RESEARCH ARTICLE

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,] *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*

Received: 07th July, 19; Revised: 08th August, 19, Accepted: 06th September, 19; Available Online: 23th September, 2019

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 the identification and determines 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 30C◦ 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.

International Journal of Pharmaceutical Quality Assurance (2019); DOI: 10.25258/ijpqa.10.3.23

How to cite this article: Jasim, H.M., Alhamadani, A.H. and Abbas, A.A. (2019). Detection of Candida *Albicans* in some enteropathy patients using PCR technique. International Journal of Pharmaceutical Quality Assurance 10(3): 66-68.

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 amplicon 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.

MARTIAL 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

**Author for Correspondence: albayatilubna@yahoo.com*

Chloramphenicol (0.05 mg/L)) and incubated for 24–48 hours at 37 C.[13] 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.[14] The PCR detection primers (PLC1) were designed by using the NCBI-Gene Bank database and Primer 3 design online and supported by Bioneer Company, South Korea. The forward sequence for PCR-PLC to *C. albicans* primer 5 '- CCTGTTAGCACCCCCTTGTT -3 "and reverse Sequence 5"- AACACATCGACACCCACGTT-3" at 459bp amplicon 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 version 16 and Microsoft Office Excel 2007.

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

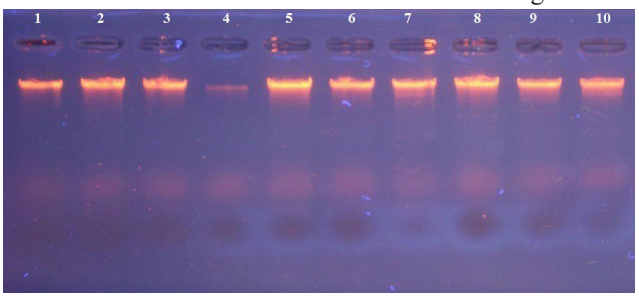

Figure 1: Gel electrophoresis image that shows DNA bands for *Candida albicans* positive isolates lane (1-10) positive DNA samples.

Candida albicans have been isolated from patients' samples and these included the *Candida albicans* were detected in 17 control subjects (42.5%), and in 51 patients (40.16%), No statistical significance was identified ($p > 0.05$), as shown in Table 1.

The DNA extracted from culture media go away with gel electrophoresis, as shown in Figure 1.

The result of amplification was performed on the DNA extracted from all studied specimens and confirmed by electrophoresis analysis. By this analysis, the strands of DNA resulted from the successful binding between specific primers of the target gene (16rRNA) and specimen extracted DNA. The successful binding appeared as single compact bands under UV—light using ethidium bromide as a specific DNA stain. The electrophoresis was also used to estimate DNA molecular size depending on DNA marker (1500-100bp DNA ladder), and the result of this estimation revealed that the amplified DNA (PCR product) was 459bp for *C. albicans* showed as Figure 2.

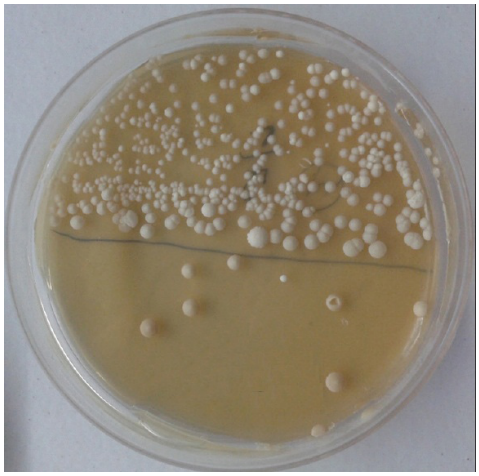

Figure 1: Show *C.albicans* growth on SDA

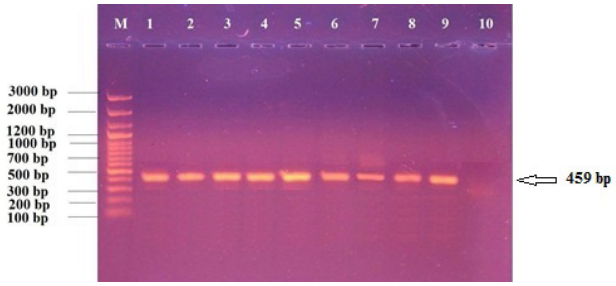

Figure 2: Gel electrophoresis image that shows the PCR product analysis of PLC1 gene (Virulence factor gene) in *Candida albicans* positive isolates. Where M: marker (3000-100bp), lanes (1-9) positive *Candida albicans* with primer at (459bp) PCR product. Lane (10) negative sample.

Table 1: Association between IBS and *C. **Albicans***

Micro-organism		*Control (n = 40)*		*p (n = 127)*		
		No.	*%*	*No.*	*%*	*p-value*
Candida (culture)	Negative	23	57.50	76	59.84	0.793
	Positive	17	42.50	51	40.16	
Candida (PCR)	Negative	23	57.50	76	59.84	0.793
	Positive	17	42.50	51	40.16	
Total		40	100.00	127	100.00	

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.[15] 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.[16] 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.[17] 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*.[18] 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

1. Vinitha Mohandas and Mamatha Ballal. Distribution of Candida Species in Different Clinical Samples and Their Virulence: Biofilm Formation, Proteinase and Phospholipase Production: A Study on Hospitalized Patients in Southern India. J Glob Infect Dis. 2011 Jan-Mar; 3(1): 4–8.
2. Jha BK, Dey S, Tamang MD, Joshy ME, Shivananda PG, Brahmadatan KN. Characterization of candida species isolated from cases of lower respiratory tract infection. Kathmandu University Medical Journal (2006), Vol. 4, No. 3, Issue 15, 290-294.
3. Shivanand Dharwad, Saldanha Dominic R.M. Species Identification of Candida Isolates in Various Clinical Specimens with Their Antifungal Susceptibility Patterns. Journal of Clinical and Diagnostic Research. 2011 November (Suppl-1), Vol-5(6): 1177-1181.
4. Clayton YM, Nobel WC - Observation on the epidemiology of *Candida albicans*. J.Clin. Pathol 1966; 19: 76-78.
5. Sakamoto S, Miura Y. Improved survival from fungaemia in patients with haematological malignancies: Analysis of risk factor for death and usefulness of early antifungal therapy. Eur J Haematol 1993; 51: 156-160.
6. Merlino J, Tambosis E, Veal. Chromogenic tube test for presumptive identification or confirmation of isolates as *Candida albicans*. J Clin Microbiol 1998; 36: 1157-1159.
7. Herent, P., D. Stynen, F. Hernando, J. Fruit, and D. Poulain. 1992. Retrospective
8. Komshian, S. V., A. K. Uwaydah, J. D. Sobel, and L. R. Crane. 1989. Fungemia caused by *Candida* species and *Torulopsis glabrata* in the hospitalized patient: frequency, characteristics, and evaluation of factors influencing outcome. Rev. Infect. Dis. 3:379–390.
9. Buchman, T. G., M. Rossier, W. G. Merz, and P. Charache. 1990. Detection of surgical pathogens by in vitro DNA amplification. Part 1. Rapid identification of *Candida albicans* by in vitro amplification of a fungus-specific gene. Surgery 108: 338–347.
10. Kan, V. L. 1993. Polymerase chain reaction for the diagnosis of candidemia. J. Infect. Dis. 168:779–783.
11. Miyakawa, Y., T. Mabuchi, and Y. Fukazawa. 1993. New method for detection of *Candida albicans* in human blood by polymerase chain reaction. J. Clin. Microbiol. 31:3344–3347.
12. Shin, J. H., F. S. Nolte, and C. J. Morrison. 1997. Rapid identification of *Candida* species in blood cultures by a clinically useful PCR method. J. Clin. Microbiol. 35:1454–1459.
13. Collee, J.G.; Fraser, A.G.; Marmion, B.P. and Simmoms, A. (1996). Gram stain or positive, gram positive. British. Journal of biomedical. Science. 45:120-26.
14. Liguori, G.; DiOnofrio, V.; and Lucariello, A. et al (2009). Oral candidiasis: a comparison between conventional methods and multiplex polymerase chain reaction for species identification. Oral Microbiol Immunol;24:76-8.
15. Bodey, G.P. (1993). Candidiasis: Pathogenesis, Diagnosis & Treatment. 2nd. Raven press. New York. 14:161-169.
16. Applied Biosystems Timeline ArchivedSeptember 27, 2007, at the Wayback Machine., AppliedBiosystems.com
17. Sugita, Y., I. Kanaizuka, H. Nakajima, M. Ibe, S. Yokota, and S. Matsuyama. 1993. Detection of *Candida albicans* DNA in cerebrospinal fluid. J. Med. Vet. Mycol. 31:353–358.
18. Nho, S., M. J. Anderson, C. B. Moore, and D. W. Denning. 1997. Species differentiation by internally transcribed PCR and *Hha*I digestion of fluconazole- resistant *Candida krusei*, *Candida inconspicua*, and *Candida norvegensis* strains. J. Clin. Microbiol. 35:1036–1039.