ISOLATION AND IDENTIFICATION OF CANDIDA SPECIES IN THE PATIENTS OF UTI

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ABSTRACT

Fungal infections of the urinary tract especially those caused by Candida species are becoming increasingly common due to prolonged antibiotic use, indwelling urinary catheters, and increase in the number of immunocompromised individuals. Symptoms of Candida pyelonephritis, cystitis, prostatitis, or epididymo-orchitis are little different from those of the same infections produced by other pathogens. Candiduria occurring in critically ill patients should initially be regarded as a marker for the possibility of invasive Candidiasis. The first step in further evaluation is to verify funguria by repeating the urinalysis and urine culture.

MATERIALS AND METHODS

A total of 3381 clinically diagnosed cases of UTI from OPD and IPD of JAH and KRH Hospitals, G. R. Medical College, Gwalior were studied for one year from 1st Jan, 2015, to 31st Dec, 2015. Urine samples were aseptically collected. Cultures were done on blood agar, MacConkey agar, CLED agar, and Sabouraud Dextrose Agar. Both bacteria and yeast were isolated. Both bacteria and yeast were identified further as per standard protocol. The isolated yeasts were included in our study for evaluation.

RESULTS

Total of 87 (2.57%) cases were found positive for yeast growth. Among these, 4 species are identified with predominance of C. albicans 31.03% (n=27), C. krusei 29.89% (n=26), C. glabrata 24.14% (n=21), C. tropicalis 14.94% (n=13).

CONCLUSION

The finding of candiduria in a patient with or without symptoms should be neither dismissed nor hastily treated, but requires a careful evaluation, which should proceed in a logical fashion.

KEYWORDS

Candiduria, UTI, Risk Factors, Germ Tube Test, HiCrome Candida Differential Agar, Growth at 45°C.


INTRODUCTION

Candida is a normal inhabitant in the skin, mucous membrane of the mouth, respiratory track or vagina, but may invade the other parts of the body especially in immunocompromised individuals. They exist predominantly in the unicellular form with both sexual and asexual forms and show thin walled ovoid cells (blastospores) that reproduce by budding. There are more than 150 species of Candida, but most important pathogenic species are C. albicans, C. tropicalis, C. krusei, C. glabrata. They may cause simple lesions to life threatening systemic infections. The most common species is C. albicans even though there has been a striking increase in the frequency of non-albicans candida in the last few years. At present, there is increase in treatment failure maybe because of drug resistance mainly in non-albicans candida. Thus, Candida is the 6th most common isolated nosocomial pathogen especially from urinary tract.2

There is also important emerging evidence that Candida infection can be acquired from hospital environment. Among the hospitalised population, the following categories pose a great risk in acquiring candidal infection viz., transplant patients, haematological malignancies, prolonged antibiotic therapy, catheterisation of urinary tract or vascular system, renal failure, hepatic failure, prolonged hospital stay and inter hospital transfer,6 use of immunosuppressive agents,6 extremes of age and female sex7 etc. The generous use of broad-spectrum antibiotics and other molecules have paved way for new opportunistic pathogen like Candida. In addition, diabetes mellitus and HIV infection provides a soil for candidal infection.8

Guze and Harley found funguria in only 15 of 1500 patients; more than half of these 15 patients had diabetes mellitus and were receiving antibiotics.9 A study performed by Platt et al found that 26.5% of all urinary infections related to indwelling catheters were caused by fungi. Rivett et al found that 2% of urine specimens submitted to a hospital microbiology laboratory tested positive for yeast versus 11% of the urine samples obtained from patients in the leukaeemia and bone marrow transplantation unit in the same hospital. Therefore, the prevalence of candiduria varies considerably in the hospital setting and is most prevalent among patients in the Intensive Care Unit (ICU).10,11,12

Candiduria is a relatively rare finding in otherwise healthy people.13 When yeast like organisms are discovered in the urine, the major decision that must be made is whether or not this signifies infection of either the upper or lower urinary tracts, colonisation of the bladder, or contamination
4 hours samples were refrigerated and more than 4 hour, then samples discarded and fresh sample were collected along with their proper requisition form.

Isolation and Identification of Candida Spp.
All urine samples were inoculated following semi-quantitative technique by calibrated loop (0.01 mL) onto blood agar (blood agar base, HiMedia Laboratories Pvt. Ltd. Mumbai, India), MacConkey agar (HiMedia Laboratories Pvt. Ltd. Mumbai, India), and CLED agar (Cystine-Lactose-Electrolyte-Deficient, HiMedia Laboratories Pvt. Ltd. Mumbai, India) medium and incubated at 37°C and read twice at 24 hours and 48 hours of incubation. Both bacteria and yeast were isolated. Dry creamy white opaque colonies on blood agar and tiny dry lactose fermenting pink colonies on MacConkey agar medium that resembled Candida colony were confirmed by Gram Stain. These candida isolates were subcultured on Sabouraud’s Dextrose Agar (HiMedia Laboratories Pvt. Ltd. Mumbai, India) (Fig. 1. showing cream-coloured colony of Candida species on Sabouraud’s Dextrose Agar media), Corn meal agar (HiMedia Laboratories Pvt. Ltd. Mumbai, India), and HiCrome candida differential agar medium (HiMedia Laboratories Pvt. Ltd. Mumbai, India) for further identification.

Identification was carried out by performing Gram stain, germ tube test (Fig. 2. showing germ tube test under 400x.), chlamydospore production test (Fig. 3. showing chlamydospore under 400x.), growth at 45°C and HiCrome candida differential agar medium (Fig. 4. Showing Growth of candida species on HiCrome candida differential agar. (1 and 3-C. albicans, 2-C. tropicalis, 4-C. krusei, 5-C. glabrata)) as per the CLSI guidelines.
RESULTS
Total of 3381 urine sample for culture were received in the department. 2417 were culture negative, 877 were bacteriologically positive, and only 87 samples were showing yeast growth. These 87 samples were included in our study.

<table>
<thead>
<tr>
<th>Sl. No.</th>
<th>Results</th>
<th>No. of Samples</th>
<th>Percentages</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>No Growth</td>
<td>2417</td>
<td>71.49</td>
</tr>
<tr>
<td>2</td>
<td>Bacterial</td>
<td>877</td>
<td>25.94</td>
</tr>
<tr>
<td>3</td>
<td>Yeast Growth</td>
<td>87</td>
<td>2.57</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>3381</strong></td>
<td><strong>100</strong></td>
<td></td>
</tr>
</tbody>
</table>

*Table 1: Results of Urine Culture*

Sex and age wise distribution of cases under study depicted in Table 2 and Fig. 5 shows that there was a predominance of females reported with candiduria. In case of females, the maximum numbers of cases were in the age group of 31-60 years. Similarly, the majority of the case in males also fell in the age group of 31-60 years.

Out of 87 isolates, 58 (66.67%) were isolated from female patients and 29 (33.33%) from male. Male-to-female sex ratio was found to be 1:2. (Table 2).

<table>
<thead>
<tr>
<th>Sex</th>
<th>No. of Cases</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male</td>
<td>29</td>
<td>33.33</td>
</tr>
<tr>
<td>Female</td>
<td>58</td>
<td>66.67</td>
</tr>
</tbody>
</table>

*Table 2: Sex-Wise Distribution of Fungal Isolates*
Out of 87 isolates, 69 (79.31%) were recovered among the 31 to 60 years of age. (Fig. 5).

<table>
<thead>
<tr>
<th>Sl. No.</th>
<th>Risk Factors</th>
<th>No. of Isolates</th>
<th>C. albicans</th>
<th>Non-Albicans Candida</th>
<th>C. albicans</th>
<th>Non-Albicans Candida</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Urinary Catheter</td>
<td>7 (12.96%)</td>
<td>11 (12.64%)</td>
<td>18 (20.69%)</td>
<td>10 (18.52%)</td>
<td>25 (46.30%)</td>
<td>54 (61.8%)</td>
</tr>
<tr>
<td>2.</td>
<td>Antibiotic Use</td>
<td>9 (19.15%)</td>
<td>14 (29.79%)</td>
<td>11 (23.40%)</td>
<td>13 (27.66%)</td>
<td>47 (54.4%)</td>
<td></td>
</tr>
<tr>
<td>3.</td>
<td>Diabetes</td>
<td>5 (12.82%)</td>
<td>14 (29.79%)</td>
<td>7 (17.95%)</td>
<td>17 (39.5%)</td>
<td>39 (44.4%)</td>
<td></td>
</tr>
<tr>
<td>4.</td>
<td>ICU Stay</td>
<td>5 (21.73%)</td>
<td>13 (18.84%)</td>
<td>6 (26.09%)</td>
<td>6 (26.09%)</td>
<td>23 (26.4%)</td>
<td></td>
</tr>
<tr>
<td>5.</td>
<td>Age Between 31-60 Years</td>
<td>7 (10.14%)</td>
<td>13 (18.84%)</td>
<td>12 (17.39%)</td>
<td>37 (53.62%)</td>
<td>69 (79.31%)</td>
<td></td>
</tr>
</tbody>
</table>

Total of 87 candida isolates, 4 species are identified with predominance of C. albicans 31.03% (n=27), C. krusei 29.89% (n=26), C. glabrata 24.14% (n=21), C. tropicalis 14.94% (n=13). (Table 4, Fig. 6).

<table>
<thead>
<tr>
<th>Fungal Isolates</th>
<th>No. of Isolates</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>C. Albicans</td>
<td>27</td>
<td>31.03</td>
</tr>
<tr>
<td>C. Krusei</td>
<td>26</td>
<td>29.89</td>
</tr>
<tr>
<td>C. Glabrata</td>
<td>21</td>
<td>24.14</td>
</tr>
<tr>
<td>C. Tropicalis</td>
<td>13</td>
<td>14.94</td>
</tr>
<tr>
<td>Total</td>
<td>87</td>
<td>100</td>
</tr>
</tbody>
</table>

**Table 4: Percentage of Various Candida Species Isolated from Urine**

Out of 87 isolates, 27 isolates were positive for germ tube test and growth at 45°C on SDA media indicating all 27 isolates were C. albicans.

Out of 87 isolates, 27 (31.03%) were C. albicans and 60 (68.97%) were non-albicans candida (NAC). Common predisposing condition included urinary catheter 61.8% patients using antibiotics 54.4%, diabetes in 44.5%, ICU stay in 26.4%, age group of 31 to 60 year (79.31%) were more commonly affected. (Table 3).

Punjab was 5.3% as studied by Mahajan A. et al.27 In a study by Rivett et al, the incidence of candiduria among the urine specimens submitted to a hospital microbiology laboratory was 2%.11 However, studies conducted by Kobayashi, Claudia et al,29 and N. Febre V. Silva et al23 higher incidence of candiduria were reported 22% and 18.6% respectively. Our study is in accordance with the study of Rivett et al11 where incidence of candiduria was 2%. Therefore, the prevalence of candiduria varies considerably in the hospital setting.

In the present study, it was observed that incidence of candiduria was reported higher among the females (66.67%) than males (33.33%). Mahajan A. et al27 reported 74% females and 26% males had Candiduria. In a study, N. Safdar et al29 found that 77% females had candiduria. N. Jain et al30 observed that 77.4% females had candiduria. However, Kobayashi et al28 reported female incidence to be 57.8%. Kaufman CA et al37 reported 59.9% females with candiduria. Hence, all studies done in different parts of the world show that females have more predilections towards candiduria most probably due to short urethra in females.

In the present study, out of 87 Candida isolates isolated from urine specimens, C. albicans predominated 31.03% followed by C. krusei 29.89%, C. glabrata 24.14%, and C. tropicalis 14.94%. Similarly, Mahajan A. et al27 reported 34% of C. albicans followed by C. dubliniensis (31%), C. krusei (19%), C. tropicalis (15%), and C. glabrata (1%). Kobayashi et al28 reported incidence of C. albicans to be 35.6%, C. tropicalis 22%. N. Safdar et al29 in their study reported incidence of C. albicans to be 35%, C. tropicalis 1%, C. glabrata 53%, C. krusei 1%, and C. parapsilosis to be 4%. So, it is fair to assume that Candida albicans is the commonest species isolated.

In the present study, common predisposing condition included urinary catheter 61.8%, patients using antibiotics 54.4%, diabetes in 44.5%, ICU stay in 26.4%, age between 31
to 60 year and sex that was affected more is female that is 66.67%. According to Navin Paul et al.\textsuperscript{31} incidence of various predisposing factors was catheterisation 66.6%, intake of antibiotics 47.61%, diabetes 38.09%, and surgery in 38.09%. That is in accordance to the present study. Kobayashi et al.\textsuperscript{28} reported incidence of various predisposing factors was intake of antibiotics 100%, urinary catheter was present in 84.4% surgical procedure in 66.7%.

**CONCLUSION**

The present study and several similar studies show that Candida albicans was the predominant isolate. Over the last three decades, an increase in the prevalence of candidia as well as in the incidence of candida UTI are associated with certain risk factors, which are use of antibiotics, urinary catheterisation, diabetes mellitus, patients undergoing surgery, and female sex. In this study, a fair idea is obtained about the predisposing factors and epidemiological data of our study can serve as a template for the development of local guideline for making diagnosis of Candiduria.

The finding of candiduria in a patient with or without symptoms should be neither dismissed nor hastily treated, but requires a careful evaluation, which should proceed in a logical fashion.

**REFERENCES**

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