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Research Article

Biochemical, microbiological an molecular identification of *Candida spp*. based on 18s rRNA Markers

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Abstract

Candida (sometimes referred to as monilia) is a fungus that is normally present on the skin and in mucous membranes such as the vagina, mouth, or rectum. The fungus can also travel through the blood stream and affect the throat, intestines, and heart valves. *Candida* species especially *albicans* becomes an infectious agent when there is some changes in the body environment that allows it to grow out of control. Based on the above report, in our study, we isolated 20 *Candida species* and for all the cultures we did the basic biochemical tests such as carbohydrate fermentation and carbohydrate assimilation to check the ability of acid and gas production for those organisms. Before the organism underwent biochemical test, we streaked cultures in CHROM agar it offers additional evidence which is very useful medium for use in medical mycology. Application of CHROM agar to both stock and clinical cultures demonstrates its ability to identify difference in the *Candida* species. Out of 20 only 18 samples were processed for the entire test which mentioned above but only 12 samples are processed for sequencing and the results are analyzed by using bioinformatics software and the species are identified. All the *Candida species* has similar regions and during germ tube formation and it appears to be characteristic of *C. albicans, C. dubliniensis* and other isolates but not in *C. guillermondii* and other spp. such as *C. parapsilosis and C. lusitaniae.* These considerations support the use of this gene as a target to unequivocally identify *Candida species*. So finally we summarize that the method which described here is simple, cost-effective, and offers a high throughput in the correct identification of *C.albicans C. tropicalis, C. dubliniensis* from other species of C*andida* and non-*Candida* species.

Keywords: Germ tube test, Biochemical test, Isolation of DNA, Polymerase Chain Reaction

Introduction

Candida belongs to the genus of yeasts; candida species are responsible for many life threatening opportunistic infections in immune compromised patients. It is also a frequent colonizer of human skin and mucous membranes. Candida is a member of normal flora ofskin, mouth, vagina, and stool. As well as being a pathogen and a colonizer, it is found the environment, particularly on leaves, flowers, water, and soil. While most of the *Candidaspecies* is mitosporic, some have known teleomorphic state and produce sexual spores. Candida hyphae reproduce sexually since the nucleus has two copies of the chromosomes to create two separate cells through the sporangia when a male a female meet.

If you could call it male and female, it really isn't, it's more like two cells splitting themselves to join and create a new cell. They can reproduce asexually or sexually and during asexual reproduction the cell divides as a new bud to produce a new yeast cell.

The world of fungi is fascinating comprising of a wide range of macro as well as microorganisms. The importance of opportunistic fungal pathogens is increasing in recent years because of the increase in immune compromised patient population.

Human pathogenic yeasts are ubiquitous in the environment and some are normal inhabitants in the body. These yeasts are usually opportunistic organisms, causing acute to chronic infections when conditions in the host are favorable. In general, innate and acquired host defense mechanisms act in concert with the resident bacterial flora such that Candida organisms grow and survive as commensals. However, even a slight modification of the host defense system, or host ecological environment, can assist the transformation of Candida Spp. into a pathogen of causing infections that may be lethal. Among the opportunistic fungal infections in immune compromised patients, candidiasis ranks high in terms of incidence. This is due to the dramatic increase in the number of individuals with deficient cellular immunity, in particular, those with HIV and individuals receiving immunosuppressive treatments, both in organ transplantation and in anticancer therapy.

It is just one of approximately 200 species in the genus Candida, but most significantly accounts for up to 75% of all candida infections because of their capacity to cause infection in human host.

Candida albicans(also known as monelia) and related species are the principal causes of human yeast infections (Mannarelli. BM and Kurtzman.CP, 1998).Candida albicans forms White to Cream. Smooth to wrinkled Colonies on Sabrauds Dextrose Agar. On microscopic analysis it forms pseudo hyphae and true hyphae with blasto conidia (grape like clusters).In recent years, species of Candida other than Candida albicans are emerging as potential pathogens.

Materials and Methods

Sample Collection

A total of 20 samples were collected. The samples were inoculated on SDA and incubated at 37ºC. The samples were also inoculated on the differential media CHROM agar and the plates were incubated at 37ºC.The species were presumptively identified based on the colour of the colonies developed on CHROM agar media. The culture positive specimens were separated based on morphology tests such as germ tube formation and colony morphology on corn meal agar. The isolates were identified by standard biochemical tests such as carbohydrate fermentation and assimilation tests. The isolates were maintained on SDA slants at 4ºC.

Morphological tests

Germ tube test

A small amount of pure culture of organism from SDA was inoculated into 0.5ml of serum (horse/sheep/human) and incubated for about 3 hours at 37ºC. A drop of suspension was placed on a clean glass slide and covered with a cover slip and examined for the production of germ tube under light microscope.

Chlamydospore formation test

An isolated colony from the SDA plate was taken and inoculated on to cornmeal agar. The plates were incubated at 25ºC for 48-72 hours and observed for chlamydospore production under light microscope.

Growth on corn meal agar for chlamydospore or true hyphae production

To demonstrate the production of chlamydospores or true hyphae by yeasts and yeast like fungi (*Candida*)

Isolation of DNA

The isolates were grown in 50 ml of YCM medium and kept in a shaker at 150rpm at 37ºC overnight.

The cells were harvested by centrifugation at 10000g for 10 minutes at 4ºC. The culture medium was decanted and the cell pellet was drained well.

- To cell pellet in Eppendorf tube, add lysis buffer and 1:1 mix of phenol and chloroform.
- Vortex the tube at top speed for 2 min.
- Add 0.2 ml of TE (10 mmTris, 1 mm EDTA, pH 8.0) and vortex again for a few seconds.
- Spin the tubes for 5 min (room temperature) at top speed in an Eppendorf centrifuge.
- Spin the tubes for 5 min (room temperature) at top speed in an Eppendorf centrifuge.
- Transfer the aqueous (upper) phase (0.38 ml) to a fresh Eppendorf tube, using a new pipette tip for each sample. Discard the tube with the glass beads.
- Add 2 volumes of 100% ethanol at room temperature. Mix thoroughly.
- Centrifuge in Eppendorf for 2-3 min at room temperature.
- Discard the supernatant (use the aspirator; take care not to dislodge the pellet).
- Rinse the pellet with 0.5 ml of cold, and add 70% ethanol slowly down the side of the tube, then centrifuge for 3-5 sec.
- Remove the supernatant. Leave the tubes open and inverted for the pellets to dry. (Or dry the pellets under vacuum).

Isolation of Genomic DNA using gel electrophoresis

Agarose is a copolymer of D-galactose and 3, 6 anhydro L-galactose. It forms a gel by hydrogen bonding and the pore size depends on the agarose concentration. The movement of DNA fragments within the gel matrix is influenced by agarose concentration. Low agarose concentration improves the resolution of larger fragments but reduces the resolution of smaller fragments and vice versa. The DNA molecules are separated by electrophoresis on the basis of the molecular size between 500-2500bp (small pieces of DNA migrate through the gel matrix faster than larger pieces under the influence of electric current) shape or the confirmation of the molecule and the magnitude of net charges on the molecule or the applied current (DNA molecules have a net negative charge and migrate towards the anode). The ethidium bromide dye is added which intercalates between the bases of the DNA molecule and fluoresces bright orange which irradiated with UV light.

Procedure

Agarose is molten in water bath until it becomes transparent and ethidium bromide tot the agarose. Seal the boat with cellophane tape at equal size on either side. Pour the molten agarose to the boat; leave it for some time undisturbed. After the gel as left sometime undisturbed meanwhile pour the 300 ml of TAE (1X buffer) to the unit and connect the unit to power pack with cord. Place the boat which is filled with gel matrix into the buffer solution by immersing it to about one millimeter in the buffer. Take two eppendorf tubes in one eppendorf tube add 5µl sample plus 5µl tracking dye and in another eppendorf tube 1µl tracking dye +1µl marker and make up to 5µl with 3µl of water. Add the marker and samples in the well (Do not over flow well).

Electrophoresis until Bromophenol blue is near the end of the gel. The gel is removed and the bands are visualized under UV light in a transilluminator (or) in Gel Doc where it can be viewed through computer using software tools. The DNA bands are seen by comparing it with the marke (ladder) correspondingly.

Polymerase Chain Reaction

Primer details
ITS1: TCCGTAGGTGAACCTGCGG
ITS 4: TCCTCCGCTTATTGATATGC

The typical amplification cycle is represented below.

Cycling conditions

Cycle 1. 94°C for 5 minutes for initial denaturation
Cycle 2. (35 Repeats)
Step.1 94°C for 30 seconds for denaturation
Step.2 55°C for 30 seconds for anneal the primer

Step.3 72°C for 1minute for chain extension
Cycle 3. 72°C for 15 minutes final extension

Agarose Gel Electrophoresis

After PCR reaction the products were checked by electrophoresis on a 0.7% agarose gel. 350 mg of agarose was melted in 40 mL of 1X TBE buffer. 10 µL of the reaction products were loaded with 10 µL of loading dye to check for amplification.

DNA Sequencing

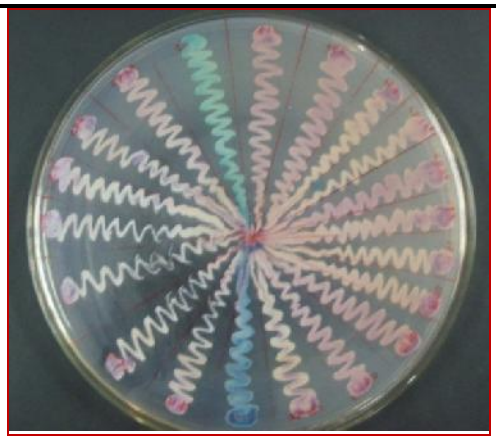
All the PCR amplified samples are purified and further subjected to sequencing which was done by using the Applied Biosystems sequencing instrument and the results were analyzed by using available bioinformatics tools such as BLAST, NJ plot and CLUSTAL-X.

Results

The emergence of *Candida* andnon-*Candida Spp.* as a potential pathogen in the environment, but still species level identification is unclear. Recent year's identification of closely related species such as *C.albicans, C. dubliniensis, C. parapsilosis, C. tropicalis, C. glabrata and other Candida species* has been identified widely. So due to increasing incidence of fungal pathogens, there is a growing need for the development of simple, rapid and accurate identification methods. Since, several non-*Candidaalbicans* are inherently less susceptible to commonly used antifungal drugs, their rapid identification is essential for the appropriate and disease management. The significant increase in the number of reports of systemic and mucosal infections caused by *Candidaspecies* and since the recognition that *Candida species* differ in the expression of putative virulence factors and susceptibilities to antifungal agents, greater emphasis has been placed on the identification of the isolates to the species level.

Interpretation of CHROM Agar

CHROM Agar plate of different *Candida spp*



Discussion

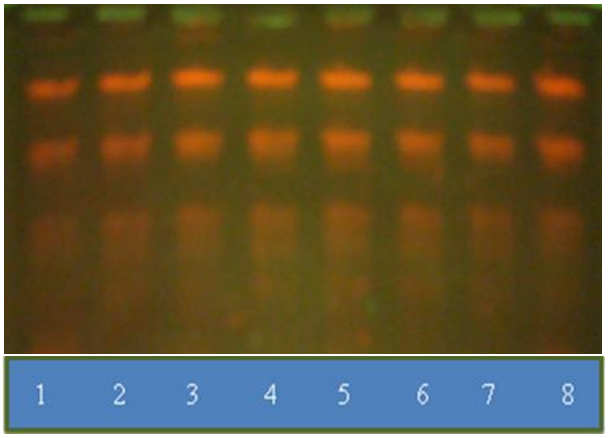
Opportunistic fungal infections are more common in HIV infected patients and previous studies have revealed that 60% to 80% of HIV patients develop one or more fungal infections at some time during their illness, the most frequent being oropharyngealcandidasis. In the present study, out of 20 oral specimens collected from HIV patients, 18 cultures of the isolates were positive for *Candida*. Generally, *C.albicans* is the most predominant causative agent. Many authors have reported *C.albicans* as the most predominant species isolated from HIV patients (Mirdha*et al.,* 1993; Walmsley*et al.,* 2001; Barcheisi*et al.,* 2002).

In the present study, out of 18 *Candida* species all the samples are underwent for basic biochemical, morphological and molecular studies. The 12 samples which taken for the molecular study were further identified 8 cultures as *C. guillermondii* or *Pichiaguilliermondii*, 2 cultures were identified as *C. parapsilosis* and 1 culture were identified as *Clavisporalusitaniae*(or) *C. lusitaniae* which was done by PCR and 28s rDNA sequencing. The sequencing results are further subjected to bioinformatics to find out the homology and phylogenetic tree was developed by available bioinformatics tool such BLAST, NJ plot and CLUSTALX. Germ tube and chlamydospore test was negative for all the three species, *C. guillermondii* also able to ferment all the sugars except Melibiose, Lactose,Maltose, Dulcitol, Raffinose and inositoland for sugar assimilation test; it shows positive results for dextrose, galactose, sucrose, cellulose, trehalose and xylose.

Table:1 Standard chart showing sugar fermentation and assimilation tests of various *Candida spp.*

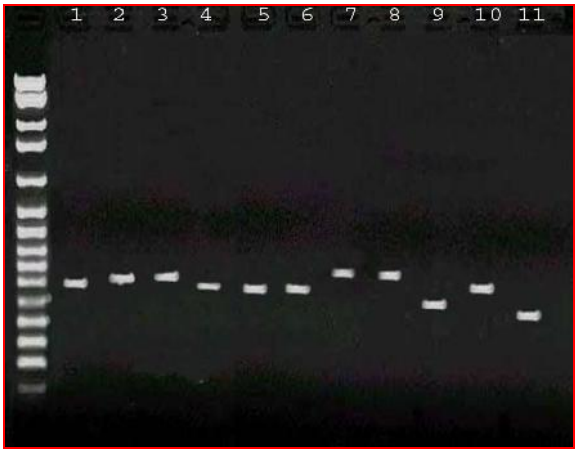
CP.No.	CP1	CP3	CP4	CP 11	CP12	CP14	CP16	CP19	CP8	CP17	CP18
Urease	-	-	-	-	-	-	-	-	-	-	-
Melibiose	-	+	+	+	+	+	+	+	+	-	-
Lactose	-	-	-	-	-	-	-	-	-	-	-
Maltose	-	-	-	-	-	-	-	-	-	-	-
Sucrose	+	+	+	+	+	+	+	+	+	-	-
Galactose	+	+	+	+	+	+	+	+	+	-	-
Cellobiose	+	+	+	+	+	+	+	+	+	-	-
Inositol	-	-	-	-	-	-	-	-	-	-	-
Xylose	+	+	+	+	+	+	+	+	+	+	+
Dulcitol	-	+	+	+	+	+	+	+	+	-	-
Raffinose	-	+	+	+	+	+	+	+	+	-	-
Trehalose	+	+	+	+	+	+	+	+	+	-	-

DNA Isolation



Lane – 1-8: DNA bands of different *Candida spp.* without marker

PCR Result



Lane: A- Marker 1.5kb; Lane 1-11 PCR products of *Candida spp.*Lane-1 to 8- *C.guillermondii*; Lane-9 and 10 *C. parapsilosis*and Lane-11- *C. lusitaniae*

>CP1_ITS4
ACTGGGGTTTTCCTACCTGATTTGAGGTCAACTTGTTTGGTTGTTGTAAGGCCGGGCCAA
CAATACCAGAAATATCCCGCCACACCATTCAACGAGTTGGATAAACCTAATACATTGAGA
GGTCGACAGCACTATCCAGTACTACCCATGCCAATACTTTTCAAGCAAACGCCTAGTCCG
ACTAAGAGTATCACTCAATACCAAACCCGGGGGTTTGAGAGAGAAATGACGCTCAAACAG
GCATGCCCTCTGGAATACCAGAGGGCGCAATGTGCGTTCAAAGATTCGATGATTCACGAA
AATCTGCAATTCATATTACTTATCGCATTTCGCTGCGTTCTTCATCGATGCGAGAACCAA
GAGATCCGTTGTTGAAAGTTTTGAAGATTAATTCAAAAGTTGACTAACTGTAAAAATAAT
TAAATTGTGTTTTGTTAAACCTCTGGCCCAACCTATCTCTAGGCCAAACCAAAGCAAGAG
TTCTGTATCAAAAAGACACTGTGTGTAAGGTTTTTCGCCGCGCAGTTAAGCGCTGGCAAA
AGAATACTGTAATGATCCTTCCGCAGGTTCACCTACGGAAACCTTGTTACGATTTTTTCT
TCCAAAGG
>CP3-ITS
TTTGGAAGAAAGTCGTACAAGTTTCGGAGGTAACCTCGGAGGATCCATACAGTATTCTTTGCCGCG
CTTACTGCGCGGCGAAAAACCTTACACACAGGTCTTTTTGATACAGAATTCTTGCTTTGGTTGGCTT
AGAGATAGGTTGGCCAGAGGTTTAACAAAACACAATTTAATTATTTTTACAGTTAGTCAAATTTTG
AATTAATCTTCAAAACTTTCAACAACGGATCTCTTGGTTCTCGCATCGATGAAGAACGCAGCGAAA
TGCGATAAGTAATATGAATTGCAGATTTTCGTGAATCATCGAATCTTTGAACGCACATTGCGCCCT
CTGGTATTCCAGAGGGCATGCCTGTTTGAGCGTCATTTCTCTCTCAAACCCCCGGGTTTGGTATTGA
GTGATACTCTTAGTCGGACTAGGCGTTTGCTTGAAAAGTATTGGCATGGGTAGTACTAGATAGTGC
TGTCGACCTCTCAATGTATTAGGTTTATCCAACTCGTTGAATGGTGTGGCGGGATATTTCTGGTATT
GTTGGCCCGGCCTTACAACAACCAAACAAGTTTGACCTCAAATCAGGTAGGAATACCCGCTGAAC
TTAAGCATATCAATAAGCGGAGGAACGA
>CP4-ITS
TGGAAGTAAAAATCGTAACAAGGTTTCCGTAGGTGAACCTGCGGAAGGATCATTACAGTATTCTTT
TGCCAGCGCTTAACTGCGCGGCGAAAAACCTTACACACAGTGTCTTTTTGATACAGAACTCTTGCT
TTGGTTTGGCCTAGAGATAGGTTGGGCCAGAGGTTTAACAAAACACAATTTAATTATTTTTACAGT
TAGTCAACTTTTGAATTAATCTTCAAAACTTTCAACAACGGATCTCTTGGTTCTCGCATCGATGAA
GAACGCAGCGAAATGCGATAAGTAATATGAATTGCAGATTTTCGTGAATCATCGAATCTTTGAAC
GCACATTGCGCCCTCTGGTATTCCAGAGGGCATGCCTGTTTGAGCGTCATTTCTCTCTCAAACCCC
CGGGTTTGGTATTGAGTGATACTCTTAGTCGGACTAGGCGTTTGCTTGAAAAGTATTGGCATGGGT
AGTACTGGATAGTGCTGTCGACCTCTCAATGTATTAGGTTTATCCAACTCGTTGAATGGTGTGGCG
GGATATTTCTGGTATTGTTGGCCCGGCCTTACAACAACCAAACAAGTTTGACCTCAAATCAGGTAG
GAATACCCGCTGAACTTAAGCATATCAATAAAGCGGAGGAA

>CP11-ITS
GTAAGGTTCCCCGGCGGGGAGGATCATTACAGTATTCTTTTGCCAGCGCTTAACTGCGCG
GCGAAAAACCTTACACACAGTGTCTTTTTGATACAGAACTCTTGCTTTGGTTTGGCCTAGAGATAG
GTTGGGCCAGAGGTTTAACAAAACACAATTTAATTATTTTTACAGTTAGTCAACTTTTGAATTAAT
CTTCAAAACTTTCAACAACGGATCTCTTGGTTCTCGCATCGATGAAGAACGCAGCGAAATGCGATA
AGTAATATGAATTGCAGATTTTCGTGAATCATCGAATCTTTGAACGCACATTGCGCCCTCTGGTAT
TCCAGAGGGCATGCCTGTTTGAGCGTCATTTCTCTCTCAAACCCCCGGGTTTGGTATTGAGTGATA
CTCTTAGTCGGACTAGGCGTTTGCTTGAAAAGTATTGGCATGGGTAGTACTGGATAGTGCTGTCGA
CCTCTCAATGTATTAGGTTTATCCAACTCGTTGAATGGTGTGGCGGGATATTTCTGGTATTGTTGGC
CCGGCCTTACAACAACCAAACAAGTTTGACCTCAAATTCAGGTAGGGAAAACCAAAATTTT
>CP12-ITS

ATTAGGGTTACTGCGGAGACATTACAGTATTCTTTTGCCAGCGCTTAACTGCGCGGCGAAAAACCT
TACACACAGTGTCTTTTTGATACAGAACTCTTGCTTTGGTTTGGCCTAGAGATAGGTTGGGCCAGA
GGTTTAACAAAACACAATTTAATTATTTTTACAGTTAGTCAAATTTTGAATTAATCTTCAAAACTTT
CAACAACGGATCTCTTGGTTCTCGCATCGATGAAGAACGCAGCGAAATGCGATAAGTAATATGAA
TTGCAGATTTTCGTGAATCATCGAATCTTTGAACGCACATTGCGCCCTCTGGTATTCCAGAGGGCA
TGCCTGTTTGAGCGTCATTTCTCTCTCAAACCCCCGGGTTTGGTATTGAGTGATACTCTTAGTCGGA
CTAGGCGTTTGCTTGAAAAGTATTGGCATGGGTAGTACTAGATAGTGCTGTCGACCTCTCAATGTA
TTAGGTTTATCCAACTCGTTGAATGGTGTGGCGGGATATTTCTGGTATTGTTGGCCCGGCCTTACA
ACAACCAAACAAGTTGACCTCAAATCAGGTTGAATTCCCATTT

>CP14-ITS
ATTTGGTACATGCGGAGACATTACAGTATTCTTTTGCCAGCGCTTAACTGCGCGGCGAAAAACCTT
ACACACAGTGTCTTTTTGATACAGAACTCTTGCTTTGGTTTGGCCTAGAGATAGGTTGGGCCAGAG
GTTTAACAAAACACAATTTAATTATTTTTACAGTTAGTCAATTTTGAAATTATTTTCAAAAATTTCA
ACAAACGGATCTCTTGGTTCTCGCATCGATGAAGAACGCAGCGAAAATGCGATAAGTAATATGAA
TTGCAGATTTTCGTGAATCATCGAATCTTTGAACGCACATTGCGCCCTCTGGTATTCCAGAGGGCA
TGCCTGTTTGAGCGTCATTTCTCTCTCAAACCCCCGGGTTTGGTATTGAGTGATACTCTTAGTCGGA
CTAGGCGTTTGCTTGAAAAGTATTGGCATGGGTAGTACTGGATAGTGCTGTCGACCTCTCAATGTA
TTAGGTTTATCCAACTCGTTGAATGGTGTGGCGGGATATTTCTGGTATTGTTGGCCCGGCCTTACA
ACAACCAAACAAGTTGACCTCAAATCAGGTTGAATACCCCGTT
>CP16-ITS
TATTGGAAGAAAAAAAGTCGTAACAAGGTTTCCGTAGGTGAACCTGCGGAAGGATCATTACAGTA
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CTTGCTTTGGTTTGGCCTAGAGATAGGTTGGGCCAGAGGTTTAACAAAACACAATTTAATTATTTT
TACAGTTAGTCAACTTTTGAATTAATCTTCAAAACTTTCAACAACGGATCTCTTGGTTCTCGCATCG
ATGAAGAACGCAGCGAAATGCGATAAGTAATATGAATTGCAGATTTTCGTGAATCATCGAATCTT
TGAACGCACATTGCGCCCTCTGGTATTCCAGAGGGCATGCCTGTTTGAGCGTCATTTCTCTCTCAA
ACCCCCGGGTTTGGTATTGAGTGATACTCTTAGTCGGACTAGGCGTTTGCTTGAAAAGTATTGGCA
TGGGTAGTACTGGATAGTGCTGTCGACCTCCTCAATGTATTAGGTTTATCCAACTCCTTGAATGGT
GTGGCGGGATATTTTCTGGTATTGTTTGGCCCGGCCTTACAAACAACCAAACAAGTTTTGACCCTC
AAATCAGGTAGGAATACCCGCCTGAACTTAAAGCATATCAATAGGCGGAAGAGAAAAA
>CP19-ITS
GGGAAGAAAAAAATCGTAACAAGGTTTCCGTAGGTGAACCTGCGGAAGGATCATTACAGTATTCT
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CTTTGGTTTGGCCTAGAGATAGGTTGGGCCAGAGGTTTAACAAAACACAATTTAATTATTTTTACA
GTTAGTCAAATTTTGAATTAATCTTCAAAACTTTCAACAACGGATCTCTTGGTTCTCGCATCGATGA
AGAACGCAGCGAAATGCGATAAGTAATATGAATTGCAGATTTTCGTGAATCATCGAATCTTTGAA
CGCACATTGCGCCCTCTGGTATTCCAGAGGGCATGCCTGTTTGAGCGTCATTTCTCTCTCAAACCC
CCGGGTTTGGTATTGAGTGATACTCTTAGTCGGACTAGGCGTTTGCTTGAAAAGTATTGGCATGGG
TAGTACTAGATAGTGCTGTCGACCTCTCAATGTATTAGGTTTATCCAACTCGTTGAATGGTGTGGC
GGGATATTTCTGGTATTGTTGGCCCGGCCTTACAACAACCAAACAAGTTTGACCTCAAATCAGGTA
GGAATACCCGCTGAACTTAAGCATATCAAAAAGCCGGAGGAAGCAT

>CP17-ITS
TTTTGGGTTACTTGCGGAGACATTACAGAATGAAAAGTGCTTAACTGCATTTTTTCTTAC
ACATGTGTTTTTCTTTTTTTGAAAACTTTGCTTTGGTAGGCCTTCTATATGGGGCCTGCCAGAGATT
AAACTCAACCAAATTTTATTTAATGTCAACCGATTATTTAATAGTCAAAACTTTCAACAACGGATC
TCTTGGTTCTCGCATCGATGAAGAACGCAGCGAAATGCGATAAGTAATATGAATTGCAGATATTC

GTGAATCATCGAATCTTTGAACGCACATTGCGCCCTTTGGTATTCCAAAGGGCATGCCTGTTTGAG
CGTCATTTCTCCCTCAAACCCTCGGGTTTGGTGTTGAGCGATACGCTGGGTTTGCTTGAAAGAAAG
GCGGAGTATAAACTAATGGATAGGTTTTTTCCACTCATTGGTACAAACTCCAAAACTTCTTCCAAA
TTCGACCTCAAATCAGGTTGACTGCCCGGTTTT

>CP18-ITS
TGGAAGAAAAAGTCGTAACAAGGTTTCCGTAGGTGAACCTGCGGAAGGATCATTACAGAATGAAA
AGTGCTTAACTGCATTTTTTCTTACACATGTGTTTTTCTTTTTTTGAAAACTTTGCTTTGGTAGGCCT
TCTATATGGGGCCTGCCAGAGATTAAACTCAACCAAATTTTATTTAATGTCAACCGATTATTTAAT
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TTCCAAAGGGCATGCCTGTTTGAGCGTCATTTCTCCCTCAAACCCTCGGGTTTGGTGTTGAGCGAT
ACGCTGGGTTTGCTTGAAAGAAAGGCGGAGTATAAACTAATGGATAGGTTTTTTCCACTCATTGGT
ACAAACTCCAAAACTTCTTCCAAATTCGACCTCAAATCAGGTAGGACTACCCGCTGAACTTAAGC
ATATCAATAAGGCGGAGGAA

>CP8-ITS
GGAAGAAAAAGTCGTAACAAGGTTTCCGTAGGTGAACCTGCGGAAGGATCATTAAAAAGAATTAT
ACACTTTGCATTTGCGAACAAAAAAATAAATTTTTTTATTCTAACAAACTAAATCAAAACTTTCAA
CAACGGATCTCTTGGTTCTCGCATCGATGAAGAACGCAGCGAATTGCGATACGTAGTATGACTTGC
AGACGTGAATCATCGAATCTTTGAACGCACATTGCGCCTCGAGGCATTCCTCGAGGCATGCCTGTT
TGAGCGTCGCATCCCCTCTAACCCCCGGTTAGGCGTTGCTCCGAAATATCAACCGCGCTGTCAAAC
ACGTTTACAGCACGACATTTCGCCCTCAAATCAGGTAGGACTACCCGCTGAACTTAAGCATATCAA
TAAGCGAGGAA

The incidence of candidiasis caused by non albicans species has been reported by many researchers (Barchiesi*et al.,* 2002; MasiaCaunto*et al.,* 1999).

The study was also attempted to screen for *C.dublinensis*, which is closely related, phenotypically similar to *C.albicans*, but we are not able to do that because of less time and sequencing reports are not reached. Despite reports of the worldwide isolation of *C.dubliniensis* from oral and systemic candidasis among immunocompromised patients, till date there has been only one report of isolation of *C. dubliniensis* from India, which was isolated from a HIV patient (Gugnani*et al.,* 2003). In the present basic study up to now stating that none of the isolates found to be either *C.albicans*or *C. dubliniensis*, but in our CHROM agar plate one culture shows apple green colonies which might be *C.albicans*and another one which shows metallic bluecolour is *C. tropicalis*. However a large number of screenings of *Candida species* in these groups of patients is required.

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