



## Original article

## Molecular identification of *Candida* species from urinary infections in Honduras



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## ABSTRACT

**Background:** Candiduria is a common infection among hospitalised patients. Although the clinical relevance of yeasts in urine is not clearly defined, fungal urinary tract infections have increased significantly in the last decades, becoming a growing public health problem. *Candida albicans* is the most commonly reported species in urinary infections, although other species of the genus are becoming particularly important, because some of them are linked with resistance to antifungal drugs.

**Aims:** This study aimed to evaluate the frequency of *Candida* species causing candiduria in a hospital in Honduras.

**Methods:** A simple and cost-effective PCR-RFLP approach was used, by amplifying a partial sequence of the ribosomal ITS1-ITS2 region and a subsequent digestion with the enzyme MspI.

**Results:** During 2016, an analysis was performed on 73 urine samples from patients of different ages. Seven species were found. *Candida albicans/dubliniensis* was the most frequent species (30%); *Candida glabrata* (28.8%) was the most isolated among the rest of the species. *Candida kefyr* was the least frequent species found (2.5%).

**Conclusions:** This study shows, for the first time in Honduras, the frequency of the *Candida* species isolated from urine using PCR-RFLP for their identification. This approach could be applied in future epidemiological studies at local and national level.

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## Identificación molecular de especies de *Candida* de infecciones urinarias en Honduras

## RESUMEN

**Antecedentes:** La candiduria es una infección común entre pacientes hospitalizados. Aunque la relevancia clínica de las levaduras en orina aun no está del todo esclarecida, el número de infecciones urinarias por hongos se ha incrementado en las últimas décadas, convirtiéndose en un creciente problema de salud pública. *Candida albicans* es la levadura más común en las infecciones urinarias, si bien otras especies del género están adquiriendo importancia debido a la resistencia a las drogas antifúngicas asociada a algunas especies.

**Objetivos:** Este estudio pretendió evaluar la frecuencia de especies de *Candida* responsables de candiduria en un hospital nacional de Honduras.

**Métodos:** Se utilizó un análisis *in silico* y PCR-RFLP de una región parcial de la secuencia ribosomal ITS1-ITS2, con posterior digestión mediante la enzima MspI.

**Resultados:** Se analizaron 73 muestras de orina de pacientes de diferentes edades durante el año 2016. Se encontraron siete especies diferentes. *Candida albicans/dubliniensis* fue la especie más común (30%); *Candida glabrata* (28,8%) fue la más frecuente entre el resto de especies. *Candida kefyr* fue la especie menos encontrada (2,5%).

## Palabras clave:

*Candida albicans*

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**Conclusiones:** Este estudio muestra la frecuencia de especies de *Candida* aisladas a partir de orina, e identificadas mediante la técnica PCR-RFLP por primera vez en Honduras. Este enfoque podría ser aplicado para futuros estudios epidemiológicos a nivel local y nacional.

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*Candida* species are the most commonly isolated fungi in urine cultures, especially among hospitalized patients.<sup>3,15</sup> *Candida* urinary infections are a growing health problem for immunosuppressed and debilitated patients, or those receiving long-term antibiotic therapy.<sup>23</sup> Although the clinical relevance of yeasts in urine is not clearly established, many authors agree that counts greater than  $10^3$ – $10^4$  CFU/ml suggest primary or disseminated infection,<sup>24</sup> rather than simple contamination or colonization.

The most common etiological agent of candiduria is *Candida albicans*<sup>1</sup>; however urinary tract infections caused by other species of the genus have increased significantly in recent years.<sup>12,19</sup> Some of the most common species causing candiduria are *Candida glabrata*, *Candida tropicalis*, *Candida parapsilosis*, *Candida krusei* and *Candida kefyr*.<sup>1,25</sup> This fact raises special concern because of the low susceptibility to antifungal drugs showed by some *Candida* species, which may lead to difficulties or failures in the treatment.<sup>5</sup>

The identification of yeast species causing candiduria and the evaluation of their susceptibility to antifungal drugs are a major problem for clinical laboratories in low-income countries due to financial constraints. Some of these laboratories are restricted, due to the lack of resources, to only report the number of CFU/ml in urine; VITEK® 2 YST ID card, API Candida, API 20C, API ID32C, or CHROMagar Candida Medium are expensive methods when there is a lack of resources. For this reason, in Honduras, the information regarding *Candida* species causing urinary infections in public hospitals is scarce. Implementing a less costly but reliable technique for the identification of *Candida* species could help to fill this knowledge gap. Several alternative strategies have been proposed,<sup>13,29</sup> however most of them require expensive equipment and a higher level of technical expertise. This study aimed to determine the frequency of species causing candiduria in hospitalized patients attending a public hospital in Honduras through a very simple molecular approach<sup>17</sup> in order to assess the frequency of infections and the possibility of its further implementation as a routine test.

## Materials and methods

### Population and culture method

A total of 73 yeast cultures from urine samples were analyzed. The samples were obtained from patients of all ages hospitalized in a national facility in Tegucigalpa, Honduras, between July and September 2016. Only urine samples revealing yeasts in the microscopic analysis were cultured by calibrated loop (0.001 ml) onto Sabouraud agar supplemented with 80 µg/ml of gentamicin. Cultures were incubated at 37 °C and read within 24 h. The number of cultivable yeasts was established by the CFU counts.

### DNA extraction

A DNA extraction protocol using organic solvents was performed in the isolated yeast colonies. Briefly, an initial cell lysis step was carried out from one loop of an axenic culture suspended in 1000 µl of buffer composed of 10 mM Tris, pH 8; 1 mM EDTA, pH 8; and 100 mM NaCl. This suspension was heated at 100 °C for 1 min and then stirred for 1 min in a micro-MiniBeadBeater® system (Bio Spec products Inc.) with 0.1 mm glass beads. Four hundred µl

were recovered and one volume of phenol – chloroform (1:1) was added, mixed and centrifuged at 10,000 rpm. The aqueous phase was transferred to a new vial and precipitated with one volume of ice cold isopropanol and 1/10 volume of sodium acetate (3 M, pH 5.2). Three washes in 70% ethanol were performed. The dried pellet was resuspended in 50 µl of nuclease-free water. The DNA concentration was calculated with a NanoDrop spectrophotometer (Thermo Fisher Scientific Inc.), diluted to a final concentration of 40 ng/µl and stored to –20 °C until further use.

### PCR-RFLP for yeast species identification

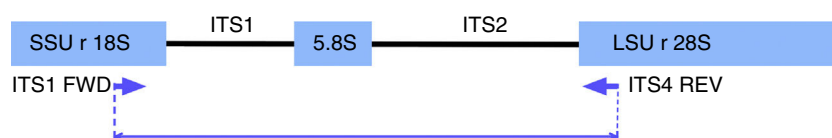
PCR reactions were performed according to Mirhendi et al.<sup>17,28</sup> with modifications. Briefly, amplifications were carried out under the following conditions in a 50 µl volume: 25 µl of PCR Master Mix (Promega Corp. Madison, WI, USA), 1 µl of 10 µM ITS1 and ITS4 primers (Fig. 1): 5'-TCCGTAGGTGAACCTGCGG-3/5'-TCCTCCGCTTATTGATATGC-3', and 1 µl of DNA (40 ng/µl). Reactions were carried out with an initial denaturation step at 95 °C for 5 min, 37 cycles of 95 °C for 30 s, 56 °C for 30 s, and 72 °C for 30 s, with a final extension at 72 °C for 5 min. Amplicons were visualized in 1.5% agarose gel electrophoresis with ethidium bromide. Ten microliters of PCR products were digested for 2 h at 37 °C with 2 µl of buffer, 0.2 µl of 10 µg/µl acetylated BSA, and 0.5 µl of the restriction enzyme MspI (10 U/µl) (Promega Corp.). The restriction fragment pattern was analyzed on 2% agarose gel in 0.5X Tris-Acetate-EDTA (TAE) buffer and visualized in a BioDoc-It Imaging System (UVP, LLC; Upland, CA).

### In silico analysis

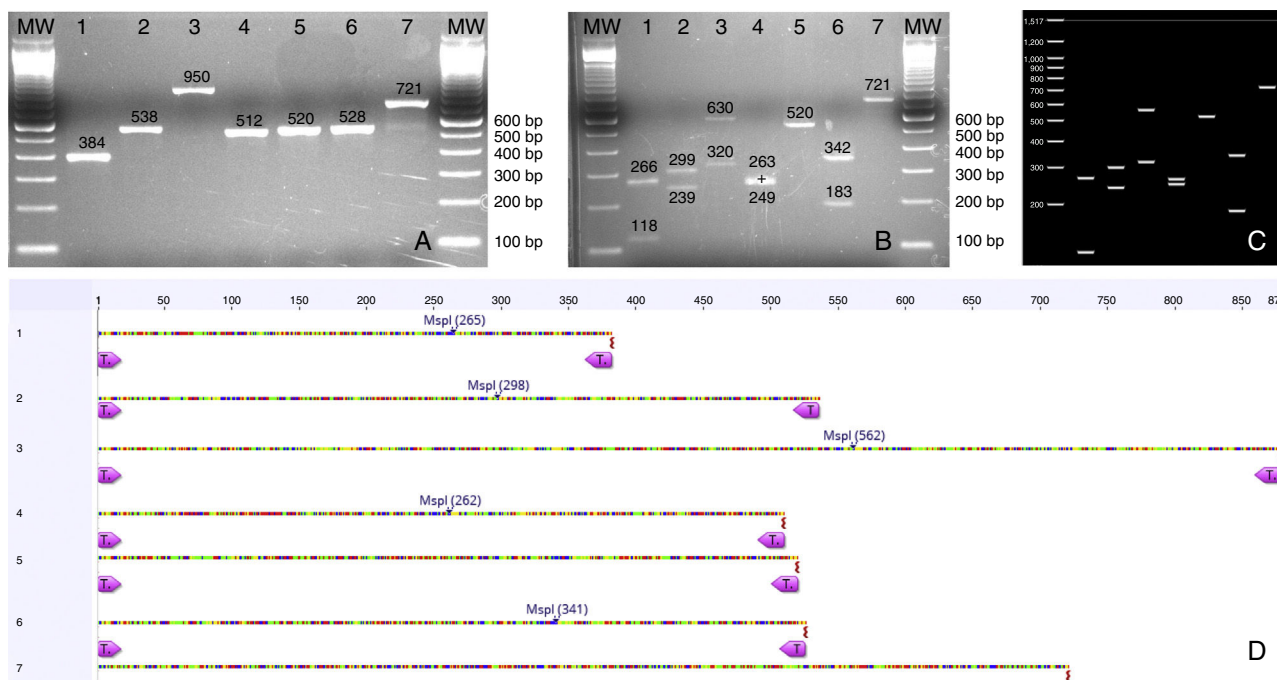
In order to confirm the restriction patterns of previously reported *Candida* species<sup>17,28</sup> as well as of other uncommon species, homologous sequences corresponding to the fungal ITS1-ITS2 region were imported from the BLAST tool of NCBI (<https://blast.ncbi.nlm.nih.gov/Blast.cgi>) into the Geneious® 7.1.5 software (Biomatters Ltd, Auckland, New Zealand). Sequences were trimmed to include a partial fragment of the small subunit ribosomal RNA gene, the internal transcribed spacer 1, the 5.8 S ribosomal RNA gene, the internal transcribed spacer 2, and a partial sequence of the large subunit ribosomal RNA gene. Primers ITS1 and ITS4 flanked all sequences. The enzyme MspI was applied in the analysis to demonstrate putative cutting sites and restriction patterns for most of the available species (Fig. 2).

### Sequencing of PCR products

To confirm the identity of the amplified products, seven gene fragments were selected according to molecular weight, and an ulterior sequencing was carried out on both strands with their respective ITS primers following standard protocols at MacroGen Corp. facilities (<https://www.macrogenusa.com>). Sequences were trimmed at both 5' and 3' ends with the Geneious® 7.1.5 software and queried against international databases contained in NCBI. The higher similarity index was recorded.



**Fig. 1.** ITS1-5.8S-ITS2 ribosomal region showing the amplification target and the amplified sequence length.



**Fig. 2.** (A) PCR products from seven *Candida* species isolated from urine samples showing differential amplicon sizes. 1: *C. lusitaniae*; 2: *C. albicans*; 3: *C. glabrata*; 4: *C. krusei*; 5: *Candida parapsilosis*; 6: *C. tropicalis*; 7: *C. kefyr*; (B, D) *In silico* analysis of restriction profiles using *MspI* for the same seven yeast species; (C) Restriction results revealing complete coincidence with the *in silico* analysis.

## Results

### Urine culture and yeast counts

Seventy-two (98.6%) urine cultures showed more than  $10^3$  CFUs, and one culture (1.36%) revealed less than 100 CFUs. Five cultures (6.84%) showed mixed candiduria and four of them (5.48%) reported two different yeast species, while the fifth culture (1.36%) revealed more than two yeast species. In none of the cases the same combination of species was reported.

### Yeast species identified through PCR-RFLP

A total of 73 yeast colonies were analyzed and putatively identified through a PCR-RFLP approach using the ITS1-5.8 S-ITS2 ribosomal region. No phenotypic method for species identification was carried out. PCR products ranging from 512 to 950 bp were obtained. The PCR-RFLP using *MspI* as restriction enzyme identified the following isolates: *C. albicans/dubliniensis*, *C. tropicalis*, *C. glabrata*, *C. krusei*, *C. parapsilosis*, *Candida lusitaniae*, and *C. kefyr*. The two most common species were *C. albicans/dubliniensis* and *C. glabrata*, with 30% and 28.8% of the isolates respectively (Table 1).

### *In silico* analysis of common and uncommon yeast species

As shown in Fig. 3 an *in silico* analysis of sequences from 21 yeast species revealed fifteen different restriction groups. Ten species show a unique RFLP pattern (*Candida blackwelliae*, *Candida buenavistaensis*, *C. glabrata*, *Candida maltosa*, *Candida oxycetoniae*,

*C. parapsilosis*, *C. tropicalis*, *C. krusei*, *Candida guilliermondii* and *C. kefyr*). The remaining 11 species shared a common restriction profile with a second or third individual as follows: Group 1 composed by *C. albicans* and *C. dubliniensis*; Group 10 (*Candida corydali*/*C. maltosa*/*Candida metapsilosis*); Group 9 (*Candida orthopsilosis*/*Candida pseudojufengensis*/*Candida viswanathii*); Group 5 (*C. lusitaniae* and *Candida pseudointermedia*), and Group 8 (*Candida pseudofoscolum* and *Candida auris*).

To demonstrate the reliability of the *in silico* analysis, more than one accession number for five species were included: *C. albicans* ( $n=3$  accession numbers), *C. glabrata* ( $n=2$ ), *C. tropicalis* ( $n=2$ ), *Clavispora lusitaniae* ( $n=2$ ), and *C. auris* ( $n=2$ ). PCR product sizes before and after restriction were similar between the same species, except for *Candida maltosa*, which showed a PCR product with two different sizes for the two analyzed accession numbers (Fig. 3). This could be due to an erroneous identification of this particular accession or to a real polymorphism within the species.

In order to evaluate the capacity of the PCR-RFLP method to identify the isolated species, amplification products that showed a unique size were further sequenced. As shown in Table 1, all sequenced isolates were correctly identified at the species level, demonstrating a perfect coincidence between both methods.

## Discussion

Presence of yeasts in urine may have different interpretations: (a) they could be contaminants with no etiological relevance in urinary tract infections<sup>14</sup>; (b) they could be the local consequence of a disseminated infection or candidemia,<sup>16</sup> or (c) they could be

**Table 1**

Yeast species identified through PCR-RFLP and sequencing. PCR product sizes, restriction profiles, and the most similar results from a BLAST search according to Max score, query cover and E-value, are shown.

| Yeast species                                              | n (%)      | PCR product in bp (ITS1-ITS4) | Restriction pattern in bp | Blast results (NCBI accession number) |
|------------------------------------------------------------|------------|-------------------------------|---------------------------|---------------------------------------|
| <i>Candida albicans</i>                                    | 24 (30%)   | 538                           | 299/239                   | <i>C. albicans</i> (KU729070)         |
| <i>Candida glabrata</i>                                    | 23 (28.8%) | 950                           | 630/320                   | <i>C. glabrata</i> (KU729050)         |
| <i>Clavispora lusitaniae</i> [ <i>Candida lusitaniae</i> ] | 11 (13.8%) | 348                           | 266/118                   | <i>C. lusitaniae</i> (KJ451657)       |
| <i>Candida tropicalis</i>                                  | 11 (13.8%) | 528                           | 342/183                   | <i>C. tropicalis</i> (KY102474)       |
| <i>Pichia kudriavzevii</i> ( <i>Candida krusei</i> )       | 5 (6.3%)   | 512                           | 263/249                   | <i>C. glabrata</i> (KU729050)         |
| <i>Candida parapsilosis</i>                                | 4 (5%)     | 520                           | 520                       | <i>C. parapsilosis</i> (KY102320)     |
| <i>Kluyveromyces marxianus</i> [ <i>Candida kefyr</i> ]    | 2 (2.5%)   | 721                           | 721                       | <i>C. kefyr</i> (CP015058)            |

| Yeast species                    | NCBI Acc. N° | PCR product (bp) | Restriction pattern (bp) | Restriction group |
|----------------------------------|--------------|------------------|--------------------------|-------------------|
| <i>Candida albicans</i>          | KY101917     | 534              | 299 / 237                | 1                 |
| <i>Candida albicans</i>          | KY101918     | 536              | 299 / 239                | 1                 |
| <i>Candida albicans</i>          | KY106276     | 539              | 299 / 242                | 1                 |
| <i>Candida glabrata</i>          | CR380958     | 879              | 563 / 318                | 2                 |
| <i>Candida glabrata</i>          | HG970737     | 881              | 563 / 320                | 2                 |
| <i>Candida parapsilosis</i>      | KY102320     | 520              | 520                      | 3                 |
| <i>Candida tropicalis</i>        | KX349477     | 526              | 342 / 186                | 4                 |
| <i>Candida tropicalis</i>        | KY102474     | 523              | 342 / 183                | 4                 |
| <i>Candida lusitaniae</i>        | KY102567     | 383              | 267 / 118                | 5                 |
| <i>Candida lusitaniae</i>        | KY102563     | 382              | 266 / 118                | 5                 |
| <i>Candida krusei</i>            | KX833114     | 510              | 263 / 249                | 6                 |
| <i>Candida kefyr</i>             | CP015058     | 607              | 372 / 157 / 82           | 7                 |
| <i>Candida dubliniensis</i>      | LT608368     | 540              | 299 / 243                | 1                 |
| <i>Candida pseudointermedia</i>  | KY102344     | 388              | 268 / 122                | 5                 |
| <i>Candida pseudofloscolorum</i> | KY102341     | 391              | 391                      | 8                 |
| <i>Candida auris</i>             | KT305977     | 398              | 398                      | 8                 |
| <i>Candida auris</i>             | KP131674     | 401              | 401                      | 8                 |
| <i>Candida orthopsilosis</i>     | KU665254     | 511              | 511                      | 9                 |
| <i>Candida pseudojifengensis</i> | KY102345     | 514              | 514                      | 9                 |
| <i>Candida viswanathii</i>       | NR_138345    | 512              | 512                      | 9                 |
| <i>Candida corydali</i>          | KY102035     | 528              | 528                      | 10                |
| <i>Candida maltosa</i>           | KY102187     | 527              | 527                      | 10                |
| <i>Candida metapsilosis</i>      | KY102203     | 531              | 531                      | 10                |
| <i>Candida blackwelliae</i>      | KY101962     | 502              | 294 / 167 / 45           | 11                |
| <i>Candida buenavistaensis</i>   | KY102007     | 491              | 491                      | 12                |
| <i>Candida maltosa</i>           | NR_138346    | 501              | 501                      | 13                |
| <i>Candida oxycetoniae</i>       | KY102281     | 546              | 546                      | 14                |
| <i>Candida guilliermondii</i>    | KY104257     | 721              | 721                      | 15                |

**Fig. 3.** *In silico* analysis showing the size of the ribosomal ITS1-ITS2 region from 21 yeast species and 28 NCBI accessions, and their restriction pattern after digestion with *MspI*.

actually the cause of a urinary infection, and in some cases even the focus of a systemic infection.<sup>8</sup> In this study, 72 urine cultures with more than  $10^3$  CFUs were included. Although the definition of candiduria remains unclear, several authors agree on yeast counts ranging from  $10^3$  to  $10^5$  CFUs/ml as clinically relevant.<sup>1,2,6,24</sup> For this reason 72 out of 73 isolated microorganisms from urine samples were considered the primary cause of genitourinary infections rather than being only colonizing the mucosa. A mixed candiduria was found in five cultures (6.84%). This result is in agreement with several authors that report around 5% of urinary infections caused by more than one yeast species.<sup>2,15</sup>

Polymorphisms in both length and sequence are the main reason why the nuclear ribosomal internal transcribed spacer (ITS) region has been currently accepted as the universal DNA barcode marker for fungi, including the Ascomycetes yeasts.<sup>22</sup> Herein, the 73 yeast colonies were putatively identified as seven different yeast species through a restriction analysis of the ITS ribosomal region. The two most common isolated species in this study were *C. albicans*/*dubliniensis* (30%) and *C. glabrata* (28.8%), followed by *C. lusitaniae* and *C. tropicalis* (13.8% each), and to a lesser extent *C. krusei*, *C. parapsilosis* and *C. kefyr*. All those seven species have been described as pathogens in urinary tract infections.<sup>20</sup> A comprehensive review sets forth 50–70% of all urine isolates are *C. albicans*, followed by *C. glabrata* and *C. tropicalis*.<sup>1</sup> A second review summarizes the most common *Candida* species in patients with candiduria from six different studies,<sup>4</sup> where three of them report *C. albicans* and *C. glabrata* as first and second etiological agents

respectively.<sup>10,15,24</sup> In contrast, *C. lusitaniae* and *C. kefyr* have been reported less frequently as cause of candiduria.<sup>26</sup>

The RFLP analysis using the *MspI* enzyme on seven yeast species showed expected DNA profiles from ribosomal ITS fragments when compared with the *in silico* analysis (Fig. 2). In addition, we included in the *in silico* analysis sequences of less common yeast species causing human infections, for example the “-psilosis group”,<sup>7</sup> *C. kefyr*, as well as *Candida* species commonly associated with plants or insects,<sup>9,21</sup> unlikely to cause human infections. *C. auris* sequences were also analyzed due to its importance as emerging yeast in invasive infections.<sup>27</sup>

Fifteen different restriction groups were obtained from the *in silico* approach, identifying 10 out of 21 species (Fig. 3). The remaining 11 species could not be completely identified through this approach. This apparent low specificity of the method could be considered a disadvantage for the correct identification of yeast species. However, this limit can be easily overcome when a second restriction enzyme is applied to differentiate between those species with a similar *MspI*-profile. For example, *C. albicans* and *C. dubliniensis* could be distinguished through an ulterior RFLP assay using the *BlnI* enzyme.<sup>18</sup> Enzymes commonly used in the laboratory can separate the species within the other 4 restriction groups described herein. *HindIII* or *HhaI* could separate the species in the restriction Group 10 (including *C. corydali*/*C. maltosa*/*C. metapsilosis*); *HaeIII* differentiated the species in Group 9 (*C. orthopsilosis*/*C. pseudojifengensis*/*C. viswanathii*); *HhaI* or *HinfI* separated the species in Group 5 (*C. lusitaniae* and *C. pseudointermedia*), and species in Group

8 (*C. pseudoflosclorum* and *C. auris*) are discriminated by AluI or TaqI. Confirmation through Sanger sequencing of seven isolates proved that a PCR-RFLP approach could be potentially useful for the identification of some of the most common yeast species causing candiduria, according to identifiable restriction profiles.

This method has also the advantage of being implementable in a hospital laboratory, and it is relatively inexpensive compared to commercial solutions based on phenotypic approaches, which are able to identify only the most common *Candida* species. Early identification of *Candida* species is also important for timely drug therapy and controlling the spread of virulent or resistant strains in a sanitary facility.<sup>17</sup> In addition, more informative molecular markers such as multilocus sequence typing have proven to be a powerful tool to find out whether a patient has a recurrence with the same strain or not,<sup>11</sup> or to demonstrate concordance between candidemia and candiduria,<sup>3</sup> which could not be evidenced by more conventional approaches.

In conclusion, as far as we know, this is the first study to describe *Candida* species isolated from patients with candiduria in Honduras through a molecular approach, where *C. albicans/dubliniensis* and *C. glabrata* were the two most common species.

### Conflict of interests

The authors declare no conflict of interests.

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### References

- Achkar JM, Fries BC. *Candida* infections of the genitourinary tract. Clin Microbiol Rev. 2010;23:253–73.
- Ayeni O, Riederer KM, Wilson F, Khatib R. Clinicians' reaction to positive urine culture for *Candida* organisms. Kliniker-Reaktionen auf *Candida*-positive Urinbefunde. Mycoses. 1999;42:285–9.
- Bougnoix ME, Kac G, Aegerter P, d'Enfert C, Fagon JY, CandiRea Study G. Candidemia and candiduria in critically ill patients admitted to intensive care units in France: incidence, molecular diversity, management and outcome. Intensive Care Med. 2008;34:292–9.
- Bukhary ZA. Candiduria: a review of clinical significance and management. Saudi J Kidney Dis Transpl. 2008;19:350–60.
- Furlaneto MC, Rota JF, Quesada RM, Furlaneto-Maia L, Rodrigues R, Oda S, et al. Species distribution and in vitro fluconazole susceptibility of clinical *Candida* isolates in a Brazilian tertiary-care hospital over a 3-year period. Rev Soc Bras Med Trop. 2011;44:595–9.
- Goldberg PK, Kozinn PJ, Wise GJ, Nouri N, Brooks RB. Incidence and significance of candiduria. JAMA. 1979;241:582–4.
- Gomez-Lopez A, Alastruey-Izquierdo A, Rodriguez D, Almirante B, Pahissa A, Rodriguez-Tudela JL, et al. Prevalence and susceptibility profile of *Candida metapsilosis* and *Candida orthopsilosis*: results from population-based surveillance of candidemia in Spain. Antimicrob Agents Chemother. 2008;52:1506–9.
- Greene MT, Chang R, Kuhn L, Rogers MA, Chenoweth CE, Shuman E, et al. Predictors of hospital-acquired urinary tract-related bloodstream infection. Infect Control Hosp Epidemiol. 2012;33:1001–7.
- Groenewald M, Robert V, Smith MT. Five novel *Wickerhamomyces*- and *Metschnikowia*-related yeast species, *Wickerhamomyces chaumierensis* sp. nov., *Candida pseudoflosclorum* sp. nov., *Candida danieliae* sp. nov., *Candida robertiae* sp. nov. and *Candida eppingiae* sp. nov., isolated from plants. Int J Syst Evol Microbiol. 2011;61:2015–22.
- Guler S, Ural O, Findik D, Arslan U. Risk factors for nosocomial candiduria. Saudi Med J. 2006;27:1706–10.
- Jain N, Kohli R, Cook E, Gialanella P, Chang T, Fries BC. Biofilm formation by and antifungal susceptibility of *Candida* isolates from urine. Appl Environ Microbiol. 2007;73:1697–703.
- Jamil S, Jamil N, Saad U, Hafiz S, Siddiqui S. Frequency of *Candida albicans* in patients with funguria. J Coll Physicians Surg Pak. 2016;26:113–6.
- Jang SJ, Han HL, Lee SH, Ryu SY, Chaulagani BP, Moon YL, et al. PFGE-based epidemiological study of an outbreak of *Candida tropicalis* candiduria: the importance of medical waste as a reservoir of nosocomial infection. Jpn J Infect Dis. 2005;58:263–7.
- Kauffman CA, Fisher JF, Sobel JD, Newman CA. *Candida* urinary tract infections—diagnosis. Clin Infect Dis. 2011;52:S452–6.
- Kauffman CA, Vazquez JA, Sobel JD, Gallis HA, McKinsey DS, Karchmer AW, et al. Prospective multicenter surveillance study of funguria in hospitalized patients. The National Institute for Allergy and Infectious Diseases (NIAID) Mycoses Study Group. Clin Infect Dis. 2000;30:14–8.
- Louria DB, Stiff DP, Bennett B. Disseminated moniliasis in the adult. Medicine. 1962;41:307–38.
- Mirhendi H, Makimura K, Khoramzadeh M, Yamaguchi H. A one-enzyme PCR-RFLP assay for identification of six medically important *Candida* species. Nihon Ishinkin Gakkai Zasshi. 2006;47:225–9.
- Mirhendi H, Makimura K, Zomorodian K, Maeda N, Ohshima T, Yamaguchi H. Differentiation of *Candida albicans* and *Candida dubliniensis* using a single-enzyme PCR-RFLP method. Jpn J Infect Dis. 2005;58:235–7.
- Occhipinti DJ, Gubbins PO, Schreckenberger P, Danziger LH. Frequency, pathogenicity and microbiologic outcome of non-*Candida albicans* candiduria. Eur J Clin Microbiol Infect Dis. 1994;13:459–67.
- Papon N, Courdavault V, Clastre M, Bennett RJ. Emerging and emerged pathogenic *Candida* species: beyond the *Candida albicans* paradigm. PLoS Pathog. 2013;9:e1003550.
- Ren YC, Xu LL, Zhang L, Hui FL. *Candida baotianmanensis* sp. nov. and *Candida pseudoviswanathii* sp. nov., two ascosporic yeast species isolated from the gut of beetles. Int J Syst Evol Microbiol. 2015;65:3580–5.
- Schoch CL, Seifert KA, Huhndorf S, Robert V, Spouge JL, Levesque CA, et al. Nuclear ribosomal internal transcribed spacer (ITS) region as a universal DNA barcode marker for Fungi. Proc Natl Acad Sci U S A. 2012;109:6241–6.
- Sobel JD, Fisher JF, Kauffman CA, Newman CA. *Candida* urinary tract infections—epidemiology. Clin Infect Dis. 2011;52 Suppl. 6:S433–6.
- Sobel JD, Kauffman CA, McKinsey D, Zervos M, Vazquez JA, Karchmer AW, et al. 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. 2000;30:19–24.
- Toka Ozer T, Durmaz S, Yula E. Antifungal susceptibilities of *Candida* species isolated from urine culture. J Infect Chemother. 2016;22:629–32.
- Tsuboi M, Uno S, Hase R, Yano Y, Sando E, Otsuka Y, et al. Acute pyelonephritis and candidemia due to *Candida lusitanae*: a case report. Kansenshogaku Zasshi. 2016;90:134–7.
- Vallabhaneni S, Kallen A, Tsay S, Chow N, Welsh R, Kerins J, et al. Investigation of the first seven reported cases of *Candida auris*, a globally emerging invasive, multidrug-resistant fungus—United States, May 2013–August 2016. Am J Transplant. 2017;17:296–9.
- Vijayakumar R, Giri S, Kindo AJ. Molecular species identification of from blood samples of intensive care unit patients by polymerase chain reaction – restricted fragment length polymorphism. J Lab Physicians. 2012;4:1–4.
- Zehm S, Schweinitz S, Wurznier R, Colvin HP, Rieder J. Detection of *Candida albicans* by mass spectrometric fingerprinting. Curr Microbiol. 2012;64:271–5.