# Emergence of Non-*albicans Candida* in Type 2 Diabetes Mellitus patients attending a tertiary care centre, Gujarat

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**Abstract:**

**Background and objectives**: *Candida* species are one of the major etiological agents causing opportunistic infections in immunocompromised patients. Presently a significant shift from *C. albicans* to Non *albicans Candida* (NAC spp.) has come into sight along with high resistance to antifungal drugs.Consequently, present study was intended to highlight the emergence of NAC spp. in patients with type 2 diabetes. **Method:** A total of fifty-eight (n=58) consecutive, symptomatic patients (30 males and 28 females; age 30-80 years) with Type 2 Diabetes Mellitus, admitted at tertiary care hospital piparia were included in the study. *Candida* species were identified from various specimens like urine, blood, pus, and sputum by techniques like culture, Germ tube test, Chlamydospore formation, Sugar assimilation test and Carbohydrate fermentation test. **Result:** Incidence of *Candidiasis* in patients with Type 2 diabetes was 48%. Infection was seen higher in category of 31-40 yrs (83.3%) with slight male predominance.*Candida*spp. isolated were *Candida tropicalis* (36%) followed by*Candida albicans* (28%), *Candida glabrata* (25%) and *Candida parapsilosis* (11%).82% of NAC spp were from the urine sample. 64% strains were from ICU and *Candida tropicalis* being the predominant one. **Conclusion**: Higher incidence of *C tropicalis*, *C. glabrata* and *C. parapsilosis* in urine of diabetic patients was

observed. The former understanding of considering NAC spp. as contaminant is no longer the trend due to the emergence of NAC in immunocompromised patients.

**Key words**: *Candida tropicalis*, Diabetes mellitus, Non albicans *Candida* (NAC spp.), Immunocompromised

**How to cite this article:Anita, Kumar S, Pandya H (2020):Emergence of non-albicans candida in type 2 diabetes mellitus patients, Ann Trop Med & Public Health; 23(S23): SP2323150. DOI: http://doi.org/10.36295/ASRO.2020.2323150**

**INTRODUCTION:**

Advances in medical care, have become inadvertently gateways for the introduction of fungal species, widely distributed in soil, plant debris & other organic material, into human body.[1]In the past few years, the fundamental nature of infections has transformed intensely, fungi were always considered as non-pathogenic but now they are recognised as a primary cause of morbidity and mortality in patients who are either chronically ill or those with compromised immune system. [2]The two most important epidemiological trends exposed recently in mycotic infections is increase in incidence of invasive fungal infections in immunocompromised patients and secondly increase in incidence of Non- albicans *Candida* (NAC spp.) causing infections that are invasive.[3]The predisposing factor of the host defence against fungi includes diverse factors like disturbance of epithelial barrier (due to either catheter, burns, ulcers, trauma, surgery, peritoneal dialysis, or use of broad spectrum antibiotics), defect or dysfunction of phagocyte and neutrophils (due to chemotherapy, aplastic anemia and Diabetes mellitus) and defect or dysfunction of T-Lymphocyte (AIDS, Leukemia, Transplantation, use of corticosteroids etc.[4]Interruption ofany of the above mentioned standard host defence can convert commensal Candida to act as pathogens.Although *C. albicans* is the most common organism associated with serious fungal infection, other *Candida*species also have emerged as clinically important opportunistic pathogen during the past decades are C. *tropicalis, C krusei, C. parapsilosis, and C. glabrata.* [5]Majority of the opportunistic infectious disease including candidiasis is common in the patients with Diabetes mellitus (DM), which is considered as one of the largest emerging threat to health in the 21st century with increase in morbidity and mortality.[5]Many researchers in past have revealed thatpatients with Type 2 Diabetes Mellitus are prone to increase in susceptibility to fungal infections especially in the urinary tract due to a high urine glucose concentration. [6]On the top, infection with *Candida* species can even complicate the control of the diabetes. [6]The diverse manifestations of candidiasis often create predicament for the physician, both in diagnosis and treatment, and are frequently the major cause of death in the patient.To explore more on this area the present

study was projected to find the incidence of *Candida albicans* and *Non-albicansCandida*(NAC) in the patients with Type 2 Diabetes Mellitus

**MATERIALS AND METHODS:**

This prospective cross-sectional study was conducted on fifty-eight (n=58) consecutive, symptomatic patients (30 males and 28females; age 30-80 years) with Type 2 Diabetes Mellitus, admitted at tertiary care hospital piparia. The Disease profile of 58 patients were following: 14 patients were having uncontrolled diabetes mellitus with fever, 12 were having UTI, 8 patients had fever with electrolyte imbalance, 8 had cerebrovascular accident with hypertension, 3 had sepsis, 3 had chronic kidney disease with gall bladder perforation, 3 had post of B/L hernia, 2 patients had acute exacerbation of COPD and 2 had fractures.

The study was carried out for a period of six months from January 2016 to June 2016. Patient's information such as age, socioeconomic status, education details, date of admission, ward, underlying medical conditions, associated risk factors such as presence of urinary catheter, respiratory ventilation, central line insertion, duration of antibiotic therapy, antifungal prophylaxis, exposure to invasive medical procedures were obtained from clinical records.

The study was approved by the Sumandeep Vidyapeeth Institutional Ethical Committee (SVIEC) and informed consent in Hindi and local language was obtained from each patient.

***Methodology*:**Total 58 clinical samples (urine,blood, pus and sputum)were collected aseptically fromconsecutive patients with type 2 diabetes mellitus admitted in Dhiraj hospital. The samples were further processed as per the standard operating Procedures.

1. **Microscopy** [7, 8]**:** Direct smears were prepared from all clinical specimens on a clean slide and were stained with standard Gram's staining. Epithelial cells, pus cells, bacteria, budding yeast cells and pseudohyphae were noted.
2. **Culture**[7, 8]
   a. All Clinical samples were directly cultured on Sabouraud's dextrose agar with chloramphenicol and chlorhexidine and Mac Conkey's agar except Blood sample. The plates were incubated at 37°C for 24-48 hours after which the colonies were studied for their morphological characters such as: colony appearance, colony colour, colony shape, colony texture, and production of hyphae and pseudohyphae.
   b. **Blood culture:** Blood samples were inoculated in BACTEC aerobic plus/F (Becton-Dickinson, New Jersey, USA) bottles (BACTEC fluorescent series 9050 instruments (Becton Dickinson, USA) was used) and were inserted in the machine within 30 minutes. Whenever the machine gave an alert signal, the

specific bottles were removed and subcultures were done on Mac Conkey's agar and Sabouraud's dextrose agar with chloramphenicol and chlorhexidine (Procured from HIMEDIA Lab, Mumbai). The colonies were further identified by Gram staining (Candida are gram positive yeast cells).

c. Hi chrom agar [7, 8]:( HI Media, Mumbai, India) for provisional species identification(*C. albicans*- Light green, *C. tropicalis*- Blue with pink halo, *C. glabrata*- Pink to purple and *C. parapsilosis*- cream to pale pink )

3. **Chlamydospore production**[7, 8]:Each *Candida* isolates were picked up with a straight wire and was streaked through deep cutting on corn meal agar plate at an angle and then covered with a sterile cover slip to produce a relative anaerobic condition. The plates were incubated at $25^0$C for 3 days. At every 24 hours interval the plates were examined under microscope for the presence of chlamydospores

4. **Germ tube production**[7, 8]:**(Raynaulds Braude phenomenon):** Raynaulds Braude phenomenon was observed by inoculating the colonies in 0.5 ml of human serum and incubating at 37 ° C for 1-2 hrs. One drop of suspension was placed on a glass slide and covered with a sterile cover slip to be observed as a wet preparation. The slide was examined for the presence of germ tubes under microscope.

5. **Sugar Assimilation tests** [7, 8] Yeast suspension was prepared in 6ml distilled water with the density of McFarland No.4 or 5 standards. Each petri-dish was labelled with isolate number. Now, yeast suspension was poured into the plate of yeast nitrogen base (15 ml). Sugar discs (Himedia) were placed in designated areas (3-4 discs per plate)and plates were incubatedfor 24 hrs at room temperature and were further observed for growth around the disc. The isolates which assimilate a particular carbohydrate grow well around that disc. The pattern of assimilation wasnoted. Outcome criteria for sugar assimilation are shown in Table 1.

6. **Carbohydrate fermentation test**[7, 8]done to detect acid and gas production. The colonies were inoculated into 2 % sugar media with Andrade's indicator and Durham's tube and incubated at 25 $^o$ C for 5 -7 days. Outcome criteria for sugar fermentation test are shown in Table 2.

**Table 1: Sugar Assimilation Tests(Auxanographic plate method: Haley and standard modification)**

| Sugar | Species | | | |
|---|---|---|---|---|
| | *C. albicans* | *C. tropicalis* | *C. parapsilosis* | *C. glabrata* |
| Glucose | + | + | + | + |
| Maltose | + | + | + | + |
| Sucrose | + | + | + | - |
| Lactose | - | - | - | - |
| Galactose | + | + | + | - |
| Melibiose | - | - | - | - |
| Cellobiose | - | + | - | - |
| Inositol | - | - | - | - |
| Xylose | + | + | + | - |
| Raffinose | - | - | - | - |
| Trehalose | + | + | + | + |
| **Dulcitol** | **-** | **-** | **-** | **-** |

**Table 2: Carbohydrate fermentation test**

| S.N. | Sugar | Species | | | |
|---|---|---|---|---|---|
| | | *C.albicans* | *C.tropicalis* | *C.parapsilosis* | *C.glabrata* |
| **1** | Glucose | + | + | + | + |
| **2** | Maltose | + | + | - | - |
| **3** | Sucrose | - | + | - | - |
| **4** | Lactose | - | - | - | - |

**RESULTS:** Fifty-eight (n=58) clinical samples were collected from consecutive type 2 Diabetic patients (30-80 yrs.) admitted at Dhiraj Hospital for diverse morbid conditions. Out of which 28 were positive for *Candida* species, which shows the incidence of 48% in diabetic patients. Most frequent NAC spp. was *Candida tropicalis* (36%) followed by *Candida glabrata* (25%) and *Candidaparapsilosis*(11%) (Figure 1).

***Association of Candida infections with various socio-demographic factors*** **(Table 3):**

Majority of the patients with *Candida* infection belonged to the category of 31-40 yrs. (83.3%), followed by 41-50 yrs. (58.3%). Gender wise, we found slight male predominance (50%) then female (46.4%). 69% of patients (n=40) belonged to rural community, while 31% (n=18) were from urban. Out of 40 rural patients, 16 (40%)were infected with *Candida,* out of which *C. albicans*(6/16) and *C. tropicalis*(6/16) were equal in number while *C. glabrata* (2/16) and *C. parapsilosis* (2/16) were less common. In urban community, out of 18 patients, 12 were infected with *Candida*, predominant species was *C. glabrata* (n=5) then by *C.tropicalis* (n=4), *C. albicans* (n=2) and *C. parapsilosis*(n=1).

***Ward wise emergence of*** **NAC (Table 4):** Majority of *Candida* infections was found in the patients from ICU (18/31, 58%), *Candida tropicalis* being the predominant one (6/18, 33%), while *Candida albicans* and *Candida glabrata* were equal in number (5/18, 28%). In medicine ward also *Non-albicans* outnumbered albicans (05/8, 62.5%). In neuro-ward and surgery ward also *Candida tropicalis* was the main one.

**Sample wise distribution of *Candida* species (Table 5):** Majority (82%) of *Candida* species were isolated from urine sample (23/28), *C. tropicalis* was the leading one, track by *C. albicans*, *C. glabrata* and *C. parapsilosis.* From sputum sample*, C. glabrata* was the chief one, while *C. albicans* and *C. tropicalis* were only 6%. There was no isolate of *Candida*from blood and pus.

**Figure 1**: **Speciation of *Candida* isolated from Type 2 Diabetes patients**

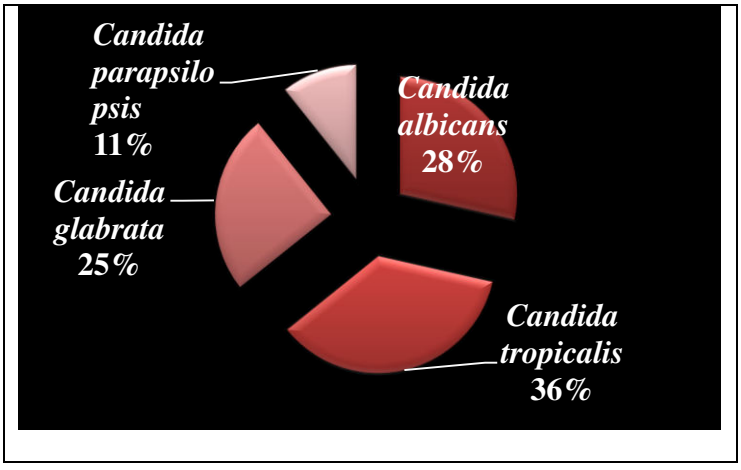

**Table -3: Correlation of Socio-demographic factors with and without *Candida* infection(n=58)**

| Variables | | With *Candida* infection | Without *Candida* infection |
|---|---|---|---|
| **Gender** | Male(n=30) | 15(50 %) | 15(50 %) |
| | Female(n=28) | 13(46.4 %) | 15(53.5 %) |
| **Age- Group** | 31-40(n=6) | 5(83.3%) | 01(16.7%) |
| | 41-50(n=12) | 7(58.3 %) | 05(41.7%) |
| | 51-60(n=17) | 8(47%) | 09(53%) |
| | 61-70(n=15) | 5(33.3%) | 10(66.7%) |
| | 71-80(n=8) | 3(37.5%) | 05(62.5%) |
| **Socioeconomic Status** | Rural (n=40, 69%) | 16(40%) | 24(60%) |
| | Urban(n=18, 31%) | 12(66.7%) | 06(33.3%) |
| **Total** | | 28(48.2%) | 30(51.7%) |

**Table 4: Isolation of *Candida* species from different ward:**

| Ward | Total Positive case | With *Candida* n=28 | | | | Without *Candida* |
|---|---|---|---|---|---|---|
| | | *C.albicans* | *C.glabrata* | *C.tropicalis* | *C.parapsilosis* | |
| **ICU(n=31)** | 18 | 5 | 5 | 6 | 2 | 13 |
| **Medicine(n=17)** | 08 | 3 | 2 | 2 | 1 | 9 |
| **NICU(n=1)** | 0 | - | - | - | - | 1 |
| **MOW(n=2)** | 0 | - | - | - | - | 2 |
| **Neuro-ward(n=1)** | 01 | - | - | 1 | - | - |
| **Surgery ward(n=6)** | 01 | - | - | 1 | - | 5 |
| **Total** | 28 | 08 | 07 | 10 | 03 | 30 |

**Table-5:Isolation of *Candida* species from various clinical specimens**

| | Culture positivity | | | | Total positive |
|---|---|---|---|---|---|
| | *C.albicans* | *C.glabrata* | *C.tropicalis* | *C.parapsilosis* | |
| **Urine (n=36)** | 7(19.4%) | 4(11%) | 9(25%) | 3(8.3%) | 23 |
| **Sputum (n=17)** | 1(6%) | 3(17.6%) | 1(6%) | - | 05 |
| **Blood (n=2)** | - | - | - | - | - |
| **Pus (n=3)** | - | - | - | - | - |
| **Total** | 8(28.5%) | 7(25%) | 10(35.7%) | 3(10%) | 28( 48%) |

**DISCUSSION**

In 2017,it was estimated that prevalence of diabetes in adults is about 425 million and by 2045 it is going to rise to 629 million as predicted by World Health Organization and the International Diabetes Federation. [9]Clinical explicationdelegates that patients with diabetes show increase in vulnerabilityto mycotic infections especially of *Candida* species. In a Host with type 2 diabetes,there are several aspects which facilitate the colonisation of *Candida* and subsequently lead to serious infections, which mainly include higher salivary glucose, reduced salivary flow, microvascular degeneration, and impaired activity of neutrophilsagainst *Candida.*[10]These factors influence the transition of *Candida* species from commensal to pathogen causing infections. [10]*C. albicans* is generally considered as the major pathogen among the *Candida*spp. Due to overuse and misuse of antimycotic drugs like azoles, emergence of *Non-albicans spp*. such as *C. glabrataand C. krusei*has been noted during the past decades and a high mortality rate of 67% was noted with patients of Diabetes. [10, 11]

In the present study, we attempted to uncover the incidence of *Candida* species in patients with Type 2 diabetes. Wefound that 48% of diabetic patients were infected with *Candida* species which is in accordance with the study done by Martinez[12]*et al*(41%), and Pallavan[13]*et al*(43%). Candida infection was found slightly higher in

males(50%) in comparison to females (46.4%) which is incongruous to many studies showing female as a higher risk of acquiring infection as its colonisation in the vulvar area is very common and poses a high risk of ascending infections causing candiduria. [14 15]Study showed that 71.4 % were NAC spp.and 28.6% were *Candida albicans*, which clearly demonstrates the emergence of Non- *albicans* over *albicans.*Most frequent Non- *albicansCandida* was *Candida tropicalis* (36%) followed by *Candida glabrata* (25%) and *Candidaparapsilosis* (11%),similar finding of emergence of NAC spp. especially *C. tropicalis* were also observed in the previous studies which showed the predominance of *C. tropicalis* followed by *C. glabrata.*[2, 5, 14]

In present study 82% of *Candida* species(n=23/28) were isolated from the patients with Urinary tract infections*while 18% Candida in sputum*, The Most frequent NAC isolated from urine was *C. Tropicalis* followed by *C. glabarata* while in sputum *C. glabrata* was predominant. The predominance of *Candida tropicalis* was an emerging NAC spp. and was recognized by many previous studies. [2, 5, 14, 16]*Non albicans Candida*species appear better adopted to the urinary tract environment and are more resistant to antifungal drugs compared to *C. albicans.* [14]Our study corroborates well with the fact that use of indwelling urinary catheters, ICU stay and diabetes mellitus is major risk factors associated with candiduria. The joint efforts of a physician and a microbiologist can confine the risk of candidiasis in immunocompromised patients.

**Conclusion:** The gamut of infections has transformed in the last few decades and has challenge the mankind. Microorganisms which were always considered as either colonizer or contaminant are now emerged as a potent pathogen with multi drug resistance. We too found the higher incidence of *C tropicalis*, *C. glabrata* and *C. parapsilosis*in urine of diabetic patients. NAC spp. now has emerged as a potential pathogen and now cannot be ignored as contaminant or considered non-pathogenic.

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