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RESEARCH ARTICLE

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ABC typing and extracellular enzyme production of *Candida albicans* isolated from *Candida* vulvovaginitis

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Abstract

Background: *Candida albicans* is the most common and virulent genus *Candida*. Detection of virulence factors in this species plays an important role in the better understanding of pathogenesis and antifungal treatment. Molecular typing investigations are important in the epidemiological interpretation of infection. This study aimed to evaluate extracellular enzyme activity and genotyping of *C. albicans* species isolated from vulvovaginal samples.

Methods: One hundred and three vaginal *C. albicans* isolates were tested for esterase, phospholipase, proteinase, and hemolysin activities by specific media. Besides, the DNA of *C. albicans* isolates was extracted and amplified for ABC genotyping.

Results: The highest enzyme production of *C. albicans* isolates was for proteinase (97.1%) and esterase (95.2%), whereas 59.2% of *C. albicans* isolates were negative for hemolysin secretion. Genotype C (83.5%) was the most frequent genotype followed by genotype B (12.6%) and genotype A (3.9%).

Conclusion: It is concluded that genotype C was the predominant genotype in all examined vulvovaginal *C. albicans* isolates. Also, there was a significant difference between enzyme production in each genotype (except for proteinase).

KEYWORDS
ABC genotyping, *Candida albicans*, extracellular enzymes, vulvovaginal candidiasis

1 | INTRODUCTION

Genus *Candida* is a compromised heterogeneous species that cause a wide variety of cutaneous, mucocutaneous, and systemic candidiasis.[1] *Candida albicans*, a commensal yeast, is a human mycoflora and remains the major pathogenic species associated with vulvovaginal candidiasis.[2,3] Although various factors are involved in the development of different types of candidiasis, some researchers believe that some genotypes of *Candida* play a greater role in causing the disease.[4-6]

Several typing methods were introduced for the genotyping of *C. albicans*. One of these methods is ABC genotyping, which is based on the presence or absence of an intron in 25s rDNA. According to this method, *C. albicans* is divided into four genotypes: genotype A (450 bp), genotype B (840 bp), genotype C (450 and 840 bp), and genotype E (1000 bp).[7] Several published studies reported that genotypes A and B are predominant genotypes among different forms of candidiasis; however, genotypes C and E are quite rare.[4,8,9]

Several reports have shown that the virulence of *C. albicans* is related to several microbial and host factors. Extracellular hydrolytic enzymes seem to play an important role in the pathogenesis of *C. albicans*.[10] These enzymes facilitated adherence, tissue penetration, invasion to host, evasion to host response, and overgrowth of *C. albicans* isolates.[11] The hydrolytic enzymes described in *C. albicans* are hemolysin, aspartyl protease, phospholipase, and esterase.[12]

In the present study, vulvovaginal *C. albicans* isolates were genotyped using ABC typing technique. Besides, extracellular enzyme secretion by isolates was evaluated. Several studies have investigated the epidemiology of vulvovaginal candidiasis, but the genotyping of agents in a different region remains uncertain. There are limited studies on the association between *C. albicans* genotypes and its virulence factors, hence the correlation of extracellular enzymes in *C. albicans* was compared with its genotypes. Therefore, this study aimed to evaluate extracellular enzyme production and genotyping of *C. albicans* species isolated from vulvovaginal samples.

2 | MATERIALS AND METHODS

2.1 | Preparation of isolates

One hundred and three vaginal *C. albicans* isolates were previously isolated from patients with vulvovaginal candidiasis and identified using the PCR-RFLP method.[13] Isolates were preserved in distilled water at room temperature in the medical mycology laboratory, affiliated with the Ahvaz Jundishapur University of Medical Sciences, Iran. These isolates were tested for ABC typing and extracellular enzyme (phospholipase, proteinase, hemolysin, and esterase activities) evaluation. All isolates were subcultured on Sabouraud dextrose agar, SDA (Sharlu, Spain), and incubated at 35°C for 24–48 h, aerobically.

2.2 | DNA extraction

DNAs were extracted according to the Look et al. method.[14] Briefly, a loop full of fresh colonies was added into a tube containing 100μL lithium acetate (Central Drug House, India) 0.2 M with SDS (Cinna Gen, Iran) 1%. Each tube was incubated in a 70°C water bath for three minutes. Afterward, ethanol 96% was added to each tube and vortexed. Tubes were centrifuged at 14,000 rpm for three minutes, and then, supernatants were discarded. Then, 300 μl of ethanol 70% was added to plates and centrifuged at 14,000 rpm for three minutes. The supernatant was discarded, and 100 μl distilled water was added to the dried plates. Finally, tubes were centrifuged at 14,000 rpm for three minutes, and supernatant (DNA) was separated and kept at −20°C.

2.3 | ABC genotyping

ABC genotyping of *C. albicans* isolates was assessed by amplification of a *Candida* 25 s rDNA gene with specific primers including CAINT-L (5′ATAAGGGAAGTCGGCAAAATAGATCCGTAA-3′) and CA-INT-R (5′-CCTTGGCTGTGGTTTCGCTAGATAGTAGAT-3′).[7] DNA was amplified in a reaction containing 12.5 μl master mix (Amplicon, Denmark), 1 μl of reverse and forward primers (10 pM), 1 μl of template DNA, and 9.5 μl distilled water. PCR amplification was performed by a thermal cycler (Bio-Rad, USA) according to the following conditions: 97°C for seven minutes for initial denaturation, 30 cycles at 94°C for 30 s, 60°C for 30 s, and 72°C for 40 s, and 72°C for five minutes for the final extension. PCR products were electrophoresed using 1.5% agarose gel in TBE buffer.

2.4 | Determination of extracellular enzyme activity

All tested isolates were screened for extracellular enzyme activities by using specific culture media.[15] Media containing specific substrates including egg yolk for phospholipase activity and bovine serum albumin agar medium (Merck, Germany) for proteinase activity were used. Sabouraud dextrose agar supplemented with defibrinated blood of the sheep (Bahar Afshan, Iran) and 3% glucose (Merck, Germany) was prepared for hemolysin activity evaluation. Esterase activity was assessed using yeast extract (Merck, Germany) and peptone (Difco, USA) supplemented with 0.5% Tween-80 (Merck, Germany). Overnight culture of each isolate was cultured on enzyme media and incubated at 37°C for phospholipase and hemolysin activity and 25°C for esterase and proteinase activity. Finally, the halo diameter and colony diameter were measured, and the activity index of each enzyme was calculated.[16]

2.5 | Statistical analysis

The independent-samples test (t test) was used for data analysis using SPSS software (version 24, USA), and *p*-value of 0.05 was considered a default value for significance.

3 | RESULTS

Out of 103 strains of *C. albicans*, the most common genotype was genotype C (86, 83.49%), followed by genotype B (9, 8.7%) and genotype A (4, 3.88%) (Figure 1). The details of extracellular enzyme secretion among different genotypes are shown in Table 1. As shown, the potency of secretion of each enzyme was varied among isolates. The highest enzyme production of *C. albicans* isolates was for proteinase (97.1%) and esterase (95.2%), whereas 59.2% of the isolates were negative for hemolysin secretion. Also, the highest index enzyme activity (+++) was obtained for esterase and proteinase activities (84.5%). Our results indicate that there are statistically significant differences between the secretion of enzymes and *C. albicans* genotypes (exception for proteinase activity, $p = 0.08$).

4 | DISCUSSION

Candida albicans is the most virulent species, and it is frequently isolated from different forms of candidiasis and animal and environmental sources. ABC genotyping analysis of 25s rDNA is one of the molecular typing methods and an important tool for epidemiological investigation.[4,17] In our study, genotype C was the most common genotype (83.5%) among vulvovaginal *C. albicans* isolates. However, in the Brazilian study, 75.7% and 24.3% of vulvovaginal *C. albicans* isolates had A and B genotypes, respectively.[4] Also, Chaves et al. reported that *C. albicans* isolates with the genotype B (from urine) have a high tendency to form invasive infections.[5] Nevertheless, in our study, 12.6% of *C. albicans* isolates were identified as B genotype. In some literature, *C. albicans* genotype A is also predominant in other series with strains obtained from different body sites, including cases of systemic infection,[6,18,19] whereas only 3.8% of our isolates had A genotypes. Also, Jafarian et al. reported that genotype C was the predominant genotype among oropharyngeal *C. albicans* isolates followed by B and A,[20] whereas genotype A was accounted as the major genotype among urinary *C. albicans* isolates in the Gharaghani et al. report.[21]

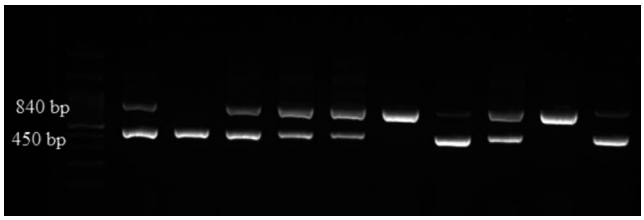

FIGURE 1 ABC genotyping profile of *Candida albicans* isolates (genotype A: 450 bp; genotype B: 840 bp; and genotype C: 450 and 840 bp)

Some virulence factors such as extracellular enzyme activities (phospholipase, esterase, hemolysin, and protease) are associated with *C. albicans* pathogenesis.[10] Tsang et al. reported that extracellular enzyme activity in *C. albicans* isolates may depend on the origin of isolates.[22] For instance, previous studies have reported phospholipase activity varies from 69% to 84.8% in *C. albicans* [12,23] isolated from blood, urine, vaginitis, skin, and oral cavity. In our study, phospholipase activity was detected in 84.5% of *C. albicans* isolates. Our results agree with reported studies[12,24] that demonstrate 84.8% and 84.7% high level of phospholipase activity in vulvovaginal *C. albicans* isolates. However, our finding does not agree with the results of Price et al. (53.8%).[16] There is a significant difference ($p = 0.00$) between phospholipase activity and each genotype.

Rossoni et al. showed that hemolysin production in different *Candida* species is dependent on isolation sites.[25] Studies on the activity of hemolysin in different genotypes of *C. albicans* are limited. We found that hemolysin activity had a statistically significant difference between each genotype ($p = 0.04$). The results of our study are completely different from the previous study of vaginitis isolates, which reported 100% hemolysin secretion in vaginitis samples.[26] This difference in results may be association with a geographical region, and sample size.

Candida protease activity plays an important role in adhesion to extracellular surfaces of host tissue.[27] In the present study, 97.1% of *C. albicans* isolates had high and medium ranges of proteinase activity. But the difference between proteinase activity in each genotype was not significant ($p = 0.08$). Despite our results, Seifi et al. reported 75% of vulvovaginal *C. albicans* isolates possess proteinase activity.[12]

TABLE 1 Frequency of extracellular enzymes between different genotypes of *Candida albicans*

Enzymes	Index		Genotype A (4)	Genotype C (86)	Genotype B (13)	Total (103)	*p*-value
Hemolysin	1	Neg.	3 (2.9)	53 (51.5)	5 (4.9)	61 (59.2)	0.047
	0.77–0.99	+	–	1 (1)	–	1 (1)	
	0.4–0.76	++	–	28 (27.2)	4 (3.9)	32 (31.1)	
	0.1–0.39	+++	1 (1)	4 (3.9)	4 (3.9)	9 (8.7)	
Phospholipase	1	Neg.	1 (0.97)	6 (5.8)	9 (8.7)	16 (15.5)	0.0
	0.77–0.99	+	–	–	–	–	
	0.4–0.76	++	3 (2.9)	53 (51.5)	3 (2.9)	59 (57.3)	
	0.1–0.39	+++	–	27 (26.2)	1 (1)	28 (27.2)	
Esterase	1	Neg.	1 (1)	4 (3.9)	–	5 (4.9)	0.035
	0.77–0.99	+	–	–	–	–	
	0.4–0.76	++	–	7 (6.8)	4 (3.9)	11 (10.7)	
	0.1–0.39	+++	3 (2.9)	75 (72.8)	9 (8.7)	87 (84.5)	
Proteinase	1	Neg.	1 (1)	2 (1.9)	–	3 (2.9)	0.085
	0.77–0.99	+	–	–	–	–	
	0.4–0.76	++	–	12 (11.7)	1 (1)	13 (12.6)	
	0.1–0.39	+++	3 (2.9)	72 (70.0)	12 (11.7)	87 (84.5)	

Yet, our results are consistent with the study by Fatahinia et al., who reported a 98.1% proteinase secretion in vulvovaginal *C. albicans* isolates.[26] In this study, we found that 95.2% of the *C. albicans* isolates had esterase activity in high and medium levels. The percentage of the secretion of esterase activity is in agreement with the studies conducted by Kumar et al.[28] and Fatahinia et al..[26] Besides, except for esterase activity, there are no significant differences between enzyme secretion and genotypes in the Gharaghani et al. study.[21]

In conclusion, in general, genotype C was the predominant genotype in all examined vulvovaginal *C. albicans* isolates. Also, there was a significant difference between enzyme production in each genotype (exception for proteinase activity).

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CONFLICTS OF INTEREST

The authors declare that they have no potential conflicts of interest.

AUTHOR CONTRIBUTIONS

AZM: study design; supervision; data interpretation; and article editing. HJ: Experimentation; data collection; analysis; literature search; and drafting of the article. MG: Experimentation; data collection; and drafting of the article. MS: Experimentation and data collection.

DATA AVAILABILITY STATEMENT

Raw data were generated at independent-samples test (t test). Derived data supporting the findings of this study are available from the corresponding author Ali Zarei Mahmoudabadi on request.

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