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RESEARCH ARTICLE

PHENOTYPIC IDENTIFICATION OF CANDIDA SPECIES AND THEIR SUSCEPTIBILITY PROFILE IN PATIENTS WITH GENITOURINARY CANDIDIASIS

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Abstract

INTRODUCTION: Among the many causes of vaginitis, VVC is the second most common after bacterial vaginosis and is diagnosed in 40 % women with vaginal complaints in the primary care setting. Candida species account for almost 10-15% nosocomial UTIs.The incidence of fungal infections has increased dramatically over the past few decades due to increase in the number of population susceptible to Candida infections. Epidemiologic data from the past decade reveal a paradigm shift in Candida infections with non - albicans Candida species. The aim of the study was to identify the distribution of Candida species among genitourinary isolates by CHROMagar and HiCandida Identification kit and their sensitivity pattern.MATERIAL AND METHODS: The samples included high vaginal swabs, and urine, those which met the selection criteria were further processed for isolation, speciation and antifungal susceptibility testing of Candida species. RESULTS: A total of 67 isolates of Candida species were obtained from 500 genitourinary specimens. Candida albicans was found to be the most frequently isolated species accounting for 31(46.26%) of the total isolates, followed by C.tropicalis 21(31.34%), C.parapsilosis 6(8.95%), C.krusei 5(7.46%), C.glabrata 3(4.47%) and C.dubliniensis 1(1.49%).Non-albicans Candida constituted 36(53.73%).Antifungal susceptibility pattern showed that Candida isolates were more sensitive to Amphotericin –B, compared to that of Clotrimazole, Fluconazole and Voriconazole. C.albicans showed more susceptibility to Azoles compared to that of non-albicans Candida. Voriconazole showed greater susceptibility compared to Fluconazole. CONCLUSION: Identification of Candida to species level and their antifungal susceptibility testing should be done to achieve better clinical results.

Introduction

The Candida species are ubiquitous yeasts which are a part of normal flora of the alimentary tract of mammals and the mucocutaneous membranes of humans(Odds FC,1988).The overall carriage rate in healthy individuals has been estimated to reach 80%(Warren NG,1999).It becomes an opportunistic pathogen as a result of one or more underlying pre-disposing factors which impair, to various degrees, the immune response to these microorganisms(metabolic diseases, AIDS, immunosuppressive chemotherapy), produce an imbalance, in favor of fungal microflora (antibacterial drugs), or damage the integrity of the integument (surgery, intravenous catheters)(Esther Segal,2005).

Among the many causes of vaginitis, VVC is the second most common after bacterial vaginosis and is diagnosed in 40% women with vaginal complaints in the primary care setting(AndersonM.R;2004).Although not associated with any mortality; VVC and RVVC are associated with considerable morbidity. Symptoms of vaginitis can cause substantial distress, resulting in time lost from work and altered self-esteem (Eckert,L.O,2006).Candida species account for almost 10-15% nosocomial UTIs(Lundstrom T,2001 ; KauffmanCA, 2000).Candiduria not properly diagnosed and treated has been source of morbidity and mortality(Manjunath GN, 2011).The incidence of fungal infections has increased dramatically over the past few decades due to increase in the number of population susceptible to Candida infections. Epidemiologic data from the past decade reveal a paradigm shift in Candida infections with non -albicans Candida species such as C.glabrata, C.tropicalis, and C.krusei as emerging important pathogens (Chander J, Textbook of Medical Mycology; Greenspan, 1994; Baradkar VP, 2010; Mane A, 2010).This transition has had a significant clinical impact due to decreased susceptibility of these non -albicans yeasts to antifungal agents (Baradkar VP, 2010). Candida albicans and non-albicans species are closely related but differ from each other with respect to epidemiology, virulence characteristics, and fungal susceptibility, therefore Candida species identification is important for successful management.

As conventional methods for speciation are more time consuming and laborious, even molecular techniques are too expensive, in the present study CHROMagar and HiCandida Identification kit has been used for easy and rapid speciation in addition to conventional methods. The present study was designed to identify the Candida isolates from genitourinary specimens to species level and to perform antifungal susceptibility.

MATERIAL AND METHODS

The present study was conducted for a period of 1year from June 2013 to May 2014, in the Department of Microbiology at Deccan College of Medical Sciences and allied hospitals, Hyderabad with prior approval of institutional ethics committee. A total of 500 clinical samples were obtained from patients attending the (OP) and patients admitted (IP). The samples included high vaginal swabs, and urine, those which met the following selection criteria were further processed for isolation, speciation and antifungal susceptibility testing of Candida species. The clinical specimens which were positive for the presence of yeast cells on KOH wet mount and Gram stain smear positive for gram positive oval yeast –like budding cells (4-8µ) and / or pseudohyphae on microscopic examination. Patients with any of the following criteria: i.e. history of prolonged hospital stay, Administration of long term, broad spectrum antibiotics, on steroid therapy, Use of oral contraceptive pills, Patients on catheters, abnormal vaginal discharge, Pregnancy, Patients with underlying clinical conditions like Diabetes. Detailed history of each case such as age, sex, site of infection, predisposing factors, and underlying clinical conditions were noted.

COLLECTION AND PROCESSING OF SAMPLES:

The samples comprised of 340 urine and 160 HVS from patients clinically diagnosed of genitourinary tract infections. Sterile speculum and swab sticks were used for collection of HVS while urine samples were collected using sterile universal containers. All urine samples were centrifuged and the sediment used for processing (Carol.A.Kauffman, 2011). Samples collected were examined microscopically by wet mount with 10 % KOH and Gram stain.

CULTURE PROCEDURE

Samples (urine sediment and HVS) collected were inoculated on Sabourauds dextrose agar (SDA) and SDA with antibiotics in duplicates at 37°C and 28° C for 24-72 hours. Growth on SDA were identified by standard methods such as Gram staining, germ tube formation, Chlamydospore formation on Corn meal agar, Sugar fermentation and Sugar assimilation by HiCandida Identification kit (Chander J, Textbook of Medical Mycology; Mondal S et al , 2013). It is a standardized colorimetric identification system utilizing twelve conventional, biochemical tests. The first well contains medium for Urease detection test. Well 2-12 has medium for Carbohydrate Utilization test. The tests are based on the principle of P^H change and substrate utilization. Candida isolates were also inoculated onto Hichrom Candida Differential Agar and incubated at 37°C for 48 hours. Species were identified on Hichrom Candida differential agar by morphology and colour of colony.

ANTIFUNGAL SUSCEPTIBILITY TESTING:

Antifungal susceptibility testing was performed by disk diffusion method using Mueller-Hinton Agar + 2% Glucose and 0.5 µg/mL Methylene Blue Dye (GMB) Medium(Mondal S, 2013; Veena Manjunath,2012;Lata R .Patel 2012) as per CLSI guidelines(C.L.S.I. document M44-A2, 2009.). 0.5 McFarland standards was used to standardize the inoculums density. Antifungal disc used: Amphotericin – B (100U), Clotrimazole (10µg), Fluconazole (25µg) and

Voriconazole (1µg).C.albicans ATCC 90028 and C.parapsilosis ATCC 22019 were used as Controls. All the culture media, Antifungal disc, and control strains were obtained from Himedia Laboratories Mumbai, India.

RESULTS

A total of 67 isolates of Candida species were obtained from 500 genitourinary specimens. Out of 340 urine samples 30(8.82%) yielded Candida species. Vaginal swabs were collected from 160 adult females, from which 37 (23.12%) yielded Candida isolates.(Table 1

The results show that majority of Candida isolates from HVS were in age group 26-35 yrs and those from urine samples were found in cases above 55 yrs. (Table 2)

Candida albicans was the most frequently isolated species accounting for 31(46.26%) of the total isolates, followed by C.tropicalis 21(31.34%), C.parapsilosis 6(8.95%), C.krusei 5(7.46%), C.glabrata 3(4.47%) and C.dubliniensis 1(1.49%). Non-albicans Candida constituted 36(53.73%) which is more than C.albicans (46.26 %). A high number of C.albicans was found in all clinical samples. C.tropicalis constituted 10(33.33%) of urine and 11(29.72%) of HVS. C. parapsilosis was isolated from 2(6.66%) urine, 4(10.81%) HVS. C.krusei was isolated from 2(6.66%) of urine and 3(8.10%) of HVS. C.glabrata constituted 3(8.10%) of HVS. C.dubliniensis was isolated from 1(2.70%) HVS. (Table3)

Analysis of risk / pre-disposing factors in patients from whom Candida spp. were isolated, showed that 26.86%had underlying Diabetes mellitus,20.89% were on prolong course of antibiotics for various ailments, 19.40%were pregnant women, that, 17.91% were on catheters and 14.92% were on OCP.(Table 4)

All the Candida albicans were 100 % susceptible to Amphotericin–B, and Voriconazole, followed by 93.33% to Fluconazole and 80% to Clotrimazole. Among non -albicans Candida spp., susceptibility to Amphotericin–B varied between 81.81% to 100 %, Clotrimazole between 50% to 100%, Fluconazole between 33.33% to 100%. C.krusei was 100% resistant to Fluconazole.(Table 5)

Candida albicans showed 100% susceptibility to Amphotericin –B, followed by 87.5% and 81.25% to Voriconazole and Fluconazole respectively. Non- albicans Candida showed 90-100% sensitivity to Amphotericin –B, Sensitivity to Fluconazole varied between 50% to 70% and that of Voriconazole varied between 50% to 100 %.(Table 6)

Figure1: KOH mount showing yeast cells and pseudohyphae of Candida species

Figure 2: Gram stain showing gram positive yeast cells with pseudohyphae

Figure 3a) Light- green colour colonies of *Candida albicans* on Chromagar

Figure 3b) Dark green colour colonies of Candida dubliniensis and purple colonies of C.krusei on Chromagar.

Figure 3c) Blue colour colonies of *Candida tropicalis on* Chromagar.

Figure3d) Cream to white colour colonies of *Candida glabrata* on Chromagar

Figure 3e) cream to pale pink colonies of *Candida parapsilosis* on Chromagar

Figure 4) Carbohydrate assimilation reactions of *Candida albicans on HiCandida identification kit.*

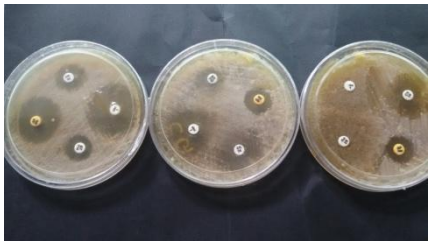

Figure 5: antifungal susceptibility pattern of Candida isolates on Mueller- Hinton Agar + GMB.

Table1: Occurrence of *Candida* isolates from various clinical samples.

Clinical specimens	Total no. screened	No positive for *Candida* isolates	Percentage %
Urine	340	30	8.82
HVS	160	37	23.12
Total	500	67	13.4

Table 2: Age distribution of patients from whom *Candida* species were isolated

Age of Patient	HVS	Urine	Total
16-25 yrs	5	-	5
26-35 yrs.	22	-	22
36-45 yrs.	7	2	9
46-55 yrs.	3	8	11
>55 yrs	-	20	20
Total	37	30	67

Table 3: Distribution of *Candida* spp.isolated among genitourinary specimens.

Species	Percentage %	Total isolates(67)	Urine	HVS
C.albicans	46.26	31	16	15
*C.*tropicalis	31.34	21	10	11
*C.*parapsilosis	8.95	6	2	4
*C.*krusei	7.46	5	2	3
C.glabrata	4.47	3	-	3
C.dubliniensis	1.49	1	-	1
Total	100	67	30	37

Table 4: Distribution of predisposing factors in patients with *Candida* isolates.

Predisposing factors	Total	Percentage %
h/o Diabetes mellitus	18	26.86
h/o prolong drug intake and 2^{o} disease	14	20.89
Pregnancy	13	19.40
On catheters	12	17.91

OCP Use	10	14.92

Table 5: Antifungal susceptibility pattern of *Candida* species.

Candida spp.	Amphotericin –B		Clotrimazole		Fluconozole		Voriconazole	
	S	R	S	R	S	R	S	R
C.albicans **n =15**	15 100%	-	12 80%	3 20%	14 93.33%	1 6.66%	15 100%	-
C.tropicalis **n =11**	9 81.81%	2 18.18%	6 54.54%	5 45.45 %	7 63.66%	4 36.63%	9 81.81%	2 18.18%
C.parapsilosis **n =4**	4 100%	-	2 50 %	2 50 %	2 50%	2 50%	3 75%	1 25%
C.krusei **n =3**	3 100%	-	3 100%	-	-	3 100%	2 66.66%	1 33.33 %
C.glabrata **n =3**	3 100%	-	2 66.67%	1 33.33%	1 33.33%	2 66.67%	2 66.67%	1 33.33%
C.dubliniensis **n =1**	1 100%	-	1 100%	-	1 100%	-	1 100%	-

Table 6:Antifungal susceptibility pattern of *Candida* species from urine samples

Candida spp.	Amphotericin –B		Fluconazole		Voriconazole	
	S	R	S	R	S	R
C.albicans **n =16**	16 100%	-	13 81.25%	3 18.75%	14 87.5%	2 12.5%
C.tropicalis **n =10**	9 90%	1 10 %	7 70%	3 30%	7 70%	3 30%
C.krusei **n =2**	2 100%	-	1 50%	1 50%	2 100%	-
C.glabrata **n =2**	2 100%	-	1 50%	1 50%	1 50%	1 50%

DISCUSSION:

The incidence of genitourinary tract infections is much higher in females during adolescence and childbearing years (Sobel, 2004).The rate of isolation of Candida from HVS in the present study was 23.12%in the present study. This is in correlation with other studies (Jindal Neerja, 2006).

The highest frequency of vaginal candidiasis was observed in age group of 26-35 yrs. This can be correlated with reports of Sehgal, who showed highest frequency of vaginal candidiasis in age group of 21-30 yrs (Sehgal, 1990).The frequency of vaginal candidiasis in women aged ≥ 40 yrs. was low which is similar to other studies(Deepa,2013).It was observed that women of child bearing age groups are more vulnerable to vaginal

candidiasis. Vaginal candidiasis is an extremely common infection in 60-70% women during their reproductive age at least once in their lives (Jacqueline, 2010). The high level of reproductive hormones and increase glycogen content of vagina favours candidiasis in pregnancy (Francisca I., 2003).Hence it is the common predisposing factor associated with vaginal candidiasis in the present study

In the present study 26.86% (18) pregnant women &14.92% (17) non-pregnant women had VVC. Progesterone has suppressive effects on the anti-Candida activity of neutrophils, Estrogen has been found to reduce the ability of vaginal epithelial cells to inhibit the growth of Candida (Paul L., 2000).This could be the reason for the narrow differential margin in the prevalence of VVC noted in pregnant and non-pregnant women who were on oral contraceptive pills (OCPs). Many investigators continue to identify OCP as predisposing factor. This might be because of similarity between the mechanism operating in pregnancy and high estrogen OCP in increasing vaginal colonization of Candida(Jindal Neerja,2006).Of the 37 vaginal isolates C.albicans 40.54% (15) was the major spp. isolated, which can be compared to other studies(Mondal S, 2013; Jindal Neerja,2006; Deepa, 2013).

In the present study it was observed that CHROMagar has the advantage of rapid identification of Candida species in 24-48 hrs and cost effective compared to technically demanding time consuming and expensive conventional method. It is superior to SDA in terms of suppressing the bacterial growth. The results on CHROMagar exactly paralleled that of conventional methods.

Among the Candida isolates C.albicans was the most frequently isolated species. In the present study, overall NAC(53.73%) had predominance over C.albicans(46.26%) which is consistent with the other studies(Mondal S, 2013; Paul L , 2000).

Though C.albicans was predominant spp. isolated, it was observed that the frequency of non-albicans spp. among the Candida isolates causing vulvovaginitis was 59.45% (22) which is more than that of C.albicans. There is an increase in frequency of non-albicans Candida spp. as potential causes of the vaginal candidiasis which can be compared with other studies. Most frequently isolated non-albicans Candida was C.tropicalis (29.72%) which can be compared with other studies (Deepa, 2013; Vijaya D, 2014). C.parapsilosis (10.81%) can be compared with 11.4% as shown by other study (Mondal S, 2013).The occurrence of C.krusei(8.10%),C.glabrata(8.10%)and C.dubliniensis(1.6%)can be compared with other studies(Jindal Neerja,2006;Deepa,2013).Candida is the most frequently isolated pathogen in nosocomial UTIs and Candiduria is usually diagnosed in elderly hospitalized patients(Jacqueline,2010). The rate of isolation of Candida isolates from urine in the present study is 8.8% (30/340). The highest isolation rate was found in age group >55yrs. which is similar to other studies (Anandkumar, 2013; Yashvanth R, 2013).Of the 30 cases having Candiduria there were 22 (73.33%) females and 8 males (26.66%). Yeast may ascend from the genital tract to the urinary tract, explaining a higher Candiduria incidence in women (Tavleen Jaggi, 2014).Of the 30 cases having Candiduria 12 (40%) were catheterized ICU patients. Catheterization process increase chances of UTIs by allowing migration of organisms into the bladder from external periurethral surfaces and by biofilm formation. Nayman Alpat had believed that long duration of ICU and hospital stay increase the incidence of Candiduria in patients (Nayman Alpat, 2011).Candiduria is rare in healthy people but relatively frequent in hospitalized patients (Jain N, 2007; Weinberger M, 2003). C.albicans 53.33% (16) was the commonest spp. isolated followed by non –albicans Candida spp. 46.66% (14). This study shows a little higher rate of isolation for C.albicans compared to non-albicans Candida, though there are studies (Sumitra Devi, 2014) which have shown that there is considerable increase in non-albicans Candida spp. in Candiduria.

According to Rippon, there is some effect of the antibiotics on the host tissue, which predisposes it to invasion by the organism, and the antibiotics itself may stimulate the growth of Candida(Rippon,1988).The most important effect of antibiotics is the elimination and alteration of the bacterial flora that holds the population of Candida in check. Use of antibiotics was also associated with prolonged hospital stay.

Diabetes mellitus was the most frequently associated risk factor accounting for 26.86% Invitro experimental studies have shown that hyperglycemia increases the growth of Candida (Warnock, 1979). This may probably be true in humans also that an increase in the concentration of glucose in the tissues, blood & urine promotes the growth of Candida. Hyperglycemic individuals may have increased risk for Candida colonization because their secretion contain glucose, which can serve as nutrients for Candida organisms Sobel & Colleagues reported a fucose (6-deoxy-galactose) vaginal epithelial cell receptor that aids in adhesion of Candida to vaginal epithelial cells(Sobel JD, 1981).

Antifungal susceptibility pattern of Candida isolates from HVS showed that C.albicans was 100% sensitive to Amphotericin –B. Similar findings were seen in other study(Deepa, 2013).80% sensitivity was found against Clotrimazole. Sensitivity to Fluconazole and Voriconazole are 93.33% and 100% respectively which is comparable with other study (Vijaya D, 2014). All the NAC spp. showed 100% sensitivity to Amphotericin-B except C.tropicalis. C.tropicalis showed sensitivity of 54.54% to Clotrimazole, 63.63% to Fluconazole and 81.81% to

Voriconazole. Deepa et al, showed 100 % Resistance of C.krusei to Fluconazole, which is similar to present study (Deepa, 2013).C.glabrata also showed greater resistance to Fluconazole.

Antifungal susceptibility of Candida isolates from urine samples showed that C.albicans was 100% susceptible to Amphotericin –B, where as sensitivity to Fluconazole and Voriconazole was 81.25% and 87.5% respectively. These findings can be compared with other study (Yahavanath, 2013). Non- albicans Candida showed maximum resistance to Fluconazole.

Conclusion:

Among various pathogenic species of fungi, Candida is the most prominent cause of fungal infections. In the present study, though C.albicans was the commonest spp. isolated, there was a slight increase in the prevalence of non-albicans Candida spp. Among the non-albicans Candida, C. tropicalis was the commonest species. Pregnancy and use of OCP were important predisposing factors for VVC, bladder catheterization, underlying DM and prolonged use of Antimicrobials along with age, were important risk factors for candidiasis of the urinary tract. CHROMagar and HiCandida Identification Kit can be used for rapid identification of Candida species. Antifungal susceptibility pattern showed that Candida isolates were more sensitive to Amphotericin –B, compared to that of Clotrimazole, Fluconazole and Voriconazole. C.albicans showed more susceptibility to Azoles compared to that of non-albicans Candida. Voriconazole showed greater susceptibility compared to Fluconazole. Invitro testing of the susceptibility of yeast to antifungal agents will play a role in appropriate selection of antifungal agents for the treatment of fungal infections.

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