

Genotypic Patterns of Secreted Aspartyl Proteinase Gene in Various *Candida* Species Isolated from Antenatal Women with Vulvovaginal Candidiasis

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Abstract

Vulvovaginal candidiasis (VVC) is a commonly found disease in antenatal women caused by *Candida* species. The usual presentations are persistent curdy white vaginal discharge with itching, bad odour, irritation, pain in the lower abdomen and local induration of vulva. Therefore this infection is an unpleasant and cause of intolerance for women. Several virulence factors are present in *Candida* species such as Secreted aspartyl proteinase (Sap), phospholipase, lipase, haemolysin, germ tube formation and production of biofilm. There are 10 SAP genes (SAP1 to SAP10) present in all *Candida* species which help for virulence. The objective of this research was to analyse the genotypic patterns of SAP genes in *Candida* species. The present research was done in SRM MCH & RC, Chennai, India, from March 2017 to December 2018. DNA was isolated and the SAP gene was detected by a polymerase chain reaction. Totally 35 *Candida* species were isolated from 92 suspected case of VVC. Out of 35 *Candida* species, 16/35 (45.7%) were from symptomatic women and 19/35 (54.3%) were from asymptomatic women. Out of 35 *Candida* species, *C. albicans* were 15(42.8%) and Non-*albicans Candida* (NAC) were 20(57.2%). Totally 28 genotypic patterns were found and 6 (21.4%) *Candida* isolates presented genotypic patterns of all the SAP genes. There is a predominance of the SAP1 gene in *Candida* isolates from asymptomatic women whereas SAP1 and SAP9 genes in *Candida* isolates from symptomatic women. The statistical analysis showed a significant difference for the SAP7 and SAP8 gene frequency among the *Candida* isolated from symptomatic and asymptomatic women.

Keywords: Vulvovaginal candidiasis, Symptomatic, Asymptomatic, Antenatal, *Candida albicans*, Secreted aspartyl proteinase.

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INTRODUCTION

In recent years there is an increased frequency of *Candida* infection and it has been the cause of a major rise in morbidity and mortality¹. Vulvovaginal candidiasis (VVC) is a commonly found disease in antenatal women. The usual presentations are persistent curdy white vaginal discharge with itching, bad odour, irritation, pain in the lower abdomen and induration of vulva. Therefore, this infection is an unpleasant, intolerant condition for women². The causative agents of this disease are *Candida* species which are yeast-like fungus present as a commensal of the skin, mucosal cavity, GI tract and vagina causing local and systemic infection in patients with the immunocompromised condition³. VVC is mostly seen in women with reproductive age group between 18 to 45 years^{2,3}. Various studies show that about 75% of all women will have at least one episode of candidiasis during their lifetime^{3,4}. The prevalence rate of vulvovaginal candidiasis in antenatal women is more than 40% worldwide and 5-10% suffers from Recurrent Vulvovaginal Candidiasis (RVVC). During pregnancy, VVC has been associated with complications such as low birth weight of babies, miscarriage, preterm birth and premature rupture of the membrane^{5,6}. *Candida* species possess several virulence factors like extracellular production of hydrolytic enzymes, hyphae formation, phenotype switching and cell adhesion^{7,8}. The emission of the extracellular hydrolytic enzyme by *Candida* species appeared to be the most important virulence factors⁹. The most vital hydrolytic enzymes produced by *C. albicans* are "Secreted aspartyl proteinases (Sap), phospholipase B, esterase and lipases". *C. albicans* isn't the only organism which produced Sap other *Candida* species have also been found to demonstrate SAP genes, including *C. dubliniensis*¹⁰, *C. tropicalis* and *C. parapsilosis*¹¹. Sap enzyme production is related to various other virulence factors of *Candida* species including, hyphal development, adhesion to the surface, phenotypic exchanging, and dimorphism¹². The sap is encoded by a multi-gene family including ten diverse highly regulated genes (*SAP1* to *SAP10*). The presence of 10 SAP genes and their controlled articulation and guideline brings up various issues regarding the role and elements of this gene amid the infective progression. Being thereof 10 SAP genes, during

their sequential activation in particular phases of infection, they organize as well as constitute a robust body and indicate that distinct members of this gene family having a significant role in the adaptive response of *Candida species* to its environment, including its host¹³. Thus the point of this investigation was to assess the genotypic patterns of SAP (*SAP1* to *SAP10*) genes in *Candida* causing VVC in antenatal women.

MATERIAL AND METHODS

It was a prospective study done in SRM tertiary healthcare Centre, from March 2017 to December 2018 after getting approval from the Institutional Ethical Committee (IEC, 1090/IEC/2017). Antenatal women were divided into two groups: (a) symptomatic patients characterised by the presence of white curdy discharge, itching, burning, oedema, erythema of vulva and vagina, (b) Asymptomatic patients. After getting informed consent from the antenatal women, two consecutive vaginal swabs were taken for direct microscopy and culture respectively. All samples were collected from the patient prior to the antifungal treatment. All samples were examined in the direct microscope by 10 % Potassium hydroxide (KOH) mount followed by Gram stain and cultured on "Sabouraud's Dextrose Agar (SDA)" with supplement (gentamycin) to prevent bacterial contamination and incubated for 48 hours at 37°C and 25°C. *Candida* was identified by Gram stain to demonstrate budding yeast cell, hyphae and pseudohyphae. Germ tube test was performed to differentiate *Candida albicans* from *Non-albicans Candida* (NAC). Subsequently, *Candida species* were identified by cultured on chrome agar (Hi-media, India) a differential media for detection of coloured colonies and corn meal agar with tween 80 for chlamydo spores and blastopores formation. Sugar fermentation and assimilation tests were done to identify *Candida species*, *C. albicans* ATCC90028 was used as a control strain. Besides the identification of *Candida species* by the conventional method, the species were also confirmed by "Matrix-Assisted Laser Desorption/Ionization Time-Of-Flight" (MALDI-TOF), for which samples were sent to Micro lab diagnostic centre, Coimbatore, Tamil Nadu, India.

Genomic DNA extraction and Purification

Candida genomic DNA of each *Candida*

isolates was extracted and purified by using PureFast® fungal DNA mini spin purification kit (Helini biomolecules, Chennai) according to the manufacturer's guidelines. Concisely, 1ml of the overnight culture was centrifuged at 6000rpm for 5min, the supernatant was discarded and the pellet was suspended in 0.2ml Phosphate Buffer Saline (PBS). 180µl of lysozyme digestion buffer and 20µl of lysozyme [10mg/ml] was added and incubated at 37°C for 15min. 400µl of Binding buffer, 5µl of internal control template and 20µl of proteinase K was added, mixed well by inverting several times and incubated at 56°C for 15min. 300µl of ethanol was added and mixed well. The entire sample was transferred into the spin column and centrifuged for 1 min. The supernatant was discarded and 500µl of wash buffer-1 was added then centrifuged for 30-60 seconds and discarded the flow-through. Again 500µl of wash buffer-2 was added and centrifuged for 30-60 seconds. The flow-through discarded and centrifuged for an additional 1 min, then transferred into a fresh 1.5 ml microcentrifuge tube. 100µl of elution buffer was added then incubated for 1 min at

room temperature and centrifuged for 2 min. The purified DNA was stored at -20°C. Quality and quantity of extracted DNA were checked by loading in 1% agarose gel and 5µl of extracted DNA was used for PCR amplification.

PCR amplification and Gel Electrophoresis

SAP Genes were identified by PCR based on the method proposed by Bassyouni *et al.*²⁰ with minor modification. Specific primers of SAP genes were used for the detection of *Candida* SAP genes by PCR as shown in table 1 (Helini biomolecules, Chennai).

Amplification program as it follows an initial denaturation at 95°C for 5 minutes, then 35 amplification cycles consisting of denaturation: at 95°C for 30 seconds, annealing: at 58°C for 30 seconds, extension: at 72°C for 30 seconds and final extension: at 72°C for 5 minutes. DNA amplification was done by thermal cycler; the final volume of the reaction mixture was 25µl. In that, sample (DNA) was 5µl, master mix- 5µl, sterile water- 11µl, primer- 2µl of each forward and reverse. After amplification 5µl of each PCR product were analysed by gel electrophoresis. For

Table 1. Primers used to identify SAP gene

Genes	Primers	Sequence (5'-3')	Size of amplified product (bp)
SAP1	SAP1F	CTGTTGGTTTTGGTGGTGCT	200
	SAP1R	CAGCATTGGGAGAGTTGAGA	
SAP2	SAP2F	GCTGTCCCAGTGACTTTACACA	409
	SAP2R	CATAACTACCACCGGCTTCA	
SAP3	SAP3F	AATGTTACTGGTCCCCAAGGT	605
	SAP3R	AAAGCAATCAATGTCCCACT	
SAP4	SAP4F	ACTGGCTCTTCTGACTTGTGG	733
	SAP4R	AAGCAGGAACGGAAATCTTG	
SAP5	SAP5F	CCCGTCGATGAGACTGGTAG	868
	SAP5R	CAGGAACGGAGATCTTGAGG	
SAP6	SAP6F	ACAAAAATCCCCTCGATGA	794
	SAP6R	CCAGCAGAATCAAATGCAC	
SAP7	SAP7F	AGCTTCCTCGGCATCTTCTT	600
	SAP7R	TAACCTTGGACAGCATCACG	
SAP8	SAP8F	TTGACCGCATTACCAATCAC	394
	SAP8R	TGGTGTCCCATCAAGATCA	
SAP9	SAP9F	TGGGTGTTATGCAACAATCG	213
	SAP9R	TGGTAGAGGTGCCAGATGAA	
SAP10	SAP10F	CAAAGTCCTGACCCATCCA	102
	SAP10R	TTCGAACCGATCTCCAATTC	

SAP= Secreted Aspartyl Proteinase, F= forward, R= reverse primer.

gel electrophoresis, 1% agarose was used. One gram of agarose was liquefied in 100 ml of 1x "Tris Borate EDTA (TBE)". 1µl of "ethidium bromide" was added. 5µl of the amplified product along with the negative control and 1 kb DNA marker was laden into the well. The gel was run in electrophoresis at 50V till the dye reached three fourth of the gel and bands were observed in "UV Transilluminator" [Figs. 1-5].

Statistical Analysis

Data were entered into an excel sheet and analysed by using R version 3.5.2 software. Z test has been used to find the difference in proportions and a 5% level of significance has been used.

RESULTS

Thirty-five *Candida* species were isolated from 92 symptomatic and asymptomatic antenatal women. Out of 35 *Candida* species 16/35 (45.7%) were from symptomatic (Infection) women and 19/35 (54.3%) were from asymptomatic (Colonization) women. Out of 35 *Candida* species, 15/35 (42.8%) were *C. albicans* and 20/35 (57.7%) were NAC as shown in table 2.

It was possible to detect the presence of *SAP1* to *SAP10* genes by PCR. Totally 16 isolates of *Candida* species from symptomatic women were subjected for *SAP* gene detection. Out of 16 isolates of *Candida* species, 9 were *C. albicans*

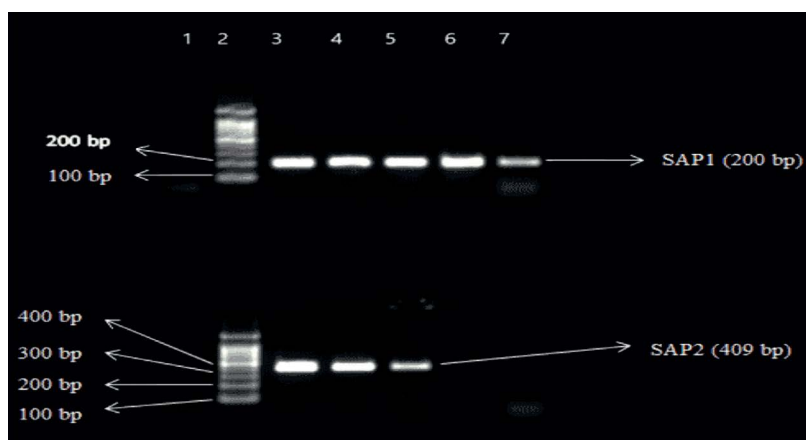


Fig. 1. Top Gel- 1-Negative control, 2-Ladder (1000bp), 3- SAP1(200bp) of *Candida albicans*, 4- SAP 1 of *Candida albicans*, 5-SAP1 of *Candida tropicalis*, 6-SAP1 of *Candida krusei*, 7-SAP1 of *Candida glabrata*. Bottom Gel- 1-Negative control, 2-Ladder (1000bp), 3- SAP2(409bp) of *Candida albicans*, 4- SAP2 of *Candida albicans*, 5- SAP2 of *Candida tropicalis*, 6- SAP2 of *Candida krusei*, 7- SAP2 of *Candida glabrata*.

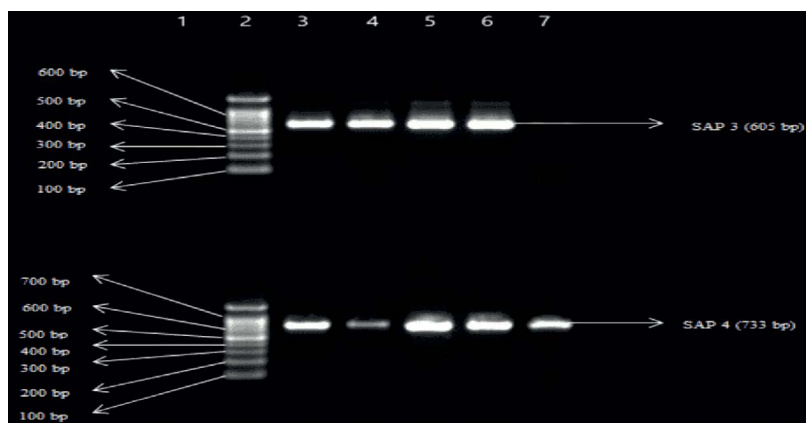


Fig. 2. Top Gel- 1-Negative control, 2-Ladder (1000bp), 3- SAP3(605bp) of *Candida albicans*, 4- SAP3 of *Candida albicans*, 5-SAP3 of *Candida tropicalis*, 6-SAP3 of *Candida krusei*, 7-SAP3 of *Candida glabrata*. Bottom Gel- 1-Negative control, 2-Ladder (1000bp), 3- SAP4(733bp) of *Candida albicans*, 4- SAP4 of *Candida albicans*, 5- SAP4 of *Candida tropicalis*, 6- SAP4 of *Candida krusei*, 7- SAP4 of *Candida glabrata*.

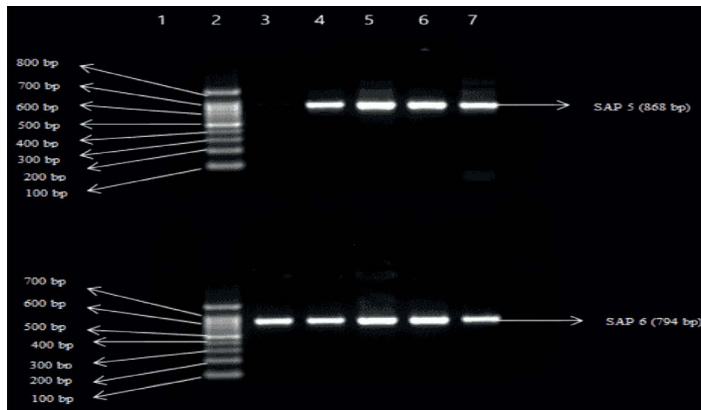


Fig. 3. Top Gel- 1-Negative control, 2-Ladder (1000bp), 3- SAP5 (868bp) of *Candida albicans*, 4- SAP5 of *Candida albicans*, 5-SAP5 of *Candida tropicalis*, 6-SAP5 of *Candida krusei*, 7-SAP5 of *Candida glabrata*. Bottom Gel- 1-Negative control, 2-Ladder (1000bp), 3- SAP6 (794bp) of *Candida albicans*, 4- SAP6 of *Candida albicans*, 5- SAP6 of *Candida tropicalis*, 6- SAP6 of *Candida krusei*, 7- SAP6 of *Candida glabrata*

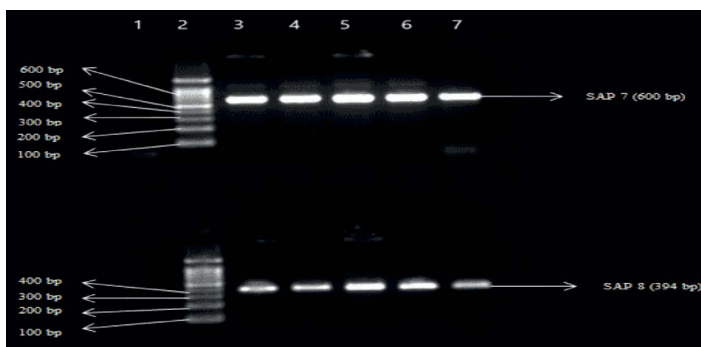


Fig. 4. Top Gel- 1-Negative control, 2-Ladder (1000bp), 3- SAP7 (600bp) of *Candida albicans*, 4- SAP7 of *Candida albicans*, 5-SAP7 of *Candida tropicalis*, 6-SAP7 of *Candida krusei*, 7-SAP7 of *Candida glabrata*. Bottom Gel- 1-Negative control, 2-Ladder (1000bp), 3- SAP8 (394bp) of *Candida albicans*, 4- SAP8 of *Candida albicans*, 5- SAP8 of *Candida tropicalis*, 6- SAP8 of *Candida krusei*, 7- SAP8 of *Candida glabrata*.

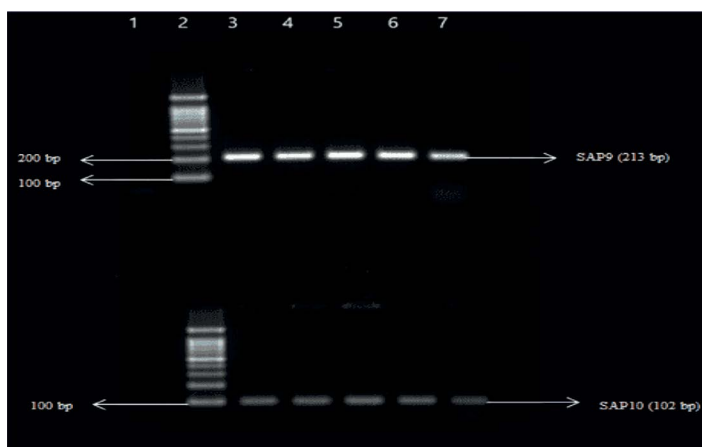


Fig. 5. Top Gel- 1-Negative control, 2-Ladder (1000bp), 3- SAP9 (213bp) of *Candida albicans*, 4- SAP9 of *Candida albicans*, 5-SAP9 of *Candida tropicalis*, 6-SAP9 of *Candida krusei*, 7-SAP9 of *Candida glabrata*. Bottom Gel- 1-Negative control, 2-Ladder (1000bp), 3- SAP10 (102bp) of *Candida albicans*, 4- SAP10 of *Candida albicans*, 5- SAP10 of *Candida tropicalis*, 6- SAP10 of *Candida krusei*, 7- SAP10 of *Candida glabrata*.

Table 2. *Candida* species distribution in symptomatic and asymptomatic women (n=35)

<i>Candida</i> species (n)	NAC*, n (%)				
	<i>C. albicans</i> n(%)	<i>C. glabrata</i> n(%)	<i>C. tropicalis</i> n(%)	<i>C. parapsilosis</i> n(%)	<i>C. krusei</i> n(%)
Symptomatic women, (n=16)	9(56.2)	2(12.5)	3(18.7)	1(6.2)	1(6.2)
Asymptomatic women, (n=19)	6(31.5)	6(31.5)	4(21)	2(10.5)	1(5.2)
Total <i>Candida</i> species (n=35)	15(42.8)	8(22.8)	7(20)	3(8.5)	2(5.7)

*NAC = Non-*albicans Candida*.

Table 3. Detection of secreted aspartyl proteases (SAP) gene in *Candida* isolates from symptomatic women (n=16)

SAP genes	SAP1 n(%)	SAP2 n(%)	SAP3 n(%)	SAP4 n(%)	SAP5 n(%)	SAP6 n(%)	SAP7 n(%)	SAP8 n(%)	SAP9 n(%)	SAP10 n(%)
<i>C. albicans</i> (n=9)	9(100)	9(100)	6(66.6)	7(77.7)	5(55.5)	7(77.7)	7(77.7)	9(100)	9(100)	6(66.6)
<i>C. tropicalis</i> (n=3)	3(100)	2(66.6)	2(66.6)	2(66.6)	2(66.6)	2(66.6)	3(100)	2(66.6)	3(100)	2(66.6)
<i>C. glabrata</i> (n=2)	2(100)	2(100)	2(100)	2(100)	1(50)	1(50)	1(50)	2(100)	2(100)	2(100)
<i>C. parapsilosis</i> (n=1)	1(100)	Nil	1(100)	1(100)	1(100)	Nil	1(100)	Nil	1(100)	1(100)
<i>C. krusei</i> (n=1)	1(100)	1(100)	1(100)	1(100)	1(100)	1(100)	1(100)	1(100)	1(100)	1(100)
Total <i>Candida</i> species (n=16)	16(100)	14(87.5)	12(75)	13(81.2)	10(62.5)	11(68.7)	13(81.2)	14(87.5)	16(100)	12(75)

SAP= Secreted Aspartyl Proteinase.

Table 4. Detection of SAP gene in *Candida* isolates from asymptomatic women (n=19)

SAP gene	SAP1 n (%)	SAP2 n (%)	SAP3 n (%)	SAP4 n (%)	SAP5 n (%)	SAP6 n (%)	SAP7 n (%)	SAP8 n (%)	SAP9 n (%)	SAP10 n (%)
<i>C. albicans</i> (n=6)	5(83.3)	6(100)	2(33.3)	4(66.6)	1(16.6)	3(50)	2(33.3)	3(50)	5(83.3)	3(50)
<i>C. glabrata</i> (n=6)	5(83.3)	3(50)	3(50)	3(50)	2(33.3)	3(50)	1(16.6)	2(33.3)	6(100)	3(50)
<i>C. tropicalis</i> (n=4)	4(100)	2(50)	2(50)	2(50)	2(50)	2(50)	2(50)	4(100)	3(75)	1(25)
<i>C. parapsilosis</i> (n=2)	1(50)	2(100)	Nil	Nil	Nil	2(100)	Nil	Nil	2(100)	Nil
<i>C. krusei</i> (n=1)	1(100)	1(100)	Nil	Nil	Nil	1(100)	1(100)	1(100)	Nil	Nil
Total <i>Candida</i> species (n=19)	16(84.2)	12(63.1)	7(36.8)	9(47.3)	5(26.3)	11(57.8)	6(31.5)	10(52.6)	16(84.2)	7(36.8)

SAP= Secreted Aspartyl Proteinase.

Table 5. Frequency of SAP genes in various *Candida* species isolated from vulvovaginal infections and colonization

Genes	Symptomatic/ Infections (n=16)n (%)	Asymptomatic/ Colonization (n=19)n (%)	Total (n=35) n (%)	P-value
SAP1	16(100)	15(78.9)	31(88.5)	0.1565
SAP2	14(87.5)	14(73.6)	28(80)	0.5527
SAP3	12(75)	7(36.8)	19(54.2)	0.0552
SAP4	13(81.2)	9(47.3)	22(62.8)	0.0863
SAP5	10(62.5)	5(26.3)	15(42.8)	0.0699
SAP6	11(68.7)	10(52.6)	21(60)	0.5331
SAP7	13(81.2)	7(36.8)	20(57.1)	0.0213*
SAP8	14(87.5)	9(47.3)	23(65.7)	0.0328*
SAP9	16(100)	14(73.6)	30(85.7)	0.0834
SAP10	12(75)	9(47.3)	21(60)	0.1882

*Statistically significant ($p < 0.05$), SAP= Secreted Aspartyl Proteinase.

followed by three *C. tropicalis*, two *C. glabrata*, one *C. parapsilosis* and *C. krusei* each. In *C. albicans* 100% (9/9) of SAP1, 100% (9/9) of SAP2, 66.6% (6/9) SAP3, 77.7% (7/9) of SAP4, 55.5% (5/9) of SAP5, 77.7% (7/9) of SAP6, 77.7% (7/9) of SAP7, 100% (9/9) of SAP8, 100% (9/9) of SAP9 and 66.6% (6/9) SAP10 genes were detected. For NAC data showed in table 3.

Totally 19 isolates of *Candida* species from asymptomatic women were subjected for SAP gene detection. Out of 19 isolates of *Candida* species, 6 were *C. albicans* and *C. glabrata* each followed by four *C. tropicalis*, two *C. parapsilosis* and one *C. krusei*. In *C. albicans* 83.3% (5/6) of SAP1, 100% (6/6) of SAP2, 33.3% (2/6) SAP3, 66.6% (4/6) of SAP4, 16.6% (1/6) of SAP5, 50% (3/6) of SAP6, 33.3% (2/6) of SAP7, 50%(3/6) of SAP8, 100% (6/6) of SAP9, 50%(3/6) of SAP10 genes were detected, for NAC data shown in table 4.

The highest frequency of SAP genes in *Candida* species isolated from antenatal women with symptomatic (infection) women of VVC was 100% (16/16) for SAP1 and SAP9 whereas SAP1 was the predominant in asymptomatic (colonization) women, for other SAP genes as data shown in table 5. Of all the genotypic patterns for the members of the SAP genes family in the *Candida* species, a total of 28 patterns were found in this present study. Out of 28 patterns 6 (21.4%) *Candida* isolates showed all the SAP genes (Table 6).

DISCUSSION

It has been four decades since the detection of Sap activity in *Candida albicans*²². Ten SAP genes have been identified till date and different expressions have been observed in vivo²³. In the midst of pregnancy, increase hormone levels and glycogen aggregation in the vagina lead to two folds rises in the frequency of VVC in antenatal women compared with non-pregnant women¹⁴. VVC is a noteworthy reason for morbidity in pregnancy which leads to preterm delivery, miscarriages, *Candida* chorioamnionitis and emotional stress¹⁵⁻¹⁶. VVC caused by the *Candida albicans* and Non-*albicans Candida* (NAC), is able to inhabit the human body without generating any signs of infection such as the asymptomatic state. Despite the fact that under immunocompromised circumstances that disturb the sensitive harmony among the host and commensal, a parasitic relationship may happen to bring about the advancement of disease¹⁷⁻¹⁹. A study showed that the increased Sap enzymes production in diabetic patients have a higher prevalence of VVC²⁰. Prolonged antibiotic treatment, diabetes mellitus and chronic stress may alter the cellular immune responses which lead to increase the susceptibility of infection by *Candida*²¹. Proteolysis activity of SAP genes of *Candida* shows vital success in pathogenicity of this opportunistic fungus. Sap production is one of the tightly regulated and controlled processes; therefore, SAP genes have

Table 6. Genotypic patterns identified in vaginal isolates of various *Candida* species

Pattern	Genotype	n (%)
1	SAP1 to SAP10	6(21.4)
2	SAP1, SAP2 and SAP4 to SAP10	2(7.1)
3	SAP1, SAP2, SAP3 and SAP5 to SAP10	2(7.1)
4	SAP1, SAP2 and SAP5 to SAP10	2(7.1)
5	SAP1, SAP2, SAP4 and SAP6 to SAP10	1(3.5)
7	SAP1 to SAP3 and SAP7 to SAP10	1(3.5)
8	SAP1, SAP3, SAP4 and SAP6 to SAP10	1(3.5)
9	SAP1 to SAP4, SAP6, SAP8 and SAP9	1(3.5)
10	SAP1, SAP3 to SAP5, SAP7, SAP9 and SAP10	1(3.5)
11	SAP1 to SAP4 and SAP8 to SAP10	1(3.5)
12	SAP1 to SAP3, SAP6, SAP7, SAP9 and SAP10	1(3.5)
14	SAP1, SAP2, SAP3, SAP4, SAP8 and SAP9	1(3.5)
15	SAP1, SAP2, SAP3, SAP6, SAP8 and SAP9	1(3.5)
16	SAP1, SAP3, SAP5, SAP7, SAP9 and SAP10	1(3.5)
17	SAP1, SAP2, SAP4, SAP6, SAP9 and SAP10	1(3.5)
18	SAP1, SAP2, SAP3, SAP7, SAP8 and SAP9	1(3.5)
19	SAP1, SAP2, SAP4, SAP5, SAP9 and SAP10	1(3.5)
20	SAP2, SAP4, SAP6, SAP8 and SAP9	1(3.5)
21	SAP1, SAP5, SAP7, SAP8 and SAP9	1(3.5)
22	SAP1, SAP2, SAP6, SAP7 and SAP8	1(3.5)
23	SAP4, SAP6, SAP9 and SAP10	1(3.5)
24	SAP1, SAP2, SAP4 and SAP9	1(3.5)
25	SAP1, SAP2, SAP4 and SAP8	1(3.5)
26	SAP1, SAP2, SAP3 and SAP8	1(3.5)
27	SAP1, SAP2 and SAP9	1(3.5)
28	SAP2, SAP6 and SAP9	1(3.5)

SAP= Secreted Aspartyl Proteinase.

multiple functions for virulence in *Candida*. SAP1 to SAP3 is essential for mucosal infection whereas SAP4 to SAP6 is responsible for systemic infections that take part in evasion of host immune response, host tissue damage and adherence to the host cell. The Sap is able to breakdown the human proteins such as mucin, collagen, keratin as well as antibodies, cytokines, complements of immune component²⁷.

There are many newly existing methods for *in vitro* identification of Sap enzyme yet it is exceptionally dubious when utilizing plate methods with protein-containing media. The pH range of Saps is 3 to 5.5. If there is alteration in pH, that enzyme gets denatured easily. Furthermore, media contains an inhibitory substance which may alter the production of proteinase by *Candida* species and *in vitro* detection of Sap becomes challenging and unreliable²⁴⁻²⁵. Kilic *et al.*²⁶ reported that detection of Sap by *in vivo* and *in vitro* may be useful. Nevertheless, molecular detection of SAP genes is more explicit than Sap enzymes detection by plate methods.

The present study revealed that the more prevalent SAP genes in *Candida* species isolated from symptomatic women were SAP1 and SAP9 (100%) followed by SAP2 and SAP8 (87.5%). Whereas SAP1 (78.9%) was more prevalent SAP gene among *Candida* species isolated from asymptomatic women. Bassyouni *et al.*²⁰ *C. albicans* was isolated from diabetic and non-diabetic women with VVC and investigated the presence of SAP1 to SAP8 genes. The author found that SAP1 and SAP2 were the most predominant detected genes. Another study done by Kalkanci *et al.*²⁸ reported that the most prevalent SAP gene in vaginal isolates were SAP1 to SAP3. A study done by Monroy *et al.*²⁹ observed that SAP4 to SAP6 genes were more frequently expressed and suggesting that SAP gene plays a significant role in the pathogenesis of the infections. Bernardis *et al.*³⁰ reported that SAP1 and SAP2 are responsible for the development of the disease. Schaller *et al.*³¹ demonstrated that SAP1, SAP2 and SAP5 were predominant genes identified in vaginal infection; the author also stated that SAP2 expression is found in both infection and colonization while SAP1 expression is higher than SAP2 in vaginal infection. The results of the earlier studies suggest the role of SAP1 for the infection process in VVC

and are in agreement with the present study.

In the present study, *SAP4* was detected 81.2% in *Candida* isolated from symptomatic VVC which is higher than the asymptomatic isolates. It is reported that *SAP4* is responsible for biofilm formation and prevent phagocytosis³³. The result of the present study does not correlate with the study done by Nas et al.³⁴ where *SAP4* was detected 40%. In this study, the *SAP6* and *SAP7* were predominantly detected in isolates of symptomatic women. The *SAP6* associated with the *SAP4* to *SAP6* subfamily, help for the development of hyphae and invasion of host cells^{12,33}. Despite the fact that *SAP6* and *SAP7* genes were associated with tissue damage in the initial phase of infection in vitro model of vaginal candidiasis based on "Reconstituted Human Vaginal Epithelium (RHVE)"³¹. In the current study, *SAP8* was detected 87.5% in isolates from symptomatic VVC and 47.3% in isolates from asymptomatic VVC. The result of the present study was higher than the study reported by Naglik et al.¹² the researcher distinguished that 75% of *C. albicans* express *SAP8* gene in the vaginal-infected group while 32% in the colonized group. Schild et al.³² proposed that *SAP9* and *SAP10* are responsible for biofilm formation, adherence and invasion into host cells.

The frequency of *SAP1* and *SAP3* are more in vaginal infection when compared with oral infection, thus indicating that the differential expression of genes varies according to the stage of infection and the anatomical site³⁵. A study done by Lian et al.³⁶ observed that *SAP1* and *SAP3* genes were present only in vaginal infection. Lin et al.³⁷ reported that *SAP2* and *SAP5* are the most common genes expressed in the vaginal mucosa. However, in the present study, *SAP1* and *SAP9* were predominantly observed in vaginal infection compared to vaginal colonization. In the present study, statistical analysis showed a significant difference ($p=0.0213$) for the *SAP7* gene frequency among the *Candida* isolated from infection (81.2%) and colonization (36.8%), similarly there is a significant difference ($p=0.0328$) for the *SAP8* gene frequency among the *Candida* isolated from infection (87.5%) and colonization (47.3%). This suggests probable participation of *SAP7* and *SAP8* associated with tissue damage in the initial phase of VVC^{12,31}.

The *SAP1* to *SAP10* genes can be expressed by *Candida* in the vaginal mucosa, both in colonization processes and in infections, but differential expression of these genes is seen when looking at the transcript levels in isolates of carriers and of active VVC, besides a predominance of certain members of the *SAP* family during vaginal infection. Medeiros et al.³⁸ did not observe the differences in the expression of *SAP* gene among *C. albicans* isolated from patients with VVC and colonization, suggesting that the capacity to express virulence factors is significant in the pathogenesis of VVC, yet it appears not to be essential for the change from colonization to disease. In the present study, we did not identify a statistical difference for the presence of *SAP1* to *SAP6*, *SAP9* and *SAP10* genes in isolates from vaginal infection and colonization.

CONCLUSION

Candida isolated from symptomatic women and asymptomatic women exhibited distinctive patterns of distribution for *SAP1* to *SAP10* genes. There is a predominance of the *SAP1* gene in *Candida* isolates from asymptomatic women whereas *SAP1* and *SAP9* genes in *Candida* isolates from symptomatic women. The statistical analysis showed a significant difference for the *SAP7* and *SAP8* gene frequency among the *Candida* isolated from symptomatic and asymptomatic women. The existence of an *SAP* gene family in *Candida* species noticeably delivers the fungus with a proficient and adaptable proteolytic framework that may demonstrate fundamental to its prosperity as a sharp pathogen. Proteinases encoded the *SAP* genes as an exceptionally flexible and multi-functional harmful gene family of *Candida*. However, we did not identify a statistical difference between the presence of *SAP1* to *SAP6*, *SAP9* and *SAP10* genes in isolates from vaginal infection and colonization. Moreover, this suggests that other factors that control the genetic fungal expression and characteristics inherent in the host are likely to influence the infection/colonization process. Though, it seems that we all know an excellent deal concerning the *Candida* *SAP* genes, further investigations involving a larger number of samples will yield a better conclusion and number of essential questions can be addressed in upcoming studies.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

AUTHORS' CONTRIBUTION

All authors have made substantial, direct and intellectual contribution to the work and approved it for publication.

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DATA AVAILABILITY

Not Applicable.

ETHICS STATEMENT

The study was approved from the Institutional Ethical Committee (IEC, 1090/IEC/2017).

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