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Candiduria in catheterized Menoufia patients: emerging microbiological trends

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Objective
The present study aimed to assess the prevalence of different *Candida* spp. as a cause of infections in the urinary tract infection (UTI) among catheterized patients and to test the susceptibility of *Candida* isolate to antifungal agents.

Background
UTIs are tied with pneumonia as the second-most common type of healthcare-associated infection. Catheter-associated UTI occurs because urethral catheters inoculate organisms into the bladder and promote colonization by providing a surface for bacterial adhesion and thus causing mucosal irritation. *Candida* spp. accounts for almost 10–15% of the nosocomial UTIs.

Patients and methods
The study was conducted on 200 catheterized inpatients from ICU. The urine specimens were examined for *Candida* by using CHROMagar and Integral System Yeasts Plus.

Results
The API system is highly effective in diagnosing fungal infections. *Candida* infection was the highest among the age group more than 45 years and in females. The most sensitive antifungal drug for *Candida* infection was flucytosmine and the least sensitive were nystain and micoconazole.

Conclusion
There is a strong relationship between host risk factors (old age, antibiotic use, catheterization, female sex, ICU stay, diabetes mellitus, hospitalization) and the expression of various virulence factors of *Candida* spp. causing candiduria and their resistance to antifungals.

Keywords:
candiduria, catheter, urinary tract infection

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Introduction

Candida fungemia may have an endogenous or exogenous origin, and in the recent years a growing proportion of episodes of candidemia have been caused by *Candida* spp. other than *C*andida *albicans* [1].

Catheter-associated urinary tract infection (UTI) occurs because urethral catheters inoculate organisms into the bladder and promote colonization by providing a surface for bacterial adhesion and thus causing mucosal irritation. The presence of a urinary catheter is the most important risk factor for candiduria [2].

Candida, especially C. albicans, is the second-most common organism that can cause catheter-associated UTI or asymptomatic colonization, although the isolation of fungi from urine rarely indicates active infection. The use of clinical signs such as fever, leukocytosis, and decreased renal function cannot reliably distinguish between asymptomatic funguria and actual infection [3].

Candiduria or presence of *Candida* spp. in the urine is rarely encountered in otherwise healthy people with structurally normal urinary tract. It is, however, of common occurrence in hospitalized patients. The *Candida* may gain entry into the bladder during insertion of the catheter, during manipulation of the catheter or the drainage system around the catheter, and after removal [4].

Candida spp. accounts for almost 10–15% of nosocomial UTIs. The clinicians always face a diagnostic dilemma as to whether the presence of candiduria in a patient represents contamination, colonization, or true infection. Furthermore, the prevalence of true infection has increased significantly over the past few years because of the presence of various predisposing factors in hospitalized patients [5].

The predisposing factors frequently associated with candiduria are urinary tract instrumentation, prior

antibiotic use, prolonged hospital stay, extremes of age, diabetes mellitus, female sex, and the use of immunosuppressive therapy [6].

The 2009 Infectious Diseases Society of America guidelines define catheter-related UTI in patients whose urinary (urethral, suprapubic, or condom) catheter has been removed within the previous 48 h by the presence of symptoms or signs compatible with UTI with no other identified source of infection along with 1000 or more colony-forming units per milliliter of one or more bacterial species [7].

It is important to know the *Candida* spp. causing the UTI before initiating the treatment as many non-*C. albicans Candida* spp. are inherently resistant to treatment with fluconazole. Although *C. albicans* is the most frequently isolated species, a few observers have emphasized the changing microbiological characteristics of yeasts as a causative agent of nosocomial UTI [6].

There are several specific risk factors for particular non-*C. albicans* species: *Candida parapsilosis* is related to foreign-body insertion, neonates, and hyperalimentation; *Candida krusei* is related to azole prophylaxis and, along with *Candida tropicalis*, to neutropenia and bone marrow transplantation; *Candida glabrata* is related to azole prophylaxis, surgery, and urinary or vascular catheters; and *Candida lusitaniae* is related to previous polyene use [8].

Chromgenic media (CHROMagar; Mast Ltd, UK) is used to differentiate *Candida* spp. The yeasts produce specific enzymes that react with chromogenic substances in the medium producing colonies of different colors [9,10].

The conventional methods of yeast identification, which mainly consist of assimilation/fermentation characteristics, are reported to be cumbersome and often beyond the expertise range in nonspecialized clinical microbiology laboratories. Packaged kit systems and automated systems are widely used, but they are expensive and are limited by the size of their databases [11].

The importance of identifying the pathogen as quickly as possible has encouraged the development of differential media for the presumptive identification of yeasts. Several chromogenic media for isolation and identification of *Candida* spp. are available. These media are based on the formation of various colored colonies with different morphology that result from the cleavage of chromogenic substrates by species-specific enzymes [11].

Antifungal drugs are important to control life-threatening fungal infections; it is very important to know the antifungal susceptibility of pathogens to select the most effective treatment method for mycosis patients [12].

Aim

The aim of the present study was to assess the prevalence of different *Candida* spp. as a cause of infections in UTI catheteized patient and to test the susceptibility of *Candida* isolate to antifungal agents.

Patients and methods

Study population

The study was conducted on 200 catheterized patients during the period from October 2013 to July 2014. The patients in this study were admitted to different wards and units of Menoufia University Hospitals. Approval of the Ethics committee of Menoufia University Hospitals was obtained. Furthermore, all patients signed an informed consent before participation in the study.

Collection of patient samples

Two-hundred urine specimens were collected from 200 (75 females and 125 males) patients aged between 6 and 80 years who were catheterized in the different wards and units of Menoufia hospitals.

Then, the catheters were sterilized in alcohol and fresh urine samples were collected by using a syringe.

Methods

Macroscopic appearance of urine

For all samples, a naked eye examination of urine for color, odor, and turbidity was performed.

Microscopical examination of clinical samples

A urine-wet film was examined for detecting leukocytes, red blood cells, crystals, or casts, and a microscopic examination of Gram-stained films from the samples was carried out to demonstrate any stainable bacteria and *Candida*.

Culture media

In the present study, following were the culture media used for cultivating the specimens: Sabouraud dextrose agar (Oxoid, UK), Blood agar (Oxoid), and MacConkey agar (Oxoid).

CHROMagar was used for the simultaneous detection and presumptive identification of *C. albicans*, *C. tropicalis*, and other *Candida* spp., and yeasts.

Principle

The chromogen mix consists of artificial substrates (chromogens) that release differently colored compounds upon degradation by specific enzymes. This permits the differentiation of certain species, or the detection of certain groups of organisms, with only a minimum of confirmatory tests for *Candida* growth on CHROMagar after incubation.

Growth of the organism was identified by coloring acquired during the growth as well as the following colonial morphololgy: green presumptive of *C. albicans*, blue presumptive of *C. tropicalis*, white to pink of other species, and *C. krusei* forms pale pink to purple crenated, rough-spreading colonies with pale edges.

Trichosporon beigelii forms pale 'dirty pink' to 'dirty green gray' small colonies that become darker and rougher on prolonged incubation (i.e. 72 h).

All samples were cultured on blood agar, MacConkey, and Sabouraud dextrose agar and incubated aerobically for 24–48 h at 37°C.

Candidal growth on Sabouraud dextrose agar was identified by its colony morphology. Colonies suspected to be *Candida* were identified by using Gram-stain.

Candida isolates were stored in distilled water at room temperature or in 30% glycerin at −80°C and subcultured onto Sabouraud dextrose agar 48 h before further study [13,14].

Identification

Morphological identification

In culture, most *Candida* spp. form smooth, white, creamy, domed colonies.

Using Gram-stain, *Candida* spp. appear as oval single cells measuring 3–6 μm in diameter and producing buds or blastoconidia. Species of *Candida* other than *C. glabrata* also produce pseudohyphae and true hyphae [15].

Germ tube test

A yeast colony from Sabouraud dextrose agar plate was inoculated into 500 μl of human serum in a small test tube.

The tube was then incubated at 37°C for 3 h.

A wet film was prepared and examined microscopically for sporulating yeast cells, that is, germ tube-like developing from one pole of some of the yeast cells. It was examined carefully to ensure that the parent cell and the germ tube were one continuous cell and that a cell wall did not separate the two as in *C. tropicalis*.

The formation of germ tubes in serum is a characteristic of clinical isolates of *C. albicans* and *Candida dubliniensis* [15].

Integral System Yeasts Plus (Liofilchem s.r.l, Italy)

Principle

Presumptive identification is based on the assimilaton reactions of sugars. The tests for the assimilation reactions are interpreted by evaluating the color change of wells 1-glucose (GLU) to 12-dulcitol (DUL). The combination of positive and negative reactions allows the formation of a numerical code that permits identification of the yeasts under examination through the use of the table of codes.

The well 13-CHR contains a chromogenic substrate that permits the differentiation of some yeasts by evaluating the color of the well.

Sensitivity to antimycotics is evaluated according to the growth or inhibition of yeasts in media containing the antimycotic and a growth indicator in wells 14-nystain (NY) to 23-fluconazole (FLU).

The color change from red to orange in the wells indicates slow growth of the yeasts under examination and intermediate sensitivity to the concentration of antimycotic in the well.

The color change from red to yellow in the wells indicates a growth of yeasts under examination and resistance to the concentration of antimycotic in the well.

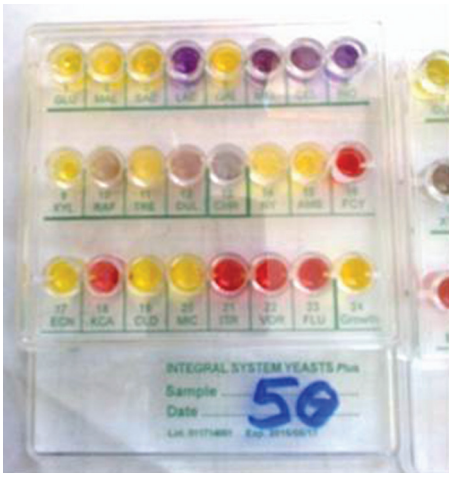
No color change in the well indicates no growth of the yeasts under examination and sensitivity to the concentration of antimycotic in the well.

The well 24-h growth does not contain antimycotics; it contains culture medium and indicator and it works as a growth control.

1-GLU(+)2-MAL(+)3-SAC(+)4-LAC(−)5-GAL(+)6-MEL(−)7-CEL(−)8-INO(−)9XYL(+)10-RAF(−)11-TRE(+)12-DUL(−)13-blue color that indicate for *C. tropicalis* (Fig. 1).

1-GLU(+)2-MAL(+)3-SAC(+)4-LAC(−)5-GAL(+)6-MEL(−)7-CEL(−)8-INO(−)9XYL(+)10-RAF(+)11-TRE(−)12-DUL(+)13-green color that indicate for *C. albicans* (Fig. 2).

Figure 1



Integral System Yeasts Plus.

Figure 2

Integral System Yeasts Plus.

Data management and statistical analysis

The data was entered on a Microsoft Office Excel worksheet and then exported to the statistical software, and analyzed using appropriate statistical tests by using the statistical package for the social services (verison17; SPSS Inc., Illinois, Chicago, USA).

Results

The prevalence of *Candida* infection among the studied patients was as follows: 75 (37.5%) of patients had no growth, 38 (19%) had *Candida* infection, 82 (41%) had bacterial isolate, and five (2.5%) had bacterial associated (Table 1).

CHROMagar diagnosed 40 (20%) patients with positive *Candida* infection and 160 (80%) patients with negative infection. As regards *Candida* species, the CHROMagar differentiated 15 (7.5%) patients infected with *C. albicans* and 17 (8.5%) infected with *Candida* (Table 2).

The diagnosis of *Candida* infection by the API system gave the following results: *C. albicans* in 18 (45%) patients, *C. tropicalis* in 19 (47.5%), *C. krusei* in one (2.5%), and *Saccharomyces cerevisiae* in one (2.5%). The API system diagnosed 38 (95%) patients with *Candida* infection and two (5.0%) with non-*Candida* infection (Table 3).

CHROMagar diagnosed 20% patients with positive infection and 80% with negative infection. On the other hand, the API system showed 95% patients with positive infection and 5% with negative infection. Therefore, the API system was highly significant in diagnosing fungal infections ($P < 0.001$) (Table 4).

Sensitivity of *Candida* infections to antifungal drugs was as follows: NY showed sensitivity in three (7.5%) patients, intermediate sensitivity in nine (22.5%), and resistance in 28 (70%); amphotericin showed sensitivity in four (10%) patients, intermediate sensitivity in 34 (85%), and resistance in two (5%); flucytosmine (FCX) showed sensitivity in 40 (100%) patients, intermediate sensitivity in 0 (0%), and resistance in 0 (0%); econazole (ECN) showed sensitivity in five (12.5%) patients, intermediate sensitivity in one (2.5%), and resistance in 34 (85%); ketonozole (KCA) showed sensitivity in 25 (62.5%) patients, intermediate sensitivity in 15 (37.5%), and resistance in 0 (0%); clotrimoxzole (CLO) showed sensitivity in five (12.5%) patients, intermediate sensistivity in 11 (27.5%), and resistance in 24 (60%); micoconazole showed sensitivity in three (7.5%) patients, intermediate sensitivity in one (27.5%), and resistance in 36 (90%); itraconazole (ITR) showed sensitivity in 26 (65%) patients, intermediate sensitivity 12 (30%), and resistance in two (5%); voriconazole showed sensitivity in 32 (80%) patients, intermediate sensitivity in six (15%), and resistance in two (5%); and FLU showed sensitivity in 30 (75%) patients, intermediate sensitivity in seven (17.5%), and resistance in three (7.5%). This shows that the most sensitive antifungal drug for *Candida* infection was FCX and the least sensitive were NY and MIC (Table 5).

Discussion

In the present study, conducted on 200 catheterized patients at Menoufia University Hospital, the prevalence of *Candida* infection among the studied patients was found to be 19%.

This is in agreement with the findings of other studies such as by Alvarez-Lerma *et al.* [16]; in their study *Candida* spp. represented 22% of the UTI cases in ICU.

Table 1 Prevalence of *Candida* infection among the studied patients by Sabaraud agar

Diagnosis	The studied patients (*N*=200) (*n* (%))
No growth	75 (37.5)
Candida	38 (19.0)
Bacterial isolate	82 (41.0)
Bacterial associated	5 (2.5)

Table 2 Identification of *Candida* spp. by CHROMagar

CHROMagar	The studied patients (*N*=200) (*n* (%))
Candida spp.	
Positive	40 (20.0)
Negative	160 (80.0)
Urinary *Candida*	
Candida albicance	15 (7.5)
Candida tropicalis	17 (8.5)
Candida kruzi	1 (0.5)
Other species	7 (3.5)
Non-*Candida*	160 (80.0)

Table 3 Identification of *Candida* spp. by API system

API system	The studied patients (*N*=40) (*n* (%))
Candida albicance	18 (45.0)
Candida tropicalis	19 (47.5)
Candida krusei	1 (2.5)
Saccharomyces cerevisiae	1 (2.5)
Crytpoccus albidus	1 (2.5)
API system	
Candida	38 (95.0)
Non-*Candida* fungal infection	2 (5.0)

Table 4 Difference between CHROMagar and API system for diagnosis of fungal infection

	CHROMagar (*N*=200) (*n* (%))	API system (*N*=40) (*n* (%))	χ^2	*P*
Candida (positive)	40 (20.0)	38 (95.0)	85.5	<0.001

Table 5 Sensitivity to antifungal drugs among the studied cases by using API

	Sensitivity to antifungal drugs (*N*=40) (*n* (%))		
	Sensitive	Intermediate	Resistant
Nystain	3 (7.5)	9 (22.5)	28 (70)
Amphotericin	4 (10)	34 (85)	2 (5)
Flucytosmine	40 (100)	0 (0)	0 (0)
Econazole	5 (12.5)	1 (2.5)	34 (85)
Ketonozole	25 (62.5)	15 (37.5)	0 (0)
Clotrimoxzole	5 (12.5)	11 (27.5)	24 (60)
Micoconazole	3 (7.5)	1 (2.5)	36 (90)
Itraconazole	26 (65)	12 (30)	2 (5)
Voriconazole	32 (80)	6 (15)	2 (5)
Fluconazole	30 (75)	7 (17.5	3 (7.5)

Detection of *Candida* on CHROMagar allows direct, more rapid, and specific identification of *C. albicans* and other species, which could decrease the time required in obtaining results. The use of chromogenic media in clinical microbiology laboratories for the isolation and presumptive identification of important *Candida* spp. is easy to perform, requires less time, and is cost effective [17].

In the present study we used CHROMagar to diagnose *Candida* infection, as did Nadeem and colleagues [17] in their study; they reported that the major advantage of 'CHROMagar *Candida*' was its ability to detect the presence of more than one yeast species.

In our study, using CHROMagar medium, 40 (20%) patients were positive with *Candida* infection: *C. tropicalis* was present in 17 out of 40 (42.5%), which was the highest occurring species, *C. albicans* was present in 15 out of 40 (37.5%), and *Candida kruzi* in one out of 40 (2.5%).

This is in agreement with Awad *et al.* [18], who found that 26.8% of the isolates were positive for *Candida* infection, and that *C. albican* was the highest occurring species with 39.1%, followed by *C. glabrata* with 21.7% [18].

Commercially available yeast identification systems, such as the API 20 C AUX and API ID32C (bioMérieux, France), which are convenient to use with an incubation period of 24–48 h, are required before biochemical reactions are to be interpreted [19].

Thus, we used the API (Integral System Yeasts Plus) to differentiate different species of *Candida*.

In our study, as regards comparison between CHROMagar and API system in the diagnosis of *Candida*, there was a highly significant difference with a *P* value of less than 0.001; this is in agreement with Pires *et al.* [20], who found that the API system was highly sensitive in the diagnosis of *Candida* compared with CHROMagar.

In our study, age was found to be a significant factor in *Candida* infection, with increased infection in the age group more than 45 years [24 (63.2%) patients] due to high susceptibility to catheterization. Other studies demonstrated that aging and age-associated physiological changes, higher rates of oropharyngeal colonization with *Candida* spp., and concomitant drug use make elderly patients more vulnerable to infections.

In this study, sex was found to be a significant factor in *Candida* infection as 20 (52.6%) patients with infection were females. This was due to anatomical differences: a female's urethera is short, and also the infection passes from the urethera during pregnancy, childbirth, menopause, and the use of contraceptives.

This is in agreement with the study by Agbagwa *et al.* [21], as they found that the pattern of UTI depends on whether the infections are acute, recurrent, nosocomial, community acquired, sex, age and presence of underlying disease are common in femle due to

anatomical differences that the female's urethera is short as well as the infection pass from urethera during pregnancy and childbirth and menopause and use of contraceptives. High incidence of infection in old age is due to diabetes mellitus and catheterization.

This was in agreement with our study, as regarding the history of antibiotics intake by the patients in our study we found that the patients with long antibiotics course had a high incidence of *Candida* infection (27.1%) than did patients with a short course.

This is in agreement with the findings of a study by Ekrem *et al.* [22]; according to them the disparity observed in the frequency of pathogen isolated from urine specimen by various researchers could be due to antibiotic as a risk factor as antibiotics taken by patients before visiting the hospital due to persistence of UTI can affect the frequency of isolated pathogens.

An increased incidence of non-*C. albicans* spp. among hospitalized and immunosuppressed patients was recorded [23].

Candiduria occurs most commonly among catheterized patients. In their study, Bouza *et al.* [24] found that the overall percentage of nosocomial UTIs among catheterized individuals was as high as 37%, of whom 16.4% had infections due to *Candida* spp. In this same study the incidence of candiduria in noncatheterized subjects was only 6.6% [24].

This is in agreement with our study as we found that catheterization was a predisposing factor for fungal infection in ICU patients as we found 19% patients with candiduria.

In our study, patients who were admitted to the hospital for long periods were more susceptible to candidal infection than were those admitted for shorter periods: 12.8% of the patients admitted to hospital for 10–30 days and 27.5% of the patients admitted to hospital for more than 30 days.

In line with our findings, Pfaller and Diekema [25] found that the most common healthcare-associated risk is long hospital or ICU stay.

In the present study, patients on corticosteroids were more vulnerable to *Candida* infection as corticosteroid was a significant factor to candidia infection: 34.3% of the patients who had candidal infection were on corticosteroids, which is in agreement with findings of a study by Djaballah. *et al.* [26] as they also found that therapy with corticosteroids or cytotoxic drugs and localized or widespread irradiation resulted in further deterioration of the host defense mechanism.

Regarding the antifungal drugs sensitivity, the most sensitive drug was FCX with 100% sensitivity and the lowest sensitivity was shown by MIC and NY with 7% sensitivity.

In line with our results, ElFeky *et al.* [27] found that the most effective antifungal agent used in their study was amphotericin, followed by ketoconazole (54 sensitive isolates, 85.7%), voriconazole (52 sensitive isolates, 82.5%), fluconazole (49 sensitive isolates, 77.8%), clotrimazole (39 sensitive isolates, 61.9%), and, finally, miconazole (32 sensitive isolates, 50.8%).

In this study, we found that the *Candida* spp. were resistant to antifungal drugs and the most resistant drug was MIC [36 (90%)] followed by econazole [34 (85%)].

In contrast, Ashour *et al.* [28] found that the most of the *C. Candida* have high rates of resistance to ITR and all *Candida* spp. have high rates of resistance to ITR, flucytosine, and fluconazole.

Conclusion

There is a strong relationship between host risk factors (old age, antibiotic use, catheterization, female sex, ICU stay, diabetes mellitus, hospitalization) and the expression of various virulence factors of *Candida* spp. causing candiduria and their resistance to antifungals.

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Conflicts of interest

There are no conflicts of interest.

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