Isolation, Speciation and Characterization of Candida Species in Clinical Isolates

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A B S T R A C T

One hundred clinical isolates of Candida were studied during a period of one year. Most common clinical sample was urine (38%) followed by sputum (17%) and blood (10%). Most common species isolated was Candida albicans (42%) followed by Candida tropicalis (34%), C. parapsilosis (11%), C. glabrata (6%), C. krusei (5%) and C. dubliniensis (2%). Non albicans candida were isolated at a higher rate (58%) than Candida albicans. To conclude the study it showed that prevalence of non albicans candida was higher than C. albicans from various clinical specimens.

Keywords
Isolation, Speciation and Characterization.

Article Info
Accepted: 04 June 2017
Available Online: 10 July 2017

Introduction

Over the last few years, the incidence of mycotic infections has progressively increased. Fungi once considered as non-pathogenic or less virulent are now recognized as a primary cause of morbidity and mortality in immune-compromised and severely ill patients (Mokaddas et al., 2007).

Candida species are among the most common fungal pathogens. They are ubiquitous yeasts found on plants and form the normal flora of alimentary tract of mammals and the mucocutaneous membranes in humans (Adhikary et al., 2011).

They are commonly found on the skin, mucosa, nails, throughout gastrointestinal tract and female genital tract particularly higher in vagina. The overall carriage rate in healthy individuals has been estimated to reach 80 %. The most commonly isolated Candida species from the gastrointestinal tract of humans is Candida albicans, followed by Candida tropicalis and Candida parapsilosis (Agarwal et al., 2011). Candida glabrata is most often isolated from the mouth.

Candidiasis is the commonest fungal disease found in humans. The infection may be acute or chronic, superficial or deep and its clinical spectrum is wide. It is found mainly as secondary infection in individuals with some underlying immune compromised condition and very rarely as primary disease.

Non albicans species are emerging pathogens and can also colonize human mucocutaneous surfaces and invades tissues; leading to life threatening diseases in patients whose cell
mediated immunity is decreased by disease or iatrogenic intervention (Ajello, 1997; Verma, 2003; Akpan, 2002 and Al- abeid et al., 2004). Now, Candida species constitute the third to fourth most common causes of nosocomial blood stream infections. In recent years HIV has been identified as one of the most important predisposing conditions for candidiasis. Other predisposing factors for Candida infections are prolonged use of antimicrobial drugs, diabetes, chemotherapy and catheterization (Ali Zarei, 2013; Anil K Paswan, 2012; Anil, 1997 and Baradkar et al., 1996). Although Candida albicans remains the most common causative agent of both superficial and deep fungal infections, an increasing incidence of Non-albicans species of Candida that cause disease has also been documented in the last few years. These include Candida tropicalis, Candida krusei, Candida parapsilosis, Candida guilliermondii, Candida dubliniensis, Candida glabrata, Candida kefyr and Candida lusitaniae.

Accurate species identification is therefore important for the treatment of the Candida infection as the non albicans species of Candida continue to be increasingly documented and as not all the species respond to the same treatment.

Hence the present study was undertaken at a Tertiary Care teaching hospital of Jaipur to isolate and speciate genus Candida from the various clinical samples and to analyse the predisposing conditions for candidiasis.

Materials and Methods

100 Clinical samples were obtained in a period of one year in the Microbiology Lab of Mahatma Gandhi Medical College and Hospital, Jaipur. A detailed history was taken with a particular emphasis on the natural receptive states like infancy, old age, pregnancy, prolonged administration of antibiotics, past history of chronic diseases such as Diabetes mellitus, tuberculosis, hospital stay duration, AIDS or immunosuppression, cancers, endocrine diseases such as hypothyroidism and post-surgical infections. Presence of signs of other opportunistic infection was looked for and associated diseases if any were recorded. Written informed consent was obtained from each patient. Relevant Clinical samples were collected with complete universal precautions. The samples collected were transported to the mycology lab as soon as possible. Samples were subjected to Gram’s stain to look for presence of Gram positive yeast like budding cells with pseudohyphae and KOH mount.

Media used for culture were Sabouraund Dextrose Agar with Chloramphenicol (5mg/ml), Brain Heart Infusion Broth and 5% Sheep Blood Agar prepared as per the standard procedures. The Lactophenol Cotton Blue (LCB) mount was used to study morphological features of fungal isolates. They were further spesicated by the germ tube test, chlamydospore formation on Corn Meal Agar and carbohydrate utilization patterns by Sugar Assimilation Tests.

Results and Discussion

In this population-based study we have shown the potential clinical importance of species-level identification as Candida species. Our results show, that in an unselected hospital population, as many as one-third of all cases of Candida infection may be attributable to nosocomial clusters, and the risk is highest in wards providing intensive care. Non Albicans Candida species are on the rise due to increasing immune compromised states. In this study of total 100 cases from various clinical isolates, 51 patients (51%) were male and 49 were female patients (49%). Majority of the male patients were in the age group of 51-60 yrs (29%), and majority of female patients were of the age group 31-40 yrs.
Out of 10 HIV positive patients the male: female ratio was 2.3:1. Higher male:female ratio shows that males are at increased risk in comparison to females because their jobs and habits entail them to be more migratory. A similar age group was reflected in studies of Maiken Arendrup (2013). Male predominance was noted in our study (51%) with Candida infection more common in 51-60 years age group males consistent with study of Deorukhkar et al., (2012). However in the study by Kandhari et al., (1969) the incidence was found to be higher in females (61.2%) than in males (38.8%). Rizwi et al., (2011) reported female preponderance in their study group with a ratio of 0.85 : 1 (M:F).

In all the 100 patients included in this study the most common clinical sample was urine in 38 (38%) patients followed by sputum 17 (17%) and blood samples 10 (10%). High vaginal swab was seen in total of 6 (6%) patients followed by 4(4 %) each of pus/wound, ascitic fluid, stool, suction tip and oral swab, 3 (3%) samples were of ear swab followed by 2 (2%) samples each of catheter tip and endotracheal tube tip. Central line tip and Broncho alveolar lavage were 1 % respectively.

Our observation is similar with the studies of Deorukhkar et al., (2014) where urine samples were in majority (34.6%) and Patel et al., (2012) where urine showed the highest number of isolates (34.5%) followed by sputum (28.9%) and blood (26%). Similar findings were seen in study by Marcia Cristina Furlaneto et al., (2011). Studies which were done earlier by Pfaller et al., (1996), have reported Candida species as the seventh most common nosocomial pathogen hospital wide and as that which caused 25% of all urinary tract infections. Most common species isolated was Candida albicans (42%) followed by Candida tropicalis (34%), Candida parapsilosis (11%), Candida glabrata (6%), Candida kruseii (5%) and Candida dublinensis (2%) in clinical samples. The present study is in agreement with the studies conducted by Dastider (72.8%), Gupta (64%) and Mokaddas et al., (2007) (39.5%) which all found Candida albicans to be the commonest isolate. In the present study Non albicans Candida were isolated at a higher rate (58%) than Candida albicans (42%) which was in agreement with the findings of the studies by Mokaddas et al., (2007) which also showed the Non albicans Candida incidence (60.5%) to be higher than that of Candida albicans (39.5%). A study by Chakrabati et al., (1996) also showed Non albicans Candida to have a higher incidence than Candida albicans (75%) and (25%) respectively. Similar findings were observed in study by Saroj Golia et al., (2013). Comparative studies on different Candida species by Manchanda showed that C. tropicalis (55.03%) was higher while it was 34% in our study.

These findings seem to suggest that the Non albicans Candida are emerging as important pathogens. In the urine sample isolates Candida tropicalis (47%) was the most common species followed by Candida albicans (34%), Candida parapsilosis (8%), Candida glabrata (5%), Candida kruseii and Candida dublinensis in 3% each. Studies conducted in Brazilian medical centres have also shown increased rates of isolation of C. tropicalis from urine.

The most common species isolated from sputum was Candida albicans (53%) followed by Candida tropicalis (24%). Blood samples showed the predominance of Candida tropicalis (30%) and Candida parapsilosis (30%) being the most common species followed by Candida albicans (20%) and Candida krusei (20%). The incidence of blood stream infection (BSI) caused by Non
albicans Candida species (80%) was higher than Candida albicans (20%). Among the NAC species C. tropicalis followed by C. parapsilosis, and C. krusei predominantly caused BSI.

In high vaginal swab samples Candida albicans was most common (50%) followed by Candida tropicalis (33%) and Candida glabrata (2%).

In study done by Gross T Norma et al., (2007), C. albicans was the predominant species (75%) followed by C. parapsilosis (12%), C. tropicalis (5.3%) and C. glabrata and C. famata 3.5% each. Similar findings were seen in studies done by Jindal et al., (2006).

In stool samples Candida tropicalis (75%) was the commonest species followed by Candida parapsilosis (25%). Candida albicans was the only species isolated from ascitic fluid, central line tip and bronchoalveolar lavage (BAL) samples.

Most number of IPD cases from the ICU/CCU (37%), followed by casualty/burn wards (12%), medicine (16%), paediatrics (5%), gynae (3%), surgical ward (8%) and remaining from other wards. In this study most frequently associated risk factor is ICU/CCU prolonged stay, being 40% of the total number of cases.

Candida albicans (16) and C. tropicalis (16) were the predominant species isolated; 40% of each, followed by C. krusei (10%), C. parapsilosis (7.5%) and C. dubliniensis (2.5%). Similar findings were seen in a study done by Paswan et al., (2012). In a study done by Giri et al., (2013) C. tropicalis was the most common isolate (74.35%).

In a study done my Chun Fang Ma et al., (2013) C. tropicalis was isolated in 30.8% of the cases followed by C. parapsilosis and C. albicans in percentage of 20% and 17.9% respectively. Incidence of candiduria was high among the patients admitted in ICU/CCU followed by patients who were diabetic and those on prolonged antibiotic intake in the present study. Similar findings were seen in a study done by Deorukhkar et al., (2014). Second most common risk factor in the present study is the immunocompromised status that is 36% of the cases. In the present study most predominant Candida species in such patients is C. albicans followed by C. tropicalis; 42% and 31% respectively. Other species were C. glabrata (11%), C. parapsilosis (8%), C. krusei (6%) and C. dubliniensis (3%).

The third most common risk factor in the present study is the long duration of antibiotic intake accounting for 20% of the total cases.

Candida species isolated from such patients in our study were C. albicans 60%, followed by C. tropicalis 30% and C. parapsilosis 10%. These findings were similar with the study done by Sajjan et al., (2014) where C. albicans, C. krusei and C. tropicalis were 69.7%, 17%, and 13% respectively.

Candida species are emerging as a potentially pathogenic fungus in patients with bronchopulmonary diseases.

In this study Candida co-infection in patients with pulmonary tuberculosis was observed in 16 patients (16%); out of which C. albicans was the most common isolate observed in 50% (8) of the cases, followed by C. tropicalis 31% (5) and C. parapsilosis 19% (3). Similar findings were observed in a study done by Arunava Kali et al., (2013), where C. albicans (50%) has been reported to be the most predominant isolate from TB patients followed by C. tropicalis and C. glabrata (20%) of each, C. parapsilosis (6.7%) and C. krusei (3.3%). The result is in keeping with other similar studies.
Image 1 Candida albicans on Corn Meal Agar

Image 2 Candida tropicalis on Corn Meal Agar

Image 3 Candida albicans (Apple Green) Candida dubliniensis (Dark Green) on chrome agar
**Image 4** *Candida krusei* (Pink Fuzzy) *Candida albicans* (Apple Green) *Candida tropicalis* (Blue)
Out of 100 patients 94% of the cases were admitted in the hospital (IPD) and remaining 6% were outdoor patients.

Table.1 Age wise distribution

<table>
<thead>
<tr>
<th>AGE</th>
<th>MALE</th>
<th>FEMALE</th>
<th>TOTAL</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt; 1 year</td>
<td>2</td>
<td>1</td>
<td>3</td>
</tr>
<tr>
<td>&lt; 10 year</td>
<td>2</td>
<td>2</td>
<td>4</td>
</tr>
<tr>
<td>10-20 year</td>
<td>-</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>21-30 year</td>
<td>4</td>
<td>9</td>
<td>13</td>
</tr>
<tr>
<td>31-40 year</td>
<td>5</td>
<td>19</td>
<td>24</td>
</tr>
<tr>
<td>41-50 year</td>
<td>7</td>
<td>7</td>
<td>14</td>
</tr>
<tr>
<td>51-60 year</td>
<td>15</td>
<td>4</td>
<td>19</td>
</tr>
<tr>
<td>61-70 year</td>
<td>8</td>
<td>4</td>
<td>12</td>
</tr>
<tr>
<td>71-80 year</td>
<td>7</td>
<td>2</td>
<td>9</td>
</tr>
<tr>
<td>&gt;80 year</td>
<td>1</td>
<td>-</td>
<td>1</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>51</strong></td>
<td><strong>49</strong></td>
<td><strong>100</strong></td>
</tr>
</tbody>
</table>

Table.2 Clinical profile of the patients

<table>
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<tr>
<th>Presenting complaints</th>
<th>No. of cases</th>
<th>%age</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fever with/without chills</td>
<td>61</td>
<td>61%</td>
</tr>
<tr>
<td>Weight loss</td>
<td>22</td>
<td>22%</td>
</tr>
<tr>
<td>Sepsis</td>
<td>16</td>
<td>16%</td>
</tr>
<tr>
<td>Cough</td>
<td>14</td>
<td>14%</td>
</tr>
<tr>
<td>Burning micturition</td>
<td>14</td>
<td>14%</td>
</tr>
<tr>
<td>Pain abdomen</td>
<td>11</td>
<td>11%</td>
</tr>
<tr>
<td>Chest pain</td>
<td>9</td>
<td>9%</td>
</tr>
<tr>
<td>White discharge/pruritis</td>
<td>6</td>
<td>6%</td>
</tr>
<tr>
<td>Diarrhoea</td>
<td>5</td>
<td>5%</td>
</tr>
<tr>
<td>Oral lesions</td>
<td>4</td>
<td>4%</td>
</tr>
</tbody>
</table>

Most common presenting complaints in the present study were fever (61%)
The prevalence of NAC spp. is increasing and maybe associated with the inadequate response to anti tubercular drugs. 14% of the total cases in the present study were diabetic. Out of these 14 cases; C. tropicalis was the predominant species [7] (50%), followed by C. albicans [3] (21%), C. krusei [2] (14%) and C. glabrata and C. dubliniensis [1] (7%) of each. NAC spp. are not only well adapted to the urinary tract but also are difficult to eradicate than C. albicans. Increase in concentration of glucose in the tissue, blood, and urine promotes growth of Candida. The present study is in keeping with studies done by Kandhari et al., with an incidence of 20.4% and Shroff with an incidence of 14.66%. The predominant species isolated in the present study in 6% cases of VVC is C. albicans (50%) followed by C. tropicalis (33%) and C. glabrata (17%).

Present study is similar to the studies done by Rylander et al., where C. albicans species were isolated in 90 out of 219 samples and C. glabrata in three and by Neeraja Jindal et al., (2006) on 400 patients, 92 cases were cultures positive for Candida of these 64 (69.57%) were C. albicans 8 (8.7%), C. glabrata, 6 (6.55%) each C. tropicalis and C. krusei, and 4(4.3%) each C. parapsilosis and C. guillimondi. In the present study occurrence of oral candidiasis was 40% out of 10 HIV positive cases. The occurrence of oral thrush ranged from 12% in Tanzania to around 94% in Zaire. The most common type of Candidiasis was the Pseudomembranous type ranging from 1% in Kenya to 70% in Peru followed by Erythematous candidiasis, the frequency of which ranged from 3% in India to 35% in Peru. In a study done by Luis Octino et al., (2005) and Diz Dias et al., (2001), the most common lesion found in HIV positive patients was Pseudomembranous which were in percentage of 97.5% and 69% respectively. Candida albicans was the most common species isolated from oral lesions/swabs of HIV positive patients accounting for 50% (2) of total 4 oral swabs. In previous studies done by Dunic et al., (2004), Mrudula Patel et al., (2006), Omar et al., (2008) C. albicans was the most common species isolated in oral swabs of HIV positive patients in the percentage of 77.7%, 83.5%, 78.6% and 84.5% respectively. Percentage of NAC spp. isolated from oral swabs was C. tropicalis and C. parapsilosis in the percentage of 25% respectively. In the present study 5% of the total cases came with the complaint of Diarrhoea. C. tropicalis was the most common species isolated from the stool samples accounting for 60%, followed by C. parapsilosis and C. krusei in the percentage of 20% respectively. This study was similar to the findings in a study by Banerjee et al., (2012) where C. tropicalis (43.8%), C. krusei and C. albicans (15.6%) of each and C. parapsilosis was (6.3%).

**Observations**

Total 100 isolates of Candida were taken from various clinical samples. The age of the patients were ranging from minimum of <1 year to maximum of >80 years (Table 1). Maximum no. of patients belonged to 31-40 years of age group, Followed by 51-60 years. Out of 100 patients 51 were males and 49 were females. The male to female ratio was M:F 1.04:1

**Sample wise distribution among cases**

Most common form of candidiasis seen was candiduria type (38). Respiratory system was involved in 17 cases, while in 10 cases blood cultures were positive for Candida species.

**Identification of Candida isolates**

Out of total 100 samples 44% Candida isolates were found to be germ tube positive while 56% were negative.
42% species produced light green coloured colonies on HiChrome Agar while blue coloured colonies were produced by 34% of the species. 2% species produced dark green colonies. Cream to pale pink, pink to purple, and pink with white edges (matt surface) colonies were produced by 11%, 6% and 5% respectively (Image 3 and 4).

Out of 100 samples 42% were Candida albicans and remaining 58% were NAC spp.

To conclude, the study showed that prevalence of Non Candida albicans were higher from various clinical specimens. It is essential that an early and accurate diagnosis be made of infecting species of Candida. This will aid the clinician in timely institution of the appropriate and accurate antifungal drug to be used and will restrict the empirical use of antifungal agents as being commonly done today.

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How to cite this article:

doi: https://doi.org/10.20546/ijcmas.2017.607.048