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Characterization of Candida Species from Clinical Isolates: Our experience at MIMSR Medical College, Latur, Maharashtra

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Abstract

Introduction: Approximately 90% of human invasive fungal infections are caused by only five species: *Candida albicans, Candida tropicalis, Candida parapsilosis, Candida glabrata and Candida krusei. Candida* is usually a commensal of digestive tract, genitourinary tract, and skin. It mainly originates endogenously and turns pathogenic because of alteration of host immunity. Varieties of factors are known to predispose superficial and deep-seated candidiasis. **Objectives:** To isolate and identify various species of candida responsible for causing clinical candidiasis. **Methodology:** Prospective study was carried out in Department of Microbiology, MIMSR Medical college, Latur from January 2015 to July 2016. One hundred and thirty-six specimens such as vaginal swab, oral swab, blood, pus, sputum, urine and body fluids were collected from clinically diagnosed cases of candidiasis. **Results:** A total 101 (74.3%) *Candida* species were isolated from 136 clinical specimens. Highest isolation rate was from vaginal swab 41 (87.2%). Vaginal candidiasis was found to be the most common form of candidiasis, contributing 41(40.6%) of Candida isolates. Out of 101 *Candida* species isolated in the present study, *Candida albicans* 48(47.5%) was the predominant species. Candidiasis was the most commonly seen in age group of 31-40 years 30 (29.7%).

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Conclusion: Maximum number of isolates were from vaginal candidiasis 41 (87.2%) followed by oral candidiasis 26(74.3%). Most common species isolated was *Candida albicans* 48 (47.5%) followed by *Candida tropicalis* 26 (25.7%). Females 53 (52.5 %) were more commonly affected than males 48(47.5%).

Key words: Candida, clinical candidiasis, fungal infection

Introduction

With the remarkable modern advances in medicine, there has been an increase in the number of immuno-compromised individuals who need extensive care in hospitals. This has resulted in a rise in the incidence of fungal infections which are also recognized as significant causes of mortality and morbidity. Factors contributing for immune-compromised status in individuals are use of steroids, antimicrobials immunosuppressive and anticancer drugs, bone marrow or solid organ transplants, HIV positivity, metabolic disorders like diabetes mellitus.[1] Among the fungal infection in human beings, candidial infections are predominantly reported.[2]

There is also alarming increase in infections caused by multi resistant bacteria leading to overuse of broad-spectrum antimicrobials, which leads to overgrowth of Candida thus enhancing opportunity to cause disease.[3] Candida infections are a problem of growing clinical importance. The incidence of infection has increased dramatically over the past two to three decades and this trend will inevitably continue into twenty first century.[4] Although Candida albicans remains the most common Candida species encountered, the morbidity and mortality caused by non-albicans Candida (NAC) species is increasing.[5]

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Approximately 90% of human invasive fungal infections are caused by only five species: *Candida albicans, Candida tropicalis, Candida parapsilosis, Candida glabrata and Candida krusei.*[2] *Candida* is usually a commensal of digestive tract, genitourinary tract, and skin. It mainly originates endogenously and turns pathogenic because of alteration of host immunity. Varieties of factors are known to predispose superficial and deep-seated candidiasis. [6] All these factors act by altering the balance of normal microbial flora of body or by lowering host resistance. Mostly numerous factors operate collectively and a full fledges infection takes place.[1]

Isolation of *C. albicans* has been associated with infections, as well as colonization, in both immunocompromised and immunocompetent patients.[7] Over the last two decades both the incidence of nosocomial candidemia and proportion of bloodstream infections due to *Candida* species other than *Candida albicans* has increased, this attributable mortality and morbidity associated with hospitalization remain significant in all age groups despite therapeutic advances. Non-*albicans Candida* is of special concern since some are associated with treatment failure due to reduced susceptibility to antifungal agents.[8,9,10]

As drug resistance in *Candida* is now common and antifungal susceptibility testing can play major role in management of resistant candid infections. Describing the unique susceptibility patterns of *Candida* species can help to identify the appropriate therapy. Thus, Increase in *Candida* infections, emergence of unusual species as pathogen and increasing resistance to antifungal agents emphasize the need to isolate, identify *Candida* species and to study antifungal susceptibility. There is also a need to monitor laboratory data for study of geographical variation of species, possible emergence of resistance and for selection of the most appropriate antifungal agents.

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Objectives

- To isolate and identify various species of candida responsible for causing clinical candidiasis.

Methodology

Prospective study was carried out in Department of Microbiology from January 2015 to July 2016. Ethical clearance was obtained from institutional ethical committee.

One hundred and thirty-six specimens such as vaginal swab, oral swab, blood, pus, sputum, urine and body fluids were collected from clinically diagnosed cases of candidiasis.

A detailed history regarding age, sex, h/o antibiotic, steroids, oral contraceptive intake was taken. Presence of associated risk factors like diabetes mellitus, HIV, malignancy, duration of hospital stay, duration of catheterization, h/o antifungal treatment and any other underlying disease was recorded.

A. Collection of specimens

Oral swab - Oral swab were collected from whitish patches on mucous membrane of mouth. Care was taken to avoid contamination of swab with saliva.

Vaginal swab - Vaginal discharge was collected with a sterile cotton wool swab.

Urine - Early morning fresh mid-stream, clean catch urine samples were collected in wide sterile, screw capped containers and sent immediately for processing. The urine samples were refrigerated at 40 C in case of delay in processing.

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Sputum - Sputum was collected in the morning, but before breakfast. Patients were instructed to rinse their mouths vigorously with water immediately before coughing and to collect sputum by deep coughing into a sterile, screw-capped container.

Pus - Pus specimens were collected with the help of a sterile cotton wool swabs.

Blood - Specimens were collected by venepuncture after cleaning the site with 1-2% tincture iodine using needle and syringe. Blood was immediately added to Aerobic Plus blood culture BACTEC bottle and processed in BACTEC 9050 system.

Body fluids - CSF- A sterile wide-bore lumbar puncture needle is inserted between the fourth and fifth lumbar vertebrae and the CSF was allowed to drip into a dry sterile container and immediately added to BACTEC blood culture bottle.

Other body fluids - Other body fluids were collected with all aseptic precautions and with suitable procedures and added to BACTEC culture bottles.

Repeat collection and isolation was done for samples such as oral swab, vaginal swab and sputum for the confirmation of pathogenicity of isolates while single isolation was considered significant for blood and body fluids.

B. Processing of specimen

I. Direct examination:

a) Wet Mount:

The specimens were examined as KOH mount. Specimen was placed on the glass slide then 10% potassium hydroxide (KOH) was added. Coverslip was placed. The slide was slightly warmed and gentle pressure was applied over the coverslip to remove the trapped air.

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Preparation was examined carefully under the low power (10x) and high power (40x) to identify the pseudohyphae and yeast cells.

b) Gram Stain:

Smears were made from specimen and also from positive BACTEC culture bottles over clean grease free slide and allowed to air dry. Material was fixed to slide by passing slide 3-4 times through the flame of Bunsen burner. Smear were stained with Gram's stain and examined for gram positive budding yeast cells and pseudohyphae.

II. Isolation of candida species:1

a. Culture:

Specimens were inoculated on two slants of Sabouraud dextrose agar (SDA) with antibacterial antibiotics (chloramphenicol, gentamicin). One was incubated at 250 C and another at 370 C. Slants were observed after 24-72 hours.

Colonies were identified by colony morphology such as cream colour, pasty, and smooth apperance

b. Microscopy:

1. Gram stain:

Smear from colonies was prepared and stained by Gram stain. Candida

species appears as Gram positive budding yeast cells and pseudohyphae.

2. India ink preparation

India ink wet mount was examined to observe capsule and to rule out cryptococcus species.

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3. Lactophenol cotton blue (LCB) mount:

LCB mount was prepared from colonies to examine yeast cells and pseudohyphae

III. Species identification

Species identification was done with the help of following tests. a Germ tube test

b Cornmeal agar culture

c Culture on CHROM agar Candida:

d Carbohydrate assimilation test.

e Sugar fermentation test.

a) Germ tube test:

Procedure:

1. A small portion of an isolated colony of the yeast to be tested was suspended in a test tube containing 0.5 ml human serum.

2. The test tube was incubated at 350 C for 2 hours.

3. A drop of yeast-serum suspension was placed on a microscopic slide, overlaid with a coverslip and examined microscopically for presence of germ tubes.

Observation: Filamentous extension from yeast cell with no constriction at the neck was considered as germ tube.

b) Cornmeal agar culture (Dalmau plate culture)1

Procedure:

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1. Isolated colonies of Candida were picked up with inoculating wire.

2. Three parallel cuts 1 cm apart were made into the surface of Cornmeal agar, holding the inoculating wire at about a 450 C angle.

3. A sterile coverslip was laid on the surface of agar, covering a portion of the inoculated streaks.

4. The inoculated plates were incubated at 300 C for 24-48hours in a closed moisturized chamber.

5. At the end of incubation period plates were examined microscopically (under 10x and 40X).

Observation -

Pattern of growth observed at edge of coverslip.

C) Culture on CHROM agar Candida:1

Procedure:

1. Isolated species were inoculated on Hi Chrome differential agar.

2. These agar plates were incubated at 370 C for 48-72hours.

Observation: characteristic colony colour was noted as per HiMedia technical data M1297 A.

a) *C. albicans* - Light green.

b) *C. tropicalis*- Blue with pink halo

c) *C. glabrata*-Pink to purple

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d) *C. krusei*- Pink.

e) *C. parapsilosis*: Cream to pale pink.

f) *C. dubliniensis*: Dark green.

D) Carbohydrate assimilation test:

Procedure:

1. Yeast suspension was made from a 24-48 hrs old culture in 4ml of distilled water. The turbidity of suspension was adjusted to match no.4 McFarland standards.

2. Yeast nitrogen-based agar plates containing bromocresol purple were covered with this suspension.

3. The excess inoculum was removed and surface of plates was allowed to dry

4. With sterile forceps, selected carbohydrate discs with 4% concentration were placed on the surface of agar 30 mm apart.

5. Glucose, maltose, sucrose, lactose, galactose, melibiose, celebiose, inositol, xylose, raffinose, trehalose discs were used.

6. Incubate Carbohydrate utilization plate at 30 degree C for 24-48hours.

Observation: Presence of growth or colour change around the disc containing carbohydrate was considered as indicator of carbohydrate assimilation.

Results

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A total of 101 (74.3%) *Candida* species were isolated from 136 clinically diagnosed cases of candidiasis.

Table 1: Isolation rate of *Candida* species from clinical specimens

Specimen	**Total No. of specimens received**	***Candida* isolated N (%)**
Vaginal swab	47	41 (87.2)
Oral swab	35	26 (74.3)
Blood	20	14 (70.0)
Urine	19	14 (73.7)
Sputum	15	06 (40.0)
Total	136	101 (74.3)

In the present study, the most common specimen received was vaginal swab followed by oral swab, blood, urine and sputum. A total 101 (74.3%) *Candida* species were isolated from 136 clinical specimens. Highest isolation rate was from vaginal swab 41 (87.2%) followed by oral swab 26(74.3%), urine 14 (73.7%) and blood 14(70.0%), sputum 06 (40.0%).

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Table 2: Distribution of *Candida* isolates from different clinical forms of candidiasis.

Clinical form of candidiasis	*Candida* isolates n=101N (%)
Vaginal candidiasis	41 (40.6)
Oral candidiasis	26 (25.7)
Candidemia	14 (13.9)
Urinary candidiasis	14 (13.9)
Bronchial candidiasis	06 (5.9)

A total 101 isolates were obtained from different clinical forms of candidiasis. Vaginal candidiasis was found to be the most common form of candidiasis, contributing 41(40.6%) of Candida isolates. The next common form seen was oral candidiasis 26 (25.7%) followed by candidemia 14 (13.9%) and urinary candidiasis 14 (13.9%). Bronchial candidiasis 6 (5.9%) was the least common form of candidiasis observed in the present study.

Table 3: Distribution of *Candida* species isolated (n=101)

***Candida* species isolated**	**No (%)**
Candida albicans	48 (47.5)
Candida tropicalis	26 (25.7)
Candida parapsilosis	13 (12.9)
Candida glabrata	7 (6.9)
Candida krusei	3 (3.0)
Candida dubliniensis	2 (2.0)
Candida guilliermondii	2 (2.0)
Total	101 (100)

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Out of 101 *Candida* species isolated in the present study, *Candida albicans* 48(47.5%) was the predominant species followed by *Candida tropicalis* 26(25.7%), *Candida parapsilosis* 13(12.9%), *Candida glabrata* 7(6.9%), *Candida. krusei* 3 (3.0%). *Candida dubliniensis* and *Candida guilliermondii* 2 (2.0%) each were the least commonly isolated species in the study.

Table 4: Age wise distribution of clinical forms.

Age	Vaginal Candidiasis N (%)	Oral Candidiasis N (%)	Candidemia N(%)	Urinary Candidiasis N (%)	Bronchial Candidiasis N (%)	Total N (%)
0 -28 days	0 (0)	3 (11.5)	6 (42.9)	1 (7.1)	0 (0.0)	10 (9.9)
29 days-1 yr	0 (0)	1 (3.9)	1 (7.1)	0 (0)	0 (0)	2 (2.0)
1-12 yrs.	0 (0)	2 (7.7)	1 (7.1)	1 (7.1)	0 (0)	4 (4.0)
13-20 yrs.	4 (9.8)	2 (7.7)	0 (0)	0 (0)	0 (0)	6 (5.9)
21-30 yrs.	19 (46.3)	5 (19.2)	0 (0)	1 (7.1)	1(16.7)	26(25.7)
31-40 yrs.	15 (36.6)	10 (38.5)	0 (0.0)	1 (7.1)	4 (66.7)	30 (29.7)
41-50 yrs.	2 (4.9)	2 (7.7)	2 (14.3)	3 (21.4)	0 (0)	9 (8.9)
51 yrs. onwards	1 (2.4)	1 (3.9)	4 (28.6)	7 (50.0)	1 (16.7)	14 (13.9)
Total	**41 (100)**	**26 (100)**	**14 (100)**	**14 (100)**	**69 (100)**	**101 (100)**

Candidiasis was the most commonly seen in age group of 31-40 years 30 (29.7%) followed by 21-30 years 26(25.7%). Age group most commonly affected by vaginal candidiasis was 21-30 years 19 (46.3%) followed by 31-40yrs 15(36.6 %). Oral candidiasis was frequently seen 10 (38.5%) in 31-40 years of age group, followed by 21-30yrs. 5(19.2%). Urinary candidiasis was most frequently 7 (50.0%) encountered in > 50 years age group, followed by 41-50 years 3

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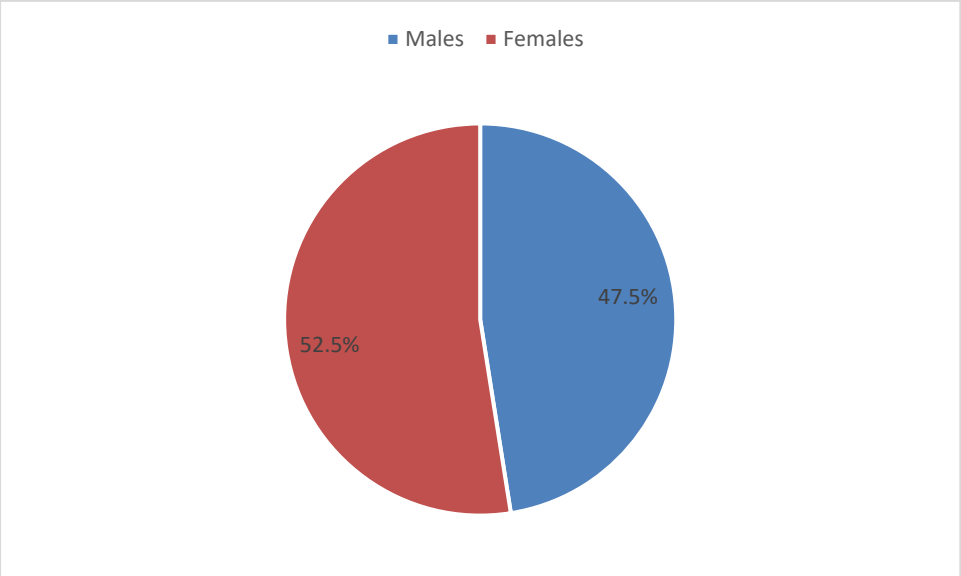
(21.4 %). Candidemia was predominantly seen 6 (42.9%) in 0-28days age group i.e. in neonates and in 4 (28.6%) of 51yrs onwards i.e.in old age group. The most common age group with bronchial candidiasis was 31-40yrs 4 (66.7%).

Figure 1: Gender wise distribution of the cases



Females 53(52.5%) were more commonly affected than males 48 (47.5%).

Discussion

Isolation rate of Candida species from clinical specimens (Table 1)

In the present study, 101 (74.3%) Candida species were isolated from 136 clinically diagnosed cases of candidiasis. Comparable observations were made by Mohandas V et al[11] who reported isolation rate as 73%. Isolation rate of Manjunath et al[12] was higher 90% while

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that of C. Roopa et al[13] was low 50%. Difference in isolation rate may be due to difference in climate, temperature.

Distribution of Candida isolates from different clinical forms (Table 2)

Clinical forms observed in study were vaginal candidiasis, oral candidiasis, candidemia urinary candidiasis and bronchial candidiasis. Vaginal candidiasis was found to be the most common form of candidiasis, contributing 41(40.6%) of Candida isolates. The most common specimen received was also vaginal swab. Similar observations were made by Dharwad et al. [14] They found vaginal candidiasis as the commonest form 38 % in their study.

Vaginal candidiasis is one of the most common infections among adult women in reproductive age group seen in general practice. About three quarter of all women suffer at least one episode of this condition during life time and around half of them have recurrence.[1]

The next common form seen in present study was oral candidiasis contributing for 26 (25.7%) of isolates. Abu-Elteem et al[15] also reported it as common form.

Oral candidiasis is a common form of disease produced by colonization of Candida species, also called as oral thrush. Upto 75% healthy individuals carry yeast Candida as part of their normal oral commensal flora. However, in past 3 decades, infections due to Candida species have increased and are of particular importance because of the rising number of immunocompromised patients78 wide spread use of antibiotics may be another factor. [6]

In our study, other forms of candidiasis seen were candidemia14 (13.9 %), urinary candidiasis 14 (13.9 %), bronchial candidiasis 06 (5.9 %). Patel LR et al[16] reported urinary candidiasis as commonest form followed by candidemia contributing 30.5% and 26 % of

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Candida isolates. Candidial bronchitis was also reported by Dalal PJ et al[17] in 12 % of the patients. Bronchial candidiasis 06 (5.9%) was the least common form of candidiasis observed in the present study.

Distribution of Candida species isolated (Table 3)

Out of 101 Candida species isolated in the present study, Candida albicans 48 (47.5%) was the predominant species. This observation correlates with C. Roopa et al[13] Dharwad et al[14] and B. Madhumati et al[18] who also reported isolation rate of Candida albicans 50.70%, 47% and 46% respectively while Manjunath et al[12] have reported slightly higher percentage 52%.

In the present study next common species is by Candida tropicalis 26 (25.7%) which was also the most common non-albicans Candida isolated. Similar findings were noted by C. Roopa et al[13], T. Jaggi et al[19] and the isolation rate was 28.6% and 27.7%, 26.4% respectively in their study. Slight lower isolation rate was reported by B. Madhumati et al[18] 22% and Manjunath et al[12] 19%. Although Candida albicans is the most common agent of candidiasis an increasing incidence of less common species of Candida has also been documented in last few years. Candida tropicalis is emerging pathogen from non- albicans Candida group globally.

In the present study next common species was Candida parapsilosis 13(12.9%). This finding is in correlation with Tavleen Jaggi et al[19] Isolation rate reported by them was 12.80%. Manjunath et al[12] and B. Madhumati et al[18] reported it in lower percentage i.e., 8%, 7.40% and 1% respectively. Use of IV catheters and lack of compliance with hand washing by health care workers were reported to increase Candida parapsilopsis infection.

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Isolation rate of Candida glabrata was 7(6.9%) in the present study. Comparable observations were also made by C. Roopa et al[13] (5.80%). High isolation rate was reported by B. Madhumati et al[18] 26%, Manjunath et al[12] 13%, Tavleen Jaggi et al[19] 11.2%

Conclusion

- In the present study total 101 (74.3%) Candida species were isolated from 136 clinically diagnosed cases of candidiasis.
- Maximum number of isolates were from patients vaginal candidiasis 41 (87.2%) followed by oral candidiasis 26(74.3%).
- Most common species isolated was Candida albicans 48 (47.5%) followed by Candida tropicalis 26 (25.7%).
- Candida tropicalis 26 (25.7%), was the commonest non-albicans Candida species isolated.
- Isolation rate of non-albicans Candida species was higher 53 (52.5%) than Candida albicans 48(47.5%).
- Candidiasis was the most commonly seen in age group of 31-40 yrs.
- Females 53 (52.5 %) were more commonly affected than males 48(47.5%).

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