

Candidiasis: Prevalence and resistance profiling in a tertiary care hospital of Pakistan

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Abstract

Objective: To determine *Candida* colonisation/infection in renal transplant patients and to determine the resistance pattern against antifungal drugs.

Method: This prospective, observational study was conducted at Al-Sayyed Hospital, Rawalpindi, Pakistan, from January to October 2014, in collaboration with the Microbiology and Public Health Laboratory's, Islamabad campus..The clinical specimens investigated included respiratory tract secretions, blood, urine, high vaginal swab, skin scrapings, and plastic devices samples.

Results: Of the 7,850 samples, 164(2.08%) were positive for *Candida*. *Candida albicans* were most prevalent as they were found in 114(69%) samples. Besides, 56(34%) of the positive samples were resistant to one or more antifungal agents. Highest resistance was obtained against fluconazole. We found only 5(3.04%) positive samples of *Candida glabrata*; of them, 3(60%)were resistant. In case of *Candida* spp, 27(48%) resistance was observed. In *Candida albicans*, 23(41%) of the samples were found to be resistant. Most of the *Candida* isolates was recovered from bronchial alveolar lavage.

Conclusion: Although *Candida albicans* remained the main responsible species for *Candida* infections, but non-albican *Candida* species are also emerging.

Keywords: Chronic renal failure, Candidiasis, Non-albicans *Candida*, Candiduria, Antifungal resistance. (JPMA 67: 688; 2017)

Introduction

Genus *Candida* comprises more than 500 species, of which around twenty species are commonly recovered from human samples. It exists as saprophyte, colonising mucosal surfaces and external genitalia of human of either gender, but especially near the urethral meatus of healthy, premenopausal women.¹ *Candida* is asymptotically present in the oral cavity of many individuals, with the posterior part of the dorsum of the tongue being the favourite habitat. Significant geographical variation has been particularly observed. For example, pathogen carriage rates of as low as 7.7% have been reported in Asian children, compared to as high as 70% in children from the West. Species-wise, *Candida albicans* is the most frequently isolated species from Europeans and Americans, while non-albicans species (NAC) seem to predominate among Chinese.² These are usually part of the commensal microflora, but can cause opportunistic infections in susceptible hosts. *Candida albicans* is the most frequently isolated species; however, NAC including *Candida parapsilosis*, *C. krusei*, *C.*

tropicalis, *C. glabrata*, and *C. dubliniensis*, are increasingly being detected in clinical samples, and seem to dominate in certain populations.²

Candidiasis prevalence is increasing rapidly in immunocompromised, aged and in those patients having prolonged antibacterial therapy and in recipients of organ transplants.^{3,4} Patel et al. observed a very high carrier rate in human immunodeficiency virus(HIV) positive individuals that accounts for 81.3%.⁴ The candidiasis is particularly important in solid organ transplant recipients as it is associated with high mortality rate. Mostly, it is observed that fungal infections occur after 2-6 months of a transplant. Particularly, liver transplant recipients are more prone to candidiasis. It is of paramount importance to rule out the fungal infections early in them to start proper treatment in order to avoid transplant failure.⁵

In recent decades, there has been an increase in the survival of recipients of solid organ transplants related to the improvement of the surgical technique, the introduction of protocols for immunosuppressive therapy, and the use of antimicrobial prophylaxis. Nonetheless, invasive fungal infection (IFI) is currently the major cause of morbidity and mortality in this group of patients. Invasive candidiasis is the most common IFI found after renal transplantation and is usually associated with total

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parenteral nutrition, broad-spectrum antibiotic therapy and abdominal surgery.⁶

From the previous data, it is apparent that candiduria is an increasingly difficult problem for modern physicians to recognise and manage. NAC species are emerging pathogens and can also colonise human muco-cutaneous surfaces. Consequently, they are also isolated in the setting of candidiasis, albeit at a lower frequency.⁶ *C. albicans* and other related *Candida* species that are pathogenic are developing resistance to antifungal agents, in particular triazole compounds, by mechanisms such as expression of efflux pumps that reduce drug accumulation, alteration of the structure or concentration of antifungal target proteins and alteration of membrane sterol composition. The clinical consequences of antifungal resistance can be seen in treatment failures in patients and variations in the prevalence of *Candida* species causing disease.⁷

Though antibiotic-resistant bacterial infections are a widely recognised public health threat, less is known about the effects of antifungal resistance and the burden of drug-resistant fungal infections. This article highlights the need for an improved understanding of the reasons for their emergence, heightened awareness among medical and public health communities about these infections, and the resistance to conventional antifungal drugs. The epidemiology of candidiasis in renal transplant patients in Pakistan is not documented yet. The current study was planned to determine *Candida* colonisation/infection in renal transplant patients and to determine the resistance pattern against antifungal drugs.

Materials and Methods

This cross-sectional study was conducted at the Kidney Centre of Al-Sayyed Hospital, Rawalpindi, Pakistan, from January 2014 to October 2014, in collaboration with the Microbiology and Public Health Laboratory, COMSATS Institute of Information Technology (CIIT), Islamabad campus, and comprised specimens of blood, urine, respiratory tract secretions, high vaginal swab, skin scrapings, and plastic devices samples. The Kidney Centre is a tertiary/quaternary care hospital that is primarily meant to treat renal ailments and offers kidney transplant surgeries, liver transplants and bone marrow transplants. However, most of the patients included in the study were with chronic renal failure and kidney transplant.

The clinical specimens investigated included respiratory tract secretions (sputum, bronchoalveolar lavage (BAL), bronchial washing and tracheal aspirate), blood, urine, high vaginal swab (HVS), skin scrapings, and plastic

devices (Foley's catheters tip, endotracheal tube tip, intravascular catheter tip and renal stent) samples. All the samples were collected by standard microbiological procedures and further cultured on blood agar and MacConkey agar. After incubation at 37°C for at least 24-48 hours, plates were observed for growth. All those samples displaying characteristic *Candida* growth were isolated and further processed for confirmation and species identification.

Chromagar *Candida* selective and differential agar (Chromagar, France) was used for detection and quantification of *Candida* species in the samples. Species were presumptively identified based on colony colour-light green colonies as *C. albicans*; metallic to dark blue colonies with or without a purple halo as *C. tropicalis*; pink and rough spreading colonies with pale edges as *C. krusei*, dark pink/mauve colonies with pale edge as *C. glabrata*, and white or gray colonies as unidentified species (Figure-1).

After identification, evaluation of minimum inhibitory concentrations (MIC) of drugs against these strains was done depending upon consultant request. E-test strips of antifungals including Amphotericin B, Voriconazole, Fluconazole and Ketoconazole were used to test susceptibility of the strains (Figure-2). Briefly, candidal suspension was prepared in normal saline and turbidity was compared to that of 0.5 McFarland standard. Microbial lawn was prepared on Roswell Park Memorial Institute (RPMI) 1640 agar (1.5%) supplemented with dextrose (2%) and buffered to pH 7.0 with morpholinepropanesulfonic (MOPS) acid with sterilised cotton swab. Plates were dried for 15 minutes and E-test strips (gradient agar diffusion technique) were applied carefully with caution and incubated at 37°C. After 24-48 hours incubation, results were interpreted according to European Committee on Antimicrobial Susceptibility Testing (EUCAST) guidelines 2015.⁸ The Etest minimum inhibitory concentration (MIC) was defined as the drug concentration at which the border of the elliptical zone of complete inhibition intersected the scale on the antifungal test strip. Broth microdilution (BMD) breakpoints were applied on MICs obtained by E-test results. The resistance breakpoints were >4 for fluconazole and >0.12 for voriconazole and >0.12 ketoconazole representing the azoles, and >1 for amphotericin B. Data was entered and recorded in Microsoft Excel 2010.

To screen samples for multidrug-resistant (MDR) isolates, the most frequently used antifungal agents were selected, i.e. fluconazole and voriconazole and ketoconazole

representing the azoles, and amphotericin B from the polyene. Isolates were considered multidrug resistant if they were resistant to one or more representative antifungal(s).⁹

Results

Of the 7,850 samples, 164(2.1%) were found to be positive for Candida. Candida albicans was the most prevalent type as it was present in 114(69%) samples, followed by C.spp 37(22.5%), C.tropicalis 7(4.2%), C.glabrata 5(3%) and C.krusei1(0.6%) (Table-1).

Moreover, 63(38%) Candida samples were recovered from Bronchoalveolar lavage, 22(13.4%) from HVS and 20(12%) from throat swab. Candidemia, which is the presence of Candida in blood, was noticed in 1(0.6%) sample (Table-2).

Resistance to any one of the tested antifungal agents was observed in 56(34%) of the positive samples. Among these resistant microbes, the prevalence of fluconazole was 48(85%), Amphotericin B 4(7%) and Voriconazole and Ketoconazole 2(3.5%) each (Table-3).

C.spp was present in 27(48%) of the resistant microbesor 73% of all the C.spp samples that we recovered. It was followed by C. albicans where resistance was noted to be 23(41%) or 20% of all isolated Candida albicans. Moreover, 3(5.4%) of Candida glabrata isolates and 2(3.6%) of Candida tropicalis isolates were found to be resistant. The only Candida krusei sample was also found to be resistant to fluconazole.

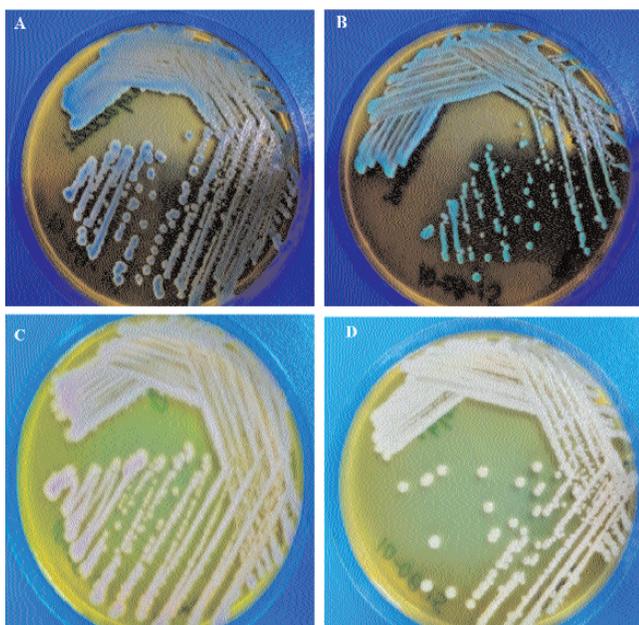


Figure-1: Identification of Candida species using differential medium. It includes Candida tropicalis (A), Candida albicans (B), Candida glabrata (C), Candida Spp (D).

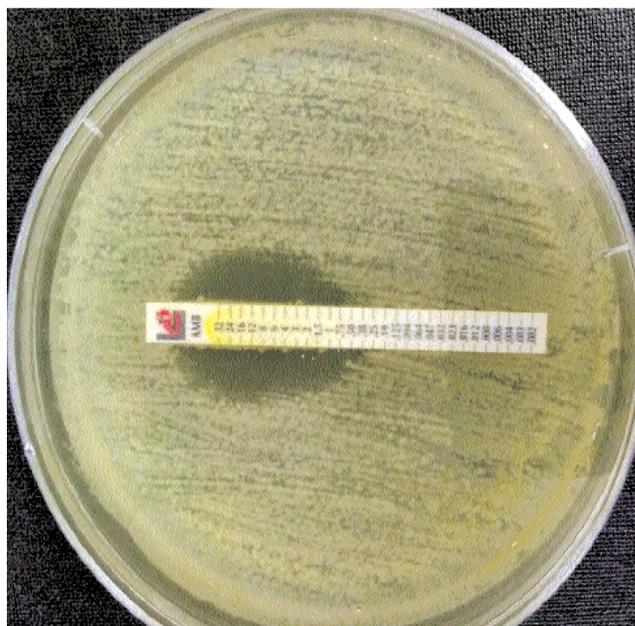


Figure-2: Evaluation of Minimum Inhibitory Concentrations (MIC) of Amphotericin B against Candida spp.

Table-1: Breakdown of total positive Candida samples into different Candida species.

S. No	Specie identified	Positive Samples (n=164)
1	C. albicans	114
2	C. glabrata	5
3	C. tropicalis	7
4	C. krusae	1
5	C. spp	37

C.: Candida.

Table-2: Candidiasis prevalence from sample sites.

S. No	Sample site	Number	Percentage
1	Bronchoalveolar lavage (BAL)	63	38
2	Blood	01	0.6
3	DJ Tip	2	1.2
4	ETT Tip	11	6.7
5	FT	12	7.3
6	HVS	22	13.4
7	Nasal swab	1	0.6
8	Pus	2	1.2
9	Sputum	14	8.5
10	Throat Swab	20	12
11	Urine	16	9.7
	Total	164	

FT: Foley catheter Tubes
 DJ: Double J
 ETT: Endotracheal tube
 HVS: High vaginal swab.

Table-3: Antifungal Resistance in Candida Isolates.

	Amphotericin B (n=56)	Voriconazole (n=56)	Fluconazole (n=56)	Ketoconazole (n=56)
<i>C. albicans</i>	1	No	22	No
<i>C. glabrata</i>	No	No	3	No
<i>C. krusei</i>	No	No	1	No
<i>C. tropicalis</i>	No	No	2	No
<i>C. spp</i>	3	2	20	2

C.: Candida.

Among all isolates, there were 8(4.9%) MDR isolates which were resistant to both fluconazole and amphotericin B. Of them, 2(25%) isolates were resistant to azoles but were susceptible to polyenes, whereas 6(75%) were found to be resistant to fluconazole and ketoconazole simultaneously.

Discussion

Certain patients undergoing solid organ transplantation have been identified to be at high risk from Candida infections, especially candidemia.^{10,11} Risk factors for candidiasis include age, female gender, antibiotic usage, diabetes, HIV infection, neutropenia, renal transplant, renal obstruction, indwelling Foley's catheter and abdominal surgery.¹² Frequent use of antibiotics alters the bacterial microflora of the vaginal and gastrointestinal tracts and thus allow for overgrowth of Candida spp.¹³ After antibiotic use, the intensification in vaginal colonisation with Candida spp., mostly *C. albicans*, is estimated to range from 10 to 30%, and Vulvovaginal candidiasis (VVC) occurs in 28% to 33% of cases. It is universally hypothesised that the reduction of Lactobacilli in the vaginal tract predisposes women to VVC as Lactobacilli play a key role in the vaginal flora through the production of hydrogen peroxide, bacteriocins and lactic acid, which protect against invasion or overgrowth of pathogenic species.¹³

We recovered Candida in approximately 2% of total samples. *Candida albicans* was the most prevailing type. Our results were found to be in accordance with a study conducted by Fraser et al. where *Candida albicans* was the most frequently isolated species (63%), followed by *Candida tropicalis* (17%), *Candida glabrata* (13%), *Candida parapsilosis* (6.5%) and *Candida krusei* (0.9%).¹⁴ In other studies, the prevalence of *Candida albicans* isolates was reported to be 53% and 55% respectively.^{15,16}

Candida glabrata is thought to be an increasing cause of candidemia, especially at cancer and bone marrow transplant centres where fluconazole is used for antifungal prophylaxis.¹⁷ However, our results are contradictory to previously reported data in a different

region of the world. We could find out *C. glabrata* only in 5 samples. Candidemia was observed only in one patient. We recovered Candida mostly from BAL, vaginal and throat swabs. According to our results, candidemia is not a common manifestation in renal patients in Pakistan.

Resistance to antifungal agents is an evolving medical problem and it complicates patient management, despite the introduction of new antifungal agents. Resistance prevails and continues to grow forth. In vitro susceptibility testing is often used to select agents with likely activity for a given infection, but perhaps its most important use is in identifying agents that will not work, i.e. to detect resistance. Standardised methods for reliable in vitro antifungal susceptibility testing are now available from the Clinical and Laboratory Standards Institute (CLSI) in the United States and EUCAST in Europe.⁸ But the antifungal testing is not a routine test, particularly susceptibility testing is not done in many medical centre.

According to Centres for Disease Control and Prevention (CDC), almost 7% of all Candida bloodstream isolates were resistant to fluconazole. Our results indicate that the resistance to fluconazole is quite high. Overall, we observed that 34% samples were resistant to various antifungal agents including fluconazole. Highest resistance was against fluconazole, followed by Amphotericin B., voriconazole and ketoconazole. Our study revealed that 14% of the Candida samples were MDR.

Candiduria, the presence of Candida species in urine, is another trivial clinical finding, particularly in hospitalised patients. Candiduria is infrequently detected in hale and hearty individuals. Contrarily, it is a matter of serious concern in admitted hospitalised patients, particularly in intensive care units (ICUs). Mostly, Candida is associated with colonisation only and no treatment is required. However, candiduria can be an indication of disseminated infection. Multiple studies indicate that at least 10%-15% of hospital acquired urinary tract infections (UTIs) are caused by Candida species. High prevalence of candiduria has also been observed in renal transplant recipients. It is evident immediately after transplant when both bladder drainage catheters and ureteral stents are present. In a study, the overall percentage of UTIs among catheterised individuals was observed to be 37%, from which 16.4% of the UTIs were caused by Candida species. However, the incidence of candiduria in non-catheterised subjects was only 6.6%.¹³ Our results indicated the prevalence of candiduria in 9.7% of total samples.

In vitro susceptibility testing for fluconazole revealed that 21.1% of vaginal isolates were resistant to fluconazole¹⁸ and the more resistant NAC species were more frequently

isolated from women.^{13,19,20} In our study, we had also observed NAC more resistant against conventional drugs of choice.

Conclusions

Although *C. albicans* remains the main responsible species for Candida infections, NAC species are emerging, highlighting the importance of improved and reliable culture techniques. Treatment of candidiasis can be effectively guided by in vitro susceptibility testing. However, susceptibility testing of fungi is not considered a routine testing procedure in many laboratories and is not always promptly available.

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Conflict of Interest: None.

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