*IOSR Journal of Pharmacy and Biological Sciences (IOSR-JPBS)*
*e-ISSN:2278-3008, p-ISSN:2319-7676. Volume 13, Issue 2 Ver. IV (Mar. – Apr. 2018), PP 52-58*
*www.iosrjournals.org*

# The Isolation and Identification of the Candida Yeast by Using Different Techniques

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***Abstract:*** *A sample of (402) of diabetic patients, pregnant women, TB patients and children patients. The Candida albicans has formulated isolation averages of (54.40%) from 142 isolates, the fallowing Candida dubliniensis has formulated isolation averages of (12.69%) of 33 isolates These sample has been collected from the beginning of October 2011 to the end of May2012This study at usingdifferent isolation media to identify the Candida albicans yeast and the Candida dubliniensis yeast because the latter kind of yeast share a lot of features with the former one .CHROM agar Candida which is one of pigmented medium, has been utilized to differentiatedifferent Candida species depending on color and appearance of the colony, Candida albicans appear light green while Candida dubliniensis appear dark green. Tobacco agar has also been utilized to identify the two yeast by the production of chlamedospores; Candida albicansis unable to produce the chlamedospores, while Candida dubliniensis is able to produce the chlamedospores. As for the Hypertonic Sabouraud Broth, it has been utilized to know the salt tolerance of the two yeast. It is worth mentioning that the Candida albicansis able to grow in this medium.In addition, the identification of Candida albicans has been reinforced by using the Polymerase Chain Reaction (PCR) which is considered as one of the most modern, developed, precise and sensitive methods of identification which depends on the DNA,the positive result occur in molecular weight 310 bp .*

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Date of Submission: 30-03-2018 Date of acceptance: 16-04-2018

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## I. Introduction

Candidiasis or Candidosis is a fungus disease caused by a yeast called *Candida* . This disease infects the skin, mucous membrane, human innards or it may infect lungs ,a type of this disease is called moniliasis[1,2,3]. Types of this species are isolated from locations of direct connection to mucocutanous tissues especially small intestine, genitals, and urinary tracks[4].These species grow on the shape of budding oval cells and formulate pseudohyphae (with creamy colour colonies) with a yeast odor of the normal flora of the body[5]. The most common species that cause diseases is the *Candida albicans* which has the ability to adapt to different environments .For instance ,it can grow inside the host or on the mucous membrane of the vagina which is characterized by the acid environment(pH4.5) and in blood of an equivalent environment (pH7.4) whereas it has the ability to grow outside the host in an environment that graded from acid to alkaline (pH2.5).It grows in a temperature between (5-46 c°)[6].One of the virulence factorsthat a *Candida albicans* has ,is the ability of adhesion ,Hydrolytic Enzyme Production **,**Germ Tube Production andPhenotypic Switching[7].

As for *Candida dubliniensis* ,it is recently added to the list of opportunist yeast that cause diseases .It shares *Candida albicans*with some features such as making chlamydospores and germ tube .*Candida dubliniensis* was first described by the scientist[8] .It is considered as a world wide spread yeast that causes oral candidiasis of the AIDS patients[9]. The *Candidadubliniensis* is isolated from different clinical samples such as stool (faeces), urine, phlegm from non AIDS patient but with diseases that weaken the immunity such as cancer[10]. The infection that causes by these yeast are:

1. CutaneausCandidiasis which infects babies. The mother's vagina is considered as a main source for this infection[3].
2. Candidal Vulvovaginitis is a disease that infects women especially pregnant ones, in this disease the yeast infect, the mucous membrane[3].
3. Respiratory Candidiasis is a disease caused when the respiratory system is infected by the *Candida* genes which infects the upper respiratory tract. This disease infects patients who stay in the hospital because they may inhale these yeast [11].
4. Oral Candidiasis (it is called thrush as well) is an infection of the mucous membrane inside nasal cavities that infects children and less immune adults such as diabetic who take antibiotics continuously. Other factors that may cause this disease are humidity and hot climate [3].

The pathogenic yeast was previously diagnosed by using special yeast media that takes some days. The chromogenic media which contains a chromogenic substance interacting with secretive enzyme by the organs to produce colonies of different colours. One of these media is CHROMagar *Candida* (CAC) which is considered one of the mediathat is utilized to diagnose the *Candida*[12].

Moreover, other differentiated media emerge such as Tobacco agar which is first described by the scientist [13] to diagnose the *Cryptocoousneoformans* yeast. Tobacco agar is a new and developed medium that is utilized to differentiate the *C. dubliniesis* and *C. albicans* yeast depending on some colonies characteristics such as the surface of the colony (soft or tough),chlamedospores ,and the colour of the colony .Tobacco agar is characterized by its adequacy and low cost [14].As for sabouraud broth salt ,it is utilized as an isolation medium by growing *C. dubliniensis* and *C. albicans* in a medium of sabouraud salt broth which contains 65 gm of Nacl .It is found that *C. albicans*(unlike *C. dubliniensis*) bear high salty substance[15] .During the 1970s, the development of gene sequence technology leads to develop gene study. Another important step happened during the 1980s represented by polymerase chain reaction by the scientist Kary Mullis who awarded Noble prize of chemistry in 1993 .The use of CaYSt1 gene is utilized to diagnose *C.albicans* and it is responsible for coding the essential protein in the translation process by using special primer[16]. The sequence of the utilized primer to discover CaYST1 gene isINT1(5'AAGTATTTGGGAGAAGGGAAAGGG-3')INT2(5'AAAATGGGCATTAAGGAAAAGAGC-3')

The primer which is utilized to discover this gene is unable to magnify the target DNA in the following yeast C.*dubliniensis,C.glabrata, C. krusei, C.kefyr, C. parapsilosis, C.tropicalisC.guilermondii*while *C.albicans* give positive result for this primer in (310 bp)[16].

## II. Material and Methods

Sterile swabcotton is utilized to take samples from the vaginal genitals of pregnancy women and oral cavity of children. Clean containers are utilized to collects samples of urine and sputum.

**Study Design:** Prospective open label observational study

**Study Location:** Babylon Teaching Hospital for Maternity and Pediatric, AL- Hilla Teaching Hospital, Merjan Teaching Hospital and Chest Diseases Center.

**Study Duration:** May 2016 until February 2017

**Sample size:** 402 patients

**Sample size calculation:** 70 diabetic patient ,36 controlled sample ,86 pregnancy women ,35 controlled sample ,55 TB patient , 20 controlled sample , and 70 children with 30 controlled sample of both genders and of different ages form less than one year until 75 years old patients is collected .

**Subjects & selection method**:Chromo agar *Candida*medium is utilized to differentiate between *Candida* species .It is prepared by melting 47.7 gm of chromo agar power in 1000 ml of Distell water, then shaking the medium and heated lightly in order to melt down the ingredients avoiding boiling. After heating, the medium is left to be cold then is distributed on petri dish to inoculate dishes that contain the yeast culture media in sabouraud agar for 48 hours .After the inoculation the dishes incubated in a 37c° for 24-48 hours [17].

As for Tobacco agar medium, it is prepared by mixing 50 gm of Tobacco in 1000 ml of Distell water, the mixture is left to boil for 30 minutes then left to be cold .The mixture is filtered by using lawn then 20 gm of agar is added then the volume completed to be 1 letter and 5.4 pH then sterilized by the autoclave. After the sterilization a 250 mg/L of chloramphenicol is added, and then the medium pour in petri dish to be inoculated with the two yeast after a 48 hours growth. The dishes are incubated for 4 days in a 28 C°, then apart of the growing yeast one a medium of tobacco agar is taken to be located in a glass slide in order to be observed using the microscope to diagnose the fragments of hyphae and chlamedospores [15].

In addition to the above mentioned media, the Hypertonic Sabouraud Broth medium is prepared by melting 10 gm of peptonand 20 gm of dextrose in 1000 ml of distilled water in addition to 6.5 of NaCl . After shaking and sterilizing the medium, a 250 mg/L of chloramphenicol is added. Then the mixture is distributed in clean tubes after culturing these two yeast on medium of sabouraudagar. In the age of 48 hours, a part of the colony is transported to tubes which contain 0.5 of distil water to formulate a suspended. A 20µl of suspended is added to the tube which contains 1 ml of Hypertonic Sabouraud Broth medium. These tubes are kept in 30 c° for 4 days to be diagnosed later [16].

**DNA Extraction of *C. albicans***

After the diagnosis of some yeast depending on explicit characteristics as well as biochemical test, a DNA extraction of some *C. albicans*isolation is utilized using the following method[18]:

1. *C. albicans*grows on sabouraud agar medium for 48 hours , the take a piece of the colony by loop and put in tube contains 400 microliter from the extraction ,this buffer contains (250Mm NaCl ,pH 8.5,Tris HCl 200Mm,SDS 0.5% , EDTA 25).Then adding little amount of sterilized sand in the tube and mixed by vortex
2. 130 microliter from 3M of Acetate Sodium is added to put component centrifuge with a 11000 cycle speed for 10 minutes .Then to put this mixture in a new eppendorof tube with an addition of protase K and leave it in a water bath with a 64 C° for one night . After this process these components should be mixed for 5 minutes and an equal amount of phenol chloroform isoamyl (25:24:1)should be and centrifuged for 5 minutes with a 7000 cycle speed.
3. The pure liquid is converted inn another tube with 80 microliter of protein residue to be shake with hands , then put the tube in the refrigerator for half an hour to be centrifuged for two minutes with a 7000 cycle speed .
4. The pure liquid is converted again in a new tube adding a double size and a half of isopropanol of the liquid which contains DNA in order to residue it .The tubes are kept in the refrigerator for an hour with a 4C° temperature then centrifuged for 10 minutes with 1400 cycle speed.
5. The DNA is rinsed by using 300Mm of 70% of alcohol then shake for 5 minutes with 1000 cycle speed .
6. 90 microliter of TE- buffer is added and centrifuge for one minute with 5000 cycle speed ,then 6 microliter of RNAase is added for 10 minutes in the temperature of the room .
7. The water bath temperature is to be raised 70C° degree for half an hour then centrifuge them with 5000 cycle speed for 30 seconds the DNA should be kept frozen to be used later.

After doing all the additions, the samples have been mixed using the vortex and then to be manipulated with the PCR thermal cycle(see table 1

Table (1) material used for reaction mixture with sizes

| size | Chemical material |
|---|---|
| 1micreliter | Primer Forward |
| 1 microliter | Primer Reverse |
| 1 microliter | DNA |
| 5microliter | Mastermix |
| 12 microliter | D.W |
| 20 microliter | Total |

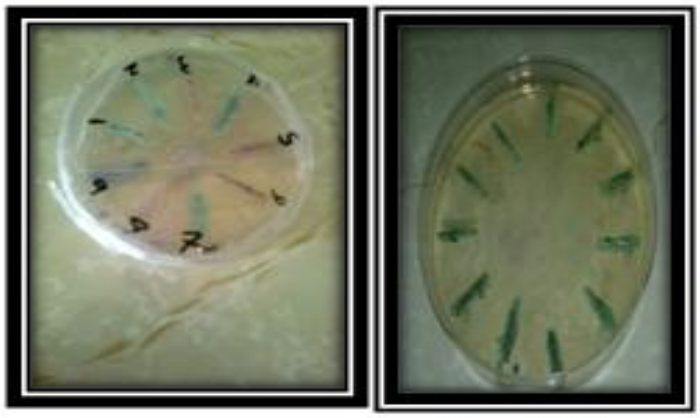
The polymerase interaction results of electrophoresis by using 1.5% of agarose gelatine then to take photo by using a digital camera (see table 2).

**Table (2) Steps of PCR special for *Candida***

| Cycle number | time | Temperature | step | Step number |
|---|---|---|---|---|
| 1 | 10minute | 95C°° | Initial denaturation | 1 |
| 30 | 30 second | 95C | Denaturation | 2 |
| | 1 minute | 55C° | Annealing | 3 |
| | 1 minute | 72C | Extension | 4 |
| 1 | 10minute | 72C° | Final Extension | 5 |

## III. Results

In this study The *C. albicans* has recorded the highest isolation average of 54.40% with (142) samples , then came the *C. dubliniensis*with an isolation range of 14.17% and 33 samples . Since there are similar characteristics between the two yeast , different media have been utilized in this study such as chromogenic that contains chromatic substance which interact with enzyme of the microbiology we want to experiment . Another medium to diagnose *Candida* was the CHROM agar *Candida* which is utilized to diagnose some types of this species such as *C. albicans*,*C. tropicalis* , *C. dubliniensis* , *C. glabrata, C. parapsilosis, C. kefyer* , *C. famata* ,*C. krusei* and other yeast depending on the colour and the shape of the colony. The results show that 142 isolation of *C. albicans* that grow on CHROM agar *Candida* appear in soft light green colonies , whereas the *C. dubliniensis* with a 26 isolation appear in crude light green colonies (see picture 1).

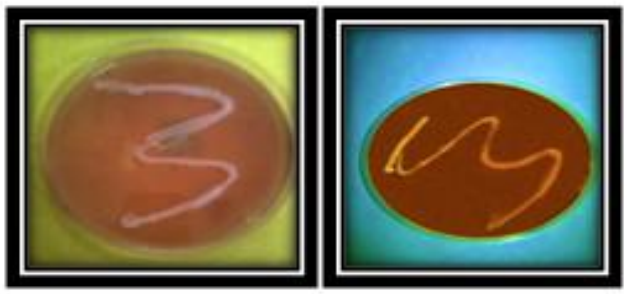


B A

Image (2)A:*C.albicans*,B:some type of *Candida* species which grow on*CHROMagar Candida*that (1,2) refer to

*C.albicans*,(3,8)*C.krusei*,(4)*C. tropicali*,(5,6)*C.parapsilosis*,(7)*C.dubliniensis* and (9)*C.glabrata*

In order to show the difference between the two yeast , a Tobacco agar medium has been utilized . Using Tobacco agar , the (142) of *C.albicans*isolation appeared as a separate yeast that do not constitutechlamedospores in the shape of soft creamy white colonies .As for *C. dubliniensis*, it appeared in a shape of crude brown yellowish colony that constitute chlamedospores when we experimented under microscope 30 isolation of *C. dubliniensis*. The rest 3 isolation of *C. dubliniensis* have the same features but they do not constitute chlamedospores (see picture 2).



A B

Image (3)Growth*C.albicans* and*C.dubliniensis* on Tobacco agar

For the abovementioned reasons we utilizedHypertonic Sabouraud Broth medium contains 65g of NaCl .All the *C. albicans* grew on this medium with turbidity in the tube (see picture 3).

Image(3): Growth*C.albicans* on Hypertonic Sabouraud Br

The *C. dubliniensis*showed a negative results in this test except for the three isolation that we doubt when they grew on the Tobacco agar medium, they returned to *C. dubliniensis*yeast because they cannot resist the high salt of the Hypertonic Sabouraud Broth medium. To make sure of the accuracy of this medium, we experimented *C. albicans*and *C. dubliniensis* isolation on the sabouraud agar under 45C° degree for 48 hour .We found out that all the *C. albicans*isolation that are tested using the previous media grow under such temperature , whereas the *C. dubliniensis* isolation did not grow under this temperature (see table 3) .

Table (3): Culture media used for different between *C.albicans* and *C.dubliniensis*

| Culture media | | | | type of yeast |
|---|---|---|---|---|
| Growth in 45C° | Hypertonic Sabouraud Broth | Tobacco agar | *CHROM agar Candida* | |
| growth | growth | White to crème color with smooth colonies and no producechlamedospores | Light green and smooth colonies | *C. albicans* |
| No growth | No growth | Brown to yellowish color with rough colonies and produce chlamedospores | Dark green with rough colonies | *C.dubliniensis* |

**Reinforcing the diagnosis of *C. albicans* using the PCR Technology**

The diagnosis of *C. albicans* yeast has been reinforced using Polymerase Chain Reaction(PCR). 32 isolation including 8 isolation we doubt whether *C. albicans* or*C. dubliniensis*electroforceswhichusing all the media utilized in this study. The results revealed that four isolation do not belong to *C. albicans* because of the nil appearance of any bunch at the molecular weight 310 bp in comparison with other isolation. Other 8 isolation that belongs to C. albicans that give a positive result are tested on media with bunch appearance at the molecular weight 310 bp , this prove the efficiency of the differentiation media . In addition , 16 isolation of the same yeast that have been taken from different disease of different part of the body are electroforced , and all of them gave position results (see picture 4 ).

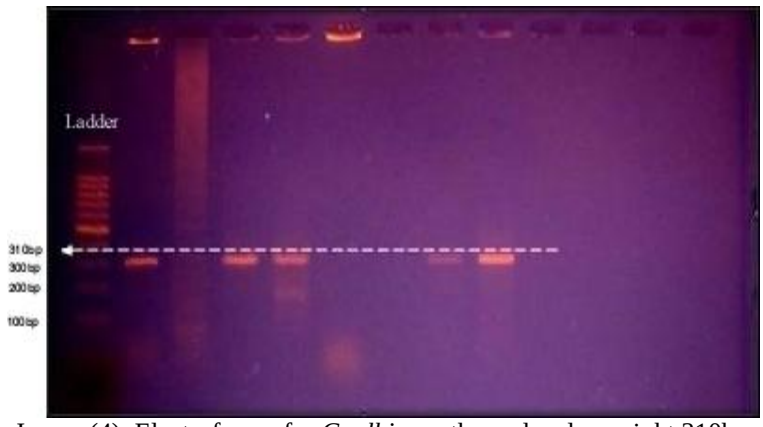



Image (4): Electroforces for *C. albicans* the molecular weight 310bp

## IV. Discussion

The fungusinfection are increased nowadays due to the less immune patients. The *Candida* species is the first responsible of opportunist infection for most of the patients in the hospitals. The *C. albicans*represent 50-70 % of the clinical isolation belongs to candida species[17, 18, and 19].

Since *C. albicans* is the main fungus that cause the *Candida*, many tests were utilized to differentiate this type from others that belong to the same species. One of these tests was the using of CHROM agar *Candida* medium to diagnose type of this species such as *C.albicans* ,*C.tropicals* , *C.dubliniensis* ,*C . glabarata* , *C. parapsilosis*, *C .kefyre* , *C.famata* , and*C.krusei* as well as other yeastdepending on the colour and the shape of the colony[20] . the isolation of the types of this species grow on this medium under 37c ° for 48 hours which make the isolation appear in different shapes and colours[21 ,22 ].

The *C.albicans* appears in soft light green colour colony (see picture 2) which explains that the medium contains a tinctorial substance interact with Hexosaminidaseenzyme excreted by the same species which in effect leads to the diagnosis .whereas*C. dublineinsis*appears in crude dark green colony[23].

The Tobacco agar medium showed a high efficiency of diagnosis, when we used it to differentiate the *C. albicans* and *C. dubliniensis* . The *C.albicans*isolation showed disability to make chlamedosporesand appeared in soft creamy white colony .As for *C.dublineinsis* , they showed the ability to make chlamedospores and appeared in crude yellowish brown colony and this come in accordance withCardenesa, C.D and *et al*[24].The Hypertonic Sabouraud broth was a confirmative medium for isolation we doubt in the Tobacco agar because it differentiate the two yeast. The *C.albicans* was able to grow on this medium because it can resists the high salty environment, whereas the *C.dublineinsis* cannot resist. This result comes in accordance withBaquero C; *et al*andGomes,S.; *et al*[16,25] .

The present study includes testing the *C.albicans* ability to produce the proteinase enzyme by growing the isolation of this yeast in a gelatine agar medium ,it is found out that they hour a high ability to produce the proteinase . these result come in accordance withMosca C; and *et al*[26] who found out the activityenzyme of this yeast with a 75%.

The sabourauds mediumthat contains blood is utilized to test the ability of thisyeast to moulder the blood and produce Proteinase . This ability due to blood mouldiness by producing Acid proteinase (the result come in accordance withAdam, H.J. and *et al*[27]. The reason behind the ability of this yeast which moulder the blood is that it have predation factors such as enzyme , so this ability might be an indicator of making disease when it grow on blood agar . This activity takes place inside the living being as well as media when there are defects in the defensive immunity system of the body. This yeast also produce proteinase in the media that contain Albumin because it is considered as a source of Hydrogen in diverse low levels. it also has the ability to be connected to many proteins such as (Fibrinogen ,Transferrin , and Abumin )[28].The proteinase is considered as a predation key in this yeast because this enzyme has a relation with its ability to colonized in organs and to attach to cells and digest the proteins of the host as food to defeat the host's defences by breaking the immunoglobulin and complementary protein. Moreover , the yeast has a high ability to moulder the casein, this result come in accordance withPage, S., and F. C. Odds[29] who refers that *C. albicans* recorded the highest enzyme activity on this medium in comparison with others, it shows a high mouldiness percent of 58.4 %.Throughout the results we notice that most isolation which produce the enzyme and totally mouldered are taken from diabetic because of the high intense of glucose in the blood and the adaptation of this yeast to grow in the presence of these substance in the body of the patient . so this yeast grow and produce the enzyme which responsible for predation on these media that contain on substance exist in the living being[30]. Moreover , the PCR result reveal a high accuracy and speed in diagnosis by using CaYST1 gene which is resposibleon coding the essential protein of the translation process at the 310 bp molecular weight. It is found that all C.albicans isolation give the same positive result at the same molecular weight, as well as confirms the efficiency of this technology in the diagnosis process . This result comes in accordance withNoumi, E.;*et al* and Mohammed , A.J. [31,32] because he utilized the some starter and the PCR results were similar at the 310bp molecular weight and this refers to the efficiency of the accuracy of the starter and media used in the present study.

## V. Conclusion

The *Candida albicans* has formulated isolation averages of (54.40%) from 142 isolates, the fallowing *Candida dubliniensis* has formulated isolation averages of (12.69%) of 33 isolates

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IOSR Journal of Pharmacy and Biological Sciences (IOSR-JPBS) is UGC approved Journal with Sl. No. 5012, Journal no. 49063.

Marwakadhimkhudhair "The Isolation and Identification of the Candida Yeast by Using Different Techniques "IOSR Journal of Pharmacy and Biological Sciences (IOSR-JPBS) 13.2 (2018): 52-58.