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Original Article

Characterization of Phenotype-Based Pathogenic Determinants of Various *Candida albicans* Strains in Jordan

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SUMMARY: Sixty-six clinical isolates of *Candida albicans* representing 14 different strain types were tested for their phospholipase and proteinase activities in correlation with adherence to buccal epithelial cells (BECs) and lethality to mice. Variations in phospholipase and proteinase production as well as adherence to BECs were observed both among isolates of the same strain type and between isolates of different strain types. All isolates tested, irrespective of strain type, produced low levels of phospholipase (0.5 mm for strain -BCD- and 2.7 mm for strain ABC--) and acid proteinase (0.6 mm for strain A --- E and 2.2 mm for strain --C--). A correlation was noted between adherence, phospholipase and proteinase production, and lethality to mice. *C. albicans* isolates, which adhered most strongly to BECs, exhibited higher levels of phospholipase and proteinase activities as well as higher pathogenicity. This was most evident in strain type --C--, which exhibited higher adherence ability (mean 717 ± 21 yeasts/100 BEC), and proteinase activity (mean 2.2 mm), and relatively higher phospholipase activity (mean 2.4 mm) compared with those of other strains. Additionally, this type was more prevalent and showed significantly higher levels of tissue colonization in the liver, kidneys, and spleen compared with most other strain types in both subjects with healthy dentates and complete denture wearers. These results clearly demonstrate the significant role of phospholipase and proteinase activities on the adherence of *C. albicans* and their overall influence on the pathogenesis of *Candida* species.

INTRODUCTION

Candida albicans, a human opportunistic fungus, is the causative agent of several localized and systemic infections especially in individuals with compromised immunity (1). The increased incidence of candidal infections, mainly due to *C. albicans*, in patients being treated for cancer, organ transplantation, and other forms of immunoincompetence (2,3), in addition to the emergence of azole antifungal drug resistance (4,5), make the effort to understand the mechanisms of *C. albicans* pathogenesis all the more important. A number of putative virulence factors have been suggested in the enhancement of *C. albicans* pathogenicity. These include yeast-to-hyphal transition, phenotype switching, thigmotropism, molecular mimicry, adhesion factors or surface hydrophobicity, phospholipase production, and the production of an extracellular secreted aspartyl proteinase (Sap) (1,6-12).

Phospholipases represent a class of lipolytic enzymes, acylhydrolases and phosphodiesterases, that have been extensively characterized (9). *C. albicans* secretes lysophospholipase, transacylase, and exhibits phospholipase A, B, and C activities (13,14). These enzymes may promote penetration of host cell membranes and influence the adherence potential of the yeast (9,12,15). It has been shown that the level of phospholipase activity secreted by each particular isolate of *C. albicans* is directly correlated with the pathogenicity of the isolate (13,16). A correlation was also found between phospholipase activity, cellular adherence, and pathogenicity of *C. albicans* (15).

Hydrolytic enzymes such as extracellular aspartyl proteinase enzyme (Saps) are believed to enhance the ability of *C. albicans* to colonize and penetrate host tissues and to evade the host immune system (8,12,17,18). The correlation between the degree of virulence and the levels of Saps production in both *C. albicans* clinical isolates (11,19) and laboratory strains with altered proteinase levels (20,21) supports a potential role of this family of enzymes in *C. albicans* virulence. It has also been suggested that highly proteolytic strains of *C. albicans* isolated from patients with AIDS are more pathogenic than strains with less proteolytic activity (22,23). The importance of Saps in establishing systemic and vaginal *C. albicans* infection is well demonstrated by several recent findings. It has been shown that the proteinase inhibitor pepstatin A (a hexa peptide from *Streptomyces*) as well as aspartic proteinase antibodies play a protective role in experimental animal models (18,24,25). Furthermore, proteinase-deficient mutants were found to have attenuated virulence in an animal model of disseminated candidiasis (20,26,28).

Adherence of *Candida* to the epithelial cells represents a crucial step in the pathogenesis of candidiasis in that it is the initial step in colonization and subsequent infection (1). Furthermore, the phenotypic variability of *Candida* spp. and strains may have some bearing on the differential pathogenic potential of such species, especially with regard to adhesion ability and toxin production (11,15,29).

In this study, we characterized 66 clinical isolates of *C. albicans*, representing 14 different strain types in terms of their phospholipase and proteinase production in correlation with the capacity to adhere to buccal epithelial cell (BEC) and their degree of lethality in mice. The influence of these putative virulence factors on the prevalence of the studied strains in denture stomatitis patients was also evaluated.

MATERIALS AND METHODS

Isolates: A total of 66 clinical isolates were obtained from

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the oral cavities of subjects with healthy dentates and complete denture wearers. Methods of isolation, identification, and strain differentiation used in this study have been described elsewhere (30,31). A resistogram, a list of the chemicals to which the isolate is resistant, of the strain types was based on resistance of isolates to sodium selenite, boric acid, cetrimide, sodium periodate, and silver nitrate. Each of the five chemicals used is assigned a letter of the alphabet (From A to E), and the letters attached to the chemicals to which an isolate is resistant are then used in the description of the strain. Thus, isolates that are resistant to chemicals B, D, and E are described as strain -B-DE (31). *Candida albicans* ATCC 36082, kindly provided by M. A. Ghannoum (Center for Medical Mycology, Mycology Reference Laboratory, University Hospital of Cleveland, Ohio, USA), was used as a control strain. All isolates were maintained on Sabouraud Dextrose Agar (SDA) (Difco, Detroit, Mich., USA) and stored at 4°C.

Phospholipase assay: Phospholipase secretion from the various isolates of *C. albicans* was measured by the method of Price et al. (32). The strains were cultured for 18 h at 30°C in Sabouraud broth containing 2% (wt/vol) glucose in an orbital shaker. Cells were harvested by centrifugation (5000 *g*, 30 min), washed twice with phosphate-buffered saline (PBS), and resuspended at a density of 1×10^8 cells ml^{-1}. Ten microliters of the yeast suspensions were spotted onto SDA plates (6.5% SDA, 5.84% [wt/vol] NaCl, 0.55% [wt/vol] $CaCl_2$, supplemented with 8% [vol/vol] egg yolk emulsion [Oxoid Ltd., Basingstoke, UK]) and the diameter of the precipitation zone around each colony was measured after incubation of the plates at 37°C for 72 h. Each assay was carried out in triplicate, and the results were expressed as the triplicate mean. Phospholipase activity (Pz) was determined as the ratio of colony diameter to the diameter of the dense white zone of precipitation using the following formula:

$$Pz = 1 - \frac{A}{B} \text{ where}$$

A = diameter of colony + dense white zone of precipitation
B = diameter of colony.

Proteinase assay: Proteinase production was evaluated by the method of Staib (33). Three flasks per isolate, each containing 50 ml of Sabouraud broth, were incubated at 26°C in an orbital shaker for 24 h. Cells were harvested by centrifugation (5000 *g*, 30 min), washed with PBS and resuspended at a density of 1×10^8 cells ml^{-1}. Ten microliters of the cell suspension were placed onto test medium (1% bactoagar [Difco], 0.1% [wt/vol] KH_2PO_4, 0.5% [wt/vol] $MgSO_4$, and 1% [wt/vol] glucose, pH 5.0) containing 0.16 (wt/vol) bovine serum albumin (Sigma, Poole, UK) as the sole nitrogen source. After 5 days of incubation at 37°C, the plates were fixed with 20% trichloroacetic acid and stained with 1.25% amido black (90% methanol, 10% acetic acid). Destaining was performed with 15% acetic acid, and the clear zones around each colony were measured and used in the determination of the Pz value as described above. Each assay was carried out in triplicate on two separate occasions, and results were expressed as the triplicate mean.

Adherence assay: Overnight culture of *C. albicans* was conducted at 37°C in a 0.67% (wt/vol) yeast nitrogen base (YNB) (Difco) supplemented with 2.5% (wt/vol) glucose. Flasks containing 50 ml of the same medium were inoculated with 1 ml of the overnight culture and grown for 24 h in a shaking water bath (180 rpm) at 37°C. The cells were harvested by centrifugation (1200 *g*, 10 min) and washed twice with sterile PBS by repeated centrifugation.

BECs were collected from healthy human volunteers by gentle rubbing of the mucosal surface of the cheeks with a sterile tongue depressor. The epithelial cells were washed twice with sterile PBS and collected by centrifugation (500 *g*, 10 min). Adherence assays were performed as previously described (34). Yeast cells were suspended in sterile PBS at 1×10^7 cells ml^{-1}; BEC were suspended in sterile PBS to a concentration of 2×10^5 cells ml^{-1}. Two ml of the yeast suspension was mixed with 2 ml of the BEC suspension in a sterile screw-capped bottle. The mixture was shaken at 37°C for 2 h then filtered through a 20 μm filter (Retsch, Idar-Oberstein, Germany) to remove non-adherent yeast cells. The BEC on the filter were washed twice with 5 ml volumes of sterile PBS and finally suspended in 5 ml of the same buffer. A drop of this suspension was mounted on a glass slide, then air-dried, heat- fixed and stained with crystal violet for 1 min. The adherence was determined microscopically by counting the mean number of yeast cells adhering to every 100 BEC. Each assay was carried out in duplicate, and student *t*-test was used to evaluate the differences in the adherence values; a P value of <0.05 was considered significant.

Mouse virulence test: Twelve-week-old Balb/c mice were purchased from the Jordan University of Science and Technology, Irbid, Jordan. Mice were used to establish a breeding colony at the Hashemite University vivarium. Ten- to fourteen-week-old Balb/c male mice with an average weight of 20 g were used. *C. albicans* cells of each isolate were grown separately at 37°C for 24 h on a rotary shaker in 50 ml quantities of YNB supplemented with 2.5% (wt/vol) glucose and adjusted to pH 6.0. Cells were then harvested by centrifugation, washed twice with sterile PBS, and re-suspended in 10 ml PBS. This suspension was serially diluted, and the cells, counted with a hemocytometer, were standardized to 1×10^7 cells ml^{-1}. Ten microliters of this suspension were used for each intravenous injection through the tail vein; a group of four mice were used for challenge by each respective isolate.

Tissue colonization: Twenty-four hours post inoculation with *C. albicans*, the liver, kidneys, and spleen of injected animals were removed under sterile conditions. Superfluous tissue was trimmed away, and the remaining tissue was then homogenized in 5 ml PBS in a sterile glass homogenizer. Serial 10-fold dilutions in PBS were prepared from these homogenates. One ml samples of appropriate dilutions were plated in triplicate onto SDA plates containing chloramphenicol (50 mg l^{-1}), and the plates were left to dry then incubated at 37°C for 24-48 h, after which the number of colony-forming units (CFU) (one colony = 1 CFU) was determined. Values were expressed as the mean CFU per organ based on data from four animals.

Statistical analyses: One-way analysis of variance (ANOVA) was carried out to test for significant differences between the means of phospholipase and acid proteinase production, and the adherence of each *C. albicans* strain type. The examined null (H_0) and alternative (H_a) hypotheses were:

H_0: $\mu 1 = \mu 2 = \mu 3$ ...and
H_a: one of the means is different from the rest.

Additionally, multiple comparison using Fisher's least significant difference (LSD) with $\alpha = 0.05$ was carried out to determine which means were significantly different from the rest.

RESULTS

Extracellular enzyme production: A total of 66 clinical isolates of *C. albicans* comprising 14 resistogram strain types were screened for secretion of phospholipase and acid proteinase. Fifty-nine isolates were tested for production of phospholipase secreted under standard conditions. Substantial variations among various *C. albicans* isolates of similar and different strain types were observed (Table 1 and Figure 1). The mean phospholipase production (Pz) of 59 *C. albicans* isolates tested was 1.91 mm irrespective of strain type; the range was 0.54 mm for strain -BCD- and 2.7 mm for strain ABC--. Overall, the level of phospholipase activities of all *C. albicans* isolates was consistently lower than that produced by the ATCC 36082 *C. albicans* control strain (Pz = 4.1 mm). As shown in Table 2, the one-way ANOVA test and LSD test of multiple comparison clearly indicated a statistically significant difference between strains ABC-- and A--D- ($P = 0.0300$), and between strains ABC-- and -BCD- ($P = 0.004$). Strain --C-- showed a statistically significant difference in comparison with strains A--D- ($P = 0.009$) and -BCD- ($P = 0.0005$). Additionally, statistically significant differences were observed

Table 1. Correlation of phospholipase and proteinase production with adherence in various strain types of *C. albicans*

Strain	Isolate No.	Phospholipase (Pz) in mm ± SE[a]	Proteinase (Pz) in mm ± SE[a]	Yeast cells adhering to 100 BEC ± SE
C. albicans ATCC 36082	--	4.3±0.2	3.1±0.1	420±16
ABCD-	1	1.9±0.01	1.7±0.2	510±23
	2	1.4±0.01	1.3±0.1	410±20
	3	1.8±0.03	1.5±0.01	540±37
ABC--	4	1.9±0.03	2.4±0.2	350±35
	5	3.0±0.2	1.8±0.01	320±36
	6	3.2±0.1	1.3±0.1	420±28
AB-D-	7	3.0±0.1	0.8±0.001	590±29
	8	1.6±0.2	1.7±0.1	580±33
	9	3.3±0.3	0.8±0.001	360±26
A-CDE	10	2.5±0.1	0.4±0.01	480±29
--C--	11	0.9±0.1	2.8±0.1	640±20
	12	1.4±0.2	3.4±0.2	430±29
	13	3.1±0.7	2.8±0.2	820±36
	14	1.1±0.04	2.3±0.01	760±24
	15	3.1±0.01	1.0±0.001	510±34
	16	1.5±0.02	2.1±0.02	730±21
	17	3.1±0.01	1.8±0.01	630±21
	18	3.4±0.1	1.2±0.01	810±24
	19	0.08±0.001	1.5±0.01	555±31
	20	1.9±0.1	2.3±0.01	720±19
	21	0.07±0.01	1.6±0.01	420±21
	22	2.6±0.2	1.8±0.01	840±31
	23	4.2±0.3	2.3±0.1	946±21
	24	4.1±0.1	1.6±0.01	819±21
	25	4.8±0.1	4.4±0.1	982±19
	26	2.4±0.01	1.5±0.01	865±26
A--DE	27	1.6±0.001	1.0±0.001	275±21
	28	0.9±0.02	0.6±0.001	NT
	29	NT	NT	250±23
	30	1.4±0.2	NT	300±24
	31	2.5±0.01	3.4±0.01	260±23
A--D-	32	1.5±0.01	2.1±0.01	220±21
	33	0.07±0.001	0.9±0.001	190±20
	34	0.9±0.002	0.1±0.001	210±23
	35	1.7±0.01	NT	NT
	36	NT	2.0±0.2	180±22
	37	1.5±0.002	2.0±0.2	175±21
	38	1.9±0.001	2.3±0.01	210±24
	39	0.08±0.001	NT	NT
	40	1.6±0.1	0.9±0.01	200±23
	41	NT	NT	195±19

Table 1.-Continued

Strain	Isolate No.	Phospholipase (Pz) in mm ± SE[a]	Proteinase (Pz) in mm ± SE[a]	Yeast cells adhering to 100 BEC ± SE
A---E	42	3.0±0.2	0.8±0.001	NT
	43	2.3±0.01	0.4±0.01	210±21
	44	1.4±0.01	0.6±0.001	200±21
-BCDE	45	0.9±0.01	0.6±0.002	190±22
	46	2.0±0.1	1.9±0.01	310±21
	47	3.0±0.06	1.0±0.02	NT
-BCD-	48	0.07±0.001	1.7±0.02	165±19
	49	NT	NT	240±20
	50	0.8±0.01	2.0±0.01	NT
	51	NT	NT	180±19
	52	0.6±0.001	NT	190±21
	53	1.1±0.04	2.3±0.5	285±27
	54	0.6±0.002	0.3±0.002	NT
	55	0.07±0.001	1.4±0.001	140±16
-B-D-	56	NT	NT	120±15
	57	0.8±0.03	0.1±0.001	140±17
-B---	58	1.4±0.2	0.9±0.003	165±16
	59	0.9±0.001	NT	NT
---D-	60	1.6±0.02	NT	195±20
	61	1.4±0.06	1.3±0.02	180±14
-----	62	NT	NT	140±18
	63	2.0±0.03	1.7±0.001	220±19
	64	0.6±0.001	3.0±0.09	160±17
	65	1.6±0.01	1.2±0.001	180±20
	66	1.5±0.02	0.4±0.001	190±22

[a] Values are the average of three separate experiments ± SE.
NT=Not tested.

between strain A--D- and the respective strains ABC-- and --C--. This strain type also showed a significant difference from strain AB-D- (P = 0.037). Overall, the correlation between phospholipase activities and adherence was good (r = 0.49, $P < 0.001$).

The mean of acid proteinase production established using a group of 53 *C. albicans* isolates representing all resistogram strain types was 1.72 mm, which ranged from 0.6 for strain A---E to 2.2 mm for strain --C--. Acid proteinase production by the ATCC 36082 *C. albicans* control strain was 3.1 mm (Table 1 and Figure 1). No statistically significant differences were found among the 14 strain types in terms of mean proteinase production (P = 0.196). The largest zone (4.4 mm) was found in strain --C--, isolate # 25; this isolate exhibited the highest levels of phospholipase production (4.8 mm) and the highest degree of adherence to BEC (982 ± 23) when compared with other isolates of the same strain type and with different strain types, respectively (Table 1). The correlation between proteinase production activity and capacity to adhere to BECs among all 53 *C. albicans* isolates tested was found to be good (r = 0.37, $P < 0.014$).

Adherence of various strain types to BECs in vitro: The adherence ability of a representative group of 58 selected *C. albicans* isolates representing all 14 resistogram strain types and having variable ranges of phospholipase and proteinase activities was determined. As was the case in both phospholipase and proteinase activities, variation in adherence was observed both among isolates of the same strain type and between isolates of different strain types (Table 1 and Figure 1). The result of the one-way ANOVA (Table 2) and LSD test of multiple comparison carried out to determine possible differences between various *C. albicans* resistogram strain types showed a statistically significant difference in adhesion between strain --C-- and all other strain types (P = 0.002). Strain ABCD- showed a statistically significant difference from respective strains A--DE (P = 0.015), A--D- (P = 0.0004), A---E (P = 0.008), -BCDE (P = 0.025), -BCD- (P = 0.0007), and strain ----- (P = 0.0005). Strain ----- showed a statistically significant difference from respective strains ABC-- (P = 0.03), AB-D- (P = 0.0001), and strain --C-- (P = 0.000). The mean adherence value of *C. albicans* isolates per 100 BECs varied from 130 adherent yeast cells in strain -B-D- to 717 adherent yeast cells in strain --C-- (Figure 1). Strains AB-D-, ABCD-, and --C-- showed high levels of adherence to BECs as compared with that of the ATCC *C. albicans* control strain (420 ± 16). Overall, the correlation between the phospholipase and proteinase activities and adherence to BECs was good. However, the correlation between the phospholipase and proteinase activities and adherence to BECs in strain --C-- was not significant, although this strain (type --C--) had a higher prevalence rate than those of most other strain types.

Pathogenicity and lethality of various *C. albicans* strains in the murine system: The pathogenicity of 23 isolates from 12 *C. albicans* strains was evaluated 24 h post intravenous inoculation into adult Balb/c mice. As shown in Table 3, the mean CFU recovered from tissues of mice inoculated with different isolates was calculated. *C. albicans* was recovered from the liver of all animals infected irrespective of the isolate

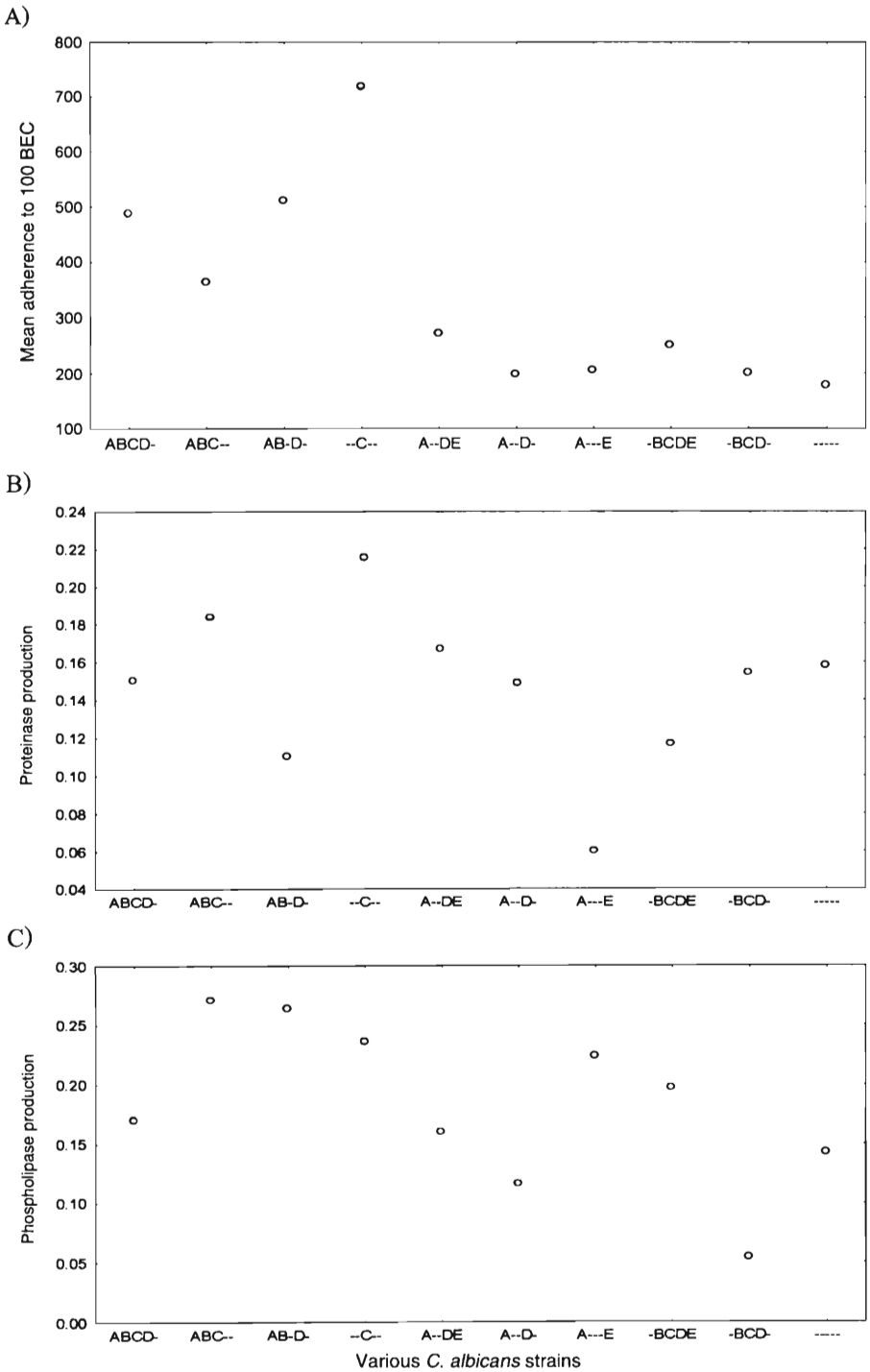

Fig. 1. Plot of adherence to buccal epithelial cells (A), proteinase production (B), and phospholipase production (C) by various *C. albicans* strains.

Table 2. One-way analysis of variance (ANOVA) results for phospholipase and proteinase production and adherence

Effect	df Effect	MS Effect	df Error	MS Error	F	*P*-level
Phospholipase	9*	0.026391*	43*	0.010314*	2.558869*	0.018790*
Proteinase	9	0.010396	40	0.007117	1.460775	0.196002
Adherence	9*	296918.2*	42*	12406.85*	23.93180*	0.000000*

df = degrees of freedom
MS= Means sum of squares
F = F-test
* = Significant difference

Table 3. Number of colony-forming units (CFU) of yeast in mouse tissues 24 h after separate intravenous challenge using 23 different isolates of *C. albicans*.

Strain type	Isolate No.[a]	Kidney[b]	Liver[b]	Spleen[b]
ABCD-	2	0.4±0.07	1±0.19	0.3±0.03
	3	0.8±0.16	3.6±0.26	0.58±0.14
ABC--	5	0.31±0.04	1±0.2	0.1±0.04
	6	0.6±0.14	5.9±0.76	0.4±0.18
--C--	13	4±1.8	9.8±0.94	3.1±0.34
	18	4.2±2.1	11±1.5	3.4±0.56
	25	5.8±2.5	20±2.9	4.2±3.5
	26	3.7±1.9	5.6±0.9	3.9±1.8
A--DE	27	0.95±0.06	1.9±0.26	0.6±0.04
	31	1.4±0.24	2.2±0.37	1.0±0.85
A--D-	33	1.6±0.3	5.1±0.22	1.9±0.28
	37	1.5±0.1	4.3±0.09	1.2±0.3
	40	0.9±0.01	4.6±0.4	0.6±0.07
A---E	43	0.35±0.09	3.0±0.48	0.28±0.04
-BCDE	46	0.39±0.11	4.3±0.55	ND
-BCD-	48	ND	1.0±0.29	ND
	53	0.8±0.06	1.6±0.29	0.6±0.07
	55	ND	0.36±0.09	ND
-B-D-	57	0.64±0.12	1.2±0.24	ND
-B---	58	ND	ND	ND
---D-	61	ND	0.3±0.1	ND
-----	63	0.4±0.14	3.1±0.45	0.01±0.025
	66	0.65±0.23	1.6±0.42	ND

[a] Isolates, refer to those in Table 1, column 2.
[b] Results are the mean CFU / organ ± SD × 10^3.
ND= Not detectable.

or strain type used for inoculation. The only exception was isolate number 58, -B---, where clearance in all tissues tested was evident. The kidneys ranked second in terms of colonization, followed by the spleen.

Mice injected with *C. albicans* strain --C-- gave the maximal yeast colony counts, followed by those recovered from mice infected with strain A--D-. Isolate number 25, strain --C--, which exhibited high levels of phospholipase and proteinase activities and had the highest adherence capability when compared with most other isolates, gave the maximal yeast colonization in the organs tested (Table 3). Only isolate number 53 from strain -BCD- showed colonization in all organs tested; the rest (isolates 48 and 55) showed colonization only in the liver. The remaining strain types showed variable degrees of tissue colonization in the liver, kidneys, and spleen.

DISCUSSION

Understanding the mechanisms of *C. albicans* pathogenesis has become more important in recent years as the incidence of serious fungal infections, particularly in immunocompromised patients, is on the rise (1). Here, we sought to determine any relationships that might exist between the production of phospholipase and proteinase and the adherence potential of various *C. albicans* isolates with the degree of prevalence of specific resistogram strain types in both healthy dentates and complete denture wearers, and, simultaneously, to attempt to characterize some of the phenotype-based pathogenic determinants of *C. albicans* prevalent in Jordan. The results showed that variations in these virulence factors exist both among isolates of the same strain type and between isolates of different resistogram strain types. All *C. albicans* isolates tested in this study, irrespective of strain type or source, were capable of secreting phospholipase. It has been shown that the quantity of this enzyme varied with the specific isolate and is correlated with the site of infection (9). Barrett-Bee et al. (15) were the first to evaluate the role of extracellular candidal phospholipases in virulence by using a murine model of candidiasis. The *C. albicans* isolates (four strains of *C. albicans*) that adhered most strongly to BEC were most pathogenic to mice and had the highest phospholipase activities (15). The role of phospholipases in the pathogenesis of *C. albicans* was explored by Ibrahim et al. (10). These authors showed that blood isolates of *C. albicans* produced higher extracellular phospholipase levels than did commensal isolates. Furthermore, it was shown that isolates with high extracellular phospholipase activity were invasive in the infant mouse candidiasis model, whereas isolates with low extracellular phospholipase activity were not (10). In this context, it was concluded that of the common virulence factors (phospholipase and proteinase production, adherence, germination, growth rate, and ability to damage endothelial cells), only extracellular phospholipase activity was found to be predictive of mortality (10). Results presented here collaborate previous findings; namely, that strain type --C-- seems to have a substantial number of isolates with relatively high phospholipase activity and greater adherence properties as compared with most strain types; this type also seems to have greater capacity to colonize in tissue. Interestingly, some isolates of this strain type had low phospholipase activity; this paradoxical finding cannot be readily explained. Nonetheless, the overall correlation between the phospholipase activity of the clinical *C. albicans* isolates representing the 14 resistogram strain types and the adherence to BEC was good ($r = 0.49$, $P = <0.001$). Although these findings suggest a correlation between phospholipase activity and candidal virulence, the strains used were not genetically identical. It is worth mentioning that future research should include an evaluation of the role of phospholipases and candidal virulence and should be confirmed by the use of an isogenic strain pair that differs only in phospholipase production. Molecular cloning of the candidal gene(s) encoding the extracellular phospholipases is the essential first step in the molecular genetic dissection of the role of these enzymes in pathogenesis.

Proteinase production is believed to enhance the ability of *C. albicans* to colonize and penetrate host tissues and to evade the host immune system (8,12). Previously it was indicated that the aspartyl proteinases (Saps) are rare or absent in non-pathogenic strains or species of *Candida* (12). Mutant strains of *C. albicans* that do not secrete the proteinase enzyme show significant reduction in lethality to mice (20,35,36). The presence of correlation between the level of proteinase activity in clinical *C. albicans* isolates (11,19,29) or *C. albicans* laboratory strains with altered proteinase levels (20,21) and *C. albicans* virulence supports the contention that this enzyme may have some role in influencing the degree of virulence of *C. albicans*. Ghannoum and Abu-Elteen (11) demonstrated that particularly strong proteolytic strains of *C. albicans* adhered more readily to human BEC in vitro. Also, variation in proteinase production and adherence has been shown to exist both among isolates of the same strain type and between different strain types. Similar findings have been reported by other researchers (29).

All *C. albicans* strains evaluated in this study were shown to be capable of secreting acid proteinase. A reasonably good correlation between proteinase levels and adherence to BEC was obtained for the 53 selected *C. albicans* isolates representing all the resistogram strain types ($r = 0.37$, $P < 0.014$). Strain type --C--, which showed relatively high enzyme activity along with greater adherence properties as compared with other strain types, showed higher levels of tissue colonization. This was clearly evident in isolates exhibiting high phospholipase and proteinase levels combined with higher adherence capabilities. In this regard, adherence of *C. albicans* to human mucosa (28,37), epidermal corneocytes (38), and epidermal keratinocytes (39) has been shown to be inhibited by pepstatin A, a hexa peptide from *Streptomyces* with strong anti-Saps potential. This suggests that surface proteins may be targets of the aspartyl proteinases. The role of Saps as virulence factors was confirmed recently by the finding that the virulence of null mutants, *SAPs 1-3* and a triple mutant of *SAPs 4-6* were all attenuated in a systemic mouse infection model, and a guinea pig infection model, and in terms of adherence to human BECs (26-28).

Strain type --C-- ranked first among the 14 strain types in terms of proteinase and adherence values and was third in terms of phospholipase production; despite this, few of the isolates belonging to this strain showed low levels with regard to all three parameters. At face value, this suggests that, for the most part, this strain type has a much higher tendency towards pathogenicity. This may also partly explain the observation that strain type --C-- is more prevalent than other strain types in both subjects with healthy dentates and complete denture wearers (31). It is worth mentioning that strain --C-- was predominant in cancer patients undergoing therapy, while the least predominant was strain A-C-- (40).

In conclusion, the results presented here indicate that a strain-dependent correlation between phospholipase and proteinase activity and adherence to BECs on one hand, and tissue colonization on the other, is present. The effect of these parameters, separate or differentially-combined, may variably contribute to the pathogenicity of *C. albicans*. That said, several other factors not addresseed here, such as host immune status, therapy (1), hydrophobicity, and environmental conditions that promote adhesion and production of phospholipase and proteinase (41), are not to be discounted.

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