

# Epidemiology of yeast species causing bloodstream infection in Tehran, Iran (2015–2017); superiority of 21-plex PCR over the Vitek 2 system for yeast identification

Mohammad Kord<sup>1</sup>, Mohammadreza Salehi<sup>2</sup>, Sadegh Khodavaisy<sup>1,\*</sup>, Seyed Jamal Hashemi<sup>1</sup>, Roshanak Daie Ghazvini<sup>1</sup>, Sassan Rezaei<sup>1</sup>, Ayda Maleki<sup>1</sup>, Ahmad Elmimoghaddam<sup>1</sup>, Neda Alijani<sup>3</sup>, Alireza Abdollahi<sup>4</sup>, Mahsa Doomanlou<sup>4</sup>, Kazem Ahmadikia<sup>1</sup>, Niloofar Rashidi<sup>1</sup>, Weihua Pan<sup>5,\*</sup>, Teun Boekhout<sup>6,7</sup> and Amir Arastehfar<sup>6,7</sup>

## Abstract

**Introduction.** Given the limited number of candidaemia studies in Iran, the profile of yeast species causing bloodstream infections (BSIs), especially in adults, remains limited. Although biochemical assays are widely used in developing countries, they produce erroneous results, especially for rare yeast species.

**Aim.** We aimed to assess the profile of yeast species causing BSIs and to compare the accuracy of the Vitek 2 system and 21-plex PCR.

**Methodology.** Yeast blood isolates were retrospectively collected from patients recruited from two tertiary care training hospitals in Tehran from 2015 to 2017. Relevant clinical data were mined. Identification was performed by automated Vitek 2, 21-plex PCR and sequencing of the internal transcribed spacer region (ITS1–5.8S–ITS2).

**Results.** In total, 137 yeast isolates were recovered from 107 patients. The overall all-cause 30-day mortality rate was 47.7%. Fluconazole was the most widely used systemic antifungal. *Candida albicans* (58/137, 42.3%), *Candida glabrata* (30/137, 21.9%), *Candida parapsilosis* sensu stricto (23/137, 16.8%), *Candida tropicalis* (10/137, 7.3%) and *Pichia kudriavzevii* (*Candida krusei*) (4/137, 2.9%) constituted almost 90% of the isolates and 10% of the species detected were rare yeast species (12/137; 8.7%). The 21-plex PCR method correctly identified 97.1% of the isolates, a higher percentage than the Vitek 2 showed (87.6%).

**Conclusion.** *C. albicans* was the main cause of yeast-derived fungaemia in this study. Future prospective studies are warranted to closely monitor the epidemiological landscape of yeast species causing BSIs in Iran. The superiority of 21-plex PCR over automated Vitek 2 indicates its potential clinical utility as an alternative identification tool use in developing countries.

## INTRODUCTION

Fungal bloodstream infections (BSIs) are a major cause of morbidity and mortality in critically ill patients [1, 2]. *Candida* species represent the fourth most common cause of BSIs in hospitalized patients in the USA [3]. It has been estimated that candidaemia is currently associated with a significant economic burden of USD \$1.4 billion in the USA annually

[4]. Depending on the yeast species, the mortality rate may vary from 30–85% [5]. Moreover, the increasing frequency of non-*albicans* *Candida* (NAC) species is not only associated with a higher mortality rate, but also a higher clinical rate of failure due to either acquired and/or intrinsic resistance to the limited number of antifungal drugs available to treat candidaemia [5, 6]. Since the antifungal susceptibility pattern

Received 21 January 2020; Accepted 24 March 2020; Published 05 May 2020

**Author affiliations:** <sup>1</sup>Department of Medical Parasitology and Mycology, School of Public Health, Tehran University of Medical Sciences, Tehran, Iran; <sup>2</sup>Department of Infectious Diseases and Tropical Medicine, Imam Khomeini Hospital Complex, Tehran University of Medical Sciences, Tehran, Iran; <sup>3</sup>Department of Infectious Diseases, Shariati Hospital, Tehran University of Medical Sciences, Tehran, Iran; <sup>4</sup>Department of Pathology, Imam Khomeini Hospital Complex, Tehran University of Medical Sciences, Tehran, Iran; <sup>5</sup>Medical Mycology, Shanghai Changzheng Hospital, Second Military Medical University, Shanghai 200003, PR China; <sup>6</sup>Westerdijk Fungal Biodiversity Institute, Utrecht, The Netherlands; <sup>7</sup>Institute of Biodiversity and Ecosystem Dynamics (IBED), University of Amsterdam, Amsterdam, The Netherlands.

\*Correspondence: Sadegh Khodavaisy, sadegh\_7392008@yahoo.com; Weihua Pan, panweihua@smmu.edu.cn

**Keywords:** bloodstream infections; candidaemia; 21-plex PCR; Vitek 2.

**Abbreviations:** AMB, Amphotericin B; BSIs, Bloodstream infections; ICUs, Intensive care units; IDSA, Infectious Diseases Society of America; MALDI-TOF MS, Matrix-assisted Laser desorption/ionization time-of-flight mass spectrometry; NAC, Non-*albicans* *Candida*; SDA, Sabouraud dextrose agar.

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varies depending on the yeast species, accurate species-level identification of yeasts causing BSIs is of high importance and may decrease the mortality rate of infected patients by guiding clinicians to administer proper antifungal treatment [5, 7].

Although sequencing of rDNA and other barcoding loci is considered to be the gold standard technique for the identification of yeast species, this technique is expensive and is not widely utilized in developing countries [8]. In contrast, time-consuming and/or less accurate phenotypic and biochemical assays, which can lead to the neglect of yeast species of high clinical importance, are widely used in developing countries [8–11]. For instance, a lack of appropriate identification tools resulted in an underestimation of *Candida auris* in developing countries [12]. In addition to manual biochemical assays, such as API 20C AUX, automated enzymatic/biochemical methods (e.g. VITEK Yeast Biochemical Card) are more comprehensive and sensitive and require a shorter turnaround time [13–15]. A recent study involving Asian countries showed the low popularity of PCR for the detection of fungal species directly from clinical samples [16], but this PCR with DNA samples obtained from pure cultures can be used to reliably identify a broad range of yeast species [8, 9, 17]. Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) is not available in Iran; therefore, most investigators identify causative agents of candidaemia using either phenotypic techniques [18–21] or PCR-RFLP assays [18, 22]. Recently, Arastehfar *et al.* developed a 21-plex PCR assay that can identify the causative agents of 95% of yeast-associated infections based on PCR product size in a stepwise manner [9]. Due to the limited number of systematic studies exploring the profile of yeast species causing BSIs in Iranian patients, we conducted the current study. Moreover, the accuracy of automated Vitek 2, one of the most popular automated biochemical assays in developing countries, was compared with that of 21-plex PCR.

## METHODS

### Yeast isolates and growth conditions

From 2015 to 2017, all patients admitted to two tertiary care training hospitals (Imam Khomeini Hospital complex and Shariati Hospital) affiliated with Tehran University of Medical Sciences, Tehran, Iran, were included in this study. We did not consider any exclusion criteria and yeast BSI was defined as occurring when a *Candida*/yeast species was isolated from at least one blood sample of a given patient. The mortality rate reported is all-cause and the 30-day mortality was defined as occurring when a yeast species was isolated from the first blood sample 30 days prior to death. Patients within the age categories of  $\leq 16$  years of age and  $> 16$  years of age were considered to be children and adults, respectively. Aerobic blood bottles were incubated in Bactec 9120 (Becton Dickinson, Spark, MD, USA) for 24 h. Blood bottles without positive signal after 1 week's incubation in Bactec device were considered negative. One hundred microlitres of positive blood bottles was streaked onto both Sabouraud's dextrose agar (SDA) and *Candida* CHROMagar (CHROMagar,

Paris, France) and incubated at 37°C for 24–48 h. Colony morphology was assessed to differentiate blood samples concurrently infected with more than one yeast species. All yeast isolates were identified retrospectively.

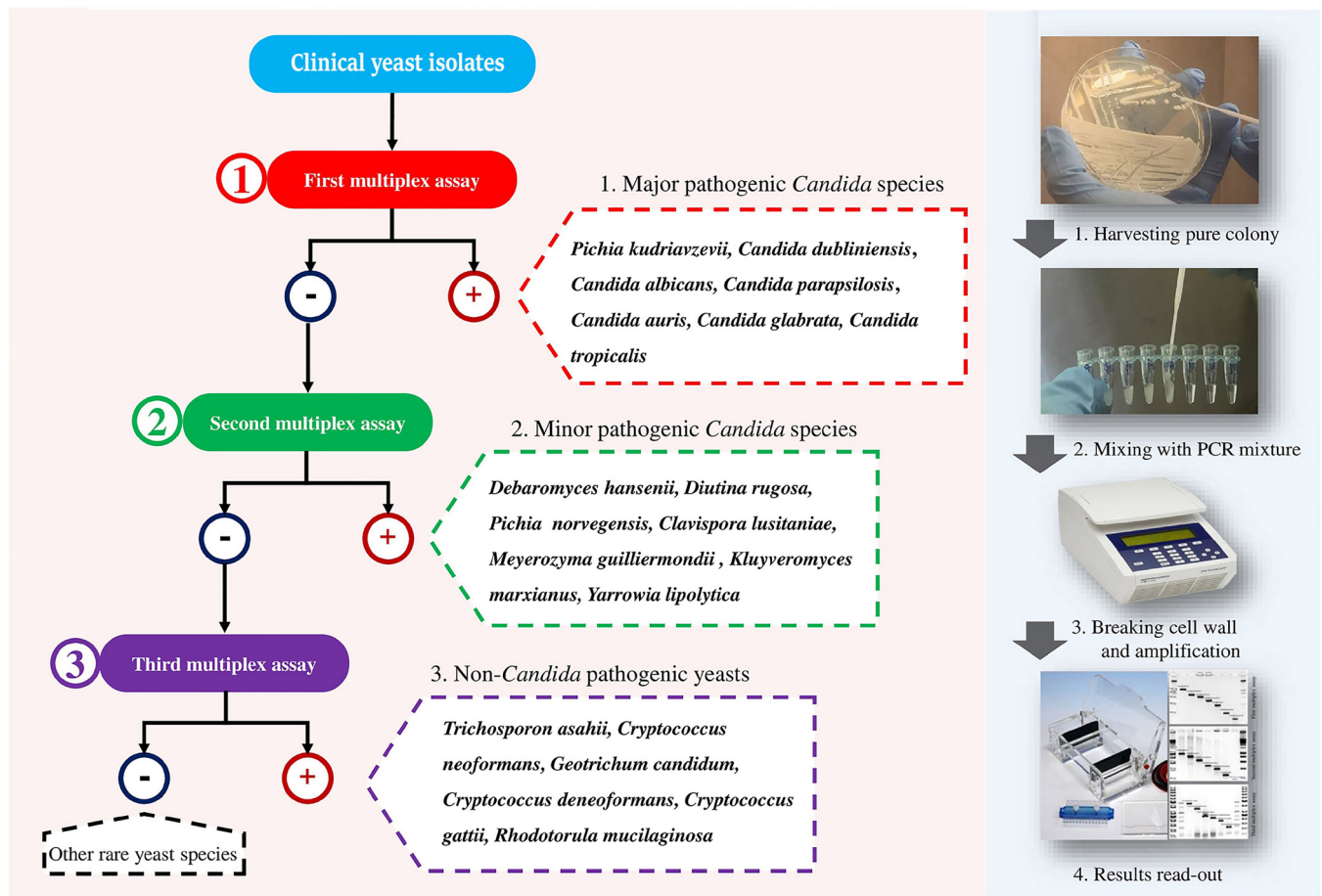
This study was approved by the local ethical committees of Tehran University of Medical Science (IR.TUMS.SPH.REC.1398.324). Collection and presentation of the patients' general clinical data were waived by the local institutional review board and patient identities were anonymized through the use of code identifiers. Moreover, clinical data were presented in clusters and as a whole to further protect the patients' privacy.

### Vitek 2 system identification

The automated Vitek 2 YST ID Card system (bioMérieux, Marcy-L'Etoile, France, database v.3.01) can identify 51 yeast species. The YST identification card is based on established biochemical methods, including newly developed substrates. According to the manufacturer's instructions, cell suspensions were adjusted to a turbidity of 1.80–2.2 McFarland and all cards were incubated at  $35.5 \pm 1.0^\circ\text{C}$  for 18 h [23]. The final profile results were compared with profiles from a database, and the identification of yeasts was based on the qualitative values of 'excellent', 'very good', 'good', 'acceptable', or 'low discrimination'. Vitek 2 identifications were performed in the Imam Khomeini Hospital Complex, Tehran, Iran.

### DNA extraction and 21-plex PCR technique

Yeast isolates were incubated for 48 h on SDA medium containing chloramphenicol and subjected to a DNA extraction method as described previously [24]. Briefly, one full loop of yeast colonies (10  $\mu\text{l}$ ) was transferred to 2 ml screw-cap tubes containing 100  $\mu\text{l}$  glass beads, 300  $\mu\text{l}$  of lysis buffer (200 mM Tris-HCl pH 8, 25 mM EDTA, 250 mM NaCl, 0.5% sodium dodecyl sulfate) and 300  $\mu\text{l}$  of phenol chloroform/isoamyl alcohol. The suspensions were vortexed for 2 min and then centrifuged for 5 min at 10000 r.p.m. Subsequently, the supernatant was transferred to a new 1.5 ml tube containing an equal volume of isopropanol, 300  $\mu\text{l}$  of chloroform and 0.1 vol of 3 M sodium acetate (pH 5.2). In the next step, the solutions were vortexed briefly and incubated at  $-20^\circ\text{C}$  for 10 min, followed by centrifugation for 15 min at 12000 r.p.m. The precipitant was washed with ice-cold 70% ethanol and centrifuged (15 min at 12000 r.p.m), the supernatant was removed and the pellet was air-dried and dissolved in 50  $\mu\text{l}$  of distilled water. Identification of yeasts with 21-plex PCR was performed in three multiplex PCRs as described previously [9]. Briefly, the first PCR reaction identifies the most prevalent *Candida* species [*C. albicans*, *C. glabrata*, *Pichia kudriavzevii* (*C. krusei*), *C. parapsilosis*, *C. tropicalis*, *C. dubliniensis* and *C. auris*], the second PCR identifies rare *Candida* species [*Diutina rugosa* (*C. rugosa*), *Clavispora lusitaniae* (*C. lusitaniae*), *Pichia norvegensis* (*C. norvegensis*), *Debaryomyces hansenii* (*C. famata*), *Yarrowia lipolytica* (*C. lipolytica*), *Meyerozyma guilliermondii* (*C. guilliermondii*) and *Kluyveromyces marxianus* (*C. kefir*)] and the third multiplex PCR identifies the most clinically important basidiomycetous



**Fig. 1.** Identification of yeast species using 21-plex PCR uses a stepwise strategy and species differentiation was based on PCR product size. Adapted with permission from [9].

yeast species, namely *Trichosporon* spp., *Cryptococcus* spp. and *Rhodotorula mucilaginosa* and one ascomycota yeast, *Geotrichum* spp. (Fig. 1). All the primers included in the 21-plex PCR, including those for *C. auris*, were comprehensively evaluated using blinded test sets in our previous studies [9, 17]. PCR products and a 100 bp ladder were run on a 2% agarose gel (100 V, 60 min), stained with Gel Red (BioTium Corporation, USA) and visualized under UV light. Yeast species identification was achieved by discrimination of the fragment size of the PCR products (Fig. 2) [9].

### ITS sequencing

The internal transcribed spacer region (ITS1-5.8S-ITS2) was selected for sequencing according to a protocol described by Leaw *et al.* [25]. Bidirectional chain terminated Sanger sequencing using referenced primers was performed. The obtained sequences were searched using the NBLAST algorithm (<https://blast.ncbi.nlm.nih.gov>) and the identity of each strain was assigned accordingly. This experiment was carried out at the Department of Dermatology, Shanghai Changzheng Hospital, Second Military Medical University, Shanghai, PR China.

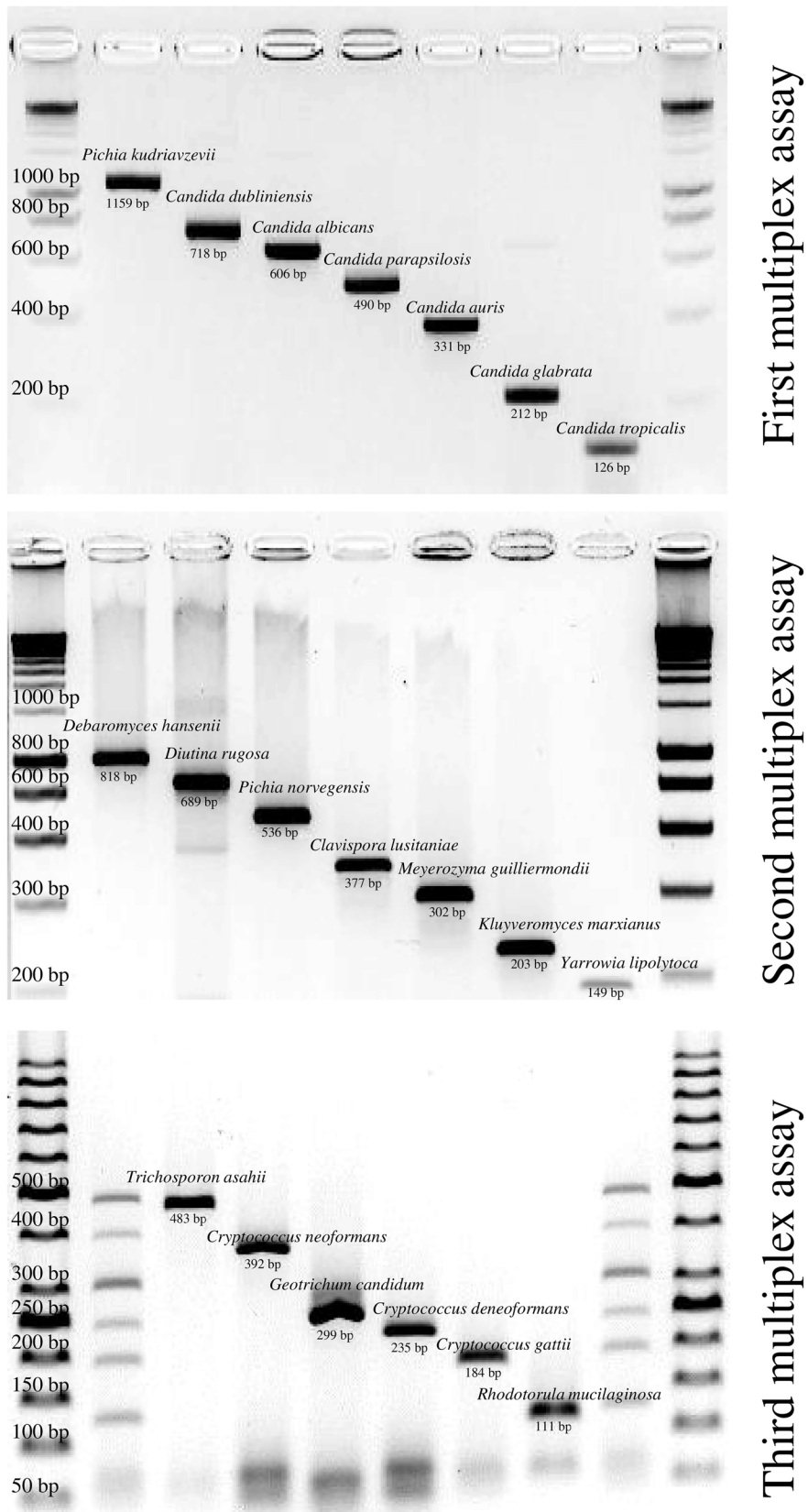
### Statistical analysis

We used SPSS software v24 (SPSS, Inc. Chicago, IL, USA) and the Kappa test; we assessed the categorical agreement between the results obtained by the Vitek 2 system, 21-plex PCR and ITS sequencing.

## RESULTS

### Clinical profiles

One hundred and thirty-seven episodes of yeast BSIs were diagnosed in 107 patients with ages ranging from 1 to 100 years old, with 92.5% of the patients ( $n=99$ ) being  $\geq 16$  years of age. The majority of patients had a single episode of candidaemia (77/107; 72.0%), while 2 isolates were recovered from 30 patients (30/107; 28.0%). There was no difference in the acquisition of candidaemia from females (55/107; 51.4%) and males (52/107; 48.6%). Intensive care units (ICUs) contained the highest number of yeast BSI patients (55/107; 51.4%). Fever (70/107; 65.5%) and dyspnea (54/107; 50.5%) were recorded as the most observed clinical presentations. Pulmonary disease (33/107;



**Fig. 2.** 21-plex PCR identifies the species based on the PCR product size. The PCR product for each species has a distinct length. Adapted with permission from [9].

**Table 1.** Demographic and clinical characteristics and outcomes for patients with yeast BSIs in this study

Variables	<i>Candida albicans</i> n (%)	<i>Candida glabrata</i> n (%)	<i>Candida parapsilosis</i> sensu stricto n (%)	<i>Candida tropicalis</i> n (%)	<i>Clavispora lusitanae</i> n (%)	<i>Meyeroczyma guilliermondii</i> n (%)	<i>Kluyveromyces marxianus</i> n (%)	<i>Pichia kudriavzevii</i> n (%)	<i>Candida dubliniensis</i> n (%)	<i>Pichia norvegensis</i> n (%)	<i>Cyberlindnera fabianii</i> n (%)	<i>Cryptococcus neoformans</i> n (%)	<i>Trichosporon asteroides</i> n (%)	Total n (%)
Age groups														
≤16 years	3 (6.3)	1 (4.5)	4 (25.0)	0	0	0	0	0	0	0	0	0	0	8/107 (7.5)
>16 years	45 (93.7)	21 (95.5)	12 (75.0)	9 (100)	3 (100)	1 (100)	1 (100)	2 (100)	1 (100)	1 (100)	1 (100)	1 (100)	1 (100)	99/107 (92.5)
Total (%)	48 (44.7)	22 (20.6)	16 (15.0)	9 (8.4)	3 (2.8)	1 (0.9)	1 (0.9)	2 (1.9)	1 (0.9)	1 (0.0)	1 (0.9)	1 (0.9)	1 (0.9)	107 (100)
Outcome														
Alive	25 (52.1)	11 (50)	10 (62.5)	4 (44.4)	1 (33.3)	1 (100)	1 (100)	1 (50)	0	0	1 (100)	0	1 (100)	56/107 (52.3)
Died	23 (47.9)	11 (50)	6 (37.5)	5 (55.6)	2 (66.7)	0	0	1 (50)	1 (100)	1 (100)	0	1 (100)	0	51/107 (47.7)
Medication														
Recipient broad-spectrum antibiotics	43 (89.5)	22 (100)	14 (87.5)	7 (77.7)	3 (100)	1 (100)	1 (100)	1 (50)	1 (100)	1 (100)	1 (100)	1 (100)	1 (100)	97/107 (90.7)
fluconazole	14 (29.1)	7 (31.8)	7 (43.7)	1 (11.1)	0	1 (100)	1 (100)	0	0	0	0	0	0	31/107 (29.0)
caspofungin	9 (18.7)	7 (31.8)	5 (31.2)	2 (22.2)	1 (33.3)	0	0	1 (50)	1 (100)	0	1 (100)	0	1 (100)	28/107 (26.2)
amphotericin B	8 (16.6)	4 (18.2)	7 (43.7)	1 (11.1)	0	0	0	0	0	0	0	1 (100)	1 (100)	22/107 (20.6)
voriconazole	2 (4.1)	1 (4.5)	3 (18.7)	0	0	0	0	0	0	0	0	0	0	6/107 (5.6)
corticosteroids	10 (20.8)	7 (31.8)	3 (18.7)	2 (22.2)	0	1 (100)	0	0	0	0	0	0	0	23/107 (21.5)

Note that duplicate yeast isolates were recovered from 30 patients.

**Table 2.** Comparison of 21-plex PCR with Vitek 2 ID-YST system for identification of 137 yeasts isolated from blood samples

Species identified by ITS sequencing (n)	21-plex PCR			Vitek 2 system			
	Correctly identified	Misidentified	Not identified	Correctly identified	Identified with low discrimination	Misidentified	Not identified
<i>Candida albicans</i> (58)	58	0	0	53	2	2	1
<i>Candida glabrata</i> (30)	30	0	0	25	2	2	1
<i>Candida parapsilosis</i> (21)	21	0	0	18	1	2	0
<i>Candida tropicalis</i> (10)	10	0	0	8	1	1	0
<i>Pichia kudriavzevii</i> (4)	4	0	0	3	0	1	0
<i>Candida dubliniensis</i> (2)	2	0	0	0	1	1	0
<i>Clavispora lusitanae</i> (3)	3	0	0	0	2	1	0
<i>Meyerozyma guilliermondii</i> (1)	1	0	0	0	1	0	0
<i>Pichia norvegensis</i> (1)	1	0	0	0	0	1	0
<i>Kluyveromyces marxianus</i> (2)	2	0	0	1	1	0	0
<i>Cryptococcus neoformans</i> (1)	1	0	0	0	0	0	1
<i>Trichosporon asteroides</i> (1)	0	1	0	0	1	0	0
<i>Candida orthopsilosis</i> (2)	0	2	0	0	0	2	0
<i>Cyberlindnera fabianii</i> (1)	0	0	1	0	0	1	0
<b>Total (137)</b>	<b>133</b>	<b>3</b>	<b>1</b>	<b>108</b>	<b>12</b>	<b>14</b>	<b>3</b>

30.8%), solid tumours (32/107; 29.9%) and diabetes (31/107; 28.9%) were the principal underlying conditions detected in the patients. Broad-spectrum antibiotics and corticosteroids were used in 91% (97/107) and 21.5% (23/107) of the patients, respectively. Fluconazole (31/107; 29%) was the main antifungal prescribed to patients, followed by caspofungin (28/107; 26.2%), amphotericin B (AMB) (22/107, 20%) and voriconazole (6/107; 5.6%), whereas the rest of the patients were not treated with any antifungal drug (Table 1). Among the patients treated with systemic antifungals, 46.7% (50/107) received a single antifungal and 34.6% (37/107) received more than one antifungal. The overall 30-day mortality was 47.7% (51/107) and no difference in mortality rate was observed for patients infected with *C. albicans* (23/48; 47.9%) and NAC yeast species as a whole (28/59; 47.5%). Patients infected with *C. tropicalis* (5/9; 55.6%) and *C. glabrata* (11/22; 50%) showed the highest mortality rate (Table 1).

### Epidemiology and distribution of yeast species causing BSIs

All yeast species isolated from the patients in this study were identified by ITS sequencing. *C. albicans* (58/137; 42.3%) was the most prevalent yeast species, and NAC species caused 56.2% of the yeast episodes (79/137) (Table 1). Among the main NAC species, *C. glabrata* (30/137; 21.9%) was the predominant species, followed by *C. parapsilosis* sensu stricto (23/137; 16.8%), *C. tropicalis* (10/137; 7.3%) and *P. kudriavzevii* (4/137; 2.9%) (Table 2). Almost 7.3% of

the species detected (10/137) were rare *Candida* and 1.5% basidiomycetous yeasts (2/137), i.e. *Trichosporon* spp. and *Cryptococcus* spp. (Table 2). *C. parapsilosis* (4/8; 50%) was the most common species isolated from the bloodstream of children, followed by *C. albicans* (3/8; 37.5%), and *C. glabrata* (1/8; 12.5%) (Table 1). However, *C. albicans* (45/107; 42.1%) and *C. glabrata* (21/107; 19.6%) were the leading agents isolated from the bloodstream of adults (Table 1). In total, single and duplicate yeast isolates were recovered from 77 patients (77/107; 72%) and 30 patients (30/107; 28%), respectively. *C. albicans* (10/30; 33.3%), *C. glabrata* (8/30; 26.6%) and *C. parapsilosis* (7/30; 23.3%) had the highest number of duplicate isolates, followed by *P. kudriavzevii* (2/30; 6.6%) and *C. tropicalis*, *C. dubliniensis* and *K. marxianus* (1/30; 1.3% each).

### Comparative analysis of rDNA sequencing and 21-plex PCR

One hundred and thirty-three yeast isolates (97.1%) were correctly identified by 21-plex PCR when compared to ITS sequencing (Table 2). All main *Candida* species, including *C. albicans*, *C. glabrata*, *C. parapsilosis*, *C. tropicalis* and *P. kudriavzevii*, were correctly identified by 21-plex PCR. Regarding the rare yeast species, *C. orthopsilosis* ( $n=2$ ) and *T. asteroides* ( $n=1$ ) were identified as *C. parapsilosis* and *T. asahii*, respectively, while *Cyberlindnera fabianii* ( $n=1$ ) was not identified (Table 2). The categorical agreement for 21-plex PCR and ITS sequencing was 0.96.

**Table 3.** Misidentified isolates using 21-plex PCR and the Vitek 2 system compared to ITS sequencing

Species	Misidentified as	
	21-plex PCR (n; %)	VITEK 2 system (n; %)
<i>Candida albicans</i>	–	<i>Candida parapsilosis</i> (1/58; 1.7) <i>Candida ciferrii</i> (1/58; 1.7)
<i>Candida glabrata</i>	–	<i>Candida parapsilosis</i> (1/30; 3.3) <i>Debaryomyces hansenii</i> (1/30; 3.3)
<i>Candida parapsilosis</i>	–	<i>Cryptococcus laurentii</i> (1/21; 4.7) <i>Debaryomyces hansenii</i> (1/21; 4.7)
<i>Candida tropicalis</i>	–	<i>Candida ciferrii</i> (1/10; 10)
<i>Pichia kudriavzevii</i>	–	<i>Yarrowia lipolytica</i> (1/4; 25)
<i>Candida orthopsilosis</i>	<i>Candida parapsilosis</i> (2/2; 100)	<i>Candida parapsilosis</i> (2/2; 100)
<i>Trichosporon asteroides</i>	<i>Trichosporon asahii</i> (1/1; 100)	–
<i>Pichia norvegensis</i>	–	<i>Debaryomyces hansenii</i> (1/1; 100)
<i>Clavispora lusitaniae</i>	–	<i>Candida ciferrii</i> (1/3; 33.3)
<i>Cyberlindnera fabianii</i>	–	<i>Cryptococcus laurentii</i> (1/1; 100)
<i>Candida dubliniensis</i>	–	<i>Candida albicans</i> (1/2; 50)

### Comparative analysis of rDNA sequencing and the VITEK 2 system

Using the Vitek 2 system, 120 isolates (87.6%) were correctly identified, including 12 isolates that were identified with low scores (low discrimination). Almost 92% of the common *Candida* species were correctly identified and the misidentified cases all involved *Candida* species (*C. albicans*, *C. parapsilosis* and *C. glabrata*, two isolates each and *C. tropicalis* and *P. kudriavzevii* one isolate each) (Table 2). Among the rare *Candida* species isolated, six isolates (50%) were misidentified (Tables 2 and 3). For the basidiomycetous yeasts, only *Cr. neoformans* was not identified, while *T. asteroides* was correctly identified (Tables 2 and 3). The categorical agreement between the Vitek 2 system (using database version 3.0.1) and ITS sequencing was 0.85.

### DISCUSSION

In this study we compared the performance of Vitek 2 and 21-plex PCR for yeast species identification in an epidemiological study of yeast BSIs in Iran. In our epidemiological

study, *C. albicans* was the most prevalent *Candida* species isolated; this result is consistent with the findings of a previous studies conducted in paediatric patients in Tehran [7, 26]. However, we found that *C. glabrata* was the second most prevalent *Candida* species, followed by *C. parapsilosis*, while Mirhendi *et al.* found that *C. parapsilosis* was the second most prevalent cause of candidaemia [26]. This is because they studied neonates and children, who are more prone to developing candidaemia due to *C. parapsilosis* [27]. In a systematic review, *C. parapsilosis* (30.8%) was the leading agent of candidaemia in Iran [28]. Although we found *C. parapsilosis* to be the leading cause of yeast-derived BSIs among children, the population size of children was small (only eight patients), and further studies are warranted to confirm this finding.

In general, the epidemiological profile observed in our study is closer to that of some Scandinavian countries [29–31], the USA [32] and Australia [33], where *C. albicans* and *C. glabrata* are the two leading causes of candidaemia. However, our epidemiological findings are different from those of India (where *C. tropicalis* is the leading cause) [34], Kuwait [35] and Southeast Asian [36] and Latin American countries [37], where *C. albicans* and *C. parapsilosis/C. tropicalis* are the two leading causes of candidaemia.

Although the Infectious Diseases Society of America (IDSA) recommends treating adult candidaemic patients with echinocandins [38], the high costs associated with these drugs discourage their use in the clinical settings of developing countries [34, 36, 39]. Therefore, most of our patients were treated with azoles (fluconazole and voriconazole) followed by caspofungin, which, along with the fact that antifungals were not used in almost 45% of our patients, may explain the all-cause 30-day mortality rate of 47.7% observed in our study. The overall mortality rate in our study is relatively similar to those observed in China (36.6%) [36], Spain (30.6%) [40] and France (41.5–56.9%) [41], but much lower than the high mortality rate of 72.2% reported in Brazil [42]. Although our isolate numbers are small, infection with *C. tropicalis* was associated with the highest mortality rate (55.6%) compared to other species, and this has been noted in other studies [43, 44]. The rise of azole-resistant *C. tropicalis* blood isolates in some studies is worrisome [34, 45, 46]. Unfortunately, we did not perform antifungal susceptibility testing, which is a major limitation of our study.

The Vitek 2 system could not identify 75% of emerging yeast species, including *C. lusitaniae*, *K. marxianus* and *P. norvegensis*, but these species were all correctly identified by 21-plex PCR. These species show variability in antifungal resistance and/or cause outbreaks in intensive care units, underscoring the importance of accurate species-level identification. For instance, it has been shown that *C. lusitaniae* can rapidly acquire multidrug resistance traits during the course of antifungal treatment with fluconazole, AMB and caspofungin [47]. *K. marxianus* can cause outbreaks, especially in leukaemic and hematological patients, with some isolates being resistant to AMB [48, 49]. *P. norvegensis*, a species close to *P. inconspicua*, is associated with azole resistance

[50]. The 21-plex PCR showed a higher degree of accuracy for the yeast species included in this study compared with the Vitek 2 system. A previous study found a higher degree of accuracy when 21-plex PCR, as the first line identification tool, was used in combination with API 20C AUX for isolates that were not identified by 21-plex PCR [17]. 21-plex PCR showed superiority over the Vitek 2 system and the vast majority of rare yeasts and even a few isolates of the main *Candida* species were not correctly identified by the Vitek 2 system. Therefore, for laboratories lacking automated Vitek 2, MALDI-TOF, or biochemical identification assays, 21-plex PCR may be a useful technique for the identification of yeasts isolated from clinical samples. In addition, a previous Iranian study assessing candidaemia in paediatric patients showed that 95.7% of the species identified were in the target list of the 21-plex PCR [26]. Hence, 21-plex PCR can be used as a useful standalone technique in the routine laboratories of developing countries lacking specific and accurate identification tools, i.e. MALDI-TOF MS and Sanger sequencing, or expensive and time-consuming biochemical and phenotypic assays.

In this study, we showed the superiority of 21-plex PCR over the Vitek 2 system for the identification of yeast isolates. The application of 21-plex PCR assays for the identification of common and rare yeasts can reduce turnaround times and costs if applied in developing countries. The lack of detailed clinical data and antifungal susceptibility profiles are limitations of this study. Moreover, the database of our Vitek 2 system was old (v.3) and using newer versions may have resulted in a lower misidentification rate.

#### Funding information

This study was part of a PhD. dissertation (grant No. 9511353003) and M.S. dissertation (grant No. 9711352002) supported by Tehran University of Medical Sciences. This study has been funded and supported by Tehran University of Medical Sciences (TUMS); Grant no. 99-1-99-46391; the National Health Department of China (grant no. 2018Z×10101003); the National Natural Science Foundation of China (grant no. 31770161); and Shanghai Science and Technology Committee (grant nos 14DZ2272900 and 14495800500).

#### Acknowledgements

The authors would like to thank Ferry Hagen, Mohammad Reza Safari, and Saeideh Mahfozi for their assistance during the study.

#### Author contributions

A. A., S. K., W. P., F. H. and T. B. designed the study. A. M., M. F., A. A. and M.D., collected the yeast isolates. A. M., M. K., M. R. S. and N.A. collected the clinical data. M. K., A. E., K. A., S. K. and M. D. performed the PCR and Vitek experiments. W. P. performed ITS rDNA sequencing. A. A., S. K., M. K., S. R., R. D. G. and S. J. H. performed the data analysis. A. A., M. K., S. K. and W. P. prepared the first draft. All authors contributed to the revision.

#### Conflicts of interest

The authors declare that there are no conflicts of interest.

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