RESEARCH ARTICLE

ISOLATION, IDENTIFICATION AND ANTIFUNGAL SUSCEPTIBILITY TESTING OF CANDIDA ISOLATES FROM VARIOUS CLINICAL SPECIMENS AT A TERTIARY CARE HOSPITAL, WESTERN RAJASTHAN

Dr. Sandeep Arora1, Dr. Smita Kulshreshtha2, Dr. Usha Verma3 and Dr. Prameshwar Lal4

1. Resident, Dept. of Microbiology, Dept. S. N. Medical College, Jodhpur, Rajasthan.
2. Professor, Head of Dept. of Microbiology, Dr. S. N. Medical College, Jodhpur, Rajasthan.
3. Senior Demonstrator, Dept. of Microbiology, Dr. S. N. Medical College, Jodhpur, Rajasthan.
4. Senior resident, Dept of Pediatric Surgery, Dept. S. N. Medical College, Jaipur, Rajasthan.

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Abstract

Background: Candida spp is a member of the normal flora of the skin, mucous membrane and gastrointestinal tract. Candida continues to be leading cause of morbidity and mortality in large population of immunocompromised and hospitalized patients. Invasive Candidiasis due to non-albicans candida has been on the rise in last few years. This study aims to Spectate Candida using chromogenic medium. The emerging pathogens are resistant to conventional antifungal therapy.

Objective: To identify the various species of candida isolated from different clinical specimens and to compare the susceptibility pattern of these isolated species towards different antifungal agents.

Methods: All Candida isolates recovered from various clinical samples during the period from September 2017 and August 2018 were studied. These isolates were subjected to gram's stain, germ tube test and inoculation on commercially available CHROM agar (HiMedia India).

Results: A total of 155 Candida species were isolated from the different clinical specimens of suspected candida infection cases. Most of the isolates obtained were from urine samples 93 (60%) followed by blood 26(16.77%). Non albicans Candida were isolated at a higher rate 101 (65.16%) than Candida albicans 54 (34.84%) . Among 101 non C. albicans, C. tropicalis 55 (35.48%) was the most common species followed by 19 (12.26%) C. parapsilosis. Among all species of Candida commonest isolate was C. tropicalis 55(35.48%) followed by C. albicans 54(34.83%). Candida species from various samples were high resistant to itraconazole (72.26%) followed by fluconazole(70.92%), voriconazole (68.39%) and ketoconazole (57.42%)while there was minimum resistance to amphotericin-B (20%). This study emphasizes the need for monitoring local epidemiologic data and antifungal susceptibility pattern of candida isolates for proper treatment.

Conclusions: Along with Candida albicans, non-albicans candida spp like C. tropicalis, parapsilosis, C. krusei and C. glabrata are increasingly being isolated from clinical samples. CHROM agar is a

Corresponding Author:- Dr. Smita Kulshreshtha
Address:- Professor, Head of Dept. of Microbiology, Dr. S. N. Medical College, Jodhpur, Rajasthan.
Introduction:-

*Candida* is yeast like fungus and ubiquitous human commensal. They become pathogens and cause infections when local or systemic host resistance lowered down. The Candida species have been recognized as the fourth commonest cause of nosocomial invasive infections. Candidiasis has emerged as an alarming opportunistic disease as there is an increase in number of patients who are aged, immune-compromised, receiving prolonged antibacterial and aggressive cancer chemotherapy or undergoing invasive surgical procedures and organ transplantation.

*Candida albicans* is the most common cause of candidiasis accounting for about 60-80% of infections. An increase in prevalence of non-albicans species has been noted during last decades. Also, in recent years non-albicans *Candida* (NAC) species are considered as major pathogens causing severe infections in human beings. Characterization to species level helps to identify those strains which might be intrinsically resistant to some antifungal agents.

The commonly used antifungal drugs show significant variation in the susceptibility pattern among the types of *Candida* species. The drug resistance scenario has been increasing during last decades due to over growing use of random antifungal agents. Several previous studies reported the emergence of drug resistance *Candida* species in global scenario. Therefore, the change in drug susceptibility pattern of *Candida* species and introduction of newer antifungal agents has made the in vitro susceptibility testing of antifungal agents more relevant for using specific and sensitive drugs. Thus, the early identification, speciation and susceptibility testing of *Candida* species in clinical specimens have become increasingly important to prevent the treatment failure using appropriate antifungal agent.

Aims and Objectives:-

1. To identify the various species of candida isolated from different clinical specimens.
2. To compare the susceptibility pattern of these isolated species towards different antifungal agents.

Materials And Methods:-

We enrolled 2997 clinical specimens for this study. This study was conducted between September 2017 and August 2018. The clinical specimens were urine, pus from ear & trachea, tracheal devices, blood, CSF, sputum, throat swab, indwelling medical devices, conjunctival swab, various body fluids and semen. These specimens were collected from OPD and IPD patients of all age and sex groups during the period of 1 year. All specimens were investigated for fungal culture and identification of species after obtaining ethical clearance from the Institutional Ethics Committee.

All clinically suspected samples were subjected to gram staining to look for presence of Gram positive yeast like budding cells with pseudohyphae indicating presence of candida species and KOH mount. The samples were inoculated on Sabouraud’s dextrose agar (SDA) with chloramphenicol & Blood agar and incubated at 37°C and 25°C 24-72 hours. Further identification and speciation of Candida on SDA were confirmed by Gram’s stain, Germ tube test, 0.1% Glucose agar test, Sugar fermentation test, Sugar assimilation test, CHROM agar according to standard microbiological techniques.

Germ tube test was carried out by inoculating isolated yeast cells into 0.5 ml of pooled human serum in a small tube and incubation at 37°C for 2 hours. Germ tubes formation was observed microscopically as tubular elongation extending from the yeast cells without constriction or septa at the point of attachment to the yeast cells. 0.1% Glucose agar test were inoculated and incubated at 30°C for 2-5 days and studied microscopically for the presence of pseudohyphae, chlamydospores and blastospores. Isolated candida species were sub-cultured on chromogenic Candida medium (HICHROME Candida agar) and incubated at 37°C for 48 hours. Presumptive species identification was done based on specific colony colors produced by the chromogenic substrates in the medium. All isolates were further identified by carbohydrate assimilation.
All the isolates were subjected to the antifungal susceptibility test according to CLSI document M 44 – A2 by disk diffusion testing method for yeasts. Muller Hinton agar supplemented with 0.5μg/ml Methylene Blue Dye and 2% Glucose (MHMB) was used for sensitivity testing. The inoculated plates were incubated at 37°C for 24 hours or longer.

**Results:**
We processed total 2997 samples during the time period of this study, in which 155 (05.17%) Candida species strains were isolated. Majority of the patients belonged to 0-10 years age group 36 (23.23%) followed by 41-50 years of age group 24 (15.48%). The male and female patients ratio of Candidiasis was 90:65 (1.38 : 1) respectively. In this study, maximum number of Candida species strains recovered from Urine samples 93 (06.97%) followed by Blood 26 (12.56%), Sputum 22 (14.76%), Indwelling medical devices 05 (06.10%), Tracheal 03 (01.17%), Pus/Ear swab 2 (00.37%), Fluid sample 2 (03.57%), Conjunctival swab 1 (01.75%) and Throat swab 01 (01.04%). Out of 155 Candida species, 54 (34.84%) were *C. albicans* and 101 (65.16%) were non *C. albicans* (Fig. no. 1). Among 101 non *C. albicans*, *C. tropicalis* 55 (35.48%) was the most common species followed by 19 (12.26%) *C. parapsilosis*, 14 (09.03%) *C. krusei*, 09 (05.81%) *C. glabrata* and 04 (02.58%) were *C. kefyr* (Table no. 2). Candida species strains were isolated 90.97% in indoor patients and 09.03% in outdoor patients. *C. tropicalis* (37.59%) is the major isolated species in IPD patients which is followed by *C. albicans* (32.62%), *C. parapsilosis* (12.77%), *C. krusei* (08.51%), *C. glabrata* (05.67%) and *C. kefyr* (02.84%) whereas in OPD patient *C. albicans* (57.14%) is the major cause of candidiasis. The isolated Candida species from various samples were high resistant to itraconazole (72.26%) followed by fluconazole (70.92%), voriconazole (68.39%) and ketoconazole (57.42%) while there was minimum resistance to amphotericin-B (20%) (Table no. 3).

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**Fig. no. 1:** Distribution of *Candida albicans* and Nonalbicans Candida isolated from different clinical specimens.

**Table 2:** Candida species isolated from different clinical samples.

| Types of Specimen | *C. albicans* (%) | *C. glabrata* (%) | *C. krusei* (%) | *C. parapsilosis* (%) | *C. tropicalis* (%) | *C. kefyr* (%) | Total (%) |
|-------------------|------------------|------------------|----------------|-----------------------|---------------------|--------------|-----------|
| Urine             | 37 (39.79%)      | 08 (08.60%)      | 06 (06.45%)    | 08 (08.60%)           | 31 (33.33%)         | 03 (03.23%)  | 93 (60.0%) |
Table 3: Antifungal susceptibility Pattern for Candida species isolated from various samples.

| Candida Species Isolated | S (%) | R (%) | S (%) | R (%) | S (%) | R (%) | S (%) | R (%) | S (%) | R (%) |
|--------------------------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|
| C. albicans              | 21 (38.8%) | 33 (61.1%) | 15 (27.7%) | 39 (72.2%) | 12 (22.2%) | 42 (77.7%) | 39 (72.2%) | 15 (27.7%) | 16 (29.6%) | 38 (70.3%) |
| C. krusei               | 03 (21.4%) | 11 (78.5%) | - | - | 03 (21.4%) | 11 (78.5%) | 08 (57.1%) | 16 (29.6%) | 03 (21.4%) | 11 (78.5%) |
| C. tropicalis          | 27 (49.1%) | 28 (50.9%) | 14 (25.4%) | 41 (74.5%) | 17 (30.9%) | 38 (69.1%) | 47 (85.4%) | 08 (14.5%) | 17 (30.9%) | 38 (69.1%) |
| C. glabrata            | 05 (55.5%) | 04 (44.4%) | 04 (44.4%) | 05 (55.5%) | 04 (44.4%) | 05 (55.5%) | 08 (88.9%) | 01 (11.1%) | 04 (44.4%) | 05 (55.5%) |
| C. kefyr               | 01 (25%) | 03 (75%) | 01 (25%) | 03 (75%) | 02 (50%) | 02 (50%) | 03 (75%) | 01 (25%) | 01 (25%) | 03 (75%) |
| C. parapsilosis is     | 09 (47.3%) | 10 (52.6%) | 07 (36.8%) | 12 (63.2%) | 05 (26.3%) | 14 (73.6%) | 19 (100%) | 00 (0%) | 08 (42.1%) | 11 (57.8%) |
| **Total**             | 99     | 89     | 41     | 100    | 43     | 112    | 124    | 31     | 49     | 106    |
Discussion:
Fungal infections, particularly those attributed to Candida species, are frequent complications for hospitalized patients contributing to increased morbidity and mortality and healthcare cost. Furthermore, there is increasing prevalence of infections caused by non-albicans Candida worldwide, with various degrees of susceptibility to routinely used antifungal agents indicating the importance of laboratory diagnoses.

In this study, it was observed that candidiasis can occur at all ages and in both sexes. The highest number of isolates were obtained in the age group of 0-10 years (23.23%), followed by the age groups of 41-50 years (15.48%). Infections were more common in the 0-10 age groups in this study. This can be attributed to the various co-morbid conditions and the health issues pertaining to the particular age groups, as is relevant in this study. However, further studies have to be carried out to justify the significance of the fact. In present study, males were more affected than females with an overall male:female ratio of (90:65) 1.38. Candida tropicalis is major isolated species. In male patients, Candida tropicalis (36.67%) is the major isolated species whereas in female patients, Candida albicans (36.92%) is the major organism. The preponderance of male patients suffering from candidiasis in this study correlates with Singh et al.

In the present study, out of total 2997 specimens, 155 (5.17%) Candida species strains isolated. Candida species were isolated mainly from urine samples 93 (60%) followed by blood 26 (16.77%), sputum 22 (14.19%), indwelling medical devices samples 05 (03.23%). The study done by Shilpa et al. shows maximum Candida species isolates from urine samples 66 (46.5%) followed by genital discharge 39 (27.5%), blood 16 (11.3%), sputum 9 (6.3%), pus 7 (4.9%) and 5 (3.5%) from plastic devices (catheter tip, central line tip). Dutta et al. also reported maximum number of Candida isolates from urine samples 31 (37%) followed by sputum 18 (21%) and blood 03 (4%) which are less than present study. These prevalence rates differ in other studies because they depend upon factors like kind and number of samples received, type of hospital, and the geographical place where the studies were conducted.

Among these 155 Candida species isolated, non-Candida albicans species were more in number than C. albicans. The most common among non-C. albicans species included Candida tropicalis (35.48%), followed by C. parapsilosis (14.09%), C. krusei (05.81%), C. glabrata, and 04 (02.58%) were C. kefyr. C. albicans accounted for 54 (34.84%) of the isolates. The studies done by Dutta et al., Shivaprakasha et al., Verma et al. also indicate a trend towards an increasing prevalence of infections caused by species of non-Candida albicans. Generally, Candida causes opportunistic infection. If a person is hospitalized, having low immunity, taking prolonged antibacterial treatment, etc., these are the conditions where one can be more susceptible to Candida infection.

The disk diffusion method, according to CLSI guidelines, was used for antifungal susceptibility testing. The isolates in the study showed lower resistance to amphotericin-B.

Among the Candida species, the rate of resistance exhibited by C. albicans is maximum in itraconazole (77.78%) followed by fluconazole (72.22%), voriconazole (70.37%), ketoconazole (61.11%) whereas amphotericin-B (27.78%) shows minimum resistance.

Candida krusei has intrinsic resistance towards fluconazole. The other antifungal like ketoconazole, itraconazole and voriconazole shows 78.57% rate of resistance whereas in amphotericin-B, it is 42.86%. The resistance to antifungals in Candida tropicalis is maximum to fluconazole (74.55%) followed by 69.09% in itraconazole and voriconazole, 50.90% in ketoconazole and minimum in amphotericin-B (14.55%).

Candida glabrata shows 55.56% of resistance to fluconazole, itraconazole and voriconazole while 44.44% to ketoconazole whereas only 11.11 % of resistance shown by amphotericin-B. Candida kefyr is mostly sensitive to amphotericin-B (75%) followed by itraconazole (50%) and 25% to ketoconazole, fluconazole, and voriconazole. The resistance pattern in Candida parapsilosis shows highest resistance towards itraconazole (73.68%) followed by fluconazole (63.16%), voriconazole (57.89%) and ketoconazole (52.63%) while amphotericin-B shows no resistance.
Overall, the non-Candida albicans species showed more resistance than C. albicans to applied antifungal agents except amphotericin-B. These similar findings correlate with study done by Dutta et al.\(^\text{17}\).

**Conclusion:**

The present study suggests an increasing prevalence of non-Candida albicans species in the various clinical samples isolated. An emergence of resistance of these Candida species isolates to the routinely used antifungals, make them difficult to treat.

Therefore, detection of distribution of Candida through presumptive identification, followed by confirmation and antifungal treatment, has an efficient effect on successful treatment as it helps to optimum selection of the therapeutic agent and use of CHROMagar is a simple, rapid and inexpensive method for identification of Candida species especially in the laboratory with limited resources.

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