RESEARCH ARTICLE

SPECIES IDENTIFICATION OF CANDIDA ISOLATES IN VARIOUS CLINICAL SAMPLES.

Rachana Mehta¹ and Anupama S. Wyawahare².

1. PG Student, Department of Microbiology, MGM’s Medical College & Hospital, Aurangabad, Maharashtra, India.
2. Professor, Department of Microbiology, MGM’s Medical College & Hospital, Aurangabad, Maharashtra, India.

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.⁵,⁷ Therefore, correct identification of Candida species is of great importance. It presents prognostic and therapeutical significance, allowing an early and...
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.  

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. 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°C and 37°C 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:
Direct examination of specimens like urine, peritoneal fluid, ascitic fluid etc. were carried out microscopically for the presence of yeast like cells.

10% KOH:
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:
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:
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°C. 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:
Candida albicans can be identified presumptively by simple germ tube test.

Cornmeal agar morphology:
Cornmeal agar is used for demonstration of chlamydomospores, blastospores and pseudohyphae.

Sugar fermentation test.
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 2 (1.73%), 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) whereas 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* isolates. |  |
|---|---|---|
| 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.\textsuperscript{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),\textsuperscript{15} Patel et al (2012),\textsuperscript{16} Kumar et al (2013),\textsuperscript{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)\textsuperscript{16} who found highest number of Candida albicans 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.\textsuperscript{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)\textsuperscript{16} Pethani et al (2013)\textsuperscript{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)\textsuperscript{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.\textsuperscript{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)\textsuperscript{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)\textsuperscript{7} and Yashvanth et al (2013)\textsuperscript{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.\textsuperscript{22}
Photographs:

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

Fig 2: Photograph showing germ tube test positive.
Chlamydospor 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.

References:
1. Gordana MBM, Momir MM, Svetlana MGK. The importance of genus Candida in human samples. Proc Nat SciMaticaSrpska Novi Sad 2008;114:79-95.
2. Segal E, Elad D. Candidiasis. 10th ed. In: Topley and Wilson’s microbiology and microbial infections. United Kingdom: John Wiley and Sons Ltd; 2005. p. 577-623.
3. Chandra J. Text book of medical mycology. 3rd ed. New Delhi: Mehta Publishers; 2008. p. 266-290
4. Dharwad S, Dominic SRM. Species identification of Candida isolates in various clinical specimens with their anti-fungal susceptibility patterns. J ClinDiag Res 2011;5(6):1177-81.
5. Fanello S, Bouchara JP, Sauteron M, Delbos V, Parot E, Marot-LeblondA et al. Predictive value of oral colonization by Candida yeasts for the onset of a nosocomial infection in elderly hospitalized patients. J Med Microbiol 2006;55:223-28
6. Devi LS, Maheshwari M. Speciation of Candida species isolated from clinical specimens by using Chrom agar and conventional methods. Int J sci Res Pub 2014;4(3):1-5.
7. Binesh LY, Kalyani M. Phenotypic characterization of Candida species and their antifungal susceptibility from a tertiary care centre. J Pharm Biomed Sci 2011;11(11):1-5
8. Collee JG, Duguid JP, Fraser AG, Marmion BP. Mackie and McCartney Practical medical microbiology. 13th ed. Hong Kong: Churchill Livingstone; 1989.
9. Cheesbrough M. District laboratory practice in tropical countries. 2nd ed. New Delhi, India: Cambridge University Press; 2006
10. Ananthnarayan R and Paniker J CK. Textbook of microbiology. Kapil A Bhaskaran CS Edr. 9th ed. Edhyerabad, India: Universities Press (India) Private Limited; 2013 p. 9-27.
11. Forbes BA, Sahm DF, Weissfeld AS. Bailey and Scott’s diagnostic microbiology. 12th ed. St. Louis, United States of America: Mosby Elsevier; 2007.
12. Koneman EW, Allen SD, Janda WM, Schreckenberger PC, Winn WC, Jr. Color atlas and textbook of diagnostic microbiology. 5th ed. Philadelphia, United States of America: J. B. Lippincott Company; 1997.
13. Parija SC. Textbook of microbiology and immunology. 2nd ed. New Delhi, India: Elsevier; 2012. p. 608-610
14. Northeast Laboratory Services. McFarland Turbidity Standards. [Online] [cited 2014 Jan 10]; Available from URL: nelabservices.com/techsheets/McFarland_Turbidity_Standards.pdf.
15. Kashid RA, Belawadi S, Devi G, Indumati. Characterisation and antifungal susceptibility testing for Candida species in a tertiary care hospital. J Health Sci Res 2011;2(2):1-7.
16. Patel LR, Pethani JD, Bhatia P, Rathod SD, Shah PD. Prevalence of Candida infection and its antifungal susceptibility pattern in tertiary care hospital, Ahmedabad. Natl J Med Sci 2012;2(4):439-441.
17. Kumar S, Vyas A, Kumar M, Mehra SK. Application of CHROMagar Candida for identification of clinically important Candida species and their antifungal susceptibility pattern. Int J Biol Med Res 2013;4(4):3600-3606.
18. Sukumaran J, Sundaram JM, Sivan RR. Changing trend in the clinical distribution of Candida species in a tertiary care hospital. J Dr NTR Univ Health Sci 2012;1:222-226
19. Pethani JD, Gusani J, Rathod S, Shah PD. Candida species identification from clinical specimens and its changing pattern in a tertiary care hospital. IJABMS Jan 2013. Available from Indian Journals.com. Accessed on 2nd Nov 2013.
20. Paswan AK, Raju DC, Singh DK, Khuba S, Dubey RK. Isolation and distribution of Candida species among different clinical situations in critically ill patients: prospective study. Int J Biomed Res 2012;3(02):120-126
21. Yashavanth R, Shiju MP, Bhaskar UA, Ronald R, Anita KB. Candiduria: Prevalence and trends in antifungal susceptibility in a tertiary care hospital of Mangalore. J ClinDiag Res 2013;7(11):2459-2461
22. Flevri A, Theodorakopolou M, Velegraki A, Armaganidis A, Dimopoulos G. Treatment of invasive candidiasis in the elderly: a review. Clin Interventions Aging 2012;8:1199-1208