicans* were grown in rabbit plasma. After 24 hours of incubation, 45.3% *C. albicans* show positive results (Yigit, 2011). Rabbit plasma is found to be the most suitable medium for screening of coagulase by yeast strains. In another study, coagulase production by Candida albicans was assessed by the classical tube test, in which most of the *C. albicans* (88.5%) were able to convert fibrinogen to fibrin clots (Isenberg, 1898). Both studies showed more frequent coagulase production. This may be due to presence of increase in factor

Table I. Frequency and percentage of coagulase positive and coagulase negative *C. albicans*.

Total No. of Strains	No. of Positive Strains	No. of Negative Strains	Percentage %	
			Positive results	Negative result
20	9	11	45	55

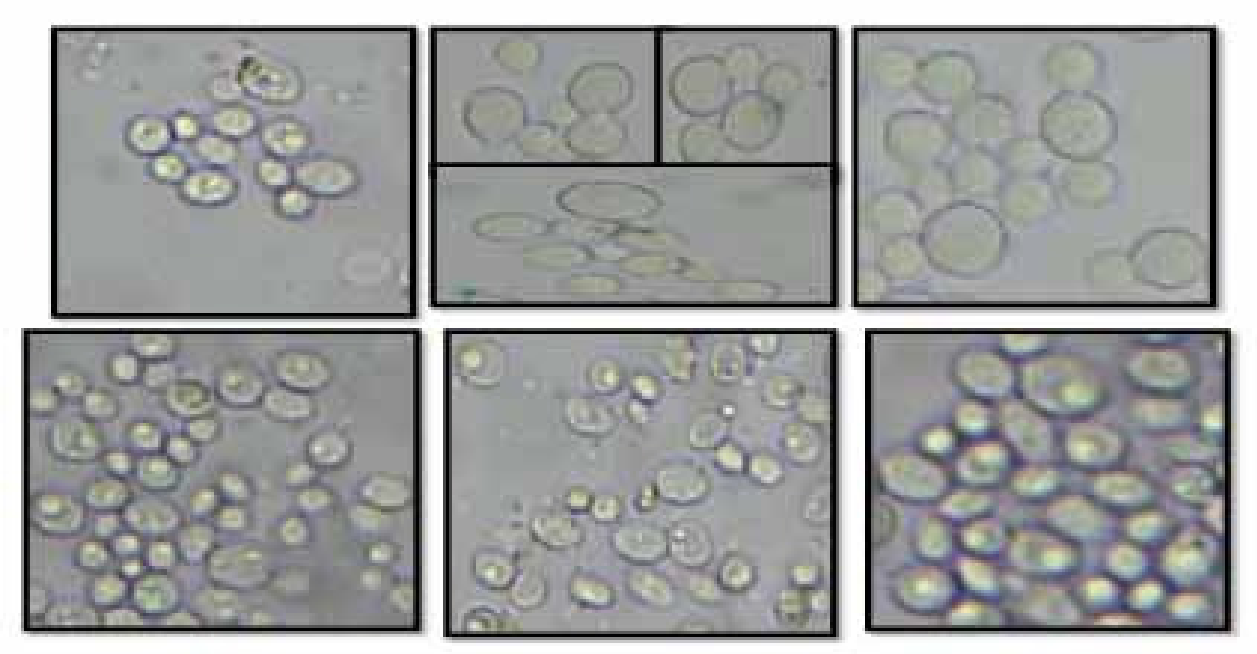

Figure 1. *Candida albicans* Showing Negative Results For Coagulase Test.

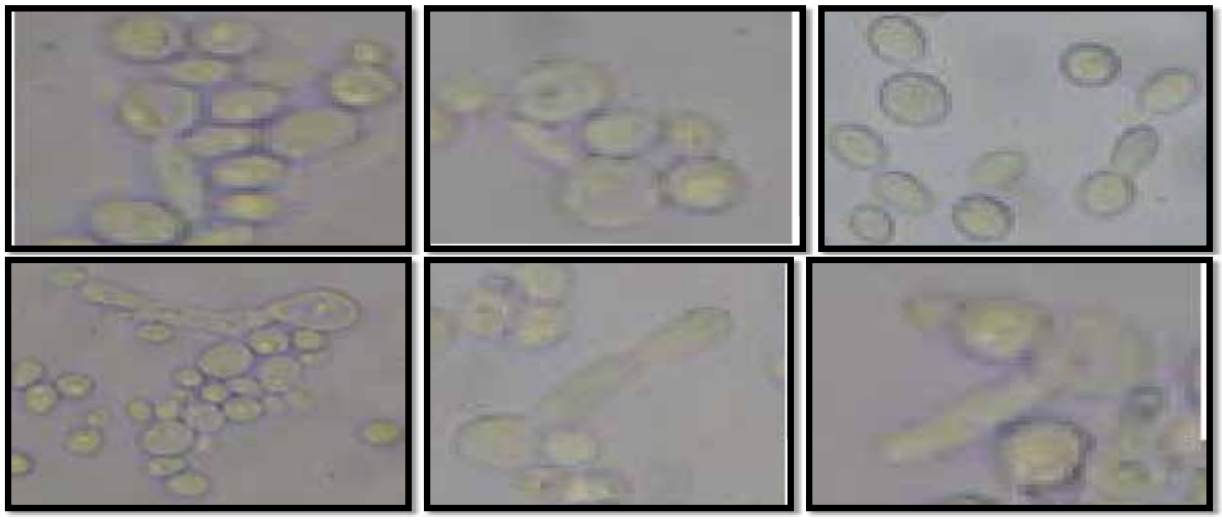

Figure 2. *Candida albicans* Showing Coagulase Positive Results .

Xa and procoagulant phospholipid which can persuade dispersed intravascular coagulation in non human primates, dogs and rabbits (Giles, 1982). Factor Xa/phospholipid complex (i.e., prothrombinase) catalyzes the conversion of prothrombin to thrombin. It may be possible due to the differences in test performance i.e: our results were based on slide test rather than tube test.

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