

DISTRIBUTION OF SECRETED ASPARTYL PROTEINASES IN VIRULENT CANDIDA ALBICANS STRAINS

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ABSTRACT

In immunocompromised people, Candida albicans is an opportunistic fungal pathogen that causes both superficial and systemic infections. Secreted aspartyl proteinases (SAPs) family of ten proteins (SAP1-10) are one of its main virulence factors and play a crucial role in pathogenicity, immunological evasion, and host tissue invasion. The purpose of this study was to invitro assessment of SAP in pathogenic strains of Candida albicans isolated from clinical material. Oral swabs, vaginal swabs, urine, blood, and wound specimens were among the 100 clinical samples taken from hospitalized patients. CHROMagar and germ tube tests were used to identify isolates. Proteolytic activity was measured using Pz values in bovine serum albumin (BSA) agar medium to determine SAP generation. Among 100 samples, 43 isolates were identified as Candida albicans. 21 (50%) isolates displayed strong SAP production, 14 (33.3%) isolates showed moderate activity, while 5 (11.9%) isolates showed weak activity, and 2 (4.8%) had no SAP activity. Higher SAP production was significantly correlated with isolates recovered from invasive infections, according to statistical analysis ($p < 0.05$). These results indicate that the synthesis of SAP is a significant virulence marker in pathogenic strains of Candida albicans and may increase the severity of the disease.

1. Introduction

Candida albicans infection is still a serious global health concern, especially for hospitalized patients, immunocompromised patients, and those on long-term antibiotic therapy[1, 2]. From frequent, mild mucocutaneous thrush to potentially fatal invasive bloodstream infections (candidemia)[4], Millions of people worldwide suffer from superficial infections; vulvovaginal candidiasis affects over 75% of women at a point in their lives. Invasive candidiasis, on the other hand, has a significant death rate and affects more than 1.5 million people each year [5]. In the oral cavity, gastrointestinal system, and genitourinary tract, *Candida albicans* is often a commensal organism, but under the right circumstances, it can become pathogenic[3]. Virulence factors, such as adhesion molecules, biofilm development, morphological switching, phospholipase synthesis, hemolysin production, and secreted aspartyl proteinases (SAPs), etc. contribute to pathogenicity of *Candida albicans* [6]. Among these, SAPs (1-10) are encoded by ten different genes allowing host tissue invasion by breaking down extracellular matrix proteins, immunological proteins, and epithelial barriers[7]. Secreted aspartyl proteinases reported to play key role in the colonization of *Candida* linked to tissue damage and aiding spread into deeper tissues exhibiting the virulence and progression of disease[18]. Thus, increased pathogenic potential was reported to be linked to increased SAP production globally. The purpose of this study was to assess production of SAP in clinical *Candida albicans* strains that were obtained from hospital laboratories of Karachi city with heavy patients load.

Methodology:

2.1. Sample collection and study design:

A cross-sectional laboratory investigation was conducted from January to December 2025. Isolates were collected from clinical samples i.e., swabs from the vagina, oral swabs, blood, Urine, and wound samples from hospitalized patients in tertiary care hospitals laboratories. A total of hundred clinical *Candida* isolates were collected primarily grown on Sabouroud's dextrose agar for the growth[4].

2.2. Morphological Characteristics:

Gram staining was performed for morphological identification of yeast isolates[1, 5].

2.3. Germ tube test:

For germ tube test the Yeast colonies were inoculated into 0.5 ml of serum and incubated at 37°C for 2.5 hrs.(10) A drop of serum was placed on clean glass slides and examined under 40X objective lens for germ tube formation [11].

2.4. presumptive identification of *Candida albicans* on CHROMagar™:

Pure colonies from SDA were subculture on CHROMagar™ *Candida*. The medium contains specific chromogenic substrates for each of fungal species and upon utilization of substrate different colored colonies produced signify the candida specie[16]. The CHROMagar™ was prepared according to the manufacturer's guidelines and distributed in Petri plates. The candida isolates were subculture on prepared medium, and incubated at 30-37°C for 24 to 48 hours [9].

2.5. Determination of Invitro SAP Production using BSA medium:

For the analysis of SAP production, the growth was adjusted to the 0.5 McFarland standard. 18–24-hour culture of yeast colonies was suspended in sterile saline, 10µL of suspension was inoculated into BSA agar plates and incubated for 48–72 hours at 37°C for clearing around colony or zone size. The zone size was measured and Pz value was calculated using the formula of Wenli Feng et Al 2021 [12].

2.6. Statistical analysis:

IBM SPSS Statistics version 26 was used to analyze the data. The chi-square test was used to determine the relationships between sample type and SAP activity. Statistical significance or p-value calculated [13].

3. Results:

3.1. Morphological identification:

On Gram stain, gram positive (purple colored) large oval single or budding yeast cells seen figure 1.

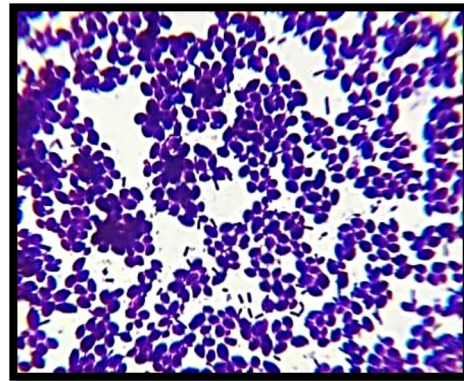


Figure 1: Yeast cells seen in gram staining.

3.2. Germ tube test:

The Germ tube test result showed production of short hyphal (filamentous) extension arising laterally from a yeast cell, with no constriction at the point of origin. *Candida albicans* also occurred

positive germ tube. The germ tube is a thin and filamentous elongation without a constriction at the point of initiation on the cell were analyzed and 75% were found positive germ tube production.

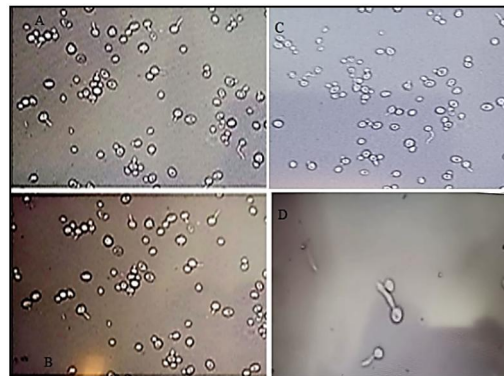


Figure 2: Candida spp (germ tube formation):

3.3. Cultural characteristics on SDA and CHROMagar™

On SDA cream colored pasty colonies observed indicated *Candida* species and among 100 *Candida* species 43 percent were confirmed as

Candida albicans. on CHROMagar™ apple green colonies, of *Candida albicans* as described by manufacturer instructions figure 2. While Non *albicans* *Candida* produced distinct colored colonies.



Figure 3: Apple green/light green colonies of Candida albicans on CHROMagar Candida medium.

Table 1: Culture Confirmed *Candida albicans*:

Sample types	No of isolates	<i>Candida albicans</i>
Vaginal swabs	25	12
Oral swabs	15	9
Urine	35	13
Blood	15	5
Wound swabs	10	4

3.4. Invitro Secreted Aspartyl Proteases on BSA medium:

Candida albicans produce secreted aspartyl proteinases (Saps), an extracellular enzyme that can hydrolyze proteins such as Bovine Serum Albumin (BSA) [7]. When *Candida albicans* is grown on a

solid medium containing BSA degrades the BSA as a result A clear zone (halo) around the colony on an opaque medium, was seen showing protein hydrolysis. The size of this zone that is 3 to 5 mm indicates the level of Sap activity[14].

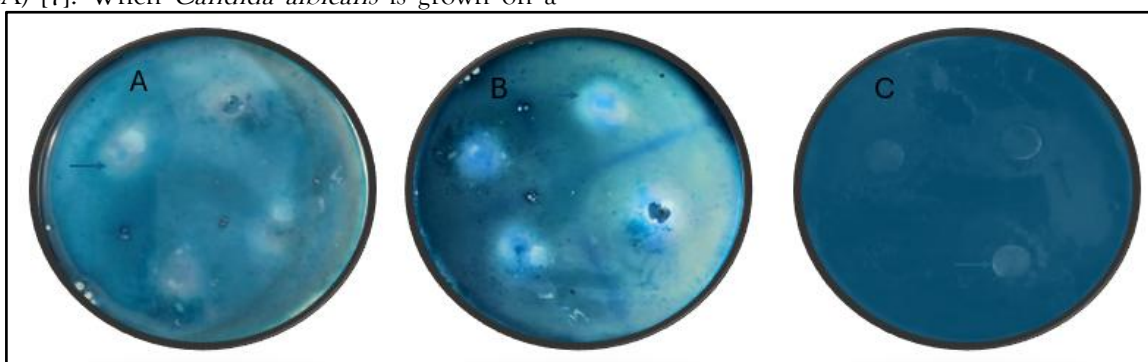


Figure 4: Figure A and B represent the SAP Production by *C. albicans*, and figure C showed the negative result.

Table 2: Production of SAP in Isolates:

SAP activity	# of isolates positive
Strong	21(50%)
Moderate	14(33.3%)
Weak	05(19.9%)
None	02(4.8%)

3.5. Relationship Between Sample Source and SAP Production:

Compared to oral isolates, blood and wound isolates produced considerably more SAP ($p = 0.03$).

Discussion:

The ability to cause tissue damage and invasion into deeper tissues attributed to production of (SAPs) secreted aspartyl proteinases that not only aid in colonization, germ tube and biofilm formation [5]. In this study 100 *Candida* isolates were identified. The purity of the isolates was performed using SDA and morphology of yeast was performed by gram stain (Rahmayanti, R., et.al. 2024 work on urine isolates and used SDA as primary culture media for the growth of *Candida*

species and 65% were confirmed as *Candida* spp. The results are following the current study. The germ tube test was used for the confirmation of the isolates and 75% of strains have germ tube positive test. The Logien, S. M. (2022) also endorsed the Germ tube test for the confirmation of *Candida albicans* from clinical specimens. All *Candida* species grown and identified on CHROMagar™ *Candida* agar. On this medium 43% of *Candida albicans* exhibited typical Apple green colored colonies as described by manufacturer while 57% of the isolates were identified as non albicans. Our results showed partial agreement with the findings of 73% of their strains identified as *Candida albicans* indicated the changing trend Ajah, H. A et.al. 2020 [17] the Invitro proteinase production

was assessed using BSA. According to current study strong proteinase activity was seen in 50% (5mm zone size) of *Candida albicans* strains followed by moderate production by 33.3%. A reduced production of proteinases seen in 19.9% of the strains while 4.8% showed no proteinase production. The results are in line with the results of Ajah, H. A et.al. 2020 who worked on only 56 isolates and reported (41) 86 % of his isolate were able to show proteinase activity. Gerges, M. A et.al. 2023 worked on 204 samples and reported that 63.2% (n = 129) *C. albicans* isolates were able to produce aspartyl proteinases with 52.0% (n = 106) being mild producers and 11.3% (n = 23) being strong producers and concluded that characterizing SAP enzymes as important pathogenic determinants of *Candida albicans*. Slight variation in results due to increase sample size of the study. [15]. The results of the current study are consistent with earlier research as discussed and highlighting the elevated SAP expression linked to pathogenic *Candida albicans* isolates. Further assays need to figure out the correlation of SAP production and pathogenesis as well as antifungal resistivity.

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