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EVALUATION OF HAEMOLYTIC ACTIVITY OF SOME *CANDIDA* SPECIES

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Abstract

The ability to produce enzymes such as hemolysins is an important virulence factor for the genus Candida the objective of this study was to compare the hemolytic activity between *C. albicans* and other *Candida* species. A total of (100) *Candida* isolates representing (4) species are examine for their respective responses to an in hemolytic test. All strains of *Candida* specie isolated from the oral cavity of patients in Jumhuri Hospital in Kirkuk city. The four species isolated were: *C. albicans, C. glabra, C. krusei* and *C. kefyr.* Production of Hemolysin was evaluated on sabouraud dextrose agar containing chloramphenicol blood and glucose. A loop full of pure *Candida* culture was spot-inoculated and incubated for 24 hours at 37°C. Hemolytic activity was defined as the formation around the colonies of a translucent halo. 10 strains of C. *albicans* four strains of *C. glabra* 43 strains of *C. krusei* and 1 strain if *C. kefyr* subsequently that were studied produced hemolysins. Over all this study showed that hemolytic activity was detected in all isolates with minor differences seen between them the highest hemolytic activity detected in *C. krusei* (20-35mm) followed by *C. albicans* produces (25-33mm) while *C. glabra* (19-31mm) and the lowest hemolytic activity was detected in *C. kefyr* (14-16mm).

Key words : *Candida* sp., Haemolytic activity, Vaginal infection.

Introduction

Candidiasis is a multiple fungal disease that includes mucosal-cutanous visceral and proliferated infections caused by *Candida* species. *Candida* infection is one of the most common human mycoses (Edward *et al.,* 2015). *Candida* species are the 3th to 4th most common bloodstream isolates in hospitalized patients with neutropenia or immunocompromised mainly from intensive, care units, (ICUs) (Pfaller *et al.,* 2007). There is a high propagation of the mucosal-cutaneous forms particularly vaginal infections. The second most common vaginal infection is vaginitis caused by *Candida* species (Sobel, 2007). *Candida* species can produce a various of hydrolytic enzymes including proteases esterase lipases phosphatases and phospholipases (Cutle, 1991; Odds, 1998; Samaranayake and MacFarlane, 1990). These enzymes have received a great deal of attention in the past since they are known to interfere to candida pathogenesis especially by facilitates the hyphal incursion particularly seen in disseminated candidiasis (Fallon *et al.,* 1997). While some of these hydrolytic enzymes such as phospholipases proteases and lipases have been explored (Hube *et al.,* 1991; Lee, 1999; Tsang *et al.,* 2007). The hemolytic activity shown by different *Candida* species is not well known (Manns and Mosser, 1994), prescribed an elegant yet simple plate assay method for observation the hemolytic activity of *Candida albicans* this method have been modified to estimate the hemolytic activity of different *Candida* species obtained from a variety of clinical manifold *Candida* species from a variety of clinical sources and to compare the species specific differences in the production of hemolysin qualitatively and quantitatively. (Watanabe *et al.,* 1997) reported that *Candida albicans* excretes a hemolytic factor that causes hemoglobin to be released and is then used by the organism as an iron source. (Luo *et al.,* 2001) reported that many species of *Candida* have two different types of hemolysins, alpha and beta hemolysin of which the nature is not yet understood. While many studies have been conducted on some of hydrolytic enzymes and hemolysin production in human isolates (Koga-Ito *et al.,* 2006; Furlaneto-Maia *et al.,* 2008). Research on famous

virulence factors particularly hemolytic activity offered by various animal-isolated *Candida* species and their products is limited Presently identifying virulence factors can play a key role in limiting pathogenesis of candidiasis and introducing new therapeutic agents (Ghannoum, 2000). Reviews have recounted that *Candida* spp. can secrete a number of exoenzymes such as hemolysin esterase proteinase and phospholipase needed for colonization and invasion host organs (Rudek, 1978; Watanabe *et al.,* 1999; Pakshir *et al.,* 2013).

Materials and Methods

Sampling and identification of yeasts

We studied (100) Candida isolated from the vaginal infection used in this study (4) species of Candida were detected. The species of these isolates were identified by vitik 2 system from May 2014 to April 2015, and the ability of these isolates to form germ tube. Colony characteristics on culture white to cream with characteristic yeast odor it grew rapidly and matured in 3 days (Mohammed and AL-Ahmadey, 2013). All *Candida* sp. strains isolation cultivation and preservation by (Kwon-Chung and Benett, 1992) on Sabouraud Dextrose Agar (SDA), they were sub-cultured on CHROM agar after the yeast colonies developed at 37°C for 48 hr. to evaluate the purity of the culture and colour of the colonies. This medium includes chromogenic substances (Staniszewska *et al.,* 2012). The method is based on the release of chromogenic breakdown products from different substrates by *candida* spp (Baker. 1967).

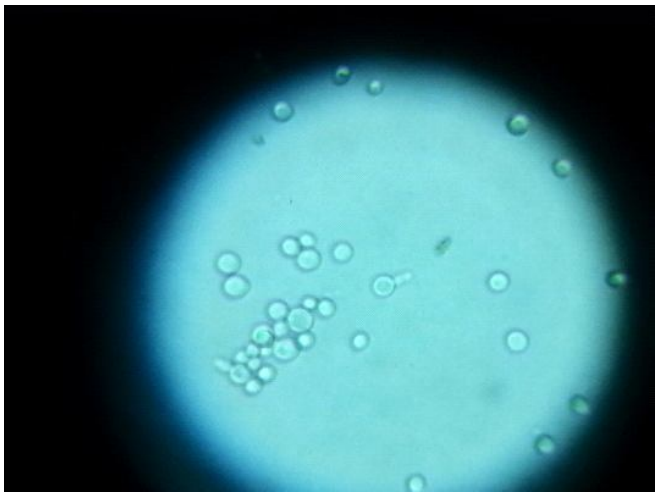


Fig. 3: Germ tube of *Candida albicans* after 3 hr incubation.

Identification using Vitik2 System

Vitik2 System has tested *Candida* isolates were tested by the reagent cards have (64) wells each with an individual test substratum. A suspension of each isolate was inoculated onto can 2 chromogenic agar plates at least twice before the testing (bioMérieux, France) and onto Sabouraud dextrose agar slants to ensure the purity and the viability of the cultures. As measured using a DensiChek instrument (bioMérieux), the inoculum suspensions for the VITEK 2 were prepared in sterile saline at a turbidity equal to a 2.0 McFarland standard. The individual test cards were automatically filled with the prepared culture suspension, sealed and incubated by the VITEK 2 instrument. The cards were incubated for 18 h at 35.5 °C and optical density readings were taken every (15) minutes automatically. The final results of the profile were compared with the database and the unknown organism the was identified.

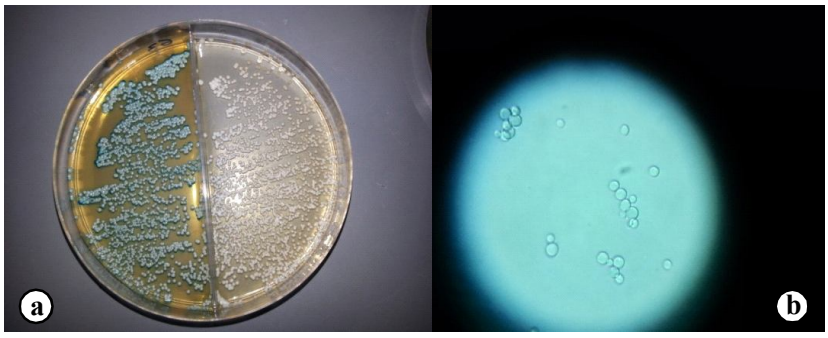
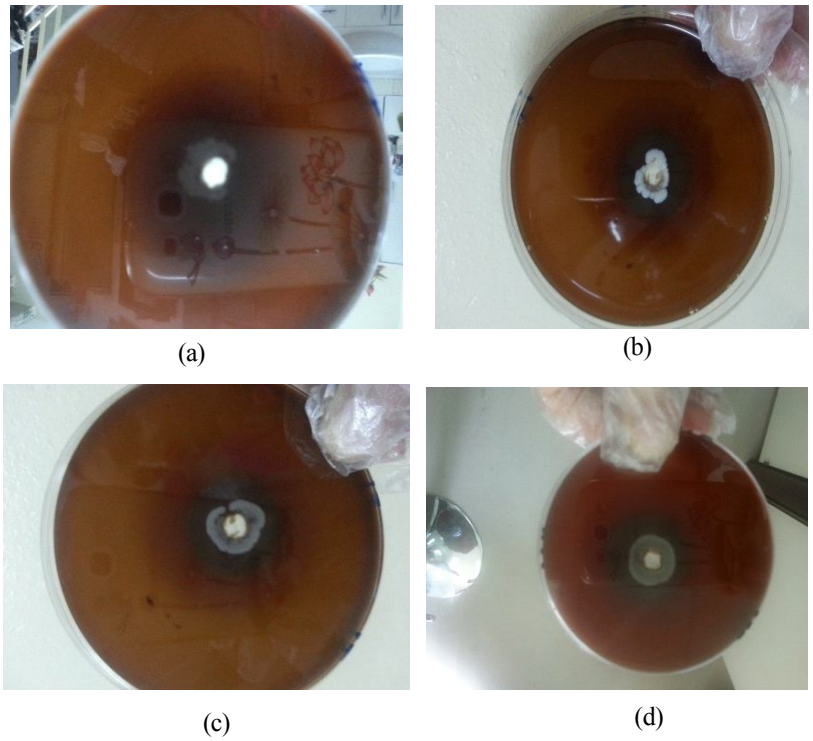


Fig. 1: (a) *C. albicans* grown on sabourauds agar (SDA) and CHROMagar. (b) Microscopy examination of *Candida albicans* showing buding and yeast cells.

Fig. 2: (a) *C. glabrata* grown on sabourauds (SDA) agar and CHROMagar. (b) Show mixed infections with more than one species of *Candida*.

Germ tube test

Candida albicans[1] ability of to form a germ tube was tested using Baker[1]s protocol which was used to identify isolates. A single colony of cells was inoculated in human serum and incubated at 37C for 2-4 hr and then examined under the microscope for detected the germ tube. Both positive and negative germ tube



(a) (b)

(c) (d)

Fig. 4: (a,b,c,d) show the heamolytic activety of *C. albicans, C. glabrata C. krusei* and *C. kefyr* respectively.

production were classified as the isolates (Baker, 1967).

Hemolysin activity

Evaluated the production of hemolysin using methods with some modifications (Manns and Mosser, 1994). A loop full culture of pure *Candida* was inoculated into SDA medium containing chloramphenicol and incubated, for 24 hours at 37°C. 10^8 cells/mL of suspension growth was prepare in sterile phosphate buffered (0.1 M, pH 7.2) saline using a spectrophotometer An aliquot (10 µL) of the standardized suspension was cultured on to blood agar with glucose this medium was prepared with (5 mL) fresh blood per (10^2 mL) SDA supplemented with chloramphenicol and (3%) glucose. The pH was adjusted to (5.6 ± 0.2) Plates were incubated for 48 hr at 37°C. Colony and halo diameters were measured using a ruler and hemolytic activity was expressed in terms of the colony's diameter ratio of the to the degradation zone's outer diameter. The assay was performed in quadruplicate for each yeast isolate tested on two separate occasions. The halo zone for each isolates shown in Fig. 4.

Statistical analysis

Statistical analysis was conducted using SPSS for concentration human- depended T-test and compared mean by using Duncan multiple ranges under the level p-value ≤ 0.05, p-value ≤ 0.01 was considered as statistically significant (Al- rawi, 2000).

Result and Discussion

Heamolytic activity of *Candida* species

(10) Strains of *C. albicans*, (4) of *C. glabrata,* (3) of *C. krusei* and (1) of *C. kefyr* species showed beta hemolysis blood SDA at 48 hours of incubation (Table 1). The quantitative data showed that *C. albicans, C. glabrata* and *C. krusei's* hemolytic activities were significantly higher than *C. kefyr* ($p<0.01$), apart from, there were no signifi-cant differences intra-species in the β-hemolytic activities between these isolates *C. albicans*, *C. glabrata* and *C. krusei* (Table 1). As putative virulence factors hemolysins are known to contribute to Candida pathogenesis in particular to facilitate hyphal incursion candidasis speard (Luo *et al.,* 2004). The hemolytic activities of yeast such as Candida genera has been investigated. (Linera*et al.,* 2007) reported a complementary hemolysis induced by *C. albicans*. (Watanabe *et al.,* 1997) reported that *Candida albicans* excretes a hemolytic factor that causes hemoglobin to be released and is then used by the organism as an iron source. (Luo *et al.,* 2001) has stadied (80) isolates of (14) species of *Candida*, these authors reported that alpha and beta hemolysis had shown by *C. albicans* and others. Recently, we reported these species of *Candida* including *C. albicans*, *C. tropi-calis C. glabrata, C. kefyr* as well as *C. krusei*, have varying skills to produce hemolysins on human rabbit and SDA enriched with blood supplemented by glucose medium (Yigit and Aktas, 2009). There are limited Studies of *Candia's* hemolysin activities isolated from oral isolates, carried out he first study of hemolytic activity from an isolated oral cavity of *C. albicans* (Tsang *et al.,* 2007) reported that the hemolytic activity of an oral *C. albicans* isolated with type 2 diabetes mellitus patients was substantially higher than those controlled (a hemolysis index of 0.764±0.08 in the non-diabetic group vs. 0.673±0.06 in the diabetic group). In this study *Candida* species isolated from vaginal infection were investigated in vitro hemolytic activities. *C. albi-cans* (n=10), *C. glabrata* (n=4), *C. krusei* (n=3) and *C. kefyr* (n=1) species exhibited β-hemolysis on blood SDA (Table 1). The quantitative data showed that the β-hemolytic activity -of *C. albicans* (25-33mm), *C. glabrata* (19-31mm) and *C. krusei* (20-35mm) showed significantly higher beta-hemolytic activities than *C. kefyr* (14-16) ($p<0.01$) (Table 1). Furthermore, there were no

Table 1: Hemolysin activity species of *Candida* sp.

Haemolysin activity mean±SD	Number of isolates	Species
25-33 (± 2.538)	10	*C.albicans*
20-35 (± 5.92)	4	*C. glabra*
19-31 (± 7.94)	3	*C. krusei*
14-16 (± 1.000)	1	*C. kyfer*

significant intra species differences in the beta-hemolytic activities between isolates *C. albicans*, *C. glabrata* and *C. krusei* (Table 1). It is still necessary to consider the possibility that species specific hemolysis may texist. These hemolysis may vary of molecules and therefore have different rates of diffusion (Luo *et al.,* 2001; Luo *et al.,* 2004). The ability of pathogeni organisms to acquire elemental iron has been shown to be of crucial importance for their survival and the ability to infect the mammalian host (Weinberg, 1978; Bullen, 1981). Because there is essentially no free iron in the human host most pathogens acquire iron – indirectly containing compounds like hemoglobin (Belanger *et al.,* 1995). To do so, However, the pathogen should be equipped with a mechanism that destroys the movement of the heme and allows the extraction of the elemental iron. The enzymes that mediate this activity are widely referred as hemolysins.

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