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Research Article

Isolation, Identification and Antifungal Susceptibility Pattern of Candida Spp Isolated From UTI Cases in a Tertiary Care Hospital

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Abstract: Majority of fungal Urinary Tract Infections (UTIs) are caused by Candida species. Because of differences in pathogenecity and drug resistance, speciation is desirable. Among the different antifungal agents, resistance to polyene compounds is uncommon while resistance to flucytosine and azoles now appears to be increasing. This study was conducted in the department of Microbiology, Adesh Institute of Medical Sciences & Research from September 2014 to July 2015 during which 100 cases of candiduria were followed. Isolation, speciation and anti fungal susceptibility of Candida spp was performed by standard procedures . 100 Candida spp were isolated from 1857 urine samples, incidence of candiduria being 5.38%. Out of the 100 Candida isolates, C. albicans was commonest 34%, followed by C.dubliniensis (31%), least common was C.glabrata (1%).The common predisposing factors associated with candiduria were intake of antibiotics (99%), followed by IV catheter (95%) Foley's catheter (90%) and female sex (74%). Out of the 100 Candida isolates, 99% were sensitive to amphotercin B, while sensitivity to ketoconazole was 20%, to fluconazole was 12%, to itraconazole was 9%. All Candida spp were sensitive to amphotercin B except C.krusei whose sensitivity was 94.7%. Sensitivity to itraconazole was maximum by C.Tropicalis (26.6%) followed by C. albicans (8.8%). C.glabrata& C.krusei were most resistant. Sensitivity to ketoconazole was maximum by C.Tropicalis (26%) followed by C. albicans (23.5%). C. Glabrata was most resistant. Sensitivity to fluconazole was maximum by C.Tropicalis (26.6%) followed by C. Albicans (14.7%). C. Glabrata was most resistant.
Keywords: Candiduria, predisposing conditions, Candida Speciation, Antifungal susceptibility.

INTRODUCTION

Majority of fungal infections of the urinary tract are caused by Candida species, and they usually present as complicated nosocomial infections. Only rarely does one encounter candiduria as a community-acquired infection in a structurally normal urinary tract [1].

The prevalence of candiduria varies considerably in the hospital setting and is most prevalent among patients in the intensive care unit. Presently, 10%-15% of nosocomial urinary tract infections are caused by Candida species [1]. Risk factors for candiduria include extremes of age, female sex, use of immunosuppressive agents, use of IV catheters, and interruption of the flow of urine, radiation therapy, and genitourinary tuberculosis.

The majority of fungal UTI involve Candida species. The most frequent organism is Candida albicans followed by Candida glabrata, Candida tropicalis, and Candida krusei. However, non-albicans Candidais increasing as the etiological cause of fungal UTI [4].

Moreover drug resistance is a major cause of treatment failure in these patients. Among the different antifungal agents, resistance to the polyene compounds has remained an uncommon problem. But resistance to flucytosine and azoles now appears to be increasingly important in some group of patients, especially after the widespread use of fluconazole for extended periods [3].

Prior use of Antibiotics is a known risk factor for candiduria [5]. Corticosteroids and other immunosuppressive agents may favors the development of candidiasis by suppressing cell-mediated immunity [6].

Glycosuria favours the growth of yeasts and their growth rate increases with the amount of glucose present at levels greater than 150 mg/dL. Below this level the glucose content of urine has no effect on the growth rate [6]. Patients with diabetes are at increased risk for UTIs, including UTIs caused by fungi. Candida

infections of the lower urinary tract occur 4 times as commonly in women as in men [6].

Candida albicans causes ~50% of cases of candiduria. Candida glabrata has been consistently responsible for ~25% of cases of candiduria. Candida tropicalis is the third most common agent. Around 5% of patients with candiduria will have 2 or more species simultaneously [7].

In a study conducted by Navin Paul and colleagues, among 12,618 urine specimens cultured, 21 were Candida isolates. Candida tropicalis was more common (9 of 21 isolates) Candida glabrata and C. albicans (each 4 of 21). In a study conducted at AIIMS, New Delhi in 1998, different species of yeasts recovered from the patients were as follows: C. albicans 49.18%, C. tropicalis 28.27%, C.krusei 4.09%, C.Pseudotropicalis 2.5%, C. Stellatoidia 0.4%, C. parapsilosis 1.22% and C. inconspicuous 0.4%.

In view of the above observations, this study was carried out with an aim to isolate and identify the various Candida species from candiduria cases, identify various predisposing factors associated and perform antifungal susceptibility testing of the isolated yeasts.

MATERIAL AND METHODS

The study was conducted in the department of Microbiology, Adesh Institute of Medical Sciences & Research Center, Bhatinda, Punjab, from September 2014 to July 2015 during which 100 cases of candiduria were followed.

Collection and Processing of Samples

Urine samples from patients admitted in various wards and intensive care units were collected and inoculated by calibrated loop (0.01 ml) onto Blood agar and Mc.Conkey agar medium and incubated at 37^0 C and read at 24 hours and 48 hours of incubation. Dry creamy white opaque colonies on Blood agar and tiny dry lactose fermenting pink colonies on Mc.Conkey agar medium that resembled Candida were confirmed by Gram Stain [10, 11]. These candida isolates were sub cultured on Sabouraoud's Dextrose Agar and HiCHROM candida agar medium.

Colour pattern of various Candida species were noted on HiCHROM Candida agar medium. C. albicans isolates impart distinctive light green colonies. C. tropicalis produce blue violet smooth colonies with halo diffusing into surrounding agar; C. kruseiisolates produce rough, fuzzy spreading big pink colonies. C. glabrata produce pink, glossy colonies with pale edges [18]. Germ Tube test was performed for preliminary identification of C.albicans & C.dublinensis. Further confirmation was by following tests:

Carbohydrate Fermentation test

An inoculums pool was prepared by emulsifying a heavily loaded loopful of the strain to be identified in 5 ml of sterile saline. The test organism was inoculated by adding one drop of the inoculums suspension into each tube sugar fermentation tube. It was incubated for 48-72 hours at 30^0C. The ability to ferment a sugar was shown by the presence of acid and gas trapped in the Durham's tube.

Candida albicans ferments glucose and maltose with gas production. Candida krusei ferments glucose with gas production. Candida tropicalis ferments glucose, sucrose and maltose with gas production. Candida dubliniensis ferments glucose and maltose with gas production. Candida guilliermondii ferments glucose and sucrose with gas production [8].

Carbohydrate Assimilation test

The organisms were inoculated on a carbohydrate free medium. Carbohydrate containing filter paper disks were added and utilization was determined by the presence of growth around the disc. It consisted sugar disk of 4% concentration [9].

Results & Interpretation

Candida albicansassimilates glucose, sucrose, maltose, trehalose, lactose, cellibiose, and galactose. Candida tropicalisassimilates glucose, maltose, sucrose, trehalose, and xylose. Candida kruseiassimilates glucose & xylose. Candida dubliniensisa ssimilates glucose, sucrose, maltose, trehalose, lactose [10].

ANTIFUNGAL DRUG SUSCEPTIBILITY TESTING

Media used for antifungal drug susceptibility

For Antifungal drug susceptibility testing Disk diffusion method was adopted. A disk contained the antifungal agent as routinely done in antibacterial sensitivity testing, which diffuses in the surrounding medium, inhibiting the growth of fungi and measurements of zone of inhibition were taken accordingly. For antifungal susceptibility testing of azoles, yeast nitrogen base with glucose and asparagine was used whereas for amphotericin B, yeast nitrogen base with glucose and without asparagine was used [19, 20].

Method for antifungal susceptibility

A suspension of an isolated colony of Candida was made in sterile saline (Nacl 0.9% w/v in water) that did not exceed the turbidity of McFarland / Stanford 1 (prepared by mixing 0.1ml of 1% barium chloride with 9.9 ml of 1% sulphuric acid). In the similar way inoculums preparation was also done for the control strain.

The swabs soaked in the inoculum were inoculated in one-half of the Petri dish from periphery to the centre. One-half of the plate was inoculated with control strain and the other half with the test strain in

such a manner which were unable to produce confluent growth [19].

Antifungal disks

Commercially available [Hi-Media] discs of amphotericin B, fluconazole, ketoconazole and itraconazole were used. Antifungal disks were placed in the centre of control as well as the test strains with the help of forceps. The plates were incubated at 35^0C for 48 hours and measurements of zone of inhibition were taken [11].

Results and Interpretation

After the measurement of zone of inhibition, the results of the antifungal susceptibility testing were interpreted according to the following criteria:

Resistant- There was no zone of inhibition.

Sensitive-The zone diameter of test strain was more than eighty percent of the control strain.

Intermediate- The zone diameter of test strain was less than eighty percent of the control strains [11, 19, 20].

RESULTS

A total of 1857 urine samples were screened and 100 Candida isolates were identified on the basis of microscopic and stained smear examination, cultural characteristics and biochemical tests. The following observations were made after data compilation:

Table 1 shows the incidence of Candiduria in our study which was 5.3%

Sex distribution of cases under study depicted in table 2 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 21-30 years. Similarly the majority of the case in males also fell in the age group of 21-30 years.

Various species of Candida shown in table 3 depicts that C. albicans was the commonest (34%) species isolated in this study. Next common was C. dubliniensis (31%) and the least common species was C. glabrata(1%).

Predisposing factors responsible for candiduria are presented in table 4. It shows the use of antibiotics was found in 99% patients. Indwelling Foley's catheter was present in 90% of patients with candiduria. Diabetes was found in 15%. IV catheter was present in 95% of the patients. Candiduria was found to be 15% in the patients admitted in Intensive Care Unit. It is also observed that 73% females reported as culture positive cases. Only 7% patients above the age of 60 years had been reported.

Antifungal susceptibility of Candida isolates is presented in table 5. It was observed that 99% Candida species were sensitive to amphotericin B followed by ketoconazole (20%), fluconazole (12%) and itraconazole (9%).

Table-6 depicts that sensitivity of all strains to amphotericin B was 100 % except C. krusei(94.7%).

Table-7 shows that C.tropicalis was most sensitive (26.6%) to itraconazole and least sensitive were C. glabrata.

Table-8 shows that C. tropicalis was most sensitive (26%) to ketoconazole followed by C. albicans (23.5%), C. krusei (21%), C. dubliniensis (12.9%) and C. glabrata was least sensitive to ketoconazole (0%).

Table-9 shows that C. tropicalis was most sensitive (26.6%) to ketoconazole followed by C. albicans (14.7%). However C. glabrata was found resistant to the drug.

Table-1: Incidence of candiduria

No. of urine samples	No. of isolates	Incidence
1857	100	5.38%

Table-2: **Sex distribution of the cases under study**

Age group (years)	Male No. of isolates	Female No. of isolates
0-10	1	2
11-20	4	7
21-30	7	25
31-40	4	18
41-50	4	12
51-60	2	7
61-70	2	3
71-80	2	0
Total	**26**	**74**

Table-3: **Various species of Candida isolated**

Candida species	No. of positive cases	Percentage
C. albicans	34	34%
C. dubliniensis	31	31%
C. krusei	19	19%
C.Tropicalis	15	15%
C. glabrata	1	1%
Total	**100**	**100%**

Table-4: **Predisposing factors for candiduria**

Predisposing factor	No. of cases	Percentage
Antibiotics	99	99%
Foley's catheter	90	90%
Diabetes	15	15%
IV catheter	95	95%
ICU	16	16%
Surgery	33	33%
Sex-females	73	73%
Age- >60 years	7	7%

Table-5: Antifungal susceptibility of Candida isolates to various antifungal drugs

Drug	Total no. of isolates	No of isolates sensitive	Percentage
Amphotericin B	100	99	99%
Itraconazole	100	9	9%
Ketoconazole	100	20	20%
Fluconazole	100	12	12%

Table-6: **Susceptibility of various Candida species to amphotericin B**

Candida Species	No.	Sensitive	Percentage
C. albicans	34	34	100%
C. dubliniensis	31	31	100%
C. krusei	19	18	94.7%
C. tropicalis	15	15	100%
C. glabrata	1	1	100%
Total	**100**	**99**	**99%**

Table-7: **Susceptibility of various Candida species to itraconazole**

Candida Species	No.	Sensitive	Percentage
C. albicans	34	3	8.8%
C. dubliniensis	31	2	6.4%
C. krusei	19	0	0%
C. tropicalis	15	4	26.6%
C. glabrata	1	0	0%
Total	**100**	**9**	**9%**

Table-8: **Susceptibility of various Candida species to ketoconazole**

Candida Species	No.	Sensitive	Percentage
C. albicans	34	8	23.5%
C. dubliniensis	31	4	12.9%
C. krusei	19	4	21%
C. tropicalis	15	4	26%
C. glabrata	1	0	0%
Total	**100**	**20**	**20%**

Table-9: **Susceptibility of various Candida species to fluconazole**

Candida Species	No.	Sensitive	Percentage
C. albicans	34	5	14.7%
C. dubliniensis	31	2	6.45%
C. krusei	19	1	5.26%
C. tropicalis	15	4	26.6%
C. glabrata	1	0	0%
Total	**100**	**12**	**12%**

DISCUSSION

In the present study the incidence of candiduria was found to be 5.37%. Rivett et al.; [12] found that 2% of urine specimens submitted to a hospital microbiology laboratory tested positive for yeasts. However in a study conducted by Kobayashi, Claudia *et al.;* [13], incidence of candiduria was 22%. N. Febre, V. silva *et al.;* [15] reported 18.6% incidence. Our study is in accordance with the study of S.C.A. Chen *et al.;* [16] where incidence of Candiduria was 4.7% .Therefore the prevalence of candiduria varies considerably in the hospital setting [1].

In the present study out of 100 Candida isolates C. albicans predominated (34%) followed by C. dubliniensis (31%), C. krusei (19%), C. tropicalis (15%) and C. glabrata (1%). Similarly Kobayashi *et al.;* [13] reported incidence of C. albicansto be 35.6%, C. tropicalis 22%.N. Safdar *et al.;* [21] in their study reported incidence of C. albicansto be 35%, C tropicalis 1%, C. Glabrata 53%, C.krusei 1%, and C. parapsilosis to be 4%.However, in a study S.C.A. Chen *et al.;* [16] observed C. albicans as 85.2%, followed by C. glabrata 27.8% and other Candida species 6.2%. So it is fair to assume that Candida albicans is the commonest species isolated.

In the present study it is observed that females reported more (74%) with candiduria cases as compared to males (26%). Similar to this study N. Jain *et al.;* [17] observed that 77.4% females had candiduria. N. Safdar *et al.;* [21] also reported 77% females with candiduria. However J.D. Sobel *et al.;* [22] reported female incidence to be 59.9% . Kobayashi *et al.;* [13] reported 57.8% 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 common predisposing conditions included the use of antibiotics (99%), Foley's catheter (90%), diabetes (15%), IV catheter (95%), ICU (16%), surgical procedures (33%), age > 60 years (7%) and sex that was affected more is female that is 74%. According to Kobayashi, Claudia *et al.;* [13] incidence of various predisposing factors was: intake of antibiotics 100%, urinary catheter was present in 84.4%, surgical procedure in 66.7%. That is in accordance to the present study. Similar to this study Francisco et al[14] reported the use of antibiotic in 98.5%, urinary catheter in 97.9%, diabetes 21.6% and IV catheter present in 55.5%.

However N Jain *et al.;* [17] reported 54.4% of patients using antibiotics, urinary catheter 61.8%, and diabetes in 54.5%, ICU stay in 26.4%. Whereas Navin Paul *et al.;* [23], in their study reported antibiotics 47.61%, urinary catheter 66.6%, diabetes 38.09% and surgery in 38.09%.

Regarding drug sensitivity of Candida species to various antifungal agents, we observed in our study that 99% Candida strains were sensitive to amphotericin B, 20% to ketoconazole and 12% to fluconazole and were least sensitive to itraconazole (9%).

As far as sensitivity of various Candida species to amphotericin B is concerned, we observed in our study that sensitivity of all species to amphotericin B was 100% except C. krusei, (94.7%). When testing for itraconazole, % sensitivity of C.Tropicalis was 26.6% followed by C. albicans 8.8% &C. dubliniensis 6.4%. C. krusei & C.glabrata were found resistant to itraconazole.

In a study in 1995, A. Chakrabarti *et al.;* [19], observed that 92% Candida species were sensitive to amphotericin B, 87% were found sensitive to fluconazole and 86 % of the Candida species were sensitive to ketoconazole. Whereas in a study conducted by A. Chakrabarti *et al.;* [20] in 1996, the sensitivity to amphotericin B was observed as 94.33%.

According to our study when ketoconazole was tested, % sensitivity of C. tropicalis was 26% followed by C. albicans 23.5%. C. krusei was 21% sensitive and C. dubliniensis 12.9%. However C. glabrata was found resistant. The sensitivity of C. glabrata to ketoconazole was found 0%.In our study, when fluconazole was tested, C. tropicalis was found most sensitive (26.6%) followed by C. Albicans, (14.7%).The sensitivity of C. dubliniensis to fluconazole was 6.45% and that of C.krusei 5.26%. However C. glabrata was 0% sensitive.

However according to A. Rokosz *et al.;* [24], 100% Candida species were sensitive to amphotericin B and Fluconazole. In a study conducted by Chakrabarti *et al.;* [20]. The sensitivity of C. tropicalis to fluconazole was 75.4%, while C. Krusei was 27.3% and the sensitivity of C.Tropicalis to ketoconazole was 94.3% while sensitivity of C. krusei was found to be 93.9%.

According to N. Febre *et al.;* [15] the specific identification of yeasts provides important help in the choice of treatment, because C. glabrata and C. krusei are naturally resistant to fluconazole and C. albicans that is initially susceptible may become resistant during treatment.

The susceptibility of the emerging and unusual yeasts to azole antifungal agents is variable. The bistriazole fluconazole appears by in vitro test to be ineffective or marginally effective against C. krusei, C. guilliermondii. C. kruseialso appears to be clinically resistant to fluconazole [25].

CONCLUSION

In view of the study conducted, a fair idea is obtained regarding the predisposing factors of Candiduria, the common species of Candida isolated and their antifungal susceptibility to commonly used antifungal drugs with which prudent empirical antifungal therapy can be started. More such studies should be carried out as the commonly isolated Candida spp and their antifungal susceptibility patterns are variable in different hospital settings.

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