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RESEARCH ARTICLE

SPECIES IDENTIFICATION OF *CANDIDA* ISOLATES IN VARIOUS CLINICAL SAMPLES.

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Abstract

Candida species especially non *albicans Candida* are increasingly being isolated from clinical specimens. The aim of our study was to detect the clinical distribution of *Candida* species in various clinical samples in a tertiary care hospital. A total of 115 *Candida* species were isolated from various clinical specimens submitted to Microbiology laboratory from different units of a tertiary care centre, Aurangabad Maharashtra from January 2014 to December 2014 using conventional yeast identification method after the approval from institutional ethical committee. The percentage of isolation of non *albicans Candida* species was 59.1% and that of *C. albicans* was 40.9%. The predominant species among non *albicans Candida* species isolated were *C. tropicalis* (40%) followed by *C. guilliermondii* (10.43%), *C. krusei* (4.34%), *C. glabrata* (2.60%), *C. kefyr* and *C. parapsilosis* (0.87%) each. As it is possible to predict the sensitivity pattern of each *Candida* species with high accuracy, the accurate species identification of *Candida* is important.

Introduction:-

Candida species are ubiquitous fungi and are the most common fungal pathogens that affect human beings. They are true opportunistic pathogens that exploit recent technological advances to gain access to the circulation and deep tissues.[1]The genus *Candida* comprises about 200 species, of which close to 20 have been associated with pathology in human and animal.[2]*Candida* species are the component of normal flora of human beings. They are commonly found on the skin throughout gastrointestinal tract and female genital tract particularly higher in vagina during pregnancy.[3] Those that are the part of normal flora can invade tissues and cause life-threatening disease in patients whose cell mediated immunity is decreased by disease or iatrogenic intervention.[4]

Candida albicans is by far, the most common in all clinical forms of Candidiasis, representing 70-80% of all yeast isolates.[5]*Candida* species especially non *albicans Candida* are increasingly being isolated from clinical specimens. Speciation of *Candida* isolates is conventionally done by germ tube test, morphology on cornmeal agar, sugar fermentation and sugar assimilation tests.[6] Non *Candida albicans* like *C. tropicalis, C. krusei, C. glabrata and C. parapsilosis* are less susceptible to azoles, particularly fluconazole.[3,7] Therefore, correct identification of *Candida* species is of great importance. It presents prognostic and therapeutical significance, allowing an early and

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appropriate antifungal therapy. Thus, the present study was undertaken for species identification of *Candida* isolates in various clinical specimens using conventional method.

Materials and Method:-

Study population:-

All the specimens collected from IPD and OPD, patients attending various clinical services at tertiary care hospital were used in the study. These specimens were processed as per standard protocol.[3]

Processing of the specimens:-

Specimens like urine, pus, vaginal discharge, blood, sputum, endotracheal tube secretion, gastric lavage and other body fluids received in the Department of Microbiology for the culture were used in the study. Brain Heart Infusion broth was used for blood culture. All the specimens were screened for growth of *Candida* species by standard protocol[3]. Relevant clinical data was recorded. In the present study, total 115 isolates of *Candida* species were obtained.

Identification of *Candida* species:-

Isolates were preliminary identified as *Candida* species by direct microscopy, colony morphology and Gram staining.

All the specimens for the fungal culture were inoculated on Sabouraud's dextrose agar with chloramphenicol and incubated at 25^0C and 37^0C for 48 hours. *Candida* species were identified by standard protocol that included germ tube formation, chlamydospore production on cornmeal agar, sugar fermentation and sugar assimilation tests.

Wet preparations :

Direct microscopy :-[8]

Direct examination of specimens like urine, peritoneal fluid, ascitic fluid etc. were carried out microscopically for the presence of yeast like cells.

10% KOH :[3,9]

The specimens like sputum and swab from lesion were examined in KOH wet mount for the presence of yeast and pseudohyphae. The yeast cells of *Candida* species were approximately 4-8 μm with budding and pseudohyphae.

Gram staining :[9-11]

For the specimens like pus, sputum etc Gram's stained smear was observed for the presence of oval budding yeast like cells..

Colony morphology on Sabouraud's dextrose agar :[3,9]

The sabouraud's dextrose agar consisting of chloramphenicol was used for isolation of *Candida* species. Cream coloured pasty colonies usually appeared after 24-48 hours incubation at 35-37^0C. The colonies had a distinctive yeast smell. Sometimes growth was observed after an overnight incubation as seen in bacteria. The lactophenol cotton blue mounts were prepared to examine detail morphological features of fungi grown on culture medium. Gram's staining was also performed from culture isolates.

ATCC strains that were used as a control were *Candida albicans ATCC 10231, Candida glabrata ATCC 15126, Candida krusei ATCC 14243 & Candida tropicalis ATCC 750*

Species identification of *Candida* isolates was done by following methods:-

Germ tube test:[3,9,11]

Candida albicans can be identified presumptively by simple germ tube test.

Cornmeal agar morphology:[3,11-13]

Cornmeal agar is used for demonstration of chlamydospores, blastospores and pseudohyphae.

Sugar fermentation test:[3,8]

For the sugar fermentation test sugars like glucose, sucrose, maltose and lactose were used.

Sugar assimilation test:[3,12,14]
Yeast Nitrogen Base Agar was used for assessing carbohydrate utilizing ability of yeasts using carbohydrate disc method. Sucrose, Lactose, Trehalose, Raffinose & cellulobiose discs were used for sugar assimilation test.

All the observations were statistically analysed.

Result:-

A total of 115 *Candida* species were isolated from various clinical specimens processed during the study period including urine 43(37.39%), sputum 34(29.56%), blood 15(13.04%), pus 10(8.69%), oral swab 4(3.47%), vaginal swab 3(2.60%), throat swab 2(1.73%), ascitic fluid 2(1.73%), central line tip 1(0.87%) and gastric lavage 1(0.87%). *Candida albicans* was the commonest species isolated 47(40.9%) followed by *C. tropicalis* 46(40%), *C. guilliermondii* 12 (10.43%), *C. krusei* 5(4.34%), *C. glabrata* 3(2.60%), *C. kefyr* 1(0.87%) and *C. parapsilosis* 1(0.87%). Isolation rate of *non albicans Candida* species were higher 68 (59.1%) as compared to *Candida albicans* 47 (40.9%) [Table 1]. *Candida* isolates were present in 60 (52.17%) males and 55 (47.82%) females with male: female ratio of 1.09:1. Table no. 2 shows age wise distribution of *Candida* isolates. Highest number of *non albicans Candida* were isolated from urine (25) followed by sputum (14) where as highest number of *Candida albicans* were isolated from sputum (20) followed by urine (18) [Table 3]

Table 1:- Distribution of *Candida albicans* and Non *Candida albicans* isolates.

***Candida* isolates**	**Number of isolates**	**Percentage**
Candida albicans	47	40.87%
Non *albicans Candida*	68	59.13%
Total	**115**	**100%**

Table 2:- Age wise distribution of *Candida* isolates.

Age	**Total No. of *Candida* isolates**	**Percentage (%)**
0-10	24	20.87%
11-20	15	13.04%
21-30	13	11.30%
31-40	13	11.30%
41-50	8	6.95%
51-60	12	10.43%
> 60	30	26.08%
Total	**115**	**100**

Table 3:- Sample wise distribution of *Candida* iso lates.

Type of specimen	**No. of *Candida albicans* isolates**	**No. of Non *albicans Candida* isolates**	**Total no. of *Candida* isolates (%)**
Urine	18	25	43 (37.39%)
Sputum	20	14	34 (29.56%)
Blood	2	13	15 (13.04%)
Pus/Discharge	3	7	10 (8.69%)
Oral swab	1	3	4 (3.47%)
Vaginal swab	0	3	3 (2.60%)
Throat swab	2	-	2 (1.73%)
Ascitic fluid	0	2	2 (1.73%)
Central line tip	1	-	1 (0.87%)
Gastric lavage	-	1	1 (0.87%)
Total	**47**	**68**	**115 (100%)**

Discussion:-

Candidiasis is the commonest fungal disease in humans affecting skin, nails, mucosa and internal organs of the body. *Candida* species is endogenous and the disease represents opportunistic infections.[15]

In our study, we found highest number of *Candida* isolates from urine 43(37.39%). Out of 43(37.39%) isolates, 18 *Candida* isolates were *Candida albicans* and 25 were *non- albicans Candida* species. Among *non albicans Candida* species, *Candida tropicalis* (21) was the most predominant species. This was in agreement with Kashid et al (2011),[15] Patel et al (2012),[16] Kumar et al (2013),[17] as they found highest number of isolates from urine and among which *Candida tropicalis* was the most predominant species.

In our study, 34(29.56%) *Candida* isolates were from sputum sample. We found highest number of *Candida albicans* 20 from sputum sample as compared to non *albicans Candida* 14. This was in agreement with Patel et al (2012)[16] who found highest number of *Candida albican*s from sputum sample. *Candida* is normal inhabitant of the mouth and can be recovered from sputum in 20% to 55% of normal subjects. The prevalence and prognosis of pulmonary candidal infection is difficult to evaluate since diagnosis were seldom confirmed. *Candida* isolated from sputum sample is mostly a colonizer of the respiratory tract. The role of *Candida* in pulmonary candidiasis and its diagnosis is still controversial.[18]

In our study, 15 (13.04%) of the *Candida* isolates were from blood. Among them 10 were *Candida guilliermondii*, 2 were *Candida albicans* and *Candida krusei* each and one was *Candida tropicalis.* We observed that *Candida guilliermondii* was the most predominant isolate from blood. This was in agreement with study done by Patel et al (2012)[16] Pethani et al (2013)[19] who reported highest number of *Candida guilliermondii* from blood

In the present study it was found that candidiasis can occur at all ages and in both sexes. In the present study, the number of *Candida* species was found to be higher in male patients 60(52.17%) as compared to female patients 55(47.82%) with male to female ratio of 1.09:1. This was in agreement with Kashid et al (2011)[15] who reported the isolation of *Candida* species was higher in males 81(55.10%) as compared to females 66(44.8%) with male to female ratio of 1:0.81.

In the present study, out of 115 isolates of *Candida* species 89(77.39%) were from IPD and 26(22.60%) were from OPD. The continuous use of invasive monitoring and surgical technologies in the intensive care unit has increased the risk of fungal infections.[20]

We observed that the frequent isolation of *Candida* species was in the age group above 60 years (26.08%) followed by age group 0-10 years (20.87%) which was similar with the study of Kashid et al (2011)[15] who reported highest incidence in the age group above 60 years (24.48%) followed by age group 0-10 years (20.40%). Our study was also in agreement with Bineshlal et al (2011)[7] and Yashvanth et al (2013)[21] who also reported maximum isolation of *Candida* species in the age group above 60 years. *Candida* species remain the most important cause of opportunistic infections worldwide, affecting predominantly patients over 65 years old.[22]

Photographs:-

Fig 1:- Morphology of *Candida* species on SDA.

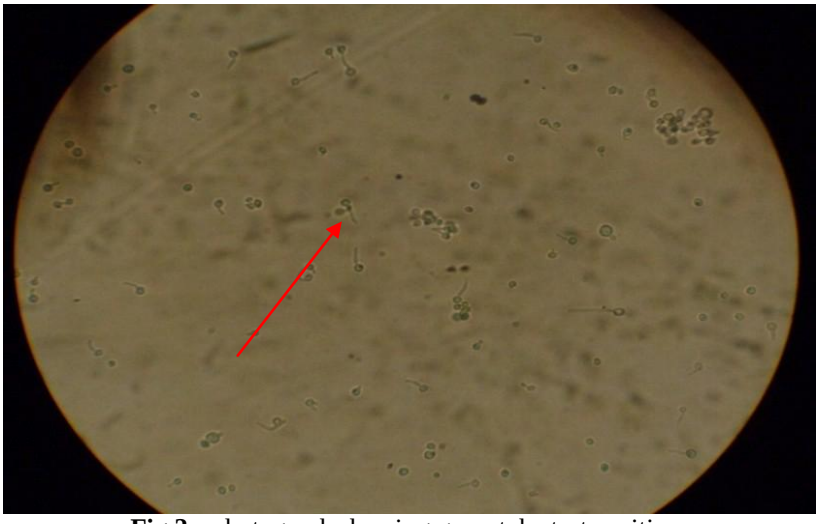
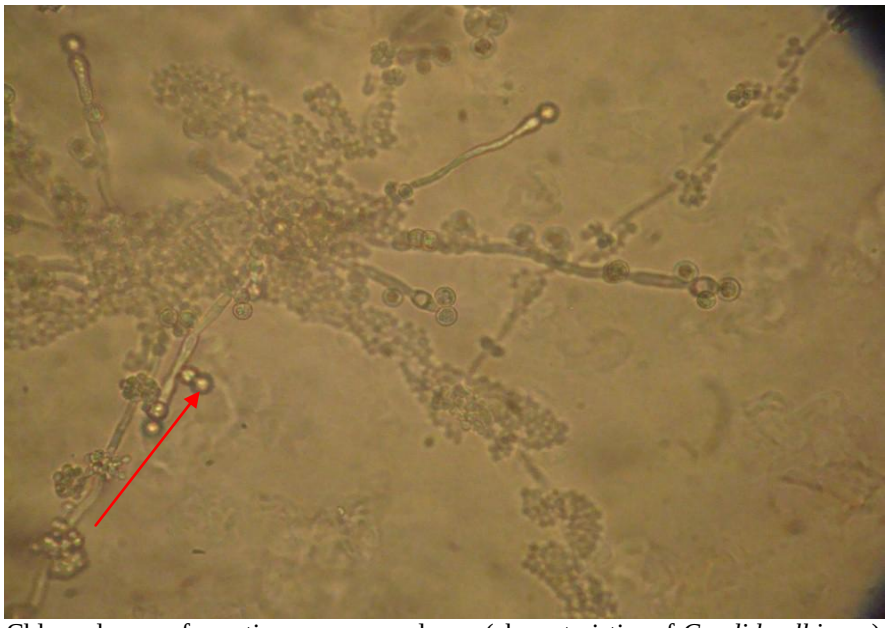
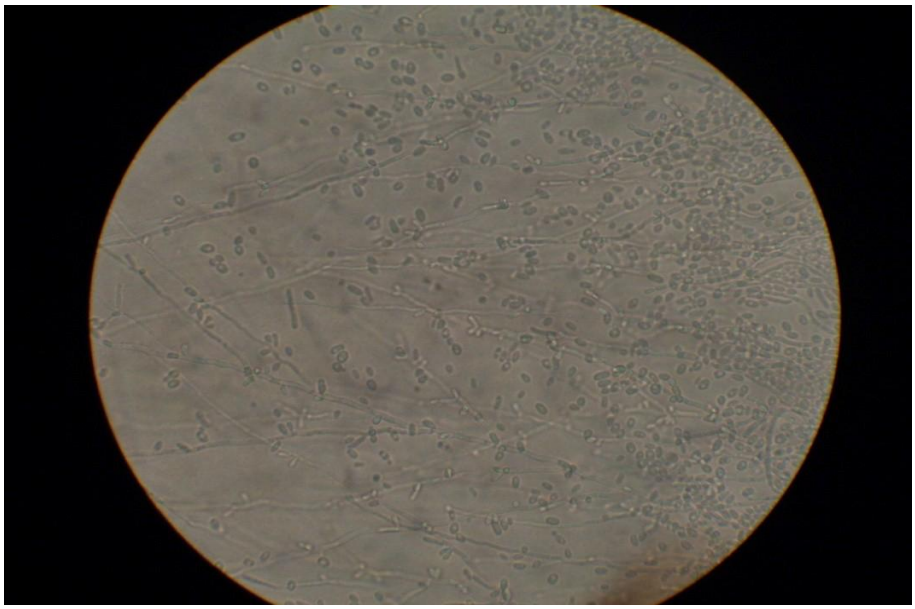


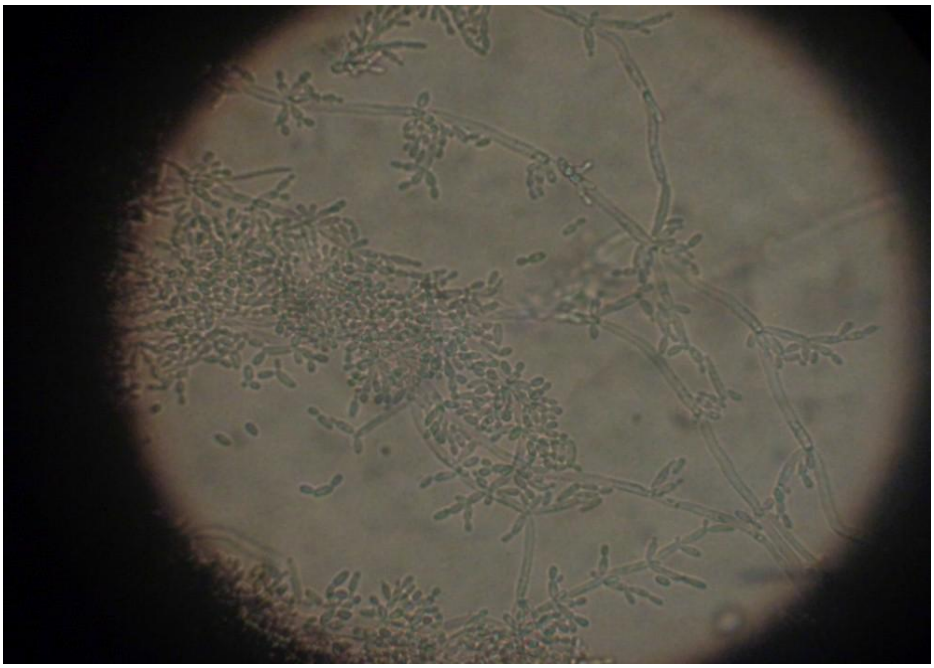
Fig 2:- photograph showing germ tube test positive.



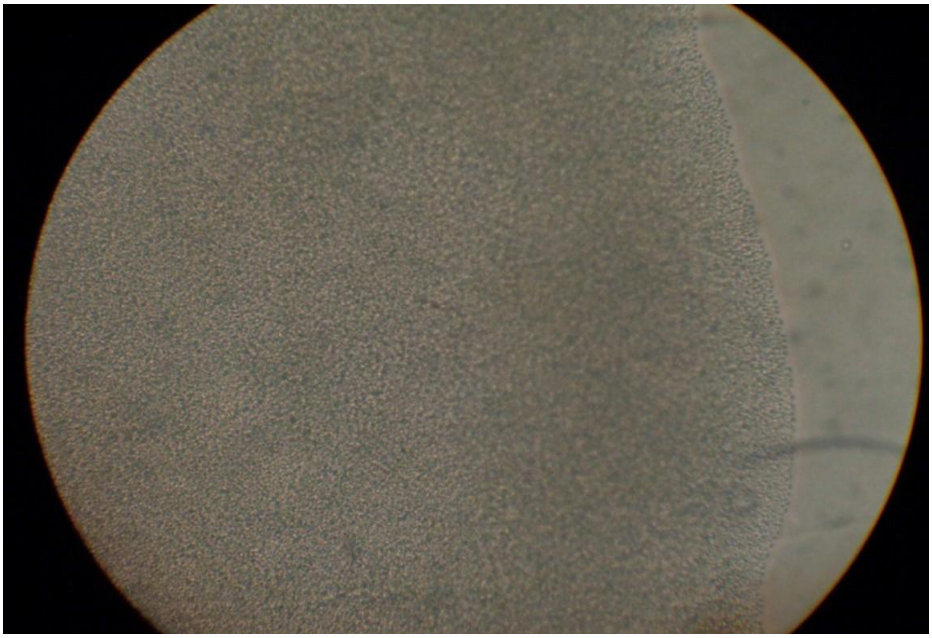
Chlamydospore formation on cornmeal agar (characteristics of *Candida albicans*)



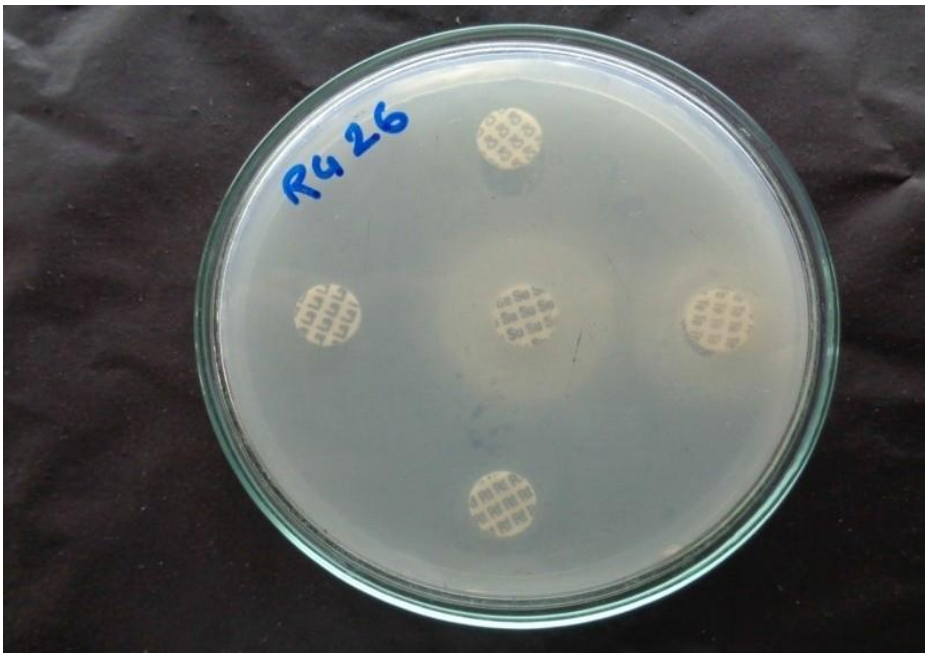
Morphology of *Candida tropicalis* on Cornmeal agar.



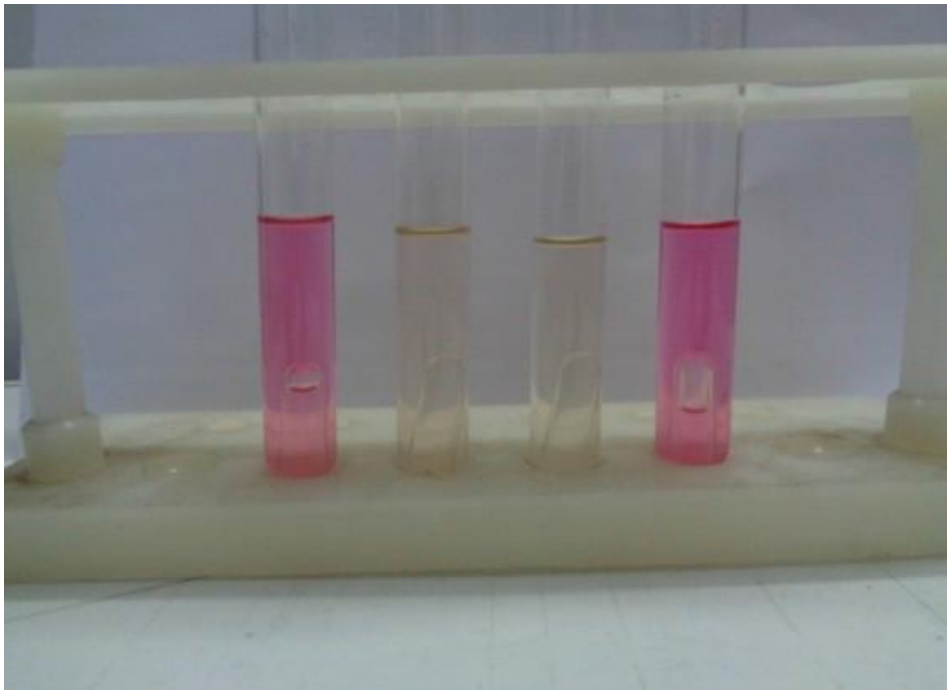
Morphology of *Candida krusei* on cornmeal agar



Morphology of *Candida glabrata* on cornmeal agar



Sugar assimilation pattern of *Candida albicans* (Sucrose: positive, Trehalose : positive Lactose : negative, Cellulobiose : negative and Raffinose : negative)



Conclusion:-

An increase in the predisposing factors in recent years has resulted in an increasing incidence of *Candida* infections. Therefore, the species level identification of the *Candida* isolates can greatly influence the treatment options for the clinician because there is variation in sensitivities of various species to different antifungals and may have an impact on the patient care.

In the present study, we found higher incidence of *non albicans Candida* as compared to *Candida albicans*. Therefore, it is necessary to identify *Candida* isolates up to species level for appropriate treatment.

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