Detection of *Candida Albicans* in some enteropathy patients using Polymerase Chain Reaction technique

Haider M Jasim\(^1\), Ali H Alhamadani\(^2\), Ahmed A Abbas\(^3\)

\(^{1,}^2,^3^) Al Muthanna University, College of Education for Pure Science/Iraqs, \(^2^) Al Muthanna University, College of Nursing/Iraq, \(^3^) Al Muthanna University, College of Medicine/Iraq

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**ABSTRACT**

Laboratory identification based on DNA amplification methods by used polymerase chain reaction (PCR) may provide an alternative and more sensitive method than traditional culture for the early detection of *Candida albicans*. This study aims to identify and determine the prevalence of *C. albicans* by use PCR method. A total of 167 samples (40 controls and 127 patients) were taken. By the use of culture techniques for isolation and identification, *C. albicans* on Sabouraud dextrose agar are characterized by cream to white and smooth at 30°C, these characteristics. *C. albicans* have been isolated from patients’ samples and these included the *C.albicans* was detected in 17 control subjects (42.5%), and 51 patients (40.16%). The results of this estimation revealed that the amplified DNA (PCR product) was 459bp for *C.albicans*.

**Keywords:** *Candida albicans*, Enteropathy, Gastrointestinal tract, Polymerase chain reaction, SDA.


**Source of support:** Nil

**Conflict of interest:** None

**INTRODUCTION**

Candida species are the predominant human commensals that can cause a wide range of diseases. A major concern is a distributed infection that occurs in post-operative and immunocompromised patients with greater precariousness. Candida is a non-pathogenic as normal flora in mucous membranes, upper respiratory tract, female genital tracts, and the gastrointestinal tract, but may become pathogenic causes candidiasis in immunocompromised individuals.\(^1^\)\(^-^\)\(^6^\) *Candida albicans*, the main reason for invasive application, has become one of the most commonly isolated pathogens in the blood of post-operative and immunocompromised patients in the last 10 years.\(^7^\)\(^,^\(^8^\)\) Laboratory diagnosis of candidemia, currently based on direct tests and blood culture, is often delayed due to the relatively slowness growth of these yeasts in clinical specimens. Since clinical presentation is not usually specific, the clinician should often take an empirical therapeutic decision before the results of the culture are known. Earlier identification of Candida in clinical specimens would be clinically and epidemiologically useful. Many studies seem to indicate that detection of *Candida albicans* based on PCR may provide an adjunct and maybe a more sensitive method than conventional culture.\(^9^\) However, the *Candida albicans* is still difficult, because the only sign of infection may be a prolonged fever that is insensitive to antibacterial treatment. Laboratory tests have been developed to detect circulating *Candida albicans* antigens for the rapid diagnosis.\(^7^\) The PCR was also used to diagnose candidiasis.\(^10^\)\(^,^\(^11^\) Detection of *C. albicans* DNA from clinical specimens, however, lacked sensitivity, even if blood culture was positive.\(^1^\) Sensitivity can be improved 10 cells per sample\(^10^\) or three cells per 0.1 mL of blood.\(^11^\) To increase the sensitivity of methods that did not include radioactivity, the amplified product was ligated to a streptavidin-coated microtiter plate using a biotinylated capture probe and the ampiclon was analyzed by an enzyme immunoassay.\(^2^,^5^,^12^\) In this study, we describe the speed method for detection of *C. albicans* in stool samples by using PCR technique.

**MATERIAL AND METHODS:**

Fecal samples were collected in appropriate, clean, dry containers; all samples were divided into three parts, the first part for microscopic test and the second part for culture and final part were fastly frozen for detection *Candida albicans* using polymerase chain reaction. The cultures media use in this study sabouraud dextrose agar (SDA) and Sabouraud Broth (SB) for *Candida albicans* and with add antibiotic to prevent the growth of bacteria. The medium was prepared according to the information on the packaging from the manufacturer company (Difco/USA). The identification was based on physiological and morphological characteristics, were streaked out on Sabouraud dextrose agar plates (SDA) modified with
Chloramphenicol (0.05 mg/L) and incubated for 24–48 hours at
37 C. Isolates with a creamy to yellowish colonies were
accepted and considered as positive specimens for Candida
spp. while isolates with different colors colonies were rejected
and considered as negative specimens for Candida albicans.
Fungal genomic DNA form of Candida albicans isolates was
extracted by using EZ-10 Spin Column Fungal Genomic DNA
Mini-Preps Kit and done according to company instructions
(BioBasic, Canada). The quantity of extracted genomic DNA
was estimated by using the Nanodrop spectrophotometer
(THERMO. USA), addition, to measuring the purity of
DNA through reading the absorbance at (260/280 nm). PCR
technique was performed for detection important virulence
factor genes in Candida albicans. The method was transported
out according to the method described by. The PCR detection
primers (PLC1) were designed by using the NCBI-Gene Bank
data base and Primer 3 design online and supported by Bioneer
Company, South Korea. The forward sequence for PCR-PLC
to C. albicans primer 5 ‘- CCTGTTAGCACCCCCCTTGTT -3
“and reverse Sequence 5 ‘- AACAATCGACCCCCACGTT-3
” at 459bp ampiclon PCR master mix reaction was prepared
using accuPower PCR PreMix Kit (Bioneer, Korea) PCR
thermocycler conditions for each gene were done using serial
PCR thermocycler system as initial denaturation at 95C for 5
minutes, also denaturation at 95C but for 30 seconds annealing
58C for 30 seconds, time for extension 45sec at 72C and final
extension 72C for 7 minutes. All these steps repeated 30
cycles. The PCR products of each gene were analyzed by using
agarose gel electrophoresis method. Data were analyzed using
Statistical Package for the Social Sciences (SPSS) program

RESULTS

By the use of culture techniques for isolation and identification,
C. albicans on Sabouraud dextrose agar are characterized by
cream to white and smooth at 30C◦ as shown in Figure 1

Table 1: Association between IBS and C. Albicans

<table>
<thead>
<tr>
<th>Micro-organism</th>
<th>Control (n = 40)</th>
<th>p (n = 127)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No.</td>
<td>%</td>
</tr>
<tr>
<td>Candida (culture)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Negative</td>
<td>23</td>
<td>57.50</td>
</tr>
<tr>
<td>Positive</td>
<td>17</td>
<td>42.50</td>
</tr>
<tr>
<td>Candida (PCR)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Negative</td>
<td>23</td>
<td>57.50</td>
</tr>
<tr>
<td>Positive</td>
<td>17</td>
<td>42.50</td>
</tr>
<tr>
<td>Total</td>
<td>40</td>
<td>100.00</td>
</tr>
</tbody>
</table>
DISCUSSION
There is no doubt that techniques according to molecular biology, and in special PCR-DNA amplification techniques, will become increasingly common in clinical microbes laboratories shortly. However, a prerequisite for the use of these techniques in the clinical laboratory should be as sensitive and specific as conventional crops and be speed, easy, and reliable. The method described in this study has all the necessary properties: it is simple, robust, sensitive, and reproducible. The total time required for the protocol is approximately 5 hours, 2.5 hours for DNA isolation, and 2.5 hours for PCR. *Giardia lamblia* was seen in 12 patients (9.45%) and also was absent in control subjects. By the use of culture techniques for isolation and identification, *C. albicans* on Sabouraud dextrose agar are characterized by cream to white and smooth at 30°C shown as Figure 3 to 5; these characteristics were identical to those described by. Based on the standard values of DNA concentration for PCR amplification, the values of the present study are considered an efficient value and suitable for the establishment of the DNA extracted with target primers or sequences amplification. Based on previous studies, adjectived a PCR assay with SAP primers according to the amplification of the SAP1 gene was the only SAP gene showed at that time. Amplification of regions a multigene in *C. albicans* SAP genes but also because one or serials of these genes are candidate genes encoding virulence factors for *C. albicans*. Thus, it is like enough to Measurement the relative standards of expression of these putative virulence factors in clinical samples directly by reverse transcription-PCR.

REFERENCE