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Phenotypic and genotypic evaluation of adherence and biofilm development in *Candida albicans* respiratory tract isolates from hospitalized patients

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Abstract

In recent years, a significant number of epidemiological variations have been observed for fungal infections. In immunocompromised patients, Candida albicans is crucially involved in invasive infections, mostly originating in respiratory tract colonization. The global rise in candidiasis has led researchers to investigate possible correlations between fungal strains virulence profiles and their pathogenic potential, among the most investigated genes being those involved in adherence and biofilm development. In this study, we established the adherence gene profiles of C. albicans strains isolated from respiratory tract secretions in patients hospitalized for cardiovascular diseases and correlated them with the ability of the respective strains to colonize the epithelial cells and form biofilms on the inert substratum. The strains isolated from the lower respiratory tract exhibited the highest adherence capacity and were intensive biofilm producers. The SAP9, ALS3, ALS5, and ALS6 genes were the most frequently detected. There was a significant association between the presence of ALS 3 gene and the cellular substrate colonizing potential of the harboring strains. We also found that the strains expressing SAP9 were more virulent in the phenotypic assays. Detecting the presence of adherence genes from different clinical isolates is a cost-effective tool that would allow researchers to predict the virulence of a certain strain and estimate its potential to adhere to host cells and develop biofilms.

***Keywords**: Candida albicans, adherence genes, adherence to cellular substratum, biofilm*

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Introduction

Candida albicans is generally a widespread fungal pathogen that is involved either in superficial or blood-disseminated infections (1,2). Oral *Candida* spp. is the most widely recognized contaminant found in the elderly or hospitalized patients, causing systemic or mucosal diseases (3-5). The oral colonization with *Candida* spp. in the elderly is favored by prostheses wearing, poor oral/denture cleaning and low salivation stream (6-9).

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The ability of *C. albicans* to form biofilms on both cellular and prosthetic device surfaces increases the severity of the infection. Biofilms are complex, three-dimensional microbial structures encased in an extracellular matrix that are connected to either a biotic or an abiotic surface (10–14). Biofilms decrease the host immune system by an increased microbial resistance to antimicrobial agents (10, 13-15) and thus play a significant role in virulence. The *C. albicans* biofilms consist of yeast and hyphae, both of which being indispensable to fungal adherence and subsequent biofilm development (16–19). The increased prevalence of candidiasis worldwide has led to investigations of the possible correlations between adherence genes profiles and the adherence and biofilm development capacity, assessed phenotypically. Recently, the detection of a variety of regulators and effectors that included biofilm formation in *C. albicans* has increased the understanding of the complex mechanisms concerned with this process [reviewed in (20)]. Various cell wall proteins, including Bcr1, a transcription factor that controls gene expression of cell wall proteins, have been revealed to play important roles in biofilm formation. In addition, Agglutinin-like sequences (*ALS1, ALS3*), and the hyphal wall protein 1 (*HWP1*) contribute to biofilm formation and maturation (21–23).

Also, a direct correlation between secreted aspartyl proteinase (SAP) production and *C. albicans* adherence capacity has been highlighted. It was observed that proteolytic *C. albicans* isolates exhibited a higher capacity of *in vitro* adherence to human oral epithelial cells compared with isolates that synthesized less proteinase (24). In mice, an isolate that produced higher levels of proteinases also exhibited increased *in vivo* tissue colonization capacity of the liver, kidneys, and spleen, and led to the death of the mice (25). The majority of the existing studies investigating the *C. albicans* biofilm production have focused on SAPs. In the anaerobic environments of the root canal systems and periodontal pockets, SAP secretion increases. This observation suggests that the virulence of *C. albicans* is significantly influenced by the concentration of oxygen in the infection site (26). It has been demonstrated that pepstatin, an inhibitor of SAP2 (and probably of SAP1 and SAP3) decreased *C. albicans* biofilm development capacity with variable efficiency (27). Pepstatin treatment decreased *C. albicans* adhesion and invasion of human epidermal cells and mucosa (28, 29, 30).

The *EAP1* gene (extracellular adhesion protein) encodes a putative cell wall adhesin (31) that contains a signal peptide and a glycosylphosphatidylinositol anchor site; *EAP1* gene is homologous to other yeast genes that code for cell wall proteins.

Cell surface hydrophobicity (CSH) plays a vital role in *C. albicans* pathogenesis. Hydrophobic cells displayed higher adherence to both epithelial and endothelial cells and to extracellular matrix proteins, as well as greater resistance to phagocytosis (32). CSH varied due to different environmental conditions and growth phases (33).

The aim of the current study was to establish the adherence gene profiles of *C. albicans* isolates from respiratory tract secretions in patients hospitalized for cardiovascular diseases and to correlate them with the ability of the respective isolates to colonize the epithelial cells and to form biofilms on the inert substratum.

Materials and Methods

Fungal strains

In this study, we analyzed twenty-nine *C. albicans* strains isolated from patients admitted for cardiovascular surgery, aged 25-89 years and diagnosed with respiratory tract infections. Nineteen specimens were isolated from sputum and 10 samples from bronchial secretions. None of the patients was dental prosthesis wearer. The *C. albicans* ATCC10231 was used as reference strain.

Sabouraud Dextrose Agar (SDA) medium was used to initially isolate the strains, which, subsequently, were biochemically identified using the VITEK II automatic analyzer (bioMérieux), after inoculating Yeast plastic cards (YPC) with the required inoculum suspension. The YBC cards were then incubated at 37°C for 24-48 h and submitted for reading.

Biofilm microtiter assay

A microtiter plate method was used to assess the ability of *C. albicans* isolates to attach to an inert substratum and to examine the subsequent biofilm production. *C. albicans* isolates were maintained overnight in Sabouraud broth at 37°C and then centrifuged at 150 rpm. The cells were collected, washed by using phosphate buffered saline (PBS), and standardized to a density of 1 × 10^7 yeast cells/ml. A volume of100 µl of the yeast cells suspension was added to the wells of a 96-well plate and incubated at 37°C for 96 h. After incubation, the liquid content of the wells was discarded, and the wells were washed three times with distilled water in order to remove the non-adherent cells. Then, the cells adherent on the plastic wells were fixed with cold methanol for 5 min and then stained with crystal violet (CV) solution for 15 min (34, 35). In order to evaluate the stained biofilms, first the wells were examined using an inverted microscope. Then, the biofilm formed on the plastic wells was re-suspended with a 33% acetic acid solution and the optical density of the biofilm cell suspension was determined by measuring the absorbance of the colored suspension at 490 nm using an enzyme linked immunosorbent assay (ELISA) plate reader, the results being interpreted according to Melo et al. (36). All tests were done in triplicate and biofilm production quantities were reported as the arithmetic mean of absorbance values of the three replicate test. The absorbance values of the negative controls (containing no cells) were subtracted from the values of the test wells to minimize background interference. The biofilm forming intensity was quantified according to their cut-offs by OD value as none absent (unable to form a biofilm), low (when< ~ ≤ 2x ODc), moderate (when< ~ ≤ 4x ODc) or high (> 4x ODc) (37).

Evaluation of the capacity of adherence to HeLa cells

An adaptation of the Cravioto method was used to determine adherence capacity of each isolate to HeLa cells. The assay was performed as previously described (38). HeLa cells were grown in 6-well plates for 24 h in Dulbecco's Modified Eagle's Medium (DMEM) involved with antibiotics; after this incubation, the cell monolayers reached 80% confluence. Wells were washed 3 times with PBS to remove the growth medium. To prepare the fungal suspension, yeast was grown in Sabouraud glucose broth supplemented with 3% glucose for 48 h at 37°C. Cells were centrifuged at 1500 rpm for 10 min, and the pellets were washed with PBS (pH ~7.2). The cell density in the final suspension was 10^7 colony forming units/ml (using a 0.5 McFarland standard). One ml of the final fungal suspension was added aseptically above each cellular monolayer. The plates were then incubated at 37°C for 2 h to allow the yeast cells to properly adhere to the cellular surface. Then, the cells were fixed with methanol for 5 min after washing with PBS three times. The wells were stained with a 1:10 Giemsa solution (Merck, Darmstadt, Germany) for 20 min, washed with tap water, used room temperature for drying, examined under microscope (2500 total magnification) with I.O., and then photographed with a Contax camera (supplied with Zeiss microscope). The adherence index (AIn) for each isolate was calculated as the percentage of Hela cell showing adherent fungal cells to the total number of HeLa cells. The average number of fungal cells adhered to one HeLa cell was also calculated.

Detection of adherence genes

The total DNA was isolated using the thermolysis method according to the protocol of Zang et al (39). All purified strains were grown on SDA medium at 37°C for 24 h. Subsequently, isolated fungal colonies were routinely subcultured in Yeast Nitrogen Base (YNB; BD Diagnostics, Cowley, UK) medium enhanced with glucose (0.5% w/v) overnight at 37 °C, under stirring at 120 rev/min. Cells were harvested by centrifugation at 3000 g for 10 min at 4°C and the pellet was washed 2 times with 10 mL PBS (pH 7). The cellular density for each strain was adjusted to 1 x 10^7 cells/ml. Pooled biofilms from each collection site were carefully transferred to Eppendorf tubes that contained 1 mL of reduced transport fluid (RTF). Immediately after collection, the samples from each site were diluted and plated onto SDA medium with chloramphenicol (bioMérieux, Paris, France). The plates were incubated in a reduced oxygen atmosphere (10% CO_2 and 90% air) for 48 h at 37°C. DNA was extracted with slight modifications from a protocol by Nascimento et al. (40). The yeast colonies were suspended in 1 mL of a solution containing 1 M sorbitol and 125 M ethylenediaminetetraacetic acid (EDTA). This suspension was centrifuged at 13000 g for 10 min at 25 °C. The pellet was resuspended in 0.5 mL lysis solution [1 M TrisHCl, pH 8.0, with 250 mM EDTA and 5% sodium dodecyl sulfate (SDS)] with 10 mL of proteinase K (Invitrogen), and incubated for 1 h at 65°C. Subsequently, 500 µl of 5 M potassium acetate was added to the mixture and incubated on ice for 2 h. The mixture was centrifuged at 13000 g for 10 min at 25 °C (40). The supernatant was transferred to an Eppendorf tube containing 1 mL absolute ethanol. The tubes were mixed by inversion centrifuged at 13000 g for 10 min at 4°C. The pellet was washed with 500 µl of ice cold 70% ethanol and then centrifuged at 13000 g for 10 min at 4°C. The final pellet was re-suspended in 0.5 mL of sterile MilliQ water. The DNA concentration was determined at 260 nm using a spectrophotometer (Genesys 10UV, Rochester, NY, USA). These data were used to prepare a standard with of 100 ng/ml concentration. The standard was stored for the next PCR reactions at 20°C. Simplex and multiplex PCR reactions using specific primers were used to identify *C. albicans* adhesion genes (41). A positive control was performed using DNA purified from the *C. albicans* ATCC 10231 reference strain. The GeneAmp PCR system 2400 (Perkin-Elmer-Applied Biosystems) was employed for PCR amplification using the following thermal conditions: 94°C for 5 min, 35 cycles of 94 °C for 30 s, 55°C for 30 s, and 72°C for 30 s, and a final extension at 72°C for 10 min. Electrophoresis was used to separate PCR products on 2% agarose gels using Tris-borate-EDTA running buffer (pH 8.0). The DNA, including the molecular mass ladder (1000 bp DNA ladder), was stained using a 0.5 µg/ml solution of ethidium bromide. The stained gel was visualized with UV illumination (Pharmacia LKB-MacroVue, San Gabriel, CA, USA).

Statistical analysis

Statistical analysis was carried out using SPSS version 16.0. For all statistical tests, $P<0.05$ was considered significant and $P<0.01$ highly significant. Inferential data analysis was performed to test. The contingency coefficients (C.C.) test was used to determine the correlation of the cause to association tables. The null hypothesis under Ho says that redistribution of the observed frequencies in the created association table through applying the new suggested method using stem-leaf technique are randomly distributed (meaningless relationship) versus the alternative hypothesis which says that the contingency coefficient of the association table is meaningful. In addition, under and upper redistribution cutoff

points were used to create 2 x 2 rank association tables and these were applied to the Steam-Leaf plotting technique.

Results

In this study, we analyzed 29 *C. albicans* isolates recently isolated from patients aged 25 to 89 years. The majority of the isolates (65%) were isolated from lower respiratory tract (bronchial) secretions. We observed no significant difference in isolation rates based on gender (male to female ratio was 1.08, $P>0.05$) (Table 1).

Most of the *C. albicans* isolates from the bronchial secretions exhibited a localized adherence pattern, while the vast majority of sputum strains exhibited an aggregative adherence pattern (Figure 1, Table 1). We observed that the isolated *C. albicans* strains adhered to HeLa cellular substratum with different intensities as revealed by the adherence index values (AIn) that ranged from 70 to 87.7% (Table 1). Bronchial-derived strains had higher AIn compared to strains isolated from sputum (Table 1).

The majority of isolates from both bronchial and sputum samples developed biofilms on the inert

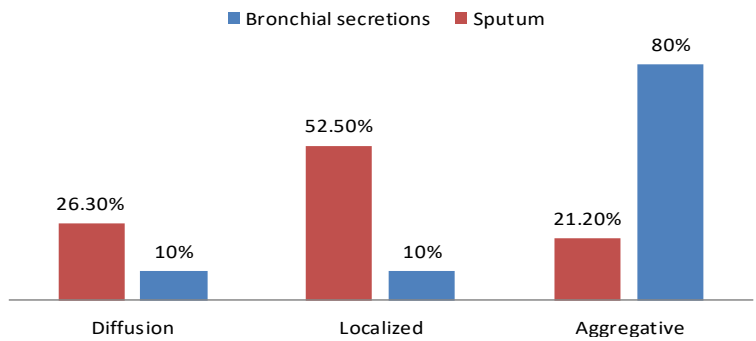

Fig. 1. Adherence patterns (%) of *C. albicans* strains isolated from sputum and bronchial secretions

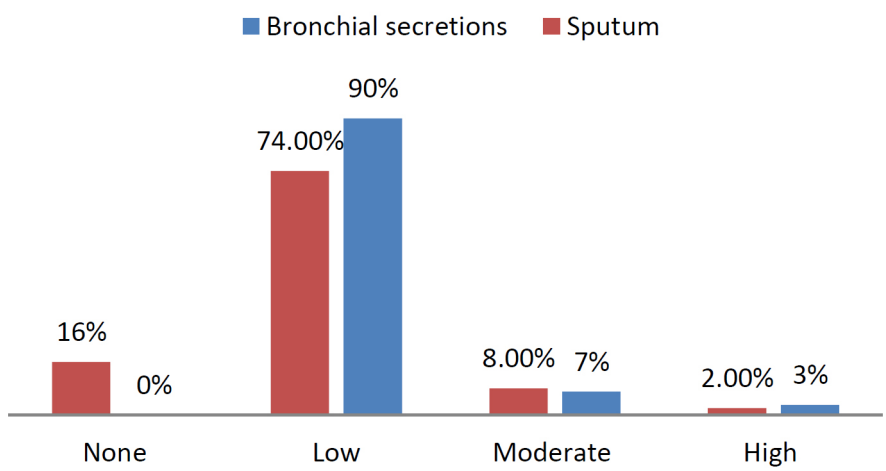

Fig. 2: Distribution of *C. albicans* strains according to source and capacity to develop a biofilm on an inert substratum. The ability to form a biofilm was classified as absent (unable to form a biofilm), low, moderate, or high.

Table 1. Phenotypic and genotypic features of *C. albicans* isolates

C. albicans strain code	Isolation source	Age	Sex	AIn	Average no. of yeast cells/ HeLa cell	Adherence pattern	Biofilm to inert substratum	Adherece genes profile
256	Sputum	89	M	73.82%	8.77	Aggregative	None	*ALS 2, 3, 4, 5,6, 7,8, SAP4, 5, 9, 10,EAP 1*
67	Sputum	62	M	66.66%	5	Localized	Low	*ALS 1, 3, 5,6, 8, SAP 9, 10*
154	Sputum	39	M	73.98%	5.12	Aggregative	None	*ALS 1,3, 4, 6, 7,8, SAP 5,6, 9, 10*
175	Sputum	85	M	65.07%	5.8	Aggregative	Low	*ALS 1,2,3, SAP 2,4, 5, 9, 10*
489	Sputum	56	M	31.31%	2.28	Localized	Low	*ALS 1, 2, 3, 5,6, 7,8, SAP 4, 6, 9, 10,*
772	Sputum	46	F	32.73%	7.42	Diffusion	Low	*ALS 3, 5, 6, SAP 6, 9*
31	Sputum	38	F	41.29%	7.87	Localized	Low	*ALS 1, 2, 3, 4, 5,6, 7,8, SAP 4, 5 ,6, 9, 10, EAP 1*
8159	Sputum	49	M	28.57%	0.7	Localized	Low	*ALS 2, 4, 5,6, 7, SAP 5, 6, 9, 10*
47	Sputum	53	F	43. 80 %	3.2	Localized	Low	*ALS 1, 2, 3, 4, 5,6, 7,8, SAP 4, 5 ,6, 9, 10, EAP 1*
67	Sputum	44	M	33.76%	5.1	Diffusion	Low	*ALS 3, 5, 7, SAP 6, 9, 10*
29	Sputum	51	M	41. 17 %	3.5	Localized	None	*ALS 1, 2, 3, 4, 5,6, 7,8, SAP 2, 5 ,6, 9, 10, EAP 1*
1166	Sputum	60	M	48. 27 %	5.6	Diffusion	low	*ALS 2, 3, 4, 5,6, 7, SAP 2, 4, 5 , 9, 10*
149	Sputum	33	F	46. 92 %	4.8	Diffusion	low	*ALS 1, 2, 3, 4, 5,6, 7, SAP 2, 5 ,6, 9, 10*
58	Sputum	31	F	44.71%	2.62	localized	low	*ALS 1, 4, 5, 6, 7, 8, SAP 4, 5 ,6, 9, 10*
81	Sputum	64	F	45.30%	3.76	localized	low	*ALS 1, 2, 3, 4, 6, SAP 4, 5 ,6, 9, 10*
103	Sputum	50	F	44.64%	3.17	localized	low	*ALS 1, 2, 3, 4, 5, 6, 7, 8, SAP 4, 5 , 9, 10,*
105	Sputum	44	M	47.29%	5.3	localized	low	*ALS 3 , 7, 8, SAP 9*
2693	Sputum	41	F	40. 0 %	3.4	Diffusion	Low	*ALS 1, 2, 4, 5, 6,7, 8, SAP 5 , 9,*
255	Sputum	37	M	82 . 79 %	5.92	Aggregative	Low	*ALS 2, 3, 4, 5, 6, 7,8, SAP 4, 5, 9, 10, EAP 1*

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C. albicans strain code	Isolation source	Age	Sex	AIn	Average no. of yeast cells/ HeLa cell	Adher-ence pattern	Biofilm to inert sub-stratum	Adherece genes profile
1776	Bronchi-al Secre-tions	64	F	28.30%	2.14	Localized	Low	*ALS 2, 3, 5, 6, 8, SAP 6, 9, 10,*
1135	Bronchi-al Secre-tions	51	M	30. 44 %	2	Diffusion	Low	*ALS 1, 3, 5, 6, SAP 2, 5, 6 , 9, 10,*
373	Bronchi-al Secre-tions	62	F	68 .10 %	6	Aggrega-tive	Low	*ALS 2, 3, 4 ,6, SAP 9, 10*
305	Bronchi-al Secre-tions	86	F	68 .50 %	6.8	Aggrega-tive	Low	*ALS 1, 5,6, 8, SAP 6*
1995	Bronchi-al Secre-tions	60	F	82.09%	8.62	Aggrega-tive	Low	*ALS 1, 2, 3, 4, 5,6, 7,8, SAP 9, 10,EAP 1*
1942	Bronchi-al Secre-tions	64	M	71 .81 %	7.9	Aggrega-tive	Low	*ALS 1, 3, 4, 5,6, SAP 6, 9, 10*
1946	Bronchi-al Secre-tions	64	F	69 .04 %	5.8	Aggrega-tive	Low	*ALS 5, 6 , SAP 9, 10*
1935	Bronchi-al Secre-tions	59	M	75 .10 %	7.18	Aggrega-tive	Low	*ALS 3, 5, 6, 7,8 , SAP 6 , 9*
1675	Bronchi-al Secre-tions	25	M	81.67%	6.15	Aggrega-tive	Low	*ALS 1, 2, 3, 4, 5,6, 7,8, SAP 4 , 5, 6, 9, 10,EAP 1*
1676	Bronchi-al Secre-tions	31	M	74. 42 %	4.57	Aggrega-tive	Low	*ALS 3 , SAP 6 , 9, 10, EAP 1*

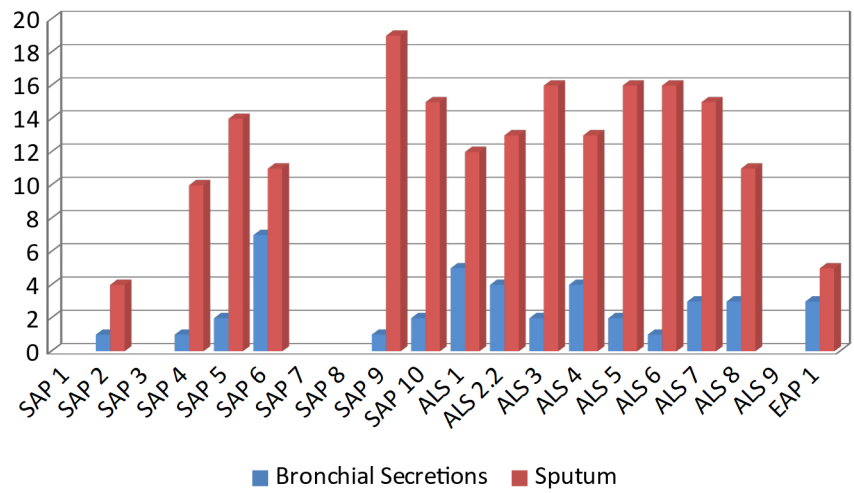

Fig. 3. Distribution of *C. albicans* adhesion genes in strains isolated from sputum and bronchial secretions

substrate (Fig. 2). Overall, isolates from the upper respiratory tract were higher biofilm producers, this feature being correlated with a high AIn (Fig. 2 insertion). Previous research studies have shown that strains isolated from invasive infections tend to produce biofilms more intensely in comparison with isolates from non-invasive infections (42).

Our results indicated a generally non-statistically significant ($P>0.05$) correlation between the presence of *ALS* and *SAP* genes and AIn for *C. albicans* isolates that were isolated from sputum and bronchial secretions (Fig. 3, Table 1). There were, however, some exceptions. The *ALS3*gene presence in strains isolated from sputum and of *SAP2* in those isolated from bronchial secretions were significantly correlated with a high AIn ($P<0.05$). However, the only significant associations between adherence genes and biofilm formation capacity were *ALS1* in tracheal and *ALS8* in sputum isolates ($P<0.05$). The *ALS3* polymorphism led to variable biofilm production in different species isolated from sources such as blood, vulvo-vaginal secretions, and urine cultures (43).

Discussion

Fungal infections due to *C. albicans* in critically ill hospitalized patients are a significant cause of morbidity and mortality worldwide. Peterson et al. (44) observed that 55% of hospitalized patients carried yeasts in their saliva. More specifically, *Candida* spp. strains were found in between 47-87% of in patients with advanced cancer, and in the oral mucosa of up to 80% of diabetic patients (40). In our study, *C. albicans* strains that are isolated from patients hospitalized for cardiovascular surgery were predominantly from bronchial secretions (65 %).

The ability to colonize host tissues is considered one of the most significant factors for *C. albicans* virulence (41). *Candida* spp. adherence to human tissues permits the fungus to reach the minimum infective threshold and colonize a specific niche environment. *Candida* has a specific feature which enables it to survive and develop within different circumstances of healthy human body and then to invade tissues. Thus, the colonized area provides the essential ground for *Candida* development, proliferation, invasion, and dissemination (45). However, any modification in the host immune system, or host environment, can lead to the switching *C. albicans* into a pathogenic form and then able of causing biofilm-associated infections were also noted to play a significant role in perpetuating these infections since these structures could allow the yeast to adhere to medical devices. However, there is limited information available as to the molecular mechanisms that mediate *C. albicans* adhesion to materials or mammalian cells. The relationship between the phenotype of a strain (e.g., the ability to adhere and/or form a biofilm) and genotype is also barely known. Our findings indicate that *C. albicans* isolated from bronchial secretions adhered better to cellular and inert substrata compared to sputum-isolated strains. These bronchial-derived strains also had a specific adherence gene profile. It has been previously described that *ALS* genes play an important role in *C. albicans* adherence and biofilm formation and growth, the *ALS1* protein product being associated with the capacity of *Candida* spp. strains to adhere to endothelial cells and form biofilms (46). We found that the presence of *ALS3*, *ALS5*, *ALS6,* and *SAP9* genes is correlated with *C. albicans* adhesion to polystyrene and human epithelial cells. The *SAP9* and *ALS3* adherence genes contribute to the overall virulence of *C. albicans* strains, being usually expressed in virulent strains isolated from symptomatic infections and enable attachment to cellular and inert substrata. Our results differed compared with a previous study (47) in which the authors report-

ed that other genes which were not identified in our study, respectively *ALS1* and *ALS9*, were detected in the clinical isolates. In our study, 90% of the analyzed strains were *SAP9* positive, the next most prevalent gene present in the strains being *SAP5*. We noticed that *C. albicans* isolates that expressed *SAP9* were more virulent in comparison with isolates that expressed *SAP5* and *SAP6*, and then the remaining analyzed genes, therefore *SAP9* plays a significant role in biofilm development, probably through favoring yeast to hyphal transition, as a response to environmental stimuli *via* a cyclic adenosine monophosphate-(cAMP) dependent signaling pathway (48). Hyphal development contributes to *Candida* invasion of epithelial cells and leads to host cell damage.

Conclusions

Our work describes the relationship between different phenotypic features that are related to cell adherence and biofilm development and the presence of adherence genes. The analyzed *C. albicans* strains were isolated from the respiratory tract of patients undergoing cardiovascular surgery. The isolates were able to adhere to cellular and inert substrata and to form biofilms. The investigation of the adherence gene profiles in our *C. albicans* isolate revealed the presence of several *ALS* genes, correlated with the respective isolates ability to colonize the cellular substratum. The isolates expressing the*SAP9* were the most virulent, likely because *SAP9* favors the yeast to hyphal transition. Overall, our study demonstrates that the PCR detection of adherence genes could represent a simple and cost effective tool for the prediction of the virulence capacity and pathogenic potential of different *C albicans* clinical isolates, replacing the more expensive and time-consuming phenotypic assays of adherence and biofilm development capacity.

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Author Contributions

OS, IG, GGP conceived and designed the experiments; IG, OS, AMH, LMD, IA, OA and DA performed the experiments; OB provided the *C. albicans* strains; IG, LMD, AMH and CC analyzed the data; OS, IG and MCC wrote and corrected the paper.

Abbreviations

ALS - Agglutinin Like Sequences
SAP - Secreted Aspartyl Proteinase
CSH - Cell Surface Hydrophobicity
AIn - Adherence Index
EAP - Extracellular Adhesion Proteins

References

1. Calderone RA, Fonzi WA. Virulence factors of *Candida albicans*. Trends Microbiol. 2001;9(7):327-35. DOI: 10.1016/S0966-842X(01)02094-7
2. Cirpaciu D, Goanţă CM, Tusaliu M, Curutiu C, Budu V. Microbial etiology of acute otitis externa-a one year study. Rom Biotech Lett. 2017;22(1):12316.
3. Fanello S, Bouchara J, Sauteron M, Delbos V, Parot E, Marot-Leblond A, et al. Predictive value of oral colonization by *Candida* yeasts for the onset of a nosocomial infection in elderly hospitalized patients. J Med Microbiol. 2006;55(2):223-8. DOI: 10.1099/jmm.0.46155-0
4. Wisplinghoff H, Bischoff T, Tallent SM, Seifert H, Wenzel RP, Edmond MB. Nosocomial bloodstream infections in US hospitals: analysis of 24,179 cases from a prospective nationwide surveillance study. Clin Infect Dis. 2004;39(3):309-17. DOI: 10.1086/421946
5. Grimoud AM, Marty N, Bocquet H, Andrieu S, Lodter JP, Chabanon G. Colonization of the oral cavity by *Candida* species: risk factors in long-term geriatric

care. J Oral Sci. 2003;45(1):51-5. DOI: 10.2334/josnusd.45.51
6. Dongari-Bagtzoglou A, Dwivedi P, Ioannidou E, Shaqman M, Hull D, Burleson J. Oral *Candida* infection and colonization in solid organ transplant recipients. Oral Microbiol Immun. 2009;24(3):249-54. DOI: 10.1111/j.1399-302X.2009.00505.x
7. Budtz-Jörgensen E, Stenderup A, Grabowski M. An epidemiologic study of yeasts in elderly denture wearers. Community Dent Oral Epidemiol. 1975;3(3):115-9. DOI: 10.1111/j.1600-0528.1975.tb00291.x
8. Shimizu C, Kuriyama T, Williams DW, Karasawa T, Inoue K, Nakagawa K, et al. Association of oral yeast carriage with specific host factors and altered mouth sensation. Oral Surg Oral Med Oral Patholo Oral Radiol Endod. 2008;105(4):445-51. DOI: 10.1016/j.tripleo.2007.11.030
9. Yano A, Abe A, Aizawa F, Yamada H, Minami K, Matsui M, et al. The effect of eating sea cucumber jelly on *Candida* load in the oral cavity of elderly individuals in a nursing home. Mar Drugs. 2013;11(12):4993-5007. DOI: 10.3390/md11124993
10. Cuéllar-Cruz M, López-Romero E, Villagómez-Castro JC, Ruiz-Baca E. *Candida* species: new insights into biofilm formation. Future Microbiol. 2012;7(6):755-71. DOI: 10.2217/fmb.12.48
11. Finkel JS, Mitchell AP. Genetic control of *Candida albicans* biofilm development. Nat Rev Microbiol. 2011;9(2):109. DOI: 10.1038/nrmicro2475
12. Harriott MM, Noverr MC. Importance of *Candida*–bacterial polymicrobial biofilms in disease. Trends Microbiol. 2011;19(11):557-63. DOI: 10.1016/j.tim.2011.07.004
13. Mayer F, Wilson D, Hube B. *Candida albicans* pathogenicity mechanisms. Virulence 4: 119–128. 2013. DOI: 10.4161/viru.22913
14. Georgescu M, Vrinceanu D, Radulescu L, Tusaliu M, Martu C, Curutiu C, et al. Microbial biofilms and implantable hearing aids. Rom Biotech Lette. 2017;22(4):12681-6.
15. Ramage G, Rajendran R, Sherry L, Williams C. Fungal biofilm resistance. Int J Microbiol. 2012;2012.
16. Mathé L, Van Dijck P. Recent insights into *Candida albicans* biofilm resistance mechanisms. Curr Genet. 2013;59(4):251-64. DOI: 10.1007/s00294-013-0400-3
17. Baillie GS, Douglas LI. Role of dimorphism in the development of *Candida albicans* biofilms. J Med Microbiol. 1999;48(7):671-9. DOI: 10.1099/00222615-48-7-671
18. Ramage G, VandeWalle K, López-Ribot JL, Wickes BL. The filamentation pathway controlled by the Efg1 regulator protein is required for normal biofilm formation and development in *Candida albicans*. FEMS Microbiol lett. 2002;214(1):95-100. DOI: 10.1111/j.1574-6968.2002.tb11330.x
19. Richard ML, Nobile CJ, Bruno VM, Mitchell AP. *Candida albicans* biofilm-defective mutants. Eukaryot Cell. 2005;4(8):1493-502. DOI: 10.1128/EC.4.8.1493-1502.2005
20. Bonhomme J, d'Enfert C. *Candida albicans* biofilms: building a heterogeneous, drug-tolerant environment. Curr Opin Microbiol. 2013;16(4):398-403. DOI: 10.1016/j.mib.2013.03.007
21. Nobile CJ, Mitchell AP. Regulation of cell-surface genes and biofilm formation by the *C. albicans* transcription factor Bcr1p. Curr Biol. 2005;15(12):1150-5. DOI: 10.1016/j.cub.2005.05.047
22. Nobile CJ, Andes DR, Nett JE, Smith Jr FJ, Yue F, Phan Q-T, et al. Critical role of Bcr1-dependent adhesins in *C. albicans* biofilm formation in vitro and in vivo. PLoS Pathog. 2006;2(7):e63. DOI: 10.1371/journal.ppat.0020063
23. Nobile CJ, Nett JE, Andes DR, Mitchell AP. Function of *Candida albicans* adhesin Hwp1 in biofilm formation. Eukaryot Cell. 2006;5(10):1604-10. DOI: 10.1128/EC.00194-06
24. Ghannoum M, Elteen KA. Correlative relationship between proteinase production, adherence and pathogenicity of various strains of *Candida albicans*. J Med Vet Mycol. 1986;24(5):407-13. DOI: 10.1080/02681218680000621
25. Abu-Elteen KH, Elkarmi AZ, Hamad M. Characterization of phenotype-based pathogenic determinants of various *Candida albicans* strains in Jordan. Jap J Infect Dis. 2001;54(6):229-36.
26. Rosa EAR, Rached RN, Ignácio SA, Rosa RT, da Silva WJ, Yau JYY, et al. Phenotypic evaluation of the effect of anaerobiosis on some virulence attributes of *Candida albicans*. J Med Microbiol. 2008;57(10):1277-81. DOI: 10.1099/jmm.0.2008/001107-0
27. Pichová I, Pavlíčková L, Dostál J, Dolejší E, Hrušková-Heidingsfeldová O, Weber J, et al. Secreted aspartic proteases of *Candida albicans*, *Candida tropicalis*, *Candida parapsilosis* and *Candida lusitaniae*: inhibition with peptidomimetic inhibitors. Eur J Biochem. 2001;268(9):2669-77. DOI: 10.1046/j.1432-1327.2001.02152.x

28. Borg M, Rüchel R. Expression of extracellular acid proteinase by proteolytic *Candida* spp. during experimental infection of oral mucosa. Infect Immun. 1988;56(3):626-31.
29. El-Maghrabi E, Dixon D, Burnett J. Characterization of *Candida albicans* epidermolytic proteases and their role in yeast-cell adherence to keratinocytes. Clin Exp Dermatol. 1990;15(3):183-91. DOI: 10.1111/j.1365-2230.1990.tb02069.x
30. Ollert M, Söhnchen R, Korting H, Ollert U, Bräutigam S, Bräutigam W. Mechanisms of adherence of *Candida albicans* to cultured human epidermal keratinocytes. Infect Immun. 1993;61(11):4560-8.
31. Li F, Palecek SP. EAP1, a *Candida albicans* gene involved in binding human epithelial cells. Eukaryot Cell. 2003;2(6):1266-73. DOI: 10.1128/EC.2.6.1266-1273.2003
32. Hazen K, Glee P. Cell surface hydrophobicity and medically important fungi. Curr Top Med Mycol. 1995;6:1.
33. Hazen KC, Wu JG, Masuoka J. Comparison of the hydrophobic properties of *Candida albicans* and *Candida dubliniensis*. Infect Immun. 2001;69(2):779-86. DOI: 10.1128/IAI.69.2.779-786.2001
34. Telcian A, Mohammed DH, Chifiriuc M, Bleotu C, Holban A, Curutiu C, et al. Assessment of the anti-biofilm activity and biocompatibility of novel PE and PVC polymers. Rom Biotech Lett. 2017;22(5):12997.
35. Stan T, Teodor ED, Gatea F, Chifiriuc C, Lazăr V. Antioxidant and antifungal activity of Romanian propolis. Rom Biotech Lett. 2017;22(6):12116-13124.
36. Melo AS, Bizerra FC, Freymüller E, Arthington-Skaggs BA, Colombo AL. Biofilm production and evaluation of antifungal susceptibility amongst clinical *Candida* spp. isolates, including strains of the *Candida parapsilosis* complex. Med Mycol. 2011;49(3):253-62. DOI: 10.3109/13693786.2010.530032
37. Hassan A, Usman J, Kaleem F, Omair M, Khalid A, Iqbal M. Evaluation of different detection methods of biofilm formation in the clinical isolates. Braz J Infect Dis. 2011;15(4):305-11. DOI: 10.1016/S1413-8670(11)70197-0
38. Konkel ME, Joens LA. Adhesion to and invasion of HEp-2 cells by *Campylobacter* spp. Infect Immun. 1989;57(10):2984-90.
39. Zhang Y, Zhang S, Liu X, Wen H, Wang M. A simple method of genomic DNA extraction suitable for analysis of bulk fungal strains. Lett Appl Microbiol. 2010;51(1):114-8. DOI: 10.1111/j.1472-765X.2010.02867.x
40. Bruder-Nascimento A, Camargo CH, Mondelli AL, Sugizaki MF, Sadatsune T, Bagagli E. *Candida* species biofilm and *Candida albicans ALS3* polymorphisms in clinical isolates. Braz J Microbiol. 2014;45(4):1371-7. DOI: 10.1590/S1517-83822014000400030
41. Soll DR. The ins and outs of DNA fingerprinting the infectious fungi. Clin Microbiol Rev. 2000;13(2):332-70. DOI: 10.1128/CMR.13.2.332
42. Samaranayake Y, Dassanayake R, Cheung B, Jayatilake J, Yeung K, Yau J, et al. Differential phospholipase gene expression by *Candida albicans* in artificial media and cultured human oral epithelium. APMIS. 2006;114(12):857-66. DOI: 10.1111/j.1600-0463.2006.apm_479.x
43. Cho T, Hamatake H, Kaminishi H, Hagihara Y, Watanabe K. The relationship between cyclic adenosine 3′, 5′-monophosphate and morphology in exponential phase *Candida albicans*. J Med Vet Mycol. 1992;30(1):35-42. DOI: 10.1080/02681219280000051
44. Ogasawara A, Odahara K, Toume M, Watanabe T, Mikami T, Matsumoto T. Change in the respiration system of *Candida albicans* in the lag and log growth phase. Biol Pharm Bull. 2006;29(3):448-50. DOI: 10.1248/bpb.29.448
45. Abad-Zapatero C, Goldman R, Muchmore SW, Hutchins C, Stewart K, Navaza J, et al. Structure of a secreted aspartic protease from *C. albicans* complexed with a potent inhibitor: implications for the design of antifungal agents. Protein Sci. 1996;5(4):640-52. DOI: 10.1002/pro.5560050408
46. O'Connor L, Lahiff S, Casey F, Glennon M, Cormican M, Maher M. Quantification of ALS1 gene expression in *Candida albicans* biofilms by RT-PCR using hybridisation probes on the LightCycler™. Mol Cell Probes. 2005;19(3):153-62. DOI: 10.1016/j.mcp.2004.10.007
47. Cheng G, Wozniak K, Wallig MA, Fidel PL, Trupin SR, Hoyer LL. Comparison between *Candida albicans* agglutinin-like sequence gene expression patterns in human clinical specimens and models of vaginal candidiasis. Infect Immun. 2005;73(3):1656-63. DOI: 10.1128/IAI.73.3.1656-1663.2005
48. Wang Y. Fungal adenylyl cyclase acts as a signal sensor and integrator and plays a central role in interaction with bacteria. PLoS Pathog. 2013;9(10):e1003612. DOI: 10.1371/journal.ppat.1003612