Molecular identification of Candida species isolated from candiduria and its risk factors in neonates and children

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ABSTRACT

Background and Purpose: The present study was performed to raise attention on the frequency of Candida spp. and evaluation of risk factors of candiduria in neonates and children.

Materials and Methods: In total, 60 urine samples were collected from the suspected neonates and children. Identification of Candida at species level was performed using the polymerase chain reaction-restriction fragment length polymorphism approach.

Results: The restriction fragment length polymorphism fingerprint analysis revealed that Candida parapsilosis (n=17; 28.33 %) is the most prevalent isolated species followed by Candida albicans (n=9; 15%), Candida tropicalis (n=4; 9.52%), and C. glabrata (n=2; 4.76%). All of the C. albicans and C. parapsilosis complex strains were identified as C. albicans with HWP1 gene primers and using the NlaIII restriction enzyme activity, respectively. In this study, none of the mentioned factors was the cause of infection, but they could be considered risk factors. The mean hospital stay was 21 days (range: 7-21 days). More than 90% of the patients had a urinary catheter, and about 26% of them received antibiotics. Regarding the risk factors, there was no significant difference between the two groups of candidiasis in terms of C. albicans and non-albicans Candida (P<0.01).

Conclusion: Candidurias has always been a challenging issue, especially in children admitted to hospitals. Outcome of candiduria in patients with generally healthy is little.

Keywords: Candiduria, Candida species, Children, Neonates, Risk factors

Introduction

Urinary tract infection (UTI) is known as one of the major nosocomial infections [1]. In total, 90% of cases are caused by bacteria and 10-15% of them are caused by fungi, particularly Candida spp. [2]. The UTI may involve the upper urinary tract (e.g., pyelonephritis) or the lower urinary tract (e.g., cystitis). It may be challenging, if not unreasonable, to separate pyelonephritis from cystitis based on clinical signs, particularly in newborns and young children. The UTIs as a result of Candida spp. have become a challenging matter in intensive care units [3].

The occurrence of Candida spp. in the urine may characterize several disorders that need precise interpretation of the report, ranging from specimen impurity to UTI, including disseminated candidiasis [4]. Some factors, such as older age, female gender, use of antibiotics, urinary catheters, diabetes, immunosuppressive treatment, and prolonged hospital stay are known as the key risk factors for candiduria [5, 6]. As soon as the presence of Candida in the urine is approved, a precise medicinal assessment should be completed to detect symptoms indicative of other disorders, such as diabetes mellitus, genitourinary structural abnormalities, decreased renal function, and metabolic syndromes [7].

Differences in Candida spp. are associated with disease severity and diversity in antifungal sensibility outlines. Therefore, the accurate identification of Candida spp., as well as the determination of sensitivity pattern to the main antifungal agents lead to choose appropriate treatment regime. Applied molecular approaches to discriminate etiological agents at the species level result in the progress of the difficulty of symptoms and limit the diffusion of the disease trough prescription of a suitable antifungal regime.

Candida can be normally diagnosed with microscopic examination and other phenotypic methods, including chlamydoconidia production and chromogenic medium (CHROMagar). Moreover, the absorption and fermentation of carbohydrates help to identify Candida spp. [8]. These methods are very time-consuming and require several days to identify various Candida spp. [8]. Other disadvantages of such tests include their low specificity and dependence on phenotypic properties. Tests based on the phenotypic property are more subject to environmental changes...
than the tests based on genotyping property.

Therefore, the present study was performed to show the frequency of Candida spp. in the urine sample of the subjects who were admitted to a hospital in Tehran, Iran during 2019. The polymerase chain reaction (PCR)- Restriction fragment length polymorphism (RFLP) method was used to evaluate the risk factors.

Materials and Methods

Study design

After eligible subjects received detailed explanations about the study, informed consent was obtained from them.

Subjects and procedure

This cross-sectional and experimental study was conducted in 2019. The hospitalized children (from Imam Hessain Hospital, Tehran, Iran) suspected of sepsis or candiduria and those with pyuria were enrolled in this study. It should be mentioned that the subjects who had positive cutler of bacteria were excluded. The Ethics Committee of Shahid Beheshti University of Medical Sciences, Tehran, Iran approved this study (Ethics Code: IR.SBMU.MSP.REC.1398.631).

In total, 60 first-void midstream urine samples or specimens were collected by urinary catheter from hospitalized patients who referred to the mycology research center. The demographic characteristics, history, and symptoms of patients were also recorded.

Initial identification of Candida spp.

Urine samples were centrifuged at 1500 rpm for 10 min and urine direct examination was performed to detect Candida fungal elements in urine sediment. Initial identification of Candida spp. was performed based on colony color and yeast counts of more than 10^5 colony-forming unit/ml on CHROM agar Candida medium (CHROM agar, France) and sabouraud dextrose agar (Merck, Germany) at 35 °C for 24 h. The ability of pseudohyphae formation in all Candida spp. has been confirmed using corn meal agar plus tween 80 culture.

Molecular identification of Candida spp.

Finally, definite identification of Candida spp. was confirmed by PCR-RFLP. This identification was based on the amplification of ITS1-5.8SrDNA-ITS2 region from RNA complex with pan fungal primers ITS1-ITS4 that were synthesized by Bioneer company (Korea). The DNA extraction from Candida isolates was performed using the DNA tissue kit (Qiagen, Germany). The PCR was carried out with a PCR reaction mixture, including 1 µl of the extracted DNA, 10 µl of Taq DNA Polymerase Master Mix RED (Ampliqon, Denmark), 1 µl of each ITS1 (5´-TCCTGATTTGAGACCTGGG-3´) and ITS4 (5´-TCCTCCGCTTATTGATATGC-3´) oligonucleotide primers, and 12 µl of water.

The PCR cycling parameters were 94 °C for 5 min, 35 cycles of denaturation for 1 min at 95 °C, annealing for 1 min at 56 °C, extension for 90 sec at 72 °C, and a final extension for 7 min at 72 °C. The PCR products were visualized by 1.5% agarose gel electrophoresis in Tris-borate-EDTA buffer (Merck, Germany) and stained.

All digestion reactions were performed in 15 µl of a mixture containing 2 µl of 10× buffer, 2 µl of the enzyme, 10 µl of topoisomerase amplicon, and enough ultrapure water (1 µl) to reach the final volume. Restriction enzyme digestions were performed with Msp I at 37 °C for 8-10 h [9]. The PCR amplicons and restriction enzyme digestion products were loaded in 2% (w/v) agarose gel in the presence of red gel (0.5 µg mL^-1). After running for 1.5 h at 90 V cm^-1. A DNA molecular weight marker and 100 bp ladder (Fermentas, USA) were used.

The C. albicans complex and C. parapsilosis complex were differentiated using the HWP1 gene amplification [10] and PCR-RFLP with NlaIII restriction enzyme [11], respectively. The PCR program was similar to ITS1-5.8 SrDNA- ITS2 PCR with an annealing temperature of 57 °C.

Data analysis

The statistical analysis was performed in SPSS software (version 22.0) for Windows (SPSS Inc., Chicago, IL, USA). Descriptive statistics are given by means and 95% confidence intervals for normally distributed data. It should be mentioned that categorical data were subsumed by relative frequencies. In analytical statistics, variables were compared between groups using the t-test. The results of the tests were considered significant for p-values of less than 0.05.

Results

In total, 32 (53.33%) out of the 60 urine samples were culture positive for Candida spp. The range age of the participants was between ≤ 1 month to 12 years. There was no significant difference between the cases in terms of age and gender (male: 53.5% vs. female: 46.5%) (P>0.05). There was evidence of empiric antifungal treatment based on medical records, including itraconazole (5%), fluconazole (20%), amphoterin B (13%), and fluconazole was not qualified for antifungal therapy. Moreover, 19 (45.23%) subjects had hematological malignancy, 2 (7.1%) of them had heart disease, and the rest of them had other non-leukemia-related diseases. The evidence of neutropenia (45%), low weight (<1500) (21%), chemotherapy (7%), history of surgery (10%), antibiotic therapy use (17%) were also observed. The mean hospital stay was 21 days; more than 90% of the patients had urinary catheters, and about 26% of them received antibiotics. Overall, C. albicans (28.125%) and non-C. albicans spp. (71.87%) were identified using conventional chromogenic media. All the primarily identified Candida on CHROM agar were precisely differentiated using the PCR method.

The bands were all located between 524 and 871 bp which suggested that they possibly belonged to Candida genus (Figure 1a). The RFLP fingerprint analysis revealed that C. parapsilosis (n=17; 53.125 %) was the most prevalent isolated species followed by C. albicans (n=9; 28.125%), C. tropicalis (n=4; 12.5%).
Figure 1. Agaros gel electrophoresis pattern of polymerase chain reaction products of Candida. a: before digestion with the restriction enzyme, lane 1-8: bands are all located between 524 and 871 bp which suggests that they possibly belonged to the Candida genus. b: enzymatic digestion pattern with MspI restriction enzyme for differentiation of Candida spp. a: C. albicans (297-238 bp): 1, 3; C. glabrata (314-557 bp): 2; C. tropicalis (184-340 bp):4; M: 100 bp DNA size marker.

Figure 2. Amplification pattern of HWP1 genes for identification of Candida albicans complex. L: 100 bp DNA size marker. The lanes in the picture belong to Candida albicans (900 bp).

and C. glabrata (n=2; 6.25%) (Figure 1b). All of C. albicans complex strains were identified as C. albicans, based on amplification of HWP1 gene (Figure 2). Further analysis of C. parapsilosis complex strains showed that all identified species were known as C. parapsilosis (Figure 3).

Figure 3. Identification of Candida parapsilosis complex after digestion with NlaIII restriction enzyme. L: 100 bp DNA size marker. Lane 1. C. parapsilosis complex before digestion; lanes 1-6. C. parapsilosis spp.

Discussion

In this study, there was no statistically significant difference between the positive cases and age (P>0.05). This finding is in line with those of a study performed by Zarei et al. [12]. In another study, the UTI of the subjects represented the most frequent diagnosis (62%) followed by fungaemia (34%) and peritonitis (4%). In the aforementioned study, UTIs were more frequent in patients with urinary tract malformation, and most of the infections were reported among neonates [13].

In this study, 53.5% and 46.5% of the patients diagnosed with candiduria were male and female, respectively. In another study, Zarei et al. reported the frequency of in males and females to be 59.2% and 40.8%, respectively [12]. Similarly, Jain et al. and Gholamipour reported that candiduria was more common in males (68%) than females (32%) [14,15]. In another study, UTI was evaluated in 301 infants (1.1% of admissions, 253 males and 48 females with a ratio of 5.3:1), and only one infant had candiduria [16].

Regarding underlying diseases in this study, 45.23% of the patients had a hematological
malignancy, 7.1% (n=2) of them had heart disease, and the rest of them had other non-leukemia-related diseases. According to Robinson et al., the most important predisposing factors in creating candiduria were hospitalization in intensive care units (76.2%) and antibiotic therapy [17]. Robinson et al. reported that the mortality rate due to candiduria in neonates in the neonatal intensive care unit was significant (30%) [17]. In this study, evidence of neutropenia, low weight (<1500 g), long hospitalization, chemotherapy, history of surgery, and antibiotic therapy could be considered risk factors. Regarding the risk factors, there was no significant difference between the two groups of candidiasis in terms of C. albicans and non-albicans Candida spp. (NAC) (P<0.01). In a study carried out by Gholamipour et al., long-term indwelling urinary drainage tools existed in 40% of the neonates with candiduria [14]. Platt et al. showed that 26.5% of all fungal urinary infections were associated with the application of indwelling catheters [18].

Regarding higher resistance to fluconazole in NAC spp., identification of Candida spp. is necessary for appropriate management of infection. Contrary to this study, in the study performed by Zarei et al., C. albicans (65.5%) was the most isolated species, followed by C. glabrata [19]. Moreover, in another research, C. albicans was the most common pathogen in neonate patients with candiduria followed by C. parapsilosis [20]. In another study, C. albicans was responsible for 97% of positive cultures in children [21]. Previously, we have reported the frequency of candidiasis in 16.5% of hospitalized patients in two general hospitals in Ahvaz whose most common agent was C. albicans [19].

Conclusion
In this study, C. parapsilosis and C. albicans were the most commonly observed species. Candida spp., which were once considered harmless, can now cause candidemia and significant morbidity and mortality in patients residing in intensive care units. Therefore, the identification of their underlying conditions and risk factors must be taken seriously. Furthermore, cases with more than one species of Candida and also the cases with bacterial infection require more attention, investigation, and consideration.

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Authors' contribution
Study concept, design, and technical supervision: A.F.; collection the samples from patients and interpretation: F.S.H.; acquisition of data and drafting of the manuscript: A.F. and F.S.H.; critical revision of the manuscript and scientific consultation: A.F.

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Conflicts of interest
The authors declare that there were no conflicts of interest.

References
1. Ma G-W, O'Neill C, Mertz DJAlOJC. Correlating incidence densities and point-prevalence for the surveillance of catheter-associated urinary tract infections. 2020.
2. Hassaneen AM, Ghonaim RA, Hassanin HM, Salama NA, Elgohary Tjmicu. Different aspects of candiduria as an important nosocomial infection. 2014;82(1):199-204.
3. Shuman EK, Chenoweth CEJcm. Recognition and prevention of healthcare-associated urinary tract infections in the intensive care unit. 2010;38:S373-S9.
4. Fisher JF. Candida urinary tract infections – epidemiology, pathogenesis, diagnosis and treatment: executive summary. Clin. Infect. Dis. 52(Suppl. 6), S429–S432 (2011). Crossref, Medline, Google Scholar
5. Harris AD, Castro J, Sheppard DC, Carmeli Y, Samore MHJcid. Risk factors for nosocomial candiduria due to Candida glabrata and Candida albicans. 1999;29(4):926-8.
6. Weinstein RA, Lundstrom T, Sobel JJCid. Nosocomial candiduria: a review. 2001;32(11):1602-7.
7. Dias V. Candida species in the urinary tract: is it a fungal infection or not? 2020 - Future Medicine.
8. Bukhary ZAJSJoKD, Transplantation. Candiduria: a review of clinical significance and management. 2008;19(3):350.
9. Safaviieh M, Coarsey C, Esibou N, Menic A, Vyas JM, Shafee H, et al. Advances in Candida detection platforms for clinical and point-of-care applications. 2017;37(4):441-58.
10. Zomorodian K, Rahimi MJ, Pakshir K, Motamedi M, Ghiassi MR, Rezashah HJFigid. Determination of antifungal susceptibility patterns among the clinical isolates of Candida species. 2011;3(4):357-63.
11. Alam MZ, Alam Q, Jiman-Fatani A, Kamal MA, Abuzenadah AM, Chaudhary AG, et al. Candida identification: a journey from conventional to molecular methods in medical mycology. 2014;30(5):1437-51.
12. Seifi Z, Azizh M, Salehi Z, Mahmoudabadi AZ, Shamshizadeh A. Candiduria in children and susceptibility patterns of recovered Candida species to antifungal drugs in Ahvaz. Journal of nephropathology. 2013 Apr;2(2):122.
13. Mesmi A, Bandettini R, Caviglia I, Fioredda F, Amoroso L, Faraci M, Mattioli G, Piaggio G, Russo FM, Moscatelli A, Loy A. Candida infections in paediatrics: Results from a prospective single-centre study in a tertiary care children's hospital. Mycoses. 2017 Feb;60(2):118-23.
14. Gholamipour P, Mahmoudi S, Pourakbari B, ASHTIANI MT, Sabouni F, Teymum M, Mamishi S. Candiduria in children: a first report from an Iranian referral pediatric hospital. Journal of Preventive Medicine and Hygiene. 2014 Jun;55(2):54.
15. Jain M, Dogra V, Mishra B, et al. Candiduria in catheterized intensive care unit patients: Emerging microbiological trends. Indian J Pathol Microbiol. 2011;54:552–552.
16. Sastre JB, Aparicio AR, Cotollo GD, Colomer BF, Hernández MC. Urinary tract infection in the newborn: clinical and radio imaging studies. Pediatric Nephrology. 2007 Oct;22(10):1735-41.
17. Robinson JL, Davies HD, Burton M, O’Brien K, Simpson K, Asztalos E. Characteristics and outcome of infants with candiduria in neonatal intensive care - a Paediatric Investigators Collaborative Network on Infections in Canada (PICNIC) study. BMC Infect Dis. 2009;9:183.
18. Platt R, Polk BF, Murdock B, et al. Risk factors for nosocomial urinary tract infection. Am J Epidemiol. 1986;124:977–985.
19. Zarei Mahmoudabadi A, Zarrin M, Ghanatir F, Vazirianzadeh B. Candiduria followed by C. albicans in children and susceptibility patterns of recovered Candida Albicans. 2011;3(4):357.
20. Elgohary TJM, Elgohary Tmjcu. Different aspects of candiduria as an emerging micr. 2010;38:S373-S9.
21. Jain M, Dogra V, Mishra B, et al. Candiduria in catheterized intensive care unit patients: Emerging microbiological trends. Indian J Pathol Microbiol. 2011;54:552–552.