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Determination of Predisposing Factors in Developing *Candida albicans* Associated Urinary Tract Infection and Antifungal Sensitivity Profile

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Authors' contributions

This work was carried out in collaboration among all authors. Author SL helped in conceptualization, designed the study, collected and assembled the data as well as approved and guarantor of the manuscript. Author OP analyze and interpret the data. Authors PK, SS and AAU wrote first draft of the manuscript. Authors ZA and KU did critical revision for important intellectual content. Author MM performed statistical evaluation. All authors read and approved the final manuscript.

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ABSTRACT

Aim: Candiduria is very common in hospitalized patients. It poses a clinical challenge for the physicians since it is usually asymptomatic. The aim of this study was to identify risk factors associated with nosocomial candiduria in urinary tract infection (UTI) suspected patients in **Methodology:** Intensive Care Unit (ICU) and to determine their antifungal sensitivity profile.
The urine specimens (168) were collected, microscopically screened for presence of yeast, cultured and analyzed for counting, isolation, phenotypic identification of *Candida albicans*. and testing antifungal resistance profile. Data regarding age, gender, use of catheter, use of antibiotics, diabetes mellitus among patients was also recorded.

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Results: Out of 168 specimens, *C. albicans* were isolated from 69 specimens, whereas 20 specimens showed other *Candida* spp. Age >45 years, gender female, previous use of antibiotics, urinary catheterization, stay in ICU >1 week were found the main predisposing factors ($p<0.05$) responsible for developing nosocomial candiduria. All *C. albicans* isolates were found either susceptible or susceptible-dose dependent to fluconazole, amphotericin B and voriconazole; however, 62.32% of the isolates were resistant to itraconazole.
Conclusion: Most frequent candiduria, possible predisposing factors in ICU patients and resistance of *C. albicans* towards itraconazole is alarming and highlights the need of candiduria surveillance.

Keywords: Candida albicans; UTI; nosocomial infection; antifungal susceptibility; resistance.

1. INTRODUCTION

The infections caused by fungi are the principal cause of morbidity and mortality in the immunocompromised people. According to Nosocomial Infections Surveillance study of the Centre for Disease Control, *Candida albicans* and other related species were rated the 6th most frequent cause of nosocomial infections [1,2].

Candida species are classified as opportunistic microflora and are very frequently found in the immunocompromised, pregnant women and diabetic patients. If these patients are not treated timely, prevalence of *Candida* could lead to systemic candidiasis, multiple organ failure or even death [3]. In the ICU, there are several risk factors that may lead to increased candiduria, such as widespread use of antibiotics, increased use of broad-spectrum antibiotics, usage of urinary tract devices (for exp. Catheter), diabetes mellitus, immunosuppressive treatment, the extent of the underlying illness, parenteral nutrition, old age, female sex, extended hospitalization and surgeries [4].

There are several species within the genus *Candida* that are responsible for pathogenicity in human. These include *C. albicans*, *C. parapsilosis*, *C. tropicalis*, *C. glabrata*, *C. krusei*, *C. lusitaniae*, *C. kefyr*, *C. guilliermondii* and *C. dubliniensis* [5]. Among these *Candida* spp., *C. albicans* is considered as most frequent cause of mucosal yeast infection.

C. albicans is present in human mouth as a commensal, vaginal and gastrointestinal tract. Nearly 80 per cent of the population do not feel its negative effect, however, overgrowth of *C. albicans* causes candidiasis [4]. In this connection, it has been reported that presence <1000CFU/mL of *Candida* cells (candiduria) in urine does not indicate clinical significance until and unless the patient is seriously ill or hospitalized. However, if urine specimens contain >1000CFU/mL of *Candida* cells, the patients are at risk of getting candidemia [6,7]. *C. albicans* possesses many virulence factors that help in host adhesion and infection. These include a) adaptation to variety of locations of body such as oropharyngeal, gastrointestinal and female genitalia; b) ability to attach with host cells; c) dimorphic form (yeast and filamentous form); d) ability to form hydrolytic enzymes (Phospholipase and proteinase) and, e) ability to form biofilm on biomaterials [8].

The biofilm forming ability of *C. albicans* is related with persistent *Candida* infection, for example, it has been reported that the *C. albicans* cells detach from an adherent biofilm on a catheter and lead to a septicemia that may not be treated with conventional treatment and these biofilm cells serve as a continuous source of infection until the catheter is removed [9]. In addition, biofilm forming ability among *C. albicans* leads to improved resistance towards antifungal agents which poses a great challenge specifically in designing therapeutic ad prophylactic measures [10,11].

The purpose of this study was to identify potential predisposing factors responsible for nosocomial candiduria in ICU patients of Civil Hospital Khairpur and to determine the antifungal sensitivity profile of *C. albicans* isolates.

2. MATERIALS AND METHODS

2.1 Type of Research, Study Area and Ethical Approval

The retrospective descriptive observational study of patients admitted to ICU of Civil Hospital Khairpur and suspected to have hospital acquired UTI was carried out from December

2018 to December 2019. Ethical approval from medical superintendent of Civil Hospital Khairpur and from Research Ethics Committee of our Institute was obtained.

2.2 Collection of Samples

The midstream morning urine specimens or urine specimens from the port of the catheter (N=168; 84 male and 84 female) were collected in sterilized urine collection bottles from only those patients who gave consent. The age, gender, use of catheter, use of antibiotics, diabetes mellitus among patients was also recorded. The specimens were analyzed for microscopic examination, *Candida* cells number (in colony forming unit (CFU)/mL), isolation and phenotypic identification of *C. albicans* and antifungal susceptibility test.

2.3 Microscopic Examination

Urine specimens were initially screened for appearance of yeast cells with or without red blood cells (RBCs). For this purpose, the urine specimens were centrifuged at 252 x g for five minutes. Subsequently, supernatant was discarded and 20µl of sediment sample was transferred on glass slide. Afterwards, specimen was covered with a cover slip and slide was observed under high power field of microscope [12]. After microscopic visualization, yeast cells positive specimens were selected for further investigation.

2.4 Isolation and Estimation of *Candida* Cell Number

For isolation and detection of yeast cell number, the urine samples were vigorously shaked for uniform distribution of urine contents and then serial dilution (10-fold) was prepared in sterilized tubes. A 100µl of specimen was inoculated on plates containing SDA and chloramphenicol (0.05% w/v) followed by incubation at 37°C for 24 h [13]. After incubation, CFU/mL were calculated by counting colonies on SDA plates and then multiplying with dilution factor.

2.5 Identification of *C. albicans*

The pure cultures of *Candida* spp. cultured on fresh SDA plates were used to identify *C. albicans* based on cultural and microscopic characteristics, germ tube test and API *Candida* test.

2.6 Cultural and Microscopic Characteristics

The colonial size, shape and color on SDA plates were recorded after 24 and 48 hours. For observing microscopic characteristics, wet mount technique was performed. Briefly, lactophenol cotton blue (20µl) was transferred on glass slide. With the help of sterilized mycological needle, a portion of colony was picked and emulsified with lactophenol cotton blue. A coverslip was placed over specimen area of slide followed by observation under compound microscope by using 40x lens [14]. The images were taken by Nikon Microscope camera (Model DS-Fi3).

2.7 Germ Tube Test

For screening *C. albicans* from other yeasts a germ tube test was performed [13]. Concisely, a 500µl of sheep serum was added in a small, sterilized tube and a small fraction of *Candida* spp. colony was emulsified in it using a sterilized Pasteur pipette. Later, inoculated tubes were incubated at 37°C for 3 hours. Afterwards, a drop of incubated serum was transferred on sterilized glass slide and coverslip was placed over it. The slide was then observed for germ tube production to identify *C. albicans* [14].

2.8 The API *Candida* Test

For the confirmation of *C. albicans*, API Candida test was performed. The API Candida strip contains 10 wells to perform 12 biochemical tests. This test was performed as per manufacturer's guidelines. Briefly, few yeast colonies were emulsified in 0.85% saline. With the help of sterilized syringe inoculum was transferred into each well containing dehydrated media and reagents. The API strip was incubated for 24 h at 37°C. Positive and negative reactions were recorded and analyzed for the identification of *C. albicans* using manufacturer's website, APIWEB™.

2.9 Antifungal Susceptibility Test

Antifungal susceptibility test for *C. albicans* isolates was performed using disk diffusion method as per CLSI M44 series for yeast [15]. The *C. albicans* inoculum was standardized to 0.5 McFarland assay and inoculated on Mueller-Hinton agar (supplemented with 2% glucose, and 0.5 mg/L methylene blue dye). The commercial antifungal discs of Fluconazole, Amphotericin B,

Voriconazole and Itraconazole were placed over fungal lawn at certain distance in petri plates. This was followed by incubation of plates at 35℃ for 24 h. After incubation size of growth inhibition zones around antifungal discs were measured in millimeter (mm) and isolates were classified in following categories: susceptible, susceptible dose dependent and resistant as per standard zone size suggested by CLSI (Table 1).

2.10 Statistical Analysis

Data for predisposing factors were analyzed using XLSTAT365-Freemium. Student's *t*-test or Mann–Whitney *U*-test was performed to compare means of two data sets. Significance was considered at $p<0.05$.

3. RESULTS

3.1 Microscopic Examination

Out of 168 urine specimens tested, candiduria was detected in 89 samples (53%), whereas 79 (47%) urine samples were found negative for *Candida* as confirmed by microscopy (Table 2). The presence of *Candida* cells along with RBCs can be seen in Fig. 1.

3.2 Candida Cells Number in Urine Specimens

Candida cells number in urine specimens were ranged from 2.2×10^2 to 5.5×10^5 CFU/mL. High number of *Candida* cells was found in catheterized patients followed by patients using antibacterial antibiotics. Specimens with high number of red blood cells in microscopy also revealed increased *Candida* cells on culture.

3.3 Identification of *C. albicans*

On SDA plates smooth, small and whitish to creamy colored colonies were observed after 24 h (Fig. 2A) suggesting the presence of *C. albicans* in urine specimens. Wet mount technique showed ovoid yeast-like cells with some producing bud and daughter cells which provided another clue for the presence of *C. albicans* (Fig. 2B). Out of 89 isolates, germ tube was observed in 69 samples distinguishing *C. albicans* from other *Candida* spp. (Fig. 2C). API Candida test was performed for the confirmation of *C. albicans* showed positive reaction for four sugar assimilation tests including glucose, galactose, sucrose and trehalose and two enzymatic activity tests including α-amylase and N-acetyl β-glucosaminidase. All other tests including raffinose, β-maltosidase, β-xylosidase, β-glucuronidase, urea hydrolysis and β-galactosidase were found negative (Fig. 2D). The score (Profile) generated from APIWEB™ analyzer was 7 1 1 2 (Fig. 2E) that confirms 99.9% identity of *C. albicans*.

3.4 Demographic Profile and Risk Factors in Patients With Candiduria

Statistical analysis showed that p value was significant ($p<0.05$) for age >45 years, gender female, previous use of antibiotics, urinary catheterization and stay in ICU >1 week, suggesting that these could be the main risk factors for developing candiduria caused by *C. albicans* (Table 2).

3.5 Susceptibility Profile of *C. albicans* to Antifungal Agents

Measurement of zone of inhibition demonstrated that 62.32% *C. albicans* showed resistance to itraconazole, whereas all the isolates were either susceptible or susceptible dose dependent to fluconazole, Amphotericin B and voriconazole investigated in this study (Table 3).

4. DISCUSSION

UTI is one of the most common infection in ICU patient. This disorder accounts for 20-50% of nosocomial infections each year [16]. *Candida* spp. have become an important causative agent of UTI in hospitalized patients. Candiduria incidents vary in the hospital settings and are most predominant in ICUs [17].

Table 1. Interpretation of disk diffusion test for yeast as per CLSI guidelines [15]

Interpretive Category	Zone Diameter (mm)
Susceptible	≥20
Susceptible dose dependent	15-19
Resistant	≤14

Table 2. Demographic profile and various risk factors in patients with candiduria

Risk factor	Specimens collected	Candiduria		Absence of candiduria	*C. albicans*	
	No. (168)%	No. (89)%	p value	No. (79)%	No. (69)%	p value
Age						
19-45	43 (25.6)	28 (31.46)	*P*=0.05	15 (18.99)	17 (24.63)	*P*=0.05
>45	125 (74.4)	61 (68.540)		64 (81.01)	52 (75.37)	
Sex						
Males	8450	37 (41.57)	*P*=0.03	47 (59.5)	21 (30.43)	*P*=0.03
Females	8450	52 (58.43)		32 (40.5)	48 (69.57)	
Antibiotics in use						
Yes	119 (70.83)	87 (97.75)	*P*=0.04	32 (40.5)	51 (73.91)	*P*=0.04
No	49 (29.17)	02 (2.25)		47 (59.5)	18 (26.09)	
Urinary catheterization						
Yes	95 (56.55)	68 (76.4)	*P*=0.04	27 (39.13)	56 (81.16)	*P*=0.04
No	73 (43.45)	21 (23.6)		52 (60.87)	13 (18.84)	
Diabetes mellitus						
Yes	23 (13.69)	13 (18.84)	*P*=0.03	10 (12.66)	07 (10.14)	*P*=0.06
No	145 (86.31)	76 (81.16)		69 (87.34)	62 (89.86)	
Duration of stay in ICU						
1week	57 (33.73)	10 (11.24)	*P*=0.04	47 (68.12)	18 (26.1)	*P*=0.05
>1week	111 (66.27)	79 (88.76)		32 (31.88)	51 (73.9)	
Total	168100	8953	*P*=0.05	7947	6941	*P*=0.05

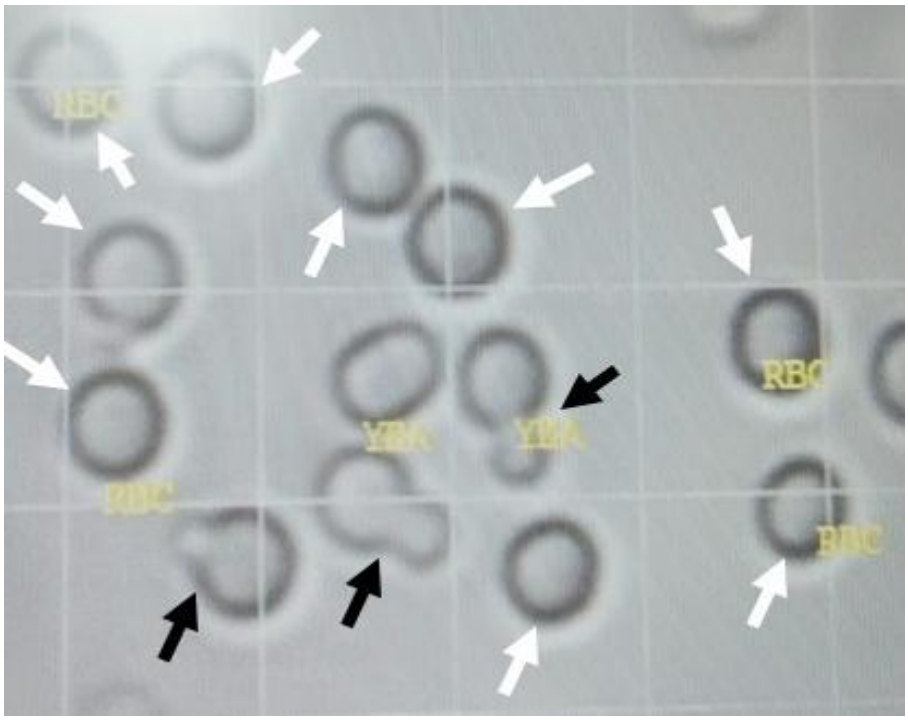

Fig. 1. Microscopic image of urine specimen showing presence of yeast cells (black arrows) and red blood cells (white arrows)

Table 3. Antifungal susceptibility pattern of *C. albicans* isolates (n=69)

Antifungal agent	Potency of disc (µg)	No. of isolates					
		Susceptible		Susceptible dose dependent		Resistant	
		No.	%	No.	%	No.	%
Fluconazole (FLU)	25	69	100	00	00	00	00
Amphotericin B (AM-B)	10	66	95.65	03	4.35	00	00
Voriconazole (VOR)	10	61	88.40	08	11.6	00	00
Itraconazole (ITR)	10	20	28.98	06	37.68	43	62.32

In this study, high incidence of candiduria with $>10^5$ CFU/ml was found in majority of ICU patients and this should be considered very important, since this high number of *Candida* cells is indication for UTI or systemic infection. These results are in consistent with other studies in which prevalence of candiduria was found 44.4% [18,19].

Among candiduria patients, *C. albicans* was more prevalent followed by other *Candida* spp. Similarly, other researchers also found same trend in candiduria [18,19].

Besides higher number of *Candida* spp. in UTI, presence of RBCs in urine was also investigated to confirm the UTI infection. In the current study, presence of RBCs and elevated number of *Candida* cells in the urine specimens (pyuria) confirmed UTI infection. Although pyuria helps in diagnosing *Candida* associated UTI, there are some drawbacks of considering pyuria for assessing the involvement in *Candida* spp. in nosocomial candiduria. For example, a) its sensitivity and specificity decrease in patients with indwelling catheters, b) concurrent presence of *Candida* spp. and bacteria also decreases the efficacy of pyuria in diagnosing whether the UTI infection is caused by *Candida* or bacteria [20].

After identification of *C. albicans*, this study also investigated variety of risk factors contributing in nosocomial UTI. These include age, sex, antibiotic therapy, urinary catheterization, diabetes mellitus and duration of stay in hospital.

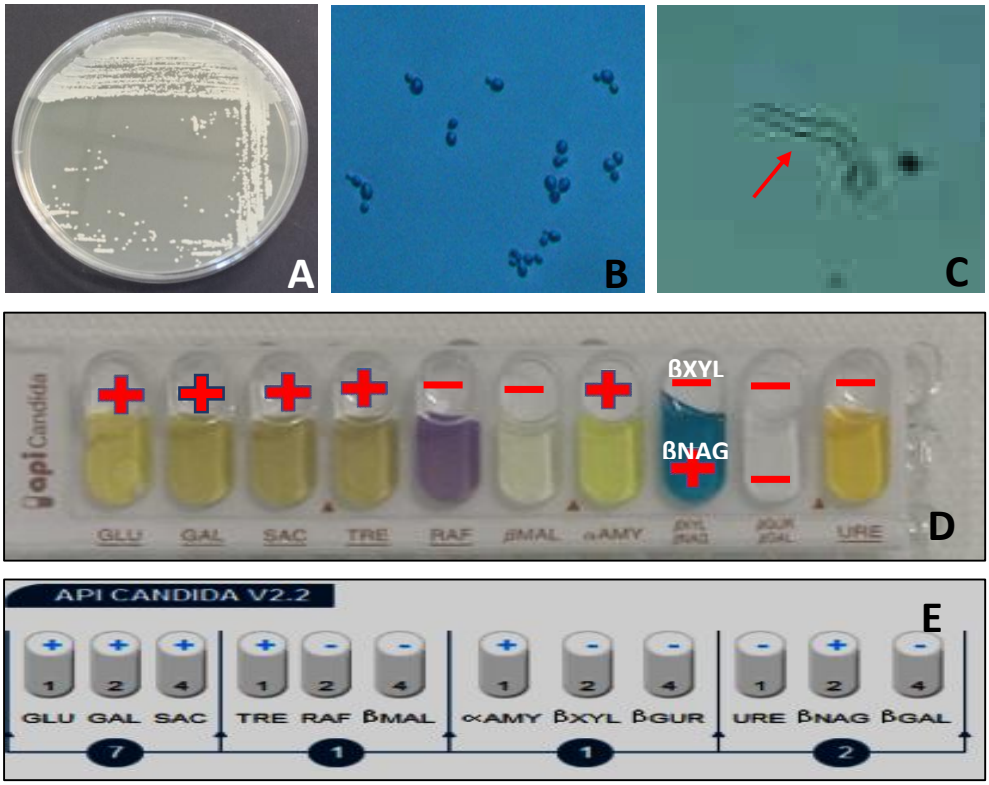

Fig. 2. Growth of yeast on SDA plate (A); Wet mount image of ovoid yeast-like cells with some producing bud and daughter cells (B); Germ tube image (pointed with arrow) (C); API Candida test for confirmation of *C. albicans* showing positive reaction for glucose, galactose, sucrose, trehalose, α-amylase and N-acetyl β-glucosaminidase (D), Score (Profile) generated from tests (E)

This study found that age play crucial role and patients aged above 45 were found more susceptible in developing *C. albicans* associated UTI. This suggest that in old age immune system is unable to fight this infection. This finding is in agreement with other studies who found over age is an important predisposing factor for *C. albicans* associated UTI [19, 21,22].

This study also found that difference in sex could make prone towards *C. albicans* associated UTI. In this study females were more vulnerable towards getting ascending UTI infection due to shorter urethral length in female [23]. Moreover, males are immune against the *C. albicans* due to anti-*Candida* activity of prostatic fluid in male [23]. Similar outcome has been reported by other authors [19,21,22].

Some researchers have reported that use of antibiotics especially broad spectrum could increase the chances of *Candida* colonization. This study verifies that almost all patients with significant candiduria (>10^5 CFU/ml) were under antibiotic treatment. The use of antibiotics has also role in causing pathogenic candiduria as antibiotics discourage the growth of sensitive commensals and encourage yeast colonization on epithelial surface. Consequently, yeast cells easily reach to urinary tract, especially in the presence of indwelling urinary catheter [24,25].

Use of catheter was also found another important predisposing factor for developing *Candida* infection [18]. In this study, high number of *Candida* cells and high frequency of *C. albicans* was found in patients with urinary catheter as *C. albicans* are more active in developing colonization on catheter due to their biofilm forming ability [26]. It is very difficult to eradicate biofilm producing *Candida* strains since they are resistant to shear force, antimicrobial agents and phagocytosis [26]. Kobayashi et al. found same outcome implying that catheter play important role in nosocomial candiduria [27].

However, this study found that diabetes had no role in developing nosocomial UTI associated with *C. albicans*. In contrast, other researchers have found 2-fold increase in nosocomial candiduria in diabetic patients [28,29]. This is because diabetic patients have weak phagocytes that could not resist invasion of fungi and due to the stasis of urine in neurogenic bladder [30]. Therefore, results in this study may be biased due to the low number 23 out of 168 specimens) of diabetic cases thus further investigation should be carried out using large number of diabetic patients.

Longer exposure in the ICU was also found one of the other risk factors in developing candiduria. Same trend has been found in previous studies as patients may come in contact with other patients, staff or objects contaminated with *C. albicans* [31,32]. However, some researchers have found that in most cases colonization appears to be originated endogenously [31,33].

In present study, majority of the *C. albicans* isolates showed resistance to itraconazole. Since itraconazole is one of the common therapeutic choices and widely used for the treatment in hospitalized patients thus *C. albicans* evolved resistance against this drug [34]. In addition, *C. albicans* were most frequently found in patients with catheter since these isolates have biofilm forming ability which could be another reason for developing resistance against itraconazole. Hence, itraconazole may not be useful for treating hospitalized patients. However, *C. albicans* isolates were found either sensitive or susceptible dose dependent to fluconazole, Amphotericin B and voriconazole therefore these drugs could be used alternatively for patients' managements. Nevertheless, surveillance of *Candida* spp. resistant to itraconazole and other antifungal agents is needed for effective antifungal therapy.

There are some limitations of this study. First, the sample size was relatively small and thus there is a potential for a Type II statistical error in the primary outcome. Randomized studies with large sample size are needed to validate the outcomes of this research. Second, the management of candiduria may vary between hospitals and be dependent on hospital-specific policies or antimicrobial stewardship interventions. In order to enhance the generalizability of our findings, future study should include different hospitals with similar numbers of beds. Third, there were less patients with diabetes in this study that showed diabetic patients were more susceptible to recurrence of candiduria. In future, large number of diabetic patients may be included in the study to confirm the linkage of diabetes with candiduria associated with *C. albicans*.

5. CONCLUSION

Considering the possible involvement of predisposing factors in Candiduria, health care workers should be informed regarding these risk factors which could be useful in mitigating the frequency of Candiduria. Health care department should conduct continuous surveillance of *C. albicans* in Candiduria which in turn will minimize this nosocomial infection. Moreover, rapid, and reliable diagnostic techniques are needed for early identification of fungi. This will help for prompt treatment and thus it could reduce the mortality rates due to *Candida* infection. Additionally, most of the *C. albicans* isolates were resistant to itraconazole which is alarming since this drug is commonly used for treating nosocomial fungal infections which underline to take the effective measures for reducing the drug resistance.

CONSENT

A written consent from patients has been collected and preserved by the authors.

ETHICAL APPROVAL

A written ethical approval from University has been obtained and preserved by the authors.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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