Species level identification and antifungal susceptibility of yeasts isolated from various clinical specimens and evaluation of Integral System Yeasts *Plus*

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SUMMARY

It is essential to use easy, standard, cost-effective and accurate methods for identification and susceptibility testing of yeasts in routine practice. This study aimed to establish the species distribution and antifungal susceptibility of yeast isolates and also to evaluate the performance of the colorimetric and commercially available Integral System Yeasts Plus (ISYP). Yeast isolates (n=116) were identified by conventional methods and ISYP. Antifungal susceptibility testing was performed by the microdilution method according to the standards of CLSI M27-A3 and ISYP. *Candida albicans* (50%) was the most common species isolated, followed by *C. parapsilosis* (25%) (mostly in blood samples). According to the CLSI M27-S3 criteria, resistance rates for amphotericin B, flucytosine, fluconazole, itraconazole, and voriconazole were 0%, 0%, 4.6%, 4.5% and 1.8%, respectively. Resistance for miconazole (MIC >1 mg/L) was found as 17.9%. Sixty-two (53.4%) of the isolates which were analyzed by ISYP showed disagreement with those identified by the conventional methods and API ID 32C identification kit or a specific identification code could not be assigned by ISYP. The performance of ISYP could be indicated as low for all antifungal drugs tested according to the ROC analysis (AUC: 0.28-0.56). As the current version of ISYP displays a poor performance, it is recommended to use the other commercial systems whose results are approved as reliable and in agreement with those of the reference methods in identification and susceptibility testing of yeasts.

Key words: Yeast, *Candida*, Identification, Susceptibility testing, Integral system.

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INTRODUCTION

Yeast infections which are commonly caused by *Candida* species comprise a wide range of clinical cases differing from superficial to systemic and potentially life-threatening infections (Morace and Borghi, 2010; Pfaller *et al.*, 2011). The microbiological diagnosis of yeast infections is based on the conventional methods (germ tube formation, colony morphology on corn meal tween 80 agar, culture) and biochemical tests which are labor intensive. Species level identification of yeasts needs experience for microscopic evaluation and this identification can be performed on the basis of assimilation/fermentation tests using automated or semiautomated systems (Fricker-Hidalgo *et al.*, 1996; Freydiere *et al.*, 2001; Kaufman, 2006).

The epidemiological data regarding *Candida* infections and antifungal drug susceptibility have been changing in recent years depending on the setting and clinical features (Pfaller *et al.*, 2011). As the *Candida* species show differences in their response to antifungal drugs, identification and an-

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timycotic susceptibility testing of causative yeasts have a critical importance for the selection of the appropriate antifungal drug. However, in most cases, antifungal therapy has been applied empirically in hospital conditions due to lack of easy, rapid, standard and cost-effective commercially available kits for susceptibility testing in diagnostic laboratories. Broth microdilution (BMD) procedures defined by the Clinical Laboratory Standards Institute (CLSI) (CLSI, 2002; CLSI, 2008a, b) and the European Committee on Antimicrobial Susceptibility Testing (EUCAST) (Rodriguez-Tudela *et al*., 2008) have been accepted as the reference methods for antifungal drug susceptibility testing of yeasts (Arikan and Rex, 2009). There are some disadvantages of the reference microdilution methods, such as long reporting time and difficulty in evaluation of minimal inhibitory concentration (MIC) results for some *Candida* strains and azoles (Park *et al*., 2006; Arikan and Rex, 2009). Various commercial systems have been developed and used as an alternative to the BMD method so far (Posteraro *et al*., 2000; Morace *et al*., 2002; Kaufman, 2006; Arikan and Rex, 2009). Integral System Yeasts *Plus* (ISYP) (Liofilchem S.r.l., Roseto D.A., Teramo (TE), Italy) is a commercially available kit for identification and drug susceptibility testing of the most clinically important yeasts and two comparative studies comprising the performance evaluation of Integral System Yeasts (ISY) in susceptibility testing of fluconazole were reported previously (Posteraro *et al*., 2000; Morace *et al*., 2002). It is important to determine the species distribution and susceptibility profile of *Candida* isolates for each institution to make a contribution to the local and nationwide surveillance data in order to guide the treatment planning of patients with *Candida* infections. The aim of the present study was to investigate the species distribution and antifungal drug susceptibilities of *Candida* isolates and to evaluate the performance of ISYP as an alternative commercial test for identification and antifungal susceptibility testing.

MATERIALS AND METHODS

Clinical isolates

A total of 116 yeast isolates recovered from specimens including 57 pulmonary (26 bronchial aspiration, 22 sputum, 5 deep tracheal aspiration [DTA], 4 bronchoalveolar lavage [BAL]) and 59 extrapulmonary (25 blood, 19 urine, 12 vaginal swab, 1 pleural fluid, 1 throat swab and 1 catheter) between April 2008 and August 2009 were analyzed in the Microbiology Laboratory of Izmir Training and Research Hospital for Chest Diseases and Chest Surgery which is located at the Aegean (West Anatolian) region of Turkey.

Identification and drug susceptibility testing

The yeasts were identified by microscopic examination, germ tube production, colony morphology on CHROMagar Candida (Becton Dickinson, Sparks MD, USA), microscopic examination of yeast morphology on cornmeal with Tween 80 agar and by using the API ID 32C (bioMerieux, Marcy l'Etoile, France) identification kit for yeasts.

MICs of amphotericin B, fluconazole, miconazole, 5-flucytosine, itraconazole and voriconazole were determined by the reference BMD method as described in the CLSI document M27-A3 (CLSI, 2008a).

Identification and antifungal susceptibility testing of yeasts for each sample were also performed with the ISYP (Liofilchem S.r.l., Roseta D.A., Teramo (TE), Italy) according to the manufacturer's recommendations. This system which contained dried biochemical and antimycotic substrates in 24 wells has been based on assimilation reactions of carbohydrates and growth or inhibition of yeasts in selective culture media with antimycotic agents (nistatin [1.25 mg/L], amphotericin [2 mg/L], flucytosine [16 mg/L], econazole [2 mg/L], ketoconazole [0.5 mg/L], clotrimoxazole [1 mg/L], miconazole [2 mg/L], itraconazole [1 mg/L], voriconazole [2 mg/L], fluconazole [16 and 64 mg/L]).

The evaluation was done according to the observation of color change in the corresponding wells for assimilation of each sugar. The species-level of identification was done by using a table of codes supplied by the manufacturer according to the differential features and morphology on corn meal agar at 25°C. The susceptibility to antimycotics was evaluated according to the color change (red=yeast growth inhibited, orange= yeast growth partially inhibited and yellow=good yeast growth) by a growth indicator in the wells. Red, orange or yellow color in the well including

an antimycotic agent was interpretated as susceptible (S), intermediate (I) or resistant (R), respectively. A well containing only culture medium and indicator was used as growth control for each test. The plate was covered with its lid and incubated at 35-37°C for 48 hours. Standard *C. albicans* ATCC 24433, *C. parapsilosis* ATCC 22019 and *C. krusei* ATCC 6258 were used as control strains in all assays.

Interpretation of antifungal susceptibility results

The susceptibility results of ISYP and BMD were compared for six antifungal drugs (amphotericin B, fluconazole, miconazole, 5-flucytosine, itraconazole and voriconazole). MIC interpretative criteria [S, susceptible dose dependent (SDD) and R] were referred to those described in the CLSI document M27-S3 for amphotericin B, 5-flucytosine, itraconazole and voriconazole and to the revised CLSI clinical breakpoint for fluconazol as reported by Pfaller *et al*. (CLSI, 2008b; Pfaller *et al*., 2011). Evaluation for voriconazole susceptibility was done according to the EUCAST (EUCAST E. DEF.7.1) (Rodriguez-Tudela *et al*., 2008) criteria as well. Since there is not a standard evaluation criteria for yeast-like species, four isolates other than *Candida* species were not considered for the comparison.

Statistical analysis

The susceptibility results obtained by ISYP (S/I < breakpoint concentration ≤ R) were compared with the MICs obtained by the reference BMD method and percent agreement between two methods was calculated. The two methods were compared by ROC analysis and the values for area under curve (AUC) and p values were calculated by using SPSS ver. 15 statistical analysis software.

RESULTS

One hundred and twelve of the isolates were *Candida* species whereas three were *Blastoschizomyces capitatus* and one *Trichosporon sp.* The distribution of *Candida* strains was as follows: *C. albicans*, 56 (50%); *C. parapsilosis*, 28 (25%); *C. tropicalis*, 18 (16.1%); *C. krusei*, 4 (3.6%); *C. kefyr,* 3 (2.7%); *C. glabrata*, 2 (1.8%) and *C. guilliermondii,* 1 (0.9%). Distribution of *Candida* species isolated from the patients according to the specimen type is shown in Table 1. When ISYP was compared with the conventional methods, 54 (46.6%) of the isolates were identified accurately by ISYP. However, 62 (53.4%) of the isolates showed disagreement with those identified by the conventional methods and API ID 32C (bioMerieux, Marcy l'Etoile, France) iden-

TABLE 1 - *Distribution of Candida species according to the type of specimens.*

Specimen type	*Candida species[1] [n (%)]*						
	Ca	*Cp*	*Ct*	*Ckr*	*Ckf*	*Cg*	*Cgm*
Pulmonary (n=54)	42 (77.8)	-	8 (14.8)	2 (3.7)	2 (3.7)	-	-
Bronchial asp. (n=23)	20 (87.0)	-	1 (4.3)	2 (8.7)	-	-	-
Sputum (n=22)	16 (72.7)	-	4 (18.2)	-	2 (9.1)	-	-
DTA (n=5)	3 (60.0)	-	2 (40.0)	-	-	-	-
BAL (n=4)	3 (75.0)	-	1 (25.0)	-	-	-	-
Extrapulmonary (n=58)	14 (24.1)	28 (48.3)	10 (17.2)	2 (3.4)	1 (1.7)	2 (3.4)	1 (1.7)
Blood (n=25)	-	22 (88.0)	2 (8.0)	-	-	-	1 (4.0)
Urine (n=19)	6 (31.6)	4 (21.1)	7 (36.8)	-	1 (5.3)	1 (5.3)	-
Vaginal swab (n=12)	7 (58.3)	1 (8.3)	1 (8.3)	2 (16.7)	-	1 (8.3)	-
Throat (n=1)	1 (100)	-	-	-	-	-	-
Catheter (n=1)	-	1 (100)	-	-	-	-	-
Total (n=112)	56 (50)	28 (25)	18 (16.1)	4 (3.6)	3 (2.7)	2 (1.8)	1 (0.9)

DTA: Deep tracheal aspiration, BAL: Bronchoalveolar lavage, Ca: *C. albicans*, Cp: *C. parapsilosis*, Ct: *C. tropicalis*, Ckr: *C. krusei*, Ckf: *C. kefyr*, Cg: *C. glabrata*, Cgm: *C. guilliermondii*, -: no isolate. [1]Among all isolates (n=116), two *Blastoschizomyces capitatus* and one *Trichosporon* sp. were identified in bronchial aspiration and one *B. capitatus* was identified in pleural fluid.

tification kit or a specific identification code could not be assigned by ISYP. The agreement rates of identification for *C. albicans* and non-*Candida albicans species* (or other yeast-like strains) were found as 91.1% and 5%, respectively. Identification results of yeast species can be examined in Table 2.

No resistant isolate was detected for amphotericin B and flucytosine by BMD method. According to the CLSI M27-S3 criteria, resistance rates for amphotericin B, flucytosine, fluconazole, itraconazole, and voriconazole were 0%, 0%, 4.6%, 4.5% and 1.8%, respectively. Resistance for miconazole (MIC >1 mg/L) was found as 17.9%. When compared with the reference method, agreement rates of ISYP results for these antimycotic agents were found within a range of 67-92.8% (Table 3). However, the performance and compliance of ISYP were indicated as low for all antifungal drugs tested (AUC: 0.28-0.56) according to the ROC analysis, and the performance of miconazole was significantly lower than that of other azoles ($p < 0.05$).

In the present study, MIC_{50} and MIC_{90} values of antifungal drugs for the most frequently isolated *Candida* species (*C. albicans*, *C. parapsilosis* and *C. tropicalis*) were found within the ranges of 0.25-0.5, 0.125-0.25, 0.125-8, 0.125-8, 0.03-16, 0.03-2 mg/L for amphotericin B, 5-flucytosine, fluconazole, miconazole, itraconazole and voriconazole, respectively. In *C. parapsilosis* and *C. tropicalis* isolates, miconazole and itraconazole showed higher MIC_{50} (≥1 mg/L) and MIC_{90} (≥8 mg/L) values in respect to the other drugs tested. MIC_{50} and MIC_{90} values of antifungal drugs for rare *Candida* species (*C. krusei*, *C. kefyr*, *C. glabrata*, *C. guilliermondii*) were found within the ranges of 0.125-0.5, 0.5-16, 0.125-64, 0.125-8, 0.125-0.5, 0.03-0.25 for amphotericin B, 5-flucytosine, fluconazole, miconazole, itraconazole and voriconazole, respectively. High MIC values for fluconazole (≥64 mg/L) and miconazole (≥8 mg/L) were determined in *C. krusei* isolates. As *C. krusei* species are intrinsically R to fluconazole, all *C. krusei* isolates were accepted as R to this agent. The rate of susceptibility of *Candida* isolates for amphotericin B, flucytosine, fluconazole, itraconazole and voriconazole was higher than 95% in this study, whilst the rate for miconazole was 82.1%.

While we analyzed the antifungal susceptibility discrepancies between the reference BMD method and ISYP in *Candida* isolates, the discrepancies were categorized as very major (R vs S), major (S vs R) and minor (S vs I; R vs I; SDD vs S and SDD vs R) errors. Thus; major errors were determined in 8, 2, 15, 27 and 11 *Candida* isolates for amphotericin B, 5-flucytosine, fluconazole, miconazole and itraconazole, respectively, whereas very major errors were determined in 4, 10 and 4 *Candida* isolates for fluconazole, miconazole and

TABLE 2 - *Comparison of ISYP and conventional methods for the identification of yeast isolates.*

Yeast species		*ISYP Results*		
		No. of isolates (%)		*No. of isolates not identified (%)*
		Positive agreement	*Negative agreement*	
C. albicans (n=56)		51 (91.1)	1 (1.8)	4 (7.1)
Non-albicans *Candida* spp. *(n=56)*	*Cp (n=28)*	-	17 (60.7)	11 (39.3)
	Ct (n=18)	-	12 (66.7)	6 (33.3)
	Ckr (n=4)	2 (50.0)	2 (50.0)	-
	Ckf (n=3)	1 (33.3)	1 (33.3)	1 (33.3)
	Cg (n=2)	-	1 (50.0)	1 (50.0)
	Cgm (n=1)	-	1 (100.0)	-
B. capitatus (n=3)		-	1 (33.3)	2 (66.6)
Trichosporon sp. (n=1)		-	-	1 (100.0)
Total (n=116)		54 (46.6)	36 (31.0)	26 (22.4)

Cp: C. parapsilosis, Ct: C. tropicalis, Ckr: C. krusei, Ckf: C. kefyr, Cg: C. glabrata, Cgm: C. guilliermondii, -: no isolate.

itraconazole, respectively. When we evaluated the susceptibility results of voriconazole, 18 major and 2 very major errors occurred with the CLSI criteria, whereas 22 major and 4 very major errors occurred with the EUCAST criteria. Additionally, minor errors occurred in 7, 15 and 1 *Candida* isolates for 5-flucytosine, itraconazole and voriconazole. Major and very major errors for miconazole and minor errors for itraconazole were more frequent among nonalbicans *Candida* species in respect to *C. albicans*.

DISCUSSION

Increasing hospital-acquired infections and drug resistance rates due to *Candida albicans* and the other *Candida* species have been reported and associated with high mortality rates (Clark and Hajjeh, 2002; Krcmery and Barnes, 2002; Kaufman, 2006; Pfaller *et al*., 2010). Yeasts, particularly *Candida* species are the fourth most common cause of nosocomial bloodstream infections among ICU patients and the third among all nosocomial bloodstream infections (Kaufman, 2006; Morace and Borghi, 2010).

The *Candida* species responsible for more than 90% of all invasive *Candida* infections including candidemias are *C. albicans*, *C. glabrata*, *C. parapsilosis*, *C. tropicalis* and *C. krusei* (Diekema *et al*., 2009; Lyon *et al*., 2010; Morace and Borghi, 2010). In the present study, *C. albicans* (50%) was the most common species isolated in total and also in pulmonary samples followed by *C. parapsilosis* (25%), *C. tropicalis* (16.1%), *C. krusei* (3.6%), *C. kefyr* (2.7%), *C. glabrata* (1.8%) and *C. guilliermondii* (0.9%). *C. parapsilosis* (48.3%) was the most frequent species among extrapulmonary samples and most of these (88%) were isolated from blood cultures of the patients hospitalized in ICU. In our study, *C. parapsilosis* was isolated from a catheter of a patient of ICU and mostly from blood samples. The high frequency of the bloodstream infections caused by *C. parapsilosis* in patients hospitalized in the ICU might be caused by catheter colonization in these patients. In the present study, two *C. tropicalis* (1.8%) and *C. guilliermondii* (0.9%) were isolated from blood samples as well. *C. guilliermondii* is known to be a normal component of the human skin and mucosal flora and is rarely associated with invasive infections. Infection with this species has com-

TABLE 3 - *Comparison of antifungal susceptibility testing results of ISYP with the standard broth microdilution method.*

		Broth Microdilution[1] [n (%)]				
Drugs		*S*	*SDD*	*R*	*Agreement (%)*	*AUC*
Amphotericin[2]	(n=111)	111 (100.0)	N/A	-	92.8	0.397
Flucytosine	(n=112)	109 (97.3)	3 (2.7)[3]	-	92	0.561
Fluconazole[4]	(n=108)	103 (95.4)	-	5 (4.6)	82.4	0.411
Voriconazole[5]	(n=112)	109 (97.3)	1 (0.9)	2 (1.8)	81.3	0.365
Voriconazole[6]	(n=112)	104 (92.9)	N/A	8 (7.1)	76.8	0.406
Itraconazole	(n=112)	95 (84.8)	12 (10.7)	5 (4.5)	73.2	0.455
Miconazole[7]	(n=112)	92 (82.1)	N/A	20 (17.9)	67	0.282

S: susceptible, R: resistant, SDD: susceptible dose dependent, N/A: not applicable, -: no isolate, AUC: area under curve. [1]Antifungal susceptibility testing of the drugs was interpreted according to the MIC values of as recommended by CLSI M27-S3 and revised clinical breakpoint was used for fluconazole as reported by Pfaller et al. (Pfaller et al., 2011) [MIC (mg/L) values: R >1, S ≤1 for amphotericin B; R ≥8, SDD = 4, S ≤2 for fluconazole; R ≥32, I = 8-16, S ≤4 for 5-flucytosine; R ≥1, SDD = 0.25-0.5, S ≤0.125 for itraconazole; R ≥4, SDD = 2, S ≤1 for voriconazole]. [2]As *C. guilliermondii* has been reported as intrinsically R to amphotericin B, one *C. guilliermondii* isolate was accepted as R. [3]Susceptibility testing results of flucytosine which were defined as "intermediate" according to the CLSI. [4]As *C. krusei* has been reported as intrinsically R to fluconazole, four *C. krusei* isolates were accepted as R. [5]Susceptibility testing results of voriconazole according to the CLSI M27-S3 criteria. [6]Susceptibility testing results of voriconazole according to the EUCAST E.DEF.7.1 criteria (S ≤0.125, R >0.125). [7]MIC >1 mg/L was accepted as R for miconazole.

monly been reported as catheter-related (Diekema *et al*., 2009).

Pulmonary candidiasis may be a secondary process arising from hematogenous dissemination or rarely a primary bronchopneumonia. Candida bronchopneumonia occurs in severely debilitated or neutropenic patients by aspiration of infected oral secretions into the tracheobronchial tree with extension into pulmonary parenchyma. Invasive medical procedures (i.e. biopsy of lung tissue, bronchial aspiration, BAL or DTA by fiberoptic bronchoscopy) are needed for diagnosis in patients with pulmonary candidiasis. However, the presence of *Candida* spp. in bronchial aspiration, BAL, DTA or sputum of a patient with pulmonary infiltrates is not as specific as biopsy to preclude a definitive diagnosis. Evaluation of colony count ($>10^4$ cfu/mL) in bronchial aspiration, DTA or BAL and pure culture or predominant growth in several sputum samples are essential to provide a supportive and available information on the clinical evaluation of colonization or infection. Treatment of *Candida* bronchopneumonia or hematogenous disseminated candidiasis is initiated with fluconazole, an echinocandin or amphotericin B (Donoghue 2009).

Multiple epidemiologic studies have indicated that *C. albicans* is responsible for most cases of vulvovaginal candidiasis, although rare reports indicate that non-albicans *Candida* species including *C. parapsilosis*, *C. glabrata*, *C. krusei*, *C. lusitaniae* are responsible in certain geographic regions (Singh *et al*., 2002; Sobel, 2007; Kennedy and Sobel, 2010). *C. kefyr* which was rarely isolated from clinical specimens in our study has been found in certain food (mainly dairy products) and has been isolated more frequently from patients with hematologic malignancies (Diekema *et al*., 2009).

The rate of susceptibility of *Candida* isolates has been reported as high (>90%) for amphotericin B, fluconazole, voriconazole and echinocandins and the increasing trend of susceptibility to fluconazole for all species has been predicted as well (Lyon *et al*., 2010). *C. krusei* and *C. glabrata* strains are innately R or easily have developed resistance against azoles and have higher rates of resistance to fluconazole (Lyon *et al*., 2010). Although there have been reduced susceptibilities to commonly used agents such as fluconazole and echinocandins in some rarely isolated strains, over 95% of clinical isolates of the rare *Candida* species were S to the available antifungals (Diekema *et al*., 2009). In a previous study, susceptibility of *C. parapsilosis* isolated from blood cultures of patients was found to be 100% to amphotericin B, fluconazole, ketoconazole, itraconazole and voriconazole (Tay *et al*., 2009).

There is a need for an accurate, cost-effective and easy method to identify the causative agent as soon as possible and initiate a proper treatment. Some automated or semi-automated systems have been developed for use in routine practice. Integral System Yeasts (ISY) is one of these systems and two studies (Posteraro *et al*., 2000; Morace *et al*., 2002) have been reported regarding the performance evaluation of this system for susceptibility testing of fluconazole, previously. However, different drug concentrations and fewer drugs were available for analysis in the former version (ISY) of ISYP. No study regarding the performance evaluation of ISYP for identification of yeasts has been reported to date. Although ISYP gave concordant results in most *C. albicans* isolates, a high rate of false *C. albicans* identification which was noted in 28 of 34 non-albicans isolates was also observed. The data in the present study has determined a low performance of ISYP for identification of yeasts other than *C. albicans*. As the primary differentiation of *C. albicans* from non-*C. albicans* has been performed easily and accurately by germ tube production, it was thought that a sole identification of *C. albicans* by a commercial test would not be necessary in routine practice.

In the study reported by Posteraro *et al*. (Posteraro *et al*., 2000), the concordance rate for ISY with the reference BMD method was found to be very low (16.6%) and it concluded that lack of standardization and non-objective interpretation schemes could be the reason of its poor performance. It was indicated that ISY had classified most S isolates as R. In another study (Morace *et al*., 2002), the concordance rates of ISY and BMD method for susceptibility testing of fluconazole were evaluated in *C. albicans* and also non-*albicans Candida* isolates. The overall concordance rate was 37.6%, whereas these values were detected as 39.1%, 24.8%, 12.3%, 77.8% and 45.3% for *C. albicans*, *C. glabrata*, *C. tropicalis*, *C. parapsilosis* and *C. krusei*, respectively.

The majority of isolates were S by BMD where as they were found to be I (as a minor error) by ISY. However, major and very major errors had also been observed in a few isolates as well. In the present study, concordance rates for fluconazole were found to be 85.7%, 100%, 55.5% and 66.7% for *C. albicans*, *C. parapsilosis*, *C. tropicalis* and the other *Candida spp*. respectively. The accuracy in susceptibility results of ISYP for fluconazole showed differences among species which is in parallel with the study by Morace *et al*. (Morace *et al*., 2002). The concordance of susceptibility testing results for all antifungal agents was found to be between 67 and 92.8% while this rate was 82.4% for fluconazole. However, ISYP showed low performance in antifungal susceptibility testing by ROC analysis in comparison with the reference method (AUC: 0.28-0.56). Most of the discrepancies were due to major errors by ISYP. As ISYP is a colorimetric system, evaluation of this system is based on visual interpretation and this could be the cause of the difference in concordance rates. In the study by Morace *et al*. (Morace *et al*., 2002), most of S isolates were evaluated as I with the ISY which could be the reason of low concordance rate obtained. On the other hand, different drug concentrations in the former ISY test could be the other reason for this difference. Numerous problems in ISYP are related to the fact that many drugs have a single concentration and that the two concentrations (16 and 64 mg/L) of fluconazole are too high based on the new clinical breakpoint revised by CLSI which leads to very major errors (R by BMD vs S by ISYP). There are also some other problems related to the breakpoints of voriconazole and flucytosine which are not in correlation with those as described by CLSI and/or EUCAST. Thus, SDD result (MIC = 2 mg/L) according to the CLSI criteria is assigned as "false" R according to the breakpoint of ISYP (≥2 mg/L). The MIC values of 0.25, 0.5 and 1 mg/L which are R according to the EUCAST criteria (>0.125 mg/L) are evaluated as "false" S according to the breakpoint of ISYP. Additionally, MIC value of 16 mg/L for flucytosine which is I according to the CLSI criteria is assigned as "false" R according to the breakpoint of ISYP (≥16 mg/L).

The epidemiological data in this study showed that *C. albicans* was the most common species isolated from pulmonary and extrapulmonary specimens in our setting, followed by *C. parapsilosis* and *C. tropicalis*. It is noteworthy that *C. parapsilosis* was the most common non-*albicans Candida* species isolated mostly in blood samples of patients hospitalized in the ICU. Most of the isolates (>95%) were S or SDD to the antifungal drugs tested, except miconazole with a resistance rate of 17.9%.

Although ISYP is cost-effective, practical and easy to apply, the performance evaluation shows that the current version of this system has unsatisfactory efficiency in identification and antifungal susceptibility testing of yeasts. Many other commercial systems for identification (i.e. API, Vitek, RapidID) and antifungal susceptibility (i.e. disk diffusion, E test, Sensititre) of yeasts have been reported as convenient alternative methods so far (Liguori *et al.* 2010; Farina *et al.* 2011). Specialists should evaluate the efficiency of each system and choose the most appropriate one whose results prove as reliable and in agreement with those of the reference methods for use in the diagnostic laboratories.

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