American Journal of Microbiological Research, 2014, Vol. 2, No. 2, 57-59
Available online at http://pubs.sciepub.com/ajnr/2/2/2

DOI:10.12691/ajmr-2-2-2

Coagulase Activity of *Candida Spp* Isolated from HIV Seropositive Patients Using Different Animal Plasma

Padmajakshi. G[1], S. Saini[1], Sachin deorukhkar[1], K V Ramana[2,*]

[1]Department of Microbiology, Pravara Medical College, Loni, Maharashtra, India
[2]Department of microbiology, Prathima Institute of Medical Sciences, Karimnagar, India
*Corresponding author: ramana_20021@rediffmail.com

Received February 24, 2014; Revised March 13, 2014; Accepted March 16, 2014

Abstract *Candida spp.* is most common opportunistic pathogen in HIV/AIDS patients causing severe and rapidly progressive, difficult to diagnose and often refractory to therapy. In the era of emergence of resistance among different microorganisms due to various factors, *Candida spp* are among those microbes that have to be extensively studied in the areas of pathogenic and virulence determinants. Coagulase emzyme is considered as a virulence factor in Staphylococci and that has been studied least in *Candida spp* as evidenced by available literature. We have attempted to evaluate the coagulase activity among *Candida spp* isolated from HIV seropositive patients. This prospective study included 179 *Candida* isolates from HIV seropositive patients presenting with various clinical conditions of candidiasis. Identificaton was carried out as per standard procedures and the coagulase activity was detected by tube coagulase method using three different animal plasmas. Among 179 clinical isolates of *Candida* tested, high coagulase activity was observed with rabbit plasma (55%) followed by sheep plasma (40.2%). Only C. albicans and C. tropicals showed coagulase activity with Human plasma.

Keywords: *Candida spp, HIV seropositive individuals, coagulase activity*

Cite This Article: Padmajakshi. G, S. Saini, Sachin deorukhkar, and K V Ramana, "Coagulase Activity of *Candida Spp* Isolated from HIV Seropositive Patients Using Different Animal Plasma." *American Journal of Microbiological Research*, vol. 2, no. 2 (2014): 57-59. doi: 10.12691/ajmr-2-2-2.

1. Introduction

In HIV infected individuals pathogenic fungi causing superficial to deep and invasive infections mostly belongs to the genus *Candida* and *Cryptococcus. Candida* is a yeast-like fungi seen as an endosymbiont, but capable of causing opportunistic infections in individuals with underlying immunocompromised conditions like HIV/AIDS. Candidiasis falls in globalized infections commonly encountered in AIDS patients and studies confirm that 60% individuals develop at least one episode per year with frequent recurrences which makes it more infectious than any other opportunistic infections (OI's). [1,2,3]. Even though its colonization doesn't always lead to infection, majority of infections caused are by the organisms that are a part of normal flora. And its risks factor increases when seen associated with immunocompromised conditions. The versatility of candidal virulence factors enhance its colonization, invasion, adherence, phenotypic switching, thigmotropism and are always been a subject of interest [3].

Candida had been slotted into so many things that it's not surprising that studies on *Candida* have revealed enormous progression of its pathogenic effects in past few decades and most importantly the effect of hydrolytic enzymes secreted by *Candida*. Compared with other enzymes coagulase activity of *candida* is least studied as revealed from the available literature with very few studies enumerated its activity. Other virulence factors attributable to *Candida spp* include its ability to form elongated budding cells resembling hyphae (pseudohyphae), phenotypic switching (budding forms to peudohyphal variants), formation of biofilims, expression of cell surface adhesins, secretion of various hydrolytic enzymes [4]. *Candida spp* also have ability to tolerate extreme environmental conditions that include pH, nutritional insufficiency and other factors [5].There is not enough scientific literature elaborating coagulase activity of *Candida spp* and its role in disease pathogenesis needs to be established.

Coagulase is a protein enzyme which enables conversion of plasma fibrinogen to fibrin and is produced by various microorganisms including S.aureus, Yersinia, Heamophilus and *candida*. Though coagulase enzyme activity is an established virulent determinant in Staphylococcus species and very little is known about candidal coagulase activity. Previous studies have demonstrated that *Candida spp* show varied coagulase activities against different animal plasma (sheep, rabbit and human) which may be attributed to the presence of hydrolytic enzymes on the cell surfaces, secretory enzymes that include proteinases, phospholipases and others but the exact mechanism of candidal coagulase activity is not clearly elucidated [6,7].

Several candidal virulence factors are under extensive evaluation to be used as potential targets in the synthesis

of pharmacological agents. Among them, antibiofilm activities of certain pharmaceutical preparations are reported in litetrature. A recent study by Silva-Dias A et al have demonstrated the antibiofilm activity of low-molecular weight chitosan hydrogel (LMWCH) against *Candida* species [8].

Candidal coagulase activity was first reported by Rodrigues et.al and very few studies are reported on candidal coagulase activity [9,10]. To our knowledge this study is the first of its kind which reports coagulase activity in *Candida spp* isolated from HIV/AIDS patients.

The Present study aims for the better understanding of coagulase activity in *Candida spp* isolated from various clinical samples of HIV infected individuals.

2. Materials and Methods

Present study was carried out between Aug'12 and Dec'13. This is a cross sectional prospective study. Samples were collected depending on clinical features after taking an informed consent. The study was approved by Institutional ethical committee. The clinical isolates of *Candida* were speciated based on Germ tube formation, sugar fermentation, sugar assimilation, and growth on chrom-agar. The pure colonies grown on Sabouraud's Dextrose Agar (SDA) were used to perform fluid-medium or tube coagulase test using three different animal plasmas. Rabbit EDTA-plasma was obtained by cardiac puncture, Sheep plasma was acquired by venipuncture and Human plasma from blood bank. The test procedure was same as routinely done for S.aureus. To 0.5ml of rabbit, sheep & human EDTA-plasma, 0.1ml of culture suspension is added and incubated at 35°C-45°C. The clot formation was observed by gently tilting and shaking the tubes at 2, 4, 6 and 24hrs of time intervals. The *S.aureus* ATCC 25923 and *S.epidermidis* ATCC 14990 served as a positive control and negative control [11,12,13,14].

Table 1. Coagulase activity of clinical isolates of *Candida spp* against different animal plasma

Species (n)	Rabbit plasma n (%)	Sheep plasma n (%)	Human plasma n (%)	Total n (%)
Candida albicans (72)	29(40.2%)	17(23.6%)	3(4.1%)	49(68%)
C.tropicalis(22)	9(40.9%)	3(13.6%)	1(4.5%)	13(59%)
C.kefyr (20)	7(35%)	1(5%)	-	8(40%)
C.glabarta(16)	6(37.5%)	2(12.5%)	-	8(50%)
C.parapsiolis (16)	4(25%)	2(12.5%)	-	6(37.5%)
C.krusei(15)	4(26.6%)	-	-	4(26.6%)
C.dubliniensis (11)	3(27.2%)	-	-	3(27.2%)
C.gullirmondi (7)	3(42.8%)	-	-	3(42.8%)
TOTAL(179)	65(36.3%)	25(13.9%)	4(2.23%)	94(52.5%)

3. Results

Total clinical isolates included in the study were 179 and all were collected from different clinical isolates of HIV seropositive patients. Major strains included were C.albicans and subspecies. Most of the isolates were positive for tube coagulase test at 2hrs and after 24hrs clot retraction was observed. The clot after 24hrs was considered to be positive and the list of them are given in the Table 1. 55.5% isolates were reactive to rabbit plasma with positive coagulase test 2 and 6hrs of incubation and at 24 hrs 40.2% showed stability in clot formation. C.krusei, C.gulliermondi and C.dubliniensis had no coagulase activity with sheep and human plasma. These isolates are from HIV seropositive patients but still its relation to the coagulase activity in these isolates is unknown. The number of isolates and their coagulase activity are detailed in the Table 1.

4. Discussion

It is an established fact that sometimes similar features are not interestingly potrayed; same is with candidal coagulase activity. Coagulase is a virulence determinant and also a species differentiation factor in Staphylococci [13]. In immunocompetent as well as HIV infected individuals, virulence factors of the microorganisms are important which determine the nature of infections (superficial/deep). There is however a need to establish lower cost diagnostic testing in resource limiting settings; hence, coagulase test falls in to one among them which is less time consuming. Yet its relation with candidal species isolated from HIV patients' remains least understood. Our study is first of its kind which has attempted to determine coagulase activity among clinical isolates of *Candida* from HIV infected population. The study was standardized and coagulase activity was tested using three different animal plasmas. This allows single test findings for comparing and differentiating organisms based on coagualse activity. In contrast to N yigit et.al study, our study showed coagulase activity in C.albicans and C.tropicalis with human plasma [7]. Earlier studies showed none/no coagulase activity with human plasma [3]. The present study outcome proves that there is a rapid change in the virulence determinants of organisms that have potential to cause infections. This major difference/variations of organisms (*Candida spp*) reacting with human plasma isolated from HIV negative and HIV positive patients is yet to be ruled out with much more research. The exact mechanism behind the variable coagulase activity of clinical isolates of *Candida spp* should be the topic of research in further studies as it is least understood

5. Conclusion

The changing scenario of candidal coagulase activity could provide critical information regarding role of virulence factors in infectious diseases among HIV/AIDS patients. Research related to this requires an urgent priority as very few studies are done.

References

[1] Raus J, Love DN: Comparision of the affinities to bovine & human prothrombin of the staphylocoagulases from S. intermedius & S. aureus of animalorigin. J Clin Microbio 1991; 29: 570-572.

[2] Luo G, Samaranayake, LP, Yau JY: Candida species exhibit differential invitro hemolytic activities. J Clin Microbiol 2001: 39: 2971-2974.

[3] Rodrigues AG, Pina-Vaz C, Costa-de-Oliveira S, et al: Expression of plasma coagulase among pathogenic candida species. J Clin Microbiol 2003; 41: 5792-5793.

[4] François L. Mayer, Duncan Wilson, and Bernhard Hube. Candida albicans pathogenicity mechanisms. Virulence. Feb 15, 2013; 4 (2): 119-128.

[5] Nicholls S, MacCallum DM, Kaffarnik FA, Selway L, Peck SC, Brown AJ. Activation of the heat shock transcription factor Hsf1 is essential for the full virulence of the fungal pathogen Candida albicans. Fungal Genet Biol. 2011; 48: 297-305.

[6] Becker K, Almasri AS, von Eiff C, et al: Systematic survey of nonspecific agglutination by Candida spp. in latex assays. J Clin Microbiol 2007; 45: 1315-1318.

[7] N Yigit, AE Aktas and A Ayyildiz. Detection of Coagulase Activity in Pathogenic Candida Species. Journal of International Medical Research 2008 36: 1378.

[8] Silva-Dias A, Palmeira-de-Oliveira A, Miranda IM, Branco J, Cobrado L, Monteiro-Soares M, Queiroz JA, Pina-Vaz C, Rodrigues AG. Anti-biofilm activity of low-molecular weight chitosan hydrogel against Candida species. Med Microbiol Immunol. 2014 Feb; 203 (1): 25-33.

[9] Richardson MD: Changing patterns and trends in systemic fungal infections. J Antimicrob Chemother 2005; 56 (suppl 1): i5-i11.

[10] Yang YL: Virulence factors of candida species. J Microbiol Immunol Infect 2003: 36: 223-228.

[11] A Forbes, Betty: Daniel F Sahm, Alice S. Weissfeld. Bailey & scott's Diagnostic Microbiology (10th edi). Don Ladig.pp: 430-431.

[12] Deorukhkar S, S. Saini: Non- albicans Candida species: It's isolation pattern, species distribution, virulence factors & anti fungal susceptibility profile. J med & pub health 2013; vol. 2: p: 511-516.

[13] Fairbrother RW: Coagulase production as a criterion for the classification of the staphylococci. J Pathol Bacteriol 1940: 50: 83-88.

[14] Dorland's Medical Dictionary. Rabbit plasma with anticoagulant.