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Epidemiological Study on Species Identification and Susceptibility Profile of *Candida* in Urine

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Abstract

Background: There have been important changes in the epidemiology of *Candida* causing urinary tract infection (UTI) over the past decades. *Candida* species other than *Candida albicans* have now emerged as an important cause of UTI and some of which exhibit reduced susceptibility to commonly used antifungal agents. The aim of this study was to investigate species distribution and antifungal susceptibility profile of *Candida* species causing candiduria in Farwania hospital in Kuwait and asses its' associated risk factors.

Materials and method: During a 12-month period, urine cultures were processed for isolation of *Candida* species. All the yeast isolates were sub-cultured on Sabouraud dextrose agar and processed for phenotypic identification by germ tube test and API ID 32C. Antifungal susceptibilities of the isolates were determined against amphotericin B, voriconazole, fluconazole and caspofungin by E-test. All the patients yielding *Candida* species in urine culture were assessed for risk factors.

Results: Of 13691 urine samples processed for culture, 2550 (18.6%) yielded microbial growth. Of these, 85 (3.3%) were identified as *Candida* UTI (CUTI) with an incidence of 3.3%. The ratio of female to male was 2:1. The average age of the patients was 54 yrs. The most common isolate was *C. albicans* (54%) followed by *C. tropicalis* (15%). The major predisposing factors were urinary tract catheterization (86.5%) and antibiotic therapy (68%).

Conclusion: The study reinforces the emerging importance of non-albicans *Candida* species in the epidemiology of candiduria. A better understanding of the associated risk factors and knowledge of susceptibility profile of the local *Candida* isolates may have therapeutic benefits.

Keywords: Community acquired candiduria; Hospital acquired candiduria; Antifungal therapy; *Candida* species

Introduction

There have been important changes to the epidemiology of *Candida* urinary tract infection over the past decades [1,2]. *Candida* species are part of normal flora and commonly colonize mucosal surfaces and external genitalia of humans. *Candida* species colonize females more often than males especially near the urethral meatus of young premenopausal women [1-3]. Up to 1% of healthy adults are reported to yield *Candida* species in measurable quantities in the urine [3]. Candiduria may represent infection, colonization or even contamination. The majority of cases however, even the true candiduria infections are asymptomatic [4].

The majority of candiduria infections are nosocomial and are associated with many risk factors, such as urinary catheter insertion, invasive procedures in the urinary tract and prolonged antibiotic therapy. Additionally, expanding population of patients with diabetes mellitus, and prolonged hospitalization of critically ill patients have also contributed to growing incidence of *Candida* UTI (CUTI). Studies from United States of America have shown that 10%– 15% of all hospital acquired UTIs are caused by *Candida* species [5,6]. Furthermore, the incidence of CUTI has alarmingly increased from 22% for the period 1986–1989 reaching 40% for the period 1992–1997 especially in ICU setting[5,6].

Although *C. albicans* is the major species involved in CUTI, other species have emerged as an important cause of infection, *C. glabrata and tropicalis* are far more common than previously thought [2,4,7]. Reports estimated that *C. albicans* causes around 50%–70% of the cases, followed by *C. glabrata* (24.4%) and *C. tropicalis* (3.7%) [5,7-9]. *C. parapsilosis* is the second most common cause of candiduria in terms of neonates and carries similar significant as its isolation from blood warranting careful evaluation [10,11]. Rarely, candiduria can be caused by more than one species [6].

First line therapy for CUTI is fluconazole according to Infectious Diseases Society of America (IDSA) guidelines, mainly because of favorable pharmacodynamics and kinetics properties over the second choice, amphotericin B [12]. Fluconazole can be taken orally, metabolized in the liver with a good concentration excreted in the urine. However, recent reports highlight the increase in high levels of resistance to fluconazole (MIC 64 µ g/ml) up to 3% particularly in *C. guilliermondii* and *C. glabrata* [11-13]. Notably, *C. krusei* is intrinsically resistant to fluconazole; as such, 100% of strains are regarded as resistant independent of the in vitro susceptibility studies.

The aim of this study was to investigate species distribution and antifungal susceptibility profile of *Candida* causing candiduria in patients in Farwania hospital and to assess its associated risk factors.

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Method

This is an observational, prospective, laboratory based study carried out in Farwania hospital, Kuwait for one year period (April 2013-March 2014). The hospital is considered a tertiary care facility with 900 beds and 4 intensive care units (ICU); two adults, a pediatric and a neonatal one. Total ICUs bed capacity are 60. The patients were eligible if the urine sample yielded the growth of *Candida* species with a count of 10^3 colony-forming units (CFU) which is considered as candiduria [14]. For the isolate to be significant causing CUTI, the patient either had symptoms suggested of UTI supported by positive urine routine in patients culture without catheters, or in catheterized patients, two consecutive cultures of urine samples were positive for *Candida* species after changing the indwelling catheters.

Candiduria was considered hospital acquired (HA-CUTI) if occurred ≥ 24 hours of admission and community acquired otherwise (CA-CUTI). Invasive candidiasis defined as outlined in recent publications [14,15]. Epidemiological data and risk factors of the patients were collected including, gender, age, clinical presentation and treatment option. Furthermore, risk factors was also assessed including; diabetes mellitus, urinary catheterization, previous antibiotic use, patient location and history of recurrent attacks.

Urine specimens were collected either by a clean catch method or from an indwelling catheters. All the yeast isolates were subcultured on Sabouraud dextrose agar and processed for phenotypic identification by germ tube test and by API ID 32C (BioMérieux, France) [6,12]. The isolates were stored at -4°C until used for identification and susceptibility testing.

All *Candida* isolates were tested for susceptibility against amphotericin B, fluconazole, voriconazole, and caspofungin by E-test (AB Biodisk, Solna, Sweden) according to manufacturer's recommendation. Briefly, each test isolate was freshly sub-cultured on SDA and five isolated colonies were suspended in sterile normal saline and turbidity adjusted to 0.5 McFarland standard. Using sterile cotton swab the suspension was inoculated on to RPMI agar (supplemented with 2% glucose and buffered with MOPS, 0.165M, and pH 7.0) plates (150 mm diameter) and allowed to dry for 10 to 15 minutes before applying the E-test strips. The plates were incubated at 35°C and minimum inhibitory concentration (MIC) was recorded after 48 hrs. The interpretive susceptibility breakpoints as recommended by Clinical Laboratory Standards Institute (CLSI) were used for fluconazole, caspofungin and voriconazole [16]. Due to lack of defined breakpoints for amphotericin B an isolate showing MIC <1.0 mg/L was considered as susceptible. Quality control ensured by testing *C. parapsilosis* ATCC 22019, *C. albicans* ATCC 90028, and *C. tropicalis* ATCC 750, as recommended by CLSI.

Results

Of the 13691-urine samples received for culture in our laboratory, 2550 were regarded as positive urinary tract infections (18.6%). Of these, 85 were identified as candiduria with an incidence of 3.3% (85/2550). Only 1.5% patients were recognized as to have clinically significant candiduria; CUTI (37/2550). The ratio of female to male was 2:1. Candiduria was common from patients in medical wards 42% (36/85), followed by ICU 23% (20/85). However, clinically significant isolates were more common in ICU setting 35% (13/37) as compared to other locations. The average age of patients with candiduria was 54 yrs while those with CUTI was 48.2 yrs.

Of the 37 CUTI cases, the majority were from catheterized patients 86.5% (32/37), mostly females 59% (22/37) and rarely community acquired 19% (7/37).

The most common isolate was *C. albicans* 65% (55/85), followed by *C. tropicalis* 15% (13/85) in candiduria cases. Similarly, *C. albicans* remained a leading species 68% (25/37), followed by *C. tropicalis* 16.2% (6/37) in the CUTI patients. Six isolates in total failed to subculture. Of these six, two were identified to species level one was *Candida* species, one was *C. albicans*, and both were considered CUTI.

In 100% of the CUTIs, cultures yielded more than 10,000 yeast colonies, and the major predisposing factors associated with CUTI were urinary catheterization (86.5%), antibiotic therapy (68%) and diabetes 51% (19/37). Seven cases were community acquired, of these all but one was catheterized patients in medical ward, and all were *C. albicans* except one *C. lusitaniae* in an ICU patient. Three urine cultures were mixed *Candida* species with bacteria, two with *Pseudomonas aeruginosa* and one *Enterococcus faecalis* representing 8.1% (3/37) of the total CUTI cases. Of all 37 significant CUTIs, eight had disseminated infection 22% (8/37) and of these six patients expired 75% (6/8) due to the infection. Both patients who survived received caspofungin. Interestingly, all the candidemia isolates were fully sensitive to all four antifungal agents tested (Table 1).

Furthermore, all the significant *Candida* in CUTI group were susceptible to amphotericin B and caspofungin. One *C. glabrata* was resistant (MIC >32 µg/ml) to fluconazole and voriconazole MIC was 4 µg/ml. One *C. tropicalis* was susceptible dose dependent to fluconazole with an MIC 5 µg/ml. All *C. krusei* were resistant to fluconazole. Amphotericin B and voriconazole had the lowest MIC 90s against *C. albicans* (0.125, 0.023) and *C. tropicalis* (0.125, 0.016).

Discussion

Despite the advances in diagnostic mycology, candiduria definition and management remains a controversial subject. Compelling evidence links antibiotic usage to the increased incidence of CUTI [17-19].

Table 1: Summary of disseminated candidemia cases.

No.	Gender	Age(years)	Location	Risk Factors	Isolate	Treatment	Outcome
1	F	73	Medical	DM/CHF/Cath	*C. tropicalis*	No treatment	Died
2	M	38	ICU	Cath	*C. tropicalis*	No treatment	Died
3	M	3	PICU	ME/ Cath	*C. albicans*	AMB	Died
4	F	1 Month	NICU	IVH-G4/premature/Cath	*C. parapsilosis*	AMB-Liposomal	Died
5	F	78	ICU	DM/ HTN/Cath	*C. albicans*	Caspofungin	Died
6	M	57	ICU	Quadreplasia/Abx/ Cath	*C. albicans*	Caspofungin	Died
7	F	40	Surgical	Kidney stone with nephrostomy	*C. glabrata*	Caspofungin	Cleared
8	M	48	Surgical	Perforated DU	*C. parapsillosis*	Caspofungin	Cleared

AMB=Amphotercin B, ICU=Intensive Care Unit, PICU=Pediatric Intensive Care Unit, NICU=Neonatal Intensive Care Unit, DM=Diabetes Mellitus, Abx=Prior Antibiotic Use (within 6 months), CHF=Congestive Heart Failure, Cath=Urinary Catheterization, ME=Meningo-Encephalitis., IVH-G4=Intraventricular Hemorrhage Grade 4, DU=Duodenal Ulcer.

Certainly, most of incidence of CUTI is likely to be underestimating, mainly because the use of standard urine culture is not very sensitive. Colony counts is considered the corner stone in the diagnosis of bacterial UTI however, it was not proven to be diagnostically useful in CUTI [4]. In addition, the isolation of *C. glabrata* represent a challenge as it grows very poorly in blood media and as such the diagnosis of CUTI may be overlooked [4].

The overall incidence of candiduria in our hospital was 3.3% and only 1.5% was considered as CUTI. Some studies have reported similar rates (4%) ,while much higher rates reaching up to 12.9 % have also been reported [17,20].

In our study, we have found that most significant cases were HA-CUTI 80% (30/37). CA-UTIs were mostly in females (5/7) and all patients but one had urinary catheter (6/7). The average age in both groups was similar, 56.2 year in HA-UTI and 48.2 year in CA-UTI. Consistent with our observations, Colodner et al also reported higher incidence of CA-UTI in females than males. However, in this study patients were significantly younger compared to the HA-UTI group in there finding [18].

Overall, about risk factors in CUTIs, the majority of clinical studies have identified parallel data to ours. These included, female gender, diabetes, indwelling urinary drainage devices, and recent antibiotic therapy [1,3,5]. Diabetes makes the female patients more prone to candiduria by colonizing the vulvo-vestibular area. Glycosuria enhances urinary fungal growth and impairs phagocytic activity thus lowering host resistance to invasion by fungi [21]. In our study, similar rates of diabetes mellitus were found in both HA and CA-CUTI (43% vs. 53%), and urinary catheterization (71% vs. 89%). Although Colonder et al. reported diabetes and urinary catheterization to be more common in HA-CUTIs than community ones (28.8% vs. 44.3%) and (15.2% vs. 32.9%) respectively. Never the less, non- reached a significant p value (Diabetes p=0.3 and urinary catheterization p=0.16) in our study similar to his. Moreover, the mortality rates in our population group were higher in the HA-CUTI patients (20% vs. 0%). Comparatively, studies have shown higher mortality in that same group [18,21](Table 2).

With reference to prior antibiotic usage, it is believed that antibiotics change the normal flora of the perineum, allowing overgrowth of yeasts in and around the urethra and progression to colonization and/or infection of the bladder [9]. Equivalently, prior antibiotic use was similar in both groups. About patient location when acquiring candiduria, we have found it most prevalent among patients in intensive care unit (ICU) similar to a study by Bougnoux et al. [22]. This is probably attributed to the fact that most patients in ICU are catheterized, critically ill and on broad-spectrum antibiotics.

Table 2: Demographic and risk factors characteristics of CA- and HA CUTIs in the significant group.

	CA (n=7)	HA (n=30)	P value
Age mean: Years (range)	56 (2 month- 93 years)	56 (1 month- 77years)	
Gender (female): n (%)	5 (71%)	17 (57%)	0.24
Diabetes mellitus: n (%)	3 (43%)	16 (53%)	0.3
ABx: n (%)	5 (71%)	20 (66%)	0.41
Cath: n (%)	5 (71%)	26 (86%)	0.16
Recurrent: n (%)	0	1 (3.3%)	0.31
***C. albicans*: n (%)**	6 (85%)	19 (63%)	0.34
Mortality: n (%)	0	6 (20%)	0.09

CA=Community-Acquired; HA=Hospital-Acquired; ABx=Antibiotic treatment during the last six months, Cath= Urinary Catheter Sample.

Recent studies highlight the changing epidemiology of community and nosocomial candiduria [6,7,14,18]. Our data showed that *C. albicans* remained as the prominent species isolated from urine cultures in both community and hospital setting, 85% and 68% respectively. Furthermore, non- *C. albicans* species accounted for almost 32% of all significant CUTIs isolates in our center. This suggests a shift to species other than *C.albicans* in causing CUTIs and should be analyzed with caution. A report by Kobayashi et al. stated high incidence of non-albicans candiduria, primarily *C. tropicalis* with a percentage of 22.2 [7]. Aubron et al. had similar distribution to our data with *C. albicans* being a major species 65% [19].

Documented candidemia occurred in eight patients (22%), and six died of disseminated candidiasis (75%). Our rates are similar to other studies as mortality associated with HA-CUTI are high [17,20]. Antifungal susceptibility in candiduric patients depends largely on the infecting strains. Traditionally, laboratories only perform antifungal susceptibility testing on non- *C. albicans* strains or in cases of repeated infections. This is based on international recommendation since the majority of the isolates are *C. albicans* and sensitive to first line agent. Recently published susceptibility demonstrate that species other than *C. albicans* are increasing and furthermore, significant numbers of *C. glabrata* strains are resistant to fluconazole. We had only one patient with disseminated *C. glabrata* infection, with an MIC of 4 µg/ml to fluconazole. Concomitant with the progress in the medical field, the incidence of UTI due to *Candida* continues to increase. Moreover, most of these cases are HA-CUTI, associated with ICU admission, urinary catheterization and diabetes. Occasionally CA-CUTI can be significant especially in diabetics and bedridden with long-term urinary-catheterization. However, majority of CA-CUTI cases are considered not significant representing colonization or contamination rather than infection. Antifungal therapy is rarely required. However, when treatment is necessary every effort should be made to identify the species and determine antifungal susceptibility.

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References

1. Nayman Alpat S, Ã–zguneÅŸ I, Ertem OT, Erben N, Doyuk Kartal E, et al. (2011) Evaluation of risk factors in patients with candiduria. Mikrobiyol Bul 45: 318-324.
2. Jain N, Kohli R, Cook E, Gialanella P, Chang T, et al. (2007) Biofilm formation by and antifungal susceptibility of Candida isolates from urine. Appl Environ Microbiol 73: 1697-1703.
3. Guze LB, Haley LD (1958) Fungus infections of the urinary tract. Yale J Biol Med 30: 292-305.
4. Kauffman CA, Fisher JF, Sobel JD, Newman CA (2011) Candida urinary tract infections--diagnosis. Clin Infect Dis 52 Suppl 6: S452-456.
5. Richards MJ, Edwards JR, Culver DH, Gaynes RP (2000) Nosocomial infections in combined medical-surgical intensive care units in the United States. Infect Control Hosp Epidemiol 21: 510-515.
6. Kauffman CA, Vazquez JA, Sobel JD, Gallis HA, McKinsey DS, et al. (2000) Prospective multicenter surveillance study of funguria in hospitalized patients. The National Institute for Allergy and Infectious Diseases (NIAID) Mycoses Study Group. Clin Infect Dis 30: 14-18.
7. Kobayashi CC, de Fernandes OF, Miranda KC, de Sousa ED, Silva Mdo R (2004) Candiduria in hospital patients: A study prospective. Mycopathologia 158: 49-52.

8. Fan-Havard P, O'Donovan C, Smith SM, Oh J, Bamberger M, et al. (1995) Oral fluconazole versus amphotericin B bladder irrigation for treatment of candidal funguria. Clin Infect Dis 21: 960-965.

9. Sobel JD, Kauffman CA, McKinsey D, Zervos M, Vazquez JA, et al. (2000) Candiduria: A randomized, double-blind study of treatment with fluconazole and placebo. The National Institute of Allergy and Infectious Diseases (NIAID) Mycoses Study Group. Clin Infect Dis 30: 19-24.

10. Wynn JL, Tan S, Gantz MG, Das A, Goldberg RN, et al. (2012) Outcomes following candiduria in extremely low birth weight infants. Clin Infect Dis 54: 331-339.

11. Wainer S, Cooper PA, Gouws H, Akierman A (1997) Prospective study of fluconazole therapy in systemic neonatal fungal infection. Pediatr Infect Dis J 16: 763-767.

12. Pappas PG, Kauffman CA, Andes D, Benjamin DK Jr, Calandra TF, et al. (2009) Clinical practice guidelines for the management of candidiasis: 2009 update by the Infectious Diseases Society of America. Clin Infect Dis 48: 503-535.

13. Arkan S, Rex JH, Murray PR, Baron EJ, Jorgensen JH, et al. (2007) Manual of Clinical Microbiology,Washington, DC: ASM Press.

14. Revankar SG, Hasan MS, Revankar VS, Sobel JD (2011) Long-term follow-up of patients with candiduria. Eur J Clin Microbiol Infect Dis 30: 137-140.

15. Ascioglu S, Rex JH, de Pauw B, Bennett JE, Bille J, et al. (2002) Defining opportunistic invasive fungal infections in immunocompromised patients with cancer and hematopoietic stem cell transplants: An international consensus. Clin Infect Dis 34: 7-14.

16. Clinical and Laboratory Standards Institute (CLSI) (2012) Reference method for broth dilution antifungal susceptibility testing of yeasts; fourth informational supplement. Wayne: Clinical and Laboratory Standards Institute; (Document M27-S4).

17. Bouza E, Juan RS, Munoz P, Voss A, Kluytmans J (2001) A European perspective on nosocomial urinary tract infections II. Report on incidence, clinical characteristics and outcome (ESGNI-004 study). European Study Group on Nosocomial Infection. Clin Microbiol Infect 7: 532–542.

18. Colodner R, Nuri Y, Chazan B, Raz R (2008) Community-acquired and hospital-acquired candiduria: comparison of prevalence and clinical characteristics. Eur J Clin Microbiol Infect Dis 27: 301-305.

19. Aubron C, Suzuki S, Glassford NJ, Garcia-Alvarez M, Howden BP, et al. (2015) The epidemiology of bacteriuria and candiduria in critically ill patients. Epidemiol Infect 143: 653-662.

20. Schaberg DR, Culver DH, Gaynes RP (1991) Major trends in the microbial etiology of nosocomial infection. Am J Med 91: 72S-75S.

21. Lundstrom T, Sobel J (2001) Nosocomial candiduria: A review. Clin Infect Dis 32: 1602-1607.

22. Bougnoux ME, Kac G, Aegerter P, d'Enfert C, Fagon JY, et al. (2008) Candidemia and candiduria in critically ill patients admitted to intensive care units in France: Incidence, molecular diversity, management and outcome. Intensive Care Med 34: 292-299.