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 assess 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. guillermondii 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 10³ 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 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 turpidity 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 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.

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

AMB=Amphotericin B, ICU=Intensive Care Unit, PICU=Pediatric Intensive Care Unit, NICU=Neonatal Intensive Care Unit, DM=Diabetes Mellitus, Abs=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 underestimated, 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 a 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 infection of the bladder [9]. Equivalently, prior antibiotic use was similar in and around the urethra and progression to colonization and/or infection. Antifungal therapy is rarely required. However, when treatment is necessary every effort should be made to identify the species and determine antifungal susceptibility.

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, treatment is necessary every effort should be made to identify the species and determine antifungal susceptibility.

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References


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

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

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


