ORIGINAL ARTICLE

Prevalence and antifungal susceptibility of Candida species in a tertiary care hospital in Islamabad, Pakistan

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Abstract

Objective: To determine the prevalence and antifungal susceptibility pattern of Candida species.

Methods: This prospective, cross-sectional study was conducted at the Quaid-e-Azam International Hospital, Islamabad, Pakistan, from January 2014 to February 2015, and comprised different clinical samples which were analysed for various types of microbial infections. Species differentiation was confirmed by biochemical and molecular methods. Antifungal susceptibility against amphotericin B, fluconazole and voriconazole was determined by Clinical and Laboratory Standards Institute M44-A disk diffusion method.

Results: Of the 219 Candida isolates, majority of them were isolated from urine 78(35.6%) and vaginal swabs 59(26.9%). Moreover, 144(65.8%) samples were of females and 75(34.2%) were of males. Candida albicans 128(58.45%) was the most predominant species followed by Candida glabrata 30(13.69%), Candida tropicalis 26(11.87%), Candida krusei 17(7.76%), Candida parapsilosis 12(5.47%), Candida dubliniensis 3(1.37%) and Candida lusitaniae 3(1.37). All isolates were least susceptible to amphotericin B with a susceptibility rate of 213(97.26%). The highest resistance was found for voriconazole 40(18.26%) compared to fluconazole 32(14.61%).

Conclusion: Candida species possessed high resistance rate against various antifungal agents.

Keywords: Candida albicans, Non-albicans (NAC), Antifungal sensitivity test, CHROMagar Candida. (JPMA 67: 986; 2017)

Introduction

The rate of Candida (C.) infections is tremendously increasing globally, ranking Candida species (spp.) as the most important opportunistic pathogens among yeasts. The spectrum of infection caused by Candida species (upon becoming a pathogen) is wide, ranging from superficial or local mucosal infections (genitourinary and non-genital) to life-threatening disseminated infections including endocarditis, peritonitis, candidemia, systemic and hepatosplenic candidiasis.[1]

Infections related to Candida can become hazardous to life for the persons possessing severely weak immunity, including persons suffering from acquired immune deficiency syndrome (AIDS), patients undergoing cancer treatment by chemotherapy and radiotherapy, patients admitted for major surgery and organ transplantation in intensive care unit (ICU).[2] Nosocomial infections due to Candida species are increasing since 1980's, leading to high morbidity and mortality rates. In the United States, Candida species are the fourth most widespread pathogens recovered from patients having systemic Candida infections. Wards where Candida infections are mostly prevalent are surgical and medical ICUs for adult, paediatric and neonatal patients.[3]

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Most of the fungal infections in humans are caused by C. albicans, which is recognised to be the most predominant pathogenic species inhabiting the skin, reproductive and gastrointestinal tract of humans. In recent years, epidemiological pattern has shifted towards the predominance of non-albicans species (NACs) such as C. glabrata, C. parapsilosis, C. krusei and C. tropicalis, accounting for 50% of the total Candida infections.[4] The emergence of antifungal drug resistance among Candida albicans and non-albicans species has resulted in the treatment failure posing a serious challenge for the effective management of candidiasis.[5]

For many years, the standard treatment for candidiasis has been amphotericin B, a polyene fungicidal agent. Nevertheless, it highly nephrotoxic nature and high cost of its lipid formulated form limit its use. Recently, azoles (fluconazole, voriconazole and itraconazole) having different spectrum of activities, with low toxicity and high efficiency are being used as initial therapy for Candida infections.[3] The rising diversity in Candida species, their varying susceptibility and resistance to antifungal agents require prompt diagnosis for the control and management of these infections. The current study was planned to determine the prevalence of Candida spp. in patients of a local hospital having mycosis symptoms and to scrutinise their susceptibility pattern against different antifungal agents.

Materials and Methods

This prospective, cross-sectional study was conducted at the Quaid-e-Azam International Hospital (QIH), Islamabad, Pakistan, from January 2014 to February 2015, and comprised different clinical samples which were analysed for various types of microbial infections. These sample were collected from Outpatient Department (OPD) and inpatients, including those in Intensive Care Unit (ICU), neonatal ICU, urology, gynaecology, surgical, operation theatre and paediatrics wards. Approval was obtained from the institutional ethics committee after written consent of patients. Different types of clinical specimens which comprised urine, blood, stool, tissue, nail clipping, sputum, nasal swab, throat swab, ear swab, pus, vaginal swab and tip of catheter were processed for isolation of Candida using Sabouraud dextrose agar (SDA) (CM0041, OXOID). Data regarding patients was recorded from patient's investigation request forms and structured questionnaires. Patients with metabolic disorder were excluded.

All types of samples were inoculated on SDA for 24-48 hours at 37ºC, and Candida colonies development was observed. After gram staining and germ tube test, further confirmation was done by employing two differential media i.e. CHROMagar Candida (BBLTM) and corn meal agar (CM0103, OXOID). In order to perform species level identification, a series of 20 biochemical tests were performed using analytical profile index (API) 20C (Bionereux, Paris, France). The results observed from API strip were noticed, four-digit figures were determined and results were checked in the analytical profile index.

Deoxyribonucleic acid (DNA) was extracted by following crude boiling method by making slight modifications.[6] The intergenic spacer region (ITS1- ITS2) of ribosomal DNA (rDNA) were amplified by employing ITS1 forward 5'-TCCGTAGGT GAA CCT GCG G-3' and ITS4 reverse 5'-TCC TCC GCT TAT TGA TAT GC-3' (Fermentas, Germany) primers. Polymerase chain reaction (PCR) amplification was carried out in a final volume of 25µl containing 5µl from 5X Prepared Master Mix (Fermentas, Germany), 3ul of template DNA, 0.4µl of each forward and reverse primer and 16µl of PCR water. Amplification was performed in thermal cycler (Biometra) consisting of 36 cycles with initial denaturation at 95°C for 5 minutes, second denaturation for 45 seconds at 95°C, primer annealing at 52.5°C for 1 minute, primary extension at 72°C for 1 minute and final extension at 72°C for 5 minutes. PCR products were analysed on 2% agarose gel photographed under ultraviolet illuminator.

Antifungal susceptibility test was performed by following Clinical and Laboratory Standards Institute (CLSI M44-A) disk diffusion method. All the isolates were tested against three antifungal impregnated disks i.e. 20µg amphotericin B (Liofilchem, Italy), 1µg voriconazole (Oxoid, England) and 25µg fluconazole (Liofilchem, Italy). Zone of inhibition for respective drug was measured after incubation at 37ºC for 24-48 hours.

Results

Of the 3,007 clinical samples, 540(17.96%) were of urine, 324(10.77%) high vaginal swab (HVS), 178(5.92%) pus, 378(12.57%) tracheal, 309(10.28%) bronchial, 167(5.55%) sputum and 1,111(36.95%) samples were from other sites. Of all, 219(7.28%) samples were positive for candida infections, including 78(35.62%) from the urine samples, 59(26.94%) HVS, 10(4.57%) pus, 20(9.13%) tracheal, 10(4.57%) bronchial, 32(14.61%) sputum and 10(4.57%)

Table-1: Specimen and ward wise distribution of Candida species.

Specimen	Candida Species							Total
	C. albicans	C. glabrata	C. tropicalis	C. krusei	C. parapsilosis	C. dubliniensis	C. lusitaniae	
Urine	47	9	10	5	7	0	0	78 (35.6%)
HVS	31	9	6	7	3	2	1	59 (26.9%)
Sputum	20	6	4	1	1	0	0	32 (14.6%)
Trachea Lavage	13	1	3	2	0	1	0	20 (9.1%)
Bronchial lavage	5	2	0	2	0	0	1	10 (4.6%)
Liquid Pus	5	1	3	0	0	0	1	10 (4.6%)
Others	7	2	0	0	1	0	0	10 (4.6%)
OPD	43	11	11	10	4	1	0	80 (36.5%)
IPD	85	19	15	7	8	2	3	139 (63.5%)
Total	128	30	26	17	12	3	3	219 (100%)

Others* include: Central venous pressure-endotracheal tube (CVP-ETT tip), Wound swab, Throat swab, Blood, Cerebral spinal fluid, Stool, Body fluid, Fungal culture, Tissues.
HVS: High vaginal swab
OPD: Outpatient department
IPD: Inpatient department.

Table-2: Gender-wise distribution of Candida albicans and non-albicans (NACs) species.

Gender	Candida species/No. of isolates								
	C. albicans (128)	C. glabrata (30)	C. tropicalis (26)	C. krusei (17)	C. parapsilosis (12)	C. dubliniensis (3)	C. lusitaniae (3)	Total NACs (91)	Total Isolates (219)
Female	81	21	17	12	8	3	2	63	144 (65.8%)
Male	47	9	9	5	4	0	1	28	75 (34.2%)

NACs: Non-albicans.

Table-3: Age-wise Candida species distribution.

Gender	Age groups						
	Children (0-11)	Teenagers (12-18)	Young adults (19-25)	Adults (26-40)	Middle aged (41-60)	Senior citizen (>60)	Total
Female	4	2	13	42	35	48	144 (65.8%)
Male	3	2	3	9	20	38	75 (34.2%)
Total	7	4	16	51	55	86	219 (100%)

from other sites. All these positive samples produced cream to white, smooth and glossy colonies - characteristic of Candida species on the SDA. These Candida-positive colonies were gram stained and only those which were round to oval with purple-coloured budding yeast cells were further processed for germ tube (GT) test. A total of 131(59.82%) strains produced germ tubes, hence were categorised as either C. albicans or C. dubliniensis, while 88(40.18%) strains which were GT-negative and were designated as Candida species.

Species level identification was performed by using CHROM agar Candida and corn meal agar. On the basis of growth on both the media, out of all the positive isolates C. albicans 128(58.45%) was the most predominant species followed by C. glabrata 30(13.69%), C. tropicalis 26(11.87%), C. krusei 17(7.76%), C. parapsilosis 12(5.47%), C. dubliniensis 3(1.37%) and C. lusitaniae 3(1.37). Among NACs, C. glabrata was the most abundant species. The Candida species were also identified through various biochemical tests using API 20C kit system and the results confirmed microscopic and morphological observations. Moreover, 139(63.5%) of the infections were acquired in hospitals compared to 80(36.5%) community-acquired infections. C. albicans was the most abundant species in both the OPD and IPD, followed by C. glabrata, C. tropicalis, C. krusei and C. parapsilosis. C. krusei was more prevalent in OPD, while other species were abundant in IPD. Highest prevalence of Candida species was in ICU (Table-1).

After amplification, species was identified on 2% agarose gel along with 1kb DNA ladder (Generular), amplicons of size 510 base-pair (bp) were identified as Candida krusei, 520 bp as Candida parapsilosis, 524 bp as Candida tropicalis, 535 bp as Candida albicans and 871 bp as Candida glabrata

Gender-wise distribution of Candida species was observed according to which more females 144(65.8%) than males 75(34.2%) were infected. It was observed that the number of C. albicans and all the NAC species was high in females as compared to males. Among the NACs, C. tropicalis, C. glabrata and C. krusei were the predominant species in females. In case of males, C. tropicalis, C. glabrata were high in number after C. albicans (Table-2).

Patients were divided into six age groups. The highest rate of Candida species was obtained from the patients aged above 60 years with highest prevalence of C. albicans followed by C. glabrata, C. tropicalis and C. krusei. In the age group 26-40 and 41-60 years, C. glabrata, and C. tropicalis were prevalent. C. krusei was most abundant within the middle-aged group, i.e. 41-60 years (Table-3).

In our study, amphotericin B was the most effective antifungal against all the Candida species with a susceptibility rate of 213(97.26%). Resistance towards amphotericin B was noted for 3(2.34%) C. albicans, 1(3.33%) C. glabrata and 1(5.88%) C. krusei species. Interestingly, the highest resistance was found for voriconazole 40(18.26%) compared to fluconazole 32(14.61%). C. krusei 4(23.5%) were the most resistant Candida species to fluconazole followed by C. albicans 24(18.75%), C. glabrata 3(10%) and C. parapsilosis 1(8.3%). However, C. parapsilosis was the most resistant to

Table-4: Antifungal susceptibility profile of Candida species.

Species/No. of isolates	AMB			FLU			VOR		
	S	I	R	S	I	R	S	I	R
C. albicans (128)	125	0	3	104	0	24	102	0	26
C. glabrata (30)	28	0	1	27	0	3	26	0	4
C. tropicalis (26)	26	0	0	26	0	0	24	0	2
C. krusei (17)	16	0	1	13	0	4	13	0	4
C. parapsilosis (12)	12	0	0	11	0	1	8	0	4
C. dubliniensis (3)	3	0	0	3/3	0	0/3	3/3	0	0/3
C. lusitaniae (3)	3	0	0	3/3	0	0/3	3/3	0	0/3
Total (219)	97.26%	0%	2.28%	85.38%	0%	14.61%	81.73	0%	18.26%

AMB: Amphotericin B
FLU: Fluconazole
VOR: Voriconazole.

voriconazole 4(33.3%), followed by C. krusei 4(23.5%), C. albicans 26(20.3%), C. glabrata 4(13.3%) and C. tropicalis 2(7.7%). A 100% susceptibility rate was noted in C. dubliniensis and C. lusitaniae for both the azole antifungals (Table-4).

According to the antifungal resistance data of this study, cross-resistance between fluconazole and voriconazole was found among 18(8.2%) of the isolates. Of them, 16(88.9%) were C. albicans while 2(11.1%) were C. glabrata. Both the C. glabrata isolates were cross-resistant to fluconazole and voriconazole. Among C. albicans, 14(87.5%) isolates were cross-resistant to fluconazole and voriconazole, 1(6.25%) isolate was resistant against amphotericin B and voriconazole while 1(6.25%) C. albicans isolate was resistant to all the three antifungals i.e., amphotericin B, fluconazole and voriconazole.

Discussion

Over the past few years, a remarkable increase in the incidence of opportunistic yeast infections, particularly caused by endogenous human commensal Candida species, has gained serious concern in the medical communities worldwide. Although Candida albicans has been the most frequent cause of infections, non-albicans species such as C. tropicalis, C. glabrata, C. krusei and C. parapsilosis have also been reported in both the systemic and mucosal candidiasis. The virulence factors and antifungal susceptibility profile of C. albicans and NACs vary which has necessitated correct and rapid species identification as this has a direct impact on the choice of treatment.[7]

In our study, C. albicans (58.4%) was the leading pathogen as compared to NACs similar to earlier reports.[8-11] Nucci et al.[12] also reported C. albicans (37.6%) as major contributor of Candida infection followed by C. parapsilosis and C. tropicalis. The order of prevalence of NACs in our study was C. glabrata (13.7%), C. tropicalis (11.9%), C. krusei (7.8%), C. parapsilosis (5.5%), C. dubliniensis (1.4%) and C. lusitaniae (1.4%). A significant finding of our study is C. glabrata among NACs being the most common species in clinical samples. This could be a perturbing threat due to high incidence of increased resistance of this species to the routinely used antifungal agents.

Patel et al.[10] isolated highest number of Candida isolates from urine and sputum, which is similar to our work where urine 78(35.6%), vagina 59(26.9%) and sputum 32(14.6%) had predominant Candida species. Especially C. albicans are found to be major pathogen in these cases, one of the reason being its strong adherence to the vaginal epithelial cells in contrast to the remaining species. Farooqi et al.[13] reported a different epidemiological trend where C. tropicalis was the most common organism followed by C. parapsilosis and C. glabrata.

Candida infection was higher in females 144(65.8%) as compared to males(30.9%) in our study, which is in accordance with findings of Nardin et al.[14] The reason of high distribution and virulence of Candida species in females is that it has a receptor for female reproductive hormones. Rashwas et al.[15] observed candiduria in 34.4% females and 14.9% in males. Aslam et al.[16] also reported nosocomial candidiasis more frequent in female patients (56%) as compared to male patients (44%). In our results, high percentage of female patients visiting the QIH may be due to problem in personal hygienic conditions. In this study, Candida infection was most prevalent within the age group of >60 years and middle aged-group, which is in accordance with studies of Furnaleto et al.[17] and Al-Hussaini et al.[18]

Candida infection may be acquired from hospital or community. In this study, 63.5% of infections were

hospital-acquired and 36.5% were community-acquired. This could be due to various factors which contribute to high incidence rate of hospital-acquired infections, including presence of immunocompromised patients, use of antibiotics, indwelling devices and hands of healthcare workers. Pregnancy is also a self-risk factor of high Candida colonisation. Chances of candidiasis are 30% high in pregnant women as compared to non-pregnant.[15] In the present study, Candida infection rate was high in gynaecology and urology wards. However, other studies reported that Candida infection was more common in ICU and surgical ward.[19]

Amphotericin B was found to be highly effective against all tested species except for C. albicans, C. glabrata and C. krusei, which is similar to report of De Almeida et al.[20] However, Patel et al.[11] observed comparatively less susceptibility of C. tropicalis (75.6%) and C. albicans (88.8%) against amphotericin.

Overall sensitivity rate against fluconazole in our study was 85.4%, which is in agreement with the study conducted by Aslam et al.[16] Literature review reported problem of increased fluconazole resistance among NACs. C. krusei and C. glabrata are regarded as intrinsically resistant species against azole antifungals. Antifungal susceptibility data of this study also observed marked rise in azole resistance in NACs as compared to C. albicans. C. krusei was the most resistant species among all the isolates followed by C. albicans, C. glabrata and C. parapsilosis. Oberoi et al.[21] reported high fluconazole sensitivity in C. tropicalis, high resistance in C. glabrata and less resistance in C. parapsilosis. All tested C. tropicalis local isolates were fluconazole sensitivity in contrast to C. parapsilosis and C. glabrata. Badiee and Alborzi[22] report 89.5% susceptibility of C. albicans to fluconazole; which is quite similar to our results. Fluconazole resistance was 18.8% similar to the Sojakova et al.,[23] which reported 13% fluconazole resistance in 227 Candida isolates. Kaya et al. reported an alarming increased fluconazole resistance in C. albicans (68.7%) and NACs (63.2%).[24]

A notable finding in our study was an increased voriconazole resistance in both the C. albicans (11.8%) and NACs (6.4%) unlike previous reports where no resistance was reported to voriconazole. At the same time, some studies observe that fluconazole-resistant isolates also develop resistance to voriconazole as a result of cross-resistance. Practically, voriconazole has been found to be more effective than itraconazole and fluconazole, however, emergence of voriconazole resistance in isolates is most likely related with crossing resistance, due to similarity in their chemical structure which is a serious matter of concern for the community. Keceli Ozcan et al. also report some degree of resistance in NACs.[25] Wiebusch et al.[26] observed 45.83% resistance to both the fluconazole and voriconazole in tested isolates. Demito et al.[27] mentioned that fluconazole and voriconazole showed equivalent efficacy in vitro. Cross-resistance and reduced susceptibility to both fluconazole and voriconazole was observed in 11.3% of isolates in a study of Oberoi et al.[21] We observed cross-resistance in 18(8.2%) of the tested isolates.

Conclusion

C. albicans was found to be the most prevalent species as compared to NACs. An increase in resistance to fluconazole and interestingly voriconazole among C. albicans and NACs was also observed. The increase of antifungal resistance in non-albicans species is an alarming situation. This emphasises that there is dire need of species level identification for the proper antifungal selection and successful treatment against mucosal and systemic candidiasis. Patients and healthcare workers should be properly educated about the growing trends of antifungal resistance. Cumulative antifungal susceptibility reports on multiple patients will help to suggest an appropriate antifungal for prophylaxis. There should be a national surveillance programme for fungal infections. The antifungal susceptibility data derived from the laboratories all over the country could be used for the reliable and rapid detection of antifungal resistance.

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