Analysis of the ALS1 and HWP1 genes from clinical isolates of *Candida albicans*

Análise de genes ALS1 e HWP1 em isolados clínicos de *Candida albicans*

Recebimento dos originais: 02/06/2018
Aceitação para publicação: 29/06/2018

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ABSTRACT

*Candida albicans* has several virulence factors including the adhesins, which are codified for *ALS1* and *HWP1* genes. Here, we evaluate the frequency of those genes in clinical isolates of *C. albicans*. We analyzed 73 clinical isolates, including 19 vaginal and 53 oral samples. PCR approach was used to detect the genes. The presence of the *ALS1* gene was identified in 26.39% of all strains, while the *HWP1* gene was present in 56.94%. The *ALS1* gene was predominant between vaginal samples and *HWP1* was more abundant in the oral samples. These results suggest distinct virulence profiles from *C. albicans* related to different sites of infection and colonization.

Keywords: *Candida albicans*. *ALS1*. *HWP1*.

RESUMO

*Candida albicans* apresenta vários fatores de virulência incluindo as adesinas, as quais são codificadas pelos genes *ALS1* e *HWP1*. Avaliamos a frequência destes genes em isolados clínicos de *C. albicans*. Foram analisados 73 isolados clínicos, incluindo 19 amostras vaginais e 53 orais. Os genes foram detectados pela técnica de PCR. O gene *ALS1* foi identificado em 26,39% de todas as linhagens, enquanto que *HWP1* esteve presente em 56,94%. O gene *ALS1* predominou entre as amostras vaginais e o *HWP1* predominou entre as amostras orais. Estes resultados sugerem distintos padrões de virulência de *C. albicans* originadas de diferentes sítios de infecção e colonização.

Palavras-chave: *Candida albicans*. *ALS1*. *HWP1*

1 INTRODUCTION

*Candida* yeasts are adaptable microorganisms, which are able to grow in a wide range of environmental conditions. This such ability reflects in its broad spectrum of clinical manifestations caused by these fungi. The most common infections caused by *Candida* species involve oral and vulvovaginal infections. The oral candidiasis is the first clinical symptom related to the HIV infection and is present in 50% to 95% of these patients during the progression of the AIDS. The vulvovaginal candidiasis affects women during their reproductive phase, which about 75% show a single episode and 9% exhibiting recurrent episodes.

*Candida albicans* adhesion to the host cells is essential for its colonization and survival. This microorganism shows a specialized group of proteins called adhesins, which are capable to attach to the eukaryotic host cells. Among those adhesins from *C. albicans*, two could be highlighted: the Als protein (Agglutinin-like Sequence) and the Hwp1 protein (Hyphal wall Protein...
These adhesins interact to each other during the formation of the biofilm playing an important role in the pathogenesis of the candidiasis. In this study, we used PCR assays to identify the presence of the genes related to the adhesins Als1 and Hwp1 in the oral and vaginal clinical isolates of *C. albicans*.

2 MATERIALS AND METHODS

A total of 72 samples of *C. albicans* were analyzed, of which 53 strains were isolated from oral and 19 strains from vaginal sites. Clinical isolates were provided by the Basic Sciences Laboratory of Mato Grosso Federal University, Rondonópolis, Brazil. The samples were obtained using oral swabs from HIV positive patients, which were asymptomatic for oral candidiasis; and also by using vaginal swabs from women symptomatic and asymptomatic for vulvovaginal candidiasis. This study was approved by the Ethics Committee of Júlio Muller Universitary Hospital (CAAE: 31905114.6.0000.5541 and 01582312.2.0000.5541).

Yeasts were kept in plates containing Sabouraud Agar (Himedia Laboratories, Mumbai, India) at 4 °C. For DNA extraction, the microorganisms were grown in Sabouraud broth at 37 °C in shaker (200 rpm) during 24h. Then the culture was centrifuged and the supernatant was discarded. The pellet containing the yeasts cells was used for DNA extraction with the Nucleo Spin Tissue kit (Macherey-Nagel GmbH & Co. KG, Duren, Germany), according the manufactory instructions. The species from the isolates was confirmed with the PCR (Polymerase Chain reaction) species-specific approach following the protocol described by Liguori *et al.*, with modifications. The *C. albicans* ATCC32354 strain was used as positive control.

To analyze the virulence genes we employed PCR methodology with specific primers. The conditions for amplification of the genes ALS1 and HWP1 were used as previously reported by Green *et al.*, and Naglik *et al.*, respectively, with minor modifications. Final volume of the reaction was 25 µL for each sample, which contain ~ 20 ng of DNA, 12.5 µL of GoTaq Hot Start Green Master Mix (Promega, Madison, Wisconsin, USA) and 1.0 µL (20 pmol/µL) of oligonucleotides (Promega, Madison, Wisconsin, USA).

The conditions for amplification of the ALS1 gene were as follow: initial denaturation at 94 °C for 5 minutes, 40 cycles of denaturation at 94 °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 7 minutes. The PCR conditions for HWP1 gene was as described: initial denaturation at 94 °C for 3 minutes, 30 cycles of denaturation at 94 °C for 30 seconds, annealing at 60 °C for 30 seconds, extension at 72 °C for 30 seconds and final extension at 72 °C for 10 minutes. The products generated were analyzes by using gel electrophoresis in agarose gel 1 % containing *Diamond Nucleic Acid Dye* (Promega, Madison,
Wisconsin, USA) and visualized with ultraviolet light (UV). The sequences regarding the oligonucleotides utilized in this study are described on Table 1.

### Table 1: Sequences of the oligonucleotides used for PCR assays for the virulence genes study.

<table>
<thead>
<tr>
<th>Gene</th>
<th>Sequence</th>
<th>Length (bp)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>ALS1</strong></td>
<td>Forward 5’-GAC TAG TGA ACC AAC AAA TAC CAG A -3’&lt;br&gt;Reverse 5’-CCA GAA GAA ACA GCA GGT GA-3’</td>
<td>318</td>
</tr>
<tr>
<td><strong>HWP1</strong></td>
<td>Forward 5’-CCATGTGATGATTACCCACA-3’&lt;br&gt;Reverse 5’-GCTGGAACAGAAGATTCAGG-3’</td>
<td>572</td>
</tr>
</tbody>
</table>

All data generated in this study were stored in the Excel 2016 program and analyzed with pro statistic program Epi-info 7.2.0. The results were analyzed by using descriptive statistics and nonparametric Fisher’s exact test with 5% of significance.

### 3 RESULTS AND DISCUSSION

The table 2 demonstrates the frequency of the virulence genes of *C. albicans* from clinical isolates. A total of 3 oral isolates showed both genes studied. The simultaneous presence of **ALS1** and **HWP1** genes was not observed in the isolates from vaginal samples.

### Table 2: Frequency of **ALS1** and **HWP1** genes in clinical isolates of *C. albicans*.

<table>
<thead>
<tr>
<th>Genes</th>
<th>Vaginal (n=19)</th>
<th>Oral (n=53)</th>
<th>Total (n=72)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>ALS1</strong></td>
<td>14 (73.68)</td>
<td>5 (9.43)</td>
<td>19 (26.39)</td>
<td>p &lt;0.001</td>
</tr>
<tr>
<td><strong>HWP1</strong></td>
<td>04 (21.05)</td>
<td>37 (69.81)</td>
<td>41 (56.94)</td>
<td>p &lt;0.001</td>
</tr>
</tbody>
</table>

The PCR assays for the **ALS1** gene showed a high frequency (73.68%) in vaginal strains of *C. albicans*, but only 9.43% from the oral samples. Unlike our results, a study that analyzed clinical isolates of *C. albicans* from Iraqi patients identified the **ALS1** gene in 11.11% (1/9) from vaginal samples and 87.5% (7/8) from oral samples. The gene **ALS1** was detected in 48% of vaginal samples from Turkish patients. A research with Mexican women showed a frequency of 100% for **ALS1** gene from vaginal mucosa. Those variations might be associated with the number of samples studied or with the virulence of the strains analyzed, and also with the methodology employed.
Several studies involving gene expression have evidenced the role of \textit{ALS1} in the pathogenesis of vaginal and oral candidiasis\textsuperscript{11}. Cheng et al.,\textsuperscript{12} by using RT-PCR technique, identified the expression of those genes from \textit{ALS} family from clinical vaginal samples and, from two models of vaginal candidiasis (reconstructed vaginal epithelium and animal infection). The results indicated that \textit{ALS1} gene was one of the most transcripted gene. Nas et al.,\textsuperscript{13} showed that \textit{ALS1} gene was expressed in \textit{C. albicans} strains isolated from women in reproductive age (67\%), pregnant (70\%) and women postmenopausal (75\%). These data highlight the participation of this gene in the pathogenesis of vaginal candidiasis independently of the vaginal estrogen levels. Monroy-Perez et al.,\textsuperscript{11} noticed that 87.5\% of \textit{C. albicans} samples expressed \textit{ALS1} gene in a model of reconstructed human vaginal epithelium. Kamai et al.,\textsuperscript{14} investigated the contribution of \textit{C. albicans ALS1} to the pathogenesis of experimental murine oropharyngeal candidiasis and showed that the \textit{ALS1} gene product is important for the adherence of the organism to the oral mucosa during the early stage of the infection. Green et al.,\textsuperscript{15} studied the expression of \textit{ALS} genes in \textit{C. albicans} in a hyposalivatory rat model of oral candidiasis and in HIV-positive human patients. \textit{ALS1} transcripts were detected in both samples, indicating the relevance of \textit{als1} in the development of oropharyngeal candidiasis.

In the present study, 69.81\% of oral and 21.05\% of the vaginal strains of \textit{C. albicans} showed positives for the \textit{HWP1} gene. Previous studies accessed the frequencies of 3.9\% and 92\% of the \textit{C. albicans} strains from oral and vaginal samples, respectively.\textsuperscript{10,11} \textit{HWP1} transcripts from \textit{C. albicans} were detected in the mucosa of asymptomatic and symptomatic patients of oral and vaginal candidiasis, which indicates that \textit{hwp1} might play a role in the establishment and maintenance of the fungus in the superficial mucosa of the host.\textsuperscript{8} The \textit{HWP1} gene was expressed in 75\% of the \textit{C. albicans} strains in an infection model of reconstructed vaginal epithelium.\textsuperscript{11} Other studies describe the expression levels of \textit{HWP1} from \textit{C. albicans} collected from vulvovaginal candidiasis such as 73\% for women of reproductive age, 60\% for pregnant and, 25\% for postmenopausal women.\textsuperscript{13}

Zakikhany et al.,\textsuperscript{16} reported that the \textit{HWP1} gene was positively regulated in \textit{C. albicans} hyphae during the infection in reconstructed oral epithelium tissue. \textit{Hwp1} was the first cell surface protein known as essential for the formation of the biofilm formed from \textit{C. albicans in vivo} and, although it could be an excellent therapeutic target.\textsuperscript{17} Detecting the presence of the \textit{ALS1} and \textit{HWP1} genes in \textit{C. albicans} strains isolated from clinical specimens will help to ascertain the roles of these genes in colonization and disease.\textsuperscript{10}
4 CONCLUSION

These results suggest distinct virulence profiles from *C. albicans* obtained from different site infections and colonization. This is the first study that evaluate the frequency of *ALS1* and *HWP1* genes in *C. albicans* strains from Brazilian patients. Future studies comparing the expression levels of those genes in vaginal and oral candidiasis should be performed.

ACKNOWLEDGEMENTS

We give our deepest thanks to the participants of the study.

References


