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ORIGINAL ARTICLE

Species Identification and Antifungal Susceptibility Profile of Candida Isolates from ICU Patients in Sohag University Hospital, Upper Egypt

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Key words:

Candida, CHROMagar Candida agar, vitek 2, PCR-RFLP

ABSTRACT

Background: *Candida species are among the most common fungal pathogens in ICU patients. Candida albicans was the predominant species, but a shift toward non-albicans Candida species has been recently observed.* ***Objectives:*** *To detect the prevalence of different Candida species and determine their antifungal susceptibility profile in ICU patients using phenotypic methods, the Vitek 2 system compared with CHROMagar Candida agar and a genotypic method; PCR-RFLP.* ***Methodology:*** *Various clinical samples were collected from 248 ICU patients in Sohag University Hospital from the period between September 2014 and May 2015. Samples were cultured on CHROMagar Candida agar. Results were compared with those of Vitek 2 system and confirmed by PCR- RFLP method and antifungal susceptibility profiles were analyzed by disc diffusion and Vitek 2 antifungal susceptibility tests.* ***Results:*** *The study revealed an overall isolation rate of Candida species among ICU patients was 29 % by PCR-RFLP. Candida albicans was the most frequent species isolated (40.3%). Non- albicans Candida species including Candida tropicalis (22.2%), Candida glabrata (18%), Candida krusei (12.5%), C. parapsilosis (4.2%), C. dubliniensis (1.4%) and Candida guilliermondii (1.4%) were also isolated. The sensitivity of vitek 2 with regard to correct identification of Candida species was 96%; the specificity was 100 % , also CHROMagar Candida agar enable the correct identification with sensitivity 89% , specificity 100 %. Vitek 2 antifungal susceptibility tests results were found to be an accurate method as it was compared with the disc diffusion method for fluconazole, voriconazole and amphotracin B.* ***Conclusion****: CHROMagar Candida agar supported by Vitek 2 system is a valuable method for identification of common Candida species, these methods are easy to interpret and give rapid results in comparison with the expensive PCR-RFLP method. Although amphotericin B and fluconazole are widely used in clinical practice, there was no evidence of enhanced resistance. Moreover, voriconazole could be used in treatment of fluconazole-resistant Candida species.*

INTRODUCTION

Fungi are now recognized as a primary cause of morbidity and mortality in immunocompromised and severely ill patients [1]. Candida species are among the most common fungal pathogens, candidiasis, hence, is called the "disease of diseased" [2].

Candida leads to a range of mucocutaneous to life-threatening invasive diseases and has become the fourth most common organism responsible for bloodstream infection in the intensive care unit (ICU). [3,4]

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Among Candida spp., *Candida albicans* is the most common infectious agent. This yeast is a commensal that colonizes skin, the gastrointestinal and the reproductive tracts. Non albicans Candida (NAC) species such as *C. tropicalis, C. glabrata* and *C. krusei* which are less susceptible to azoles derivatives have been reported with increasing frequency. [5,6]

The species distribution and antifungal susceptibility of Candida isolates varies between countries, regions, and institutions. Identification of species is essential for effective antifungal therapy with regard to the emergence of resistance to antifungal drugs. [7,8]

Several risk factors may be associated with fungal infections like neutropenia, neoplasia, decompensated diabetes mellitus, malnutrition, organ transplantation, acquired immunodeficiency syndrome, increased use of

invasive procedures and devices, widespread use of broad spectrum antibiotics and empirical use of antifungal drugs.

Accurate identification of Candida isolates from clinical specimens is vital for the establishment of etiological diagnosis, selection of appropriate antifungal therapy. Currently, Candida identification in the clinical laboratory is based on various commercial systems, utilizing mainly biochemical and occasionally morphological and physiological characteristics. [9]

The primary isolation of Candida from patients' samples is generally based on culture on Sabouraud Dextrose Agar (SDA), a medium that does not allow species identification; and differential isolation media like chromogenic media that distinguish Candida spp. on the basis of the morphology and color of the colonies. [10,11]

Automated approaches are available for the identification of Candida species as Vitek 2 system which achieves satisfactory results for all common clinical Candida, e.g., *Candida albicans, C. glabrata, C. dubliniensis, C. krusei, C. lusitaniae,* and through additional tests, identification rates could be increased, especially for *C. guilliermondii, C. parapsilosis,* and *C. tropicalis.* [12]. Molecular techniques have provided alternative methods for diagnosis and identification of fungi, especially for Candida at the species level in epidemiological studies and to determine appropriate antifungal drugs [6]. Although various methods have been reported for molecular identification of Candida species such as random amplified polymorphic DNA, DNA sequence analysis and mitochondrial large subunit ribosomal RNA gene sequencing, these tests are still time consuming and too expensive for routine use especially in medical laboratories [6,13]. Restriction fragment length polymorphism (RFLP) analysis of the ribosomal DNA (rDNA) repeats of Candida species is a simple method and easy to perform for identification of Candida isolates in the medical laboratories. [8]

METHODOLOGY

This study was conducted in the Infection Control Unit Laboratory of Sohag University Hospital and included 248 patients admitted to intensive care unit in the period from September 2014 to May 2015; written informed consent was obtained from the patients. All patients, diagnosed on the basis of clinical presentation, were subjected to the following: detailed history of associated risk factors such as underlying medical conditions, invasive medical procedures, presence of urinary catheter, respiratory ventilation, central line insertion, and use of corticosteroids, antibiotics, cancer chemotherapy, or immunosuppressive drugs. Patients are subjected to thorough clinical examination and laboratory investigations.

1- Species Identification by CHROMagar Candida agar

Various clinical samples (urine, vaginal swabs, oropharyngeal swabs, blood and sputum) were cultured on sabouraud dextrose agar (LAB M, Hey wood, United kingdom) for 24 to 48 hours at 37°C, colonies appearing pasty, flat, smooth and pale colored with a sweet smell were suspected to be colonies of Candida [2] and were identified by gram stain, sub cultured on chromogenic CHROMagar Candida agar (BD, Paris, France) and incubated at 37ºC for 48 h. The colonies were identified based upon the characteristic color & morphology of the colonies according to the chromogenic scheme of the media; *C. albicans* and *C. dubliniensis* (green), *C. tropicalis* (metallic blue), *C. krusei* and *parapsilosis* (pink,) and *C. glabrata* (white to mauve) (Figure 3a)

2- Antifungal susceptibility test (Disc diffusion method)

According to CLSI document M44-A [14], the antifungal discs: amphotericin B (20 µg), fluconazole (25 µg), voriconazole (1 µg) were used with inhibition zone diameter for fluconazole: Susceptible ≥ 19 mm; susceptible-dose dependent (SDD) 15 to 18 mm; resistant ≤ 14 mm, voriconazole: Susceptible ≥ 17 mm; susceptible-dose dependent (SDD) 14 to 16 mm; resistant ≤ 13 mm. and amphotericin B: Susceptible ≥ 15 mm; susceptible-dose dependent (SDD) 11 to 14 mm; resistant ≤ 10 mm.

3- Species Identification by Vitek 2 system

Pure subcultures of Candida species were suspended in sterile saline to achieve a turbidity of 2.0 McFarland standards, as measured by the DensiChek turbidity meter (bioMérieux) and used to inoculate the colorimetric Yeast identification cards containing the biochemical substrates. The Vitek 2 compact instrument automatically filled, sealed, and incubated the individual test cards with the prepared culture suspension. Final profile results were compared to the database, generating identification of the unknown organism. Final identifications listed as "excellent," "very good," "good," "acceptable" or "low discrimination" was considered correct (bioMérieux, Marcy l'Etoile, France).

4- Vitek 2 Antifungal susceptibility test

The Vitek 2 Yeast susceptibility cards containing serial twofold dilutions of fluconazole, voriconazole, amphotracin B, capsofungin, micafungin and flucytosine were provided by the manufacturer, and the results were expressed as MICs in ug/mL, fluconazole: Susceptible MIC ≤ 8 µg/ml, intermediate MIC = 16 to 32 µg/ml; resistant, MIC ≥ 64 µg/ml, voriconazole: Susceptible MIC ≤ 1 µg/ml; intermediate MIC = 2 µg/ml; resistant MIC ≥ 4 µg/ml and amphotericin B: Susceptible, MIC ≤ 1 µg/ml, intermediate MIC = 2 µg/ml; resistant MIC ≥ 4 µg/ml.

5- PCR-RFLP

Polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) was performed using specific primers for the molecular identification of Candida species. [8]

PCR

Genomic DNA was extracted using QIAamp DNA Mini Kits (QIAGEN, Milan, Italy) and PCR assay was performed using 1 µL of the test sample in a final volume of 50 µL. The PCR mix consisted of 10 mM Tris-HCl; master mix 1×; 1.5 mM MgCl2; 50 mM KCl; 10 mM each of dATP, dCTP, dGTP, and dTTP;0.2 mM each of primers (ITS1: 5′-TCC-GTA-GGT-GAA-CCTGCG-G-3′ and ITS4: 5′-TCC-TCC-GCT-TAT-TGA-TAT-GC-3′); [15]and 1–2 µL of Taq DNA polymerase in thermal cycler (T Gradient - Biometra). The amplified products were visualized on 1.5% agarose gel stained with ethidium bromide. The stained gels were visualized and documented with a gel documentation system and analyzed visually to determine the number of fragments present. [8]

RFLP Assay

On the basis of the sequence of ITS1–ITS4 of various Candida species, the restriction sites of different restriction enzymes were determined and the most ideal enzymes were selected. For each restriction digestion reaction, 8.5 µL of the amplified PCR product was digested with 1 µL of restriction enzyme buffer, 0.5 µl (10 U) of the restriction enzyme MspI (Biolabs, New England), the reaction mixture (10 µL) was incubated at 37°C for 180 min. Separation of the digested fragments was visualized on 2% agarose gel run in TBE buffer and stained with ethidium bromide. The size of DNA fragments determined directly by comparison with molecular size marker and distinct banding patterns which demonstrated in similar studies [6,16]. The sizes of DNA fragments of different Candida species are shown in table 2

Statistical Analysis:

Mann-Whitney test was used to determine statistical significance using SPSS version 12.0

RESULTS

A total of 72 (29 %) samples from 248 patients (154 males 62%, and 94 females 38% with mean age of 51 years) were positive for Candida by molecular identification method PCR-RFLP. These samples consisted of 24 urine (33.3%), 14 vaginal swabs (19.4%), 29 oropharyngeal swabs (40.3%) 3 blood (4.2%) and 2 sputum (2.8%) samples. The distribution of Candida species were *C. albicans* 29 (40.3%), *C. tropicalis* 16 (22.2%), *C. glabrata* 13 (18%), *C.krusei* 9 (12.5%), *C. parapsilosis* 3 (4.2%), *C. dubluniensis* 1 (1.4%), *C. guilliermondii* 1 (1.4%) (Table 1, Figure 1, 2)

Single type of Candida species was isolated from each patient except for 6 patients who had 2 different types of Candida species, 5 patients had *C. albicans* in addition to *C. tropicalis, C. glabrata* or *C. parapsilosis,* 1 patient had *C. glabrata* with *C. guilliermondii.*

Table 1: Candida species identified from different samples by PCR-RFLP

Candida species	*Urine*	*Vaginal swab*	*Oral swab*	*Blood*	*Sputum*	*Total*
C. albicans	10	7	10	0	2	**29 (40.3%)**
C. tropicalis	7	2	5	2	0	**16 (22.2%)**
C. glabrata	2	2	8	1	0	**13 (18%)**
C. krusei	4	3	2	0	0	**9 (12.5%)**
C. parapsilosis	0	0	3	0	0	**3 (4.2%)**
C. dubliniensis	0	0	1	0	0	**1 (1.4%)**
C. guilliermondii	1	0	0	0	0	**1 (1.4%)**
Total	**24(33.3%)**	**14(19.4%)**	**29(40.3%)**	**3(4.2%)**	**2(2.8%)**	**72 (100%)**

Although *Candida albicans* (40.3 %) was the predominant species in this study, Non albicans Candida (NAC) species (59.7%) have been isolated with increasing frequency, *Candida albicans* to non-albicans ratio was 29:43

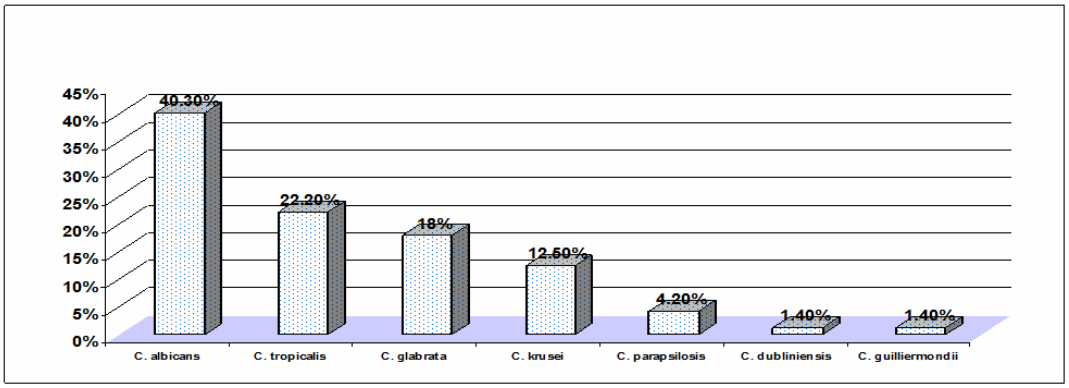

Fig. 1: Candida species identified by PCR-RFLP

Table 2: Size of DNA fragments of different Candida species before & after RFLP assay

Candida species	*Size of ITS1- 5.8S- ITS2 before digestion with MspI*	*Size of restriction fragments after digestion with MspI*
C. albicans	535 bp	297-238 bp
C. krusei	510 bp	261-249 bp
C. tropicalis	524 bp	340-184
C. glabrata	871 bp	557-314 bp
C. dublinensis	540 bp	300-240 bp
C. guilliermondii	608 bp	371-155-82 bp
C. parapsillosis	520 bp	520 bp

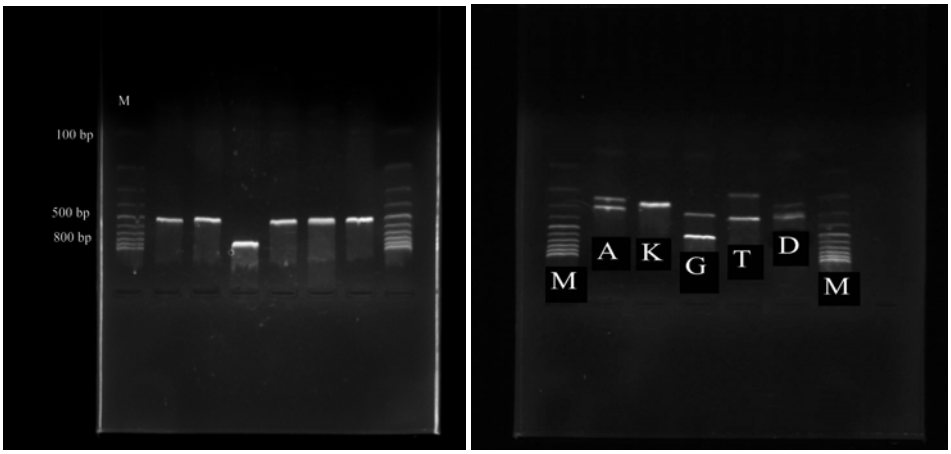

Fig. 2: DNA fragments of different Candida species before & after RFLP assay

The Vitek 2 system identified 69 (95.8%) of Candida isolates correctly within 15 hours. Of these, 42 were placed in the identification category 'excellent', 20 in the category 'very good', 1 in the category 'good', and 4 in the category 'acceptable'. In total, 67 (93 %) of 72 Candida isolates were identified correctly. A low discrimination result was obtained with 2 (2.8 %) isolates, they were identified correctly following the use of additional software tests suggested by Vitek 2; thus, the final number of correct identifications was 69 (95.8%). However, vitek 2 misidentified 3 isolates; 1 isolate of *C. albicans* and 1 isolate of *C. krusei* were not identified at all. The third isolate was misidentified, comprising an isolate of *Candida tropicalis* identified as *Candida parapsilosis.* When the test was repeated, the *C. tropicalis* isolate was identified correctly with 'good identification' (Table 3).

Table 3: Candida species identified by VITEK 2

Candida species	*Correct Identification*	*Misidentification*	*Unidentified*	*Total*
C. albicans	28	0	1	29
C. tropicalis	15	1	0	16
C. glabrata	13	0	0	13
C. krusei	8	0	1	9
C. parapsilosis	3	0	0	3
C. dubliniensis	1	0	0	1
C. guilliermondii	1	0	0	1
Total	**69(95.8)**	**1 (1.4)**	**2 (2.8)**	**72(100%)**

The sensitivity of vitek 2 with regard to correct identification of Candida species was 96%; the specificity was 100 % with diagnostic accuracy about 98%. Also CHROMagar Candida agar enables the correct identification of Candida species (Figure 3a) with sensitivity 89%, specificity 100 % and diagnostic accuracy about 95% but misidentified considerable number belonging to different species: 2 isolates of *C. albicans* and 1 isolate of *C. tropicalis,* 1 isolate of *C. glabrata*, 2 isolates of *C. krusei*, 1 isolate of *C. parapsilosis* and 1 isolate of *C. dubliniensis.* (Table 4, Figure 4)

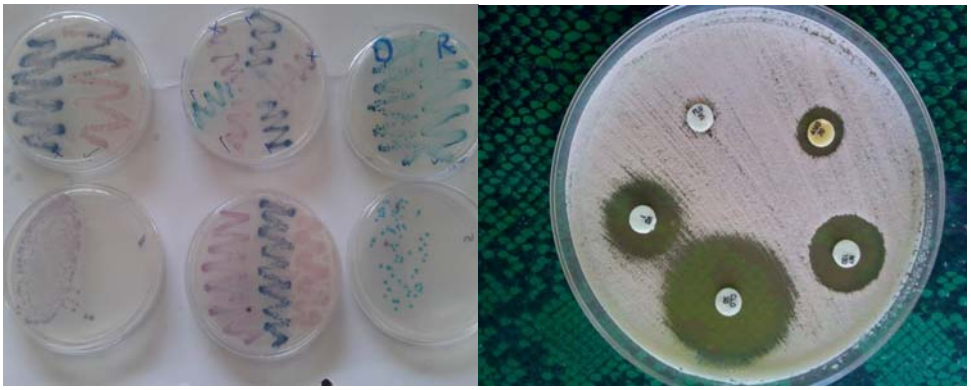

Fig. 3: a) Candida species on CHROMagar Candida agar; b) Antifungal susceptibility test (Disc diffusion method)

Table 4: Candida species identified by VITEK 2 and CHROMagar Candida agar

Candida species	*CHROMagar*		*Vitek 2*	
	Agreement (%)	**Misidentification (%)**	**Agreement (%)**	**Misidentification (%)**
C. albicans	27(93%)	2	28(97%)	1
C. tropicalis	15(94%)	1	15(100%)	1
C. glabrata	12(92%)	1	13(100%)	0
C. krusei	7(78%)	2	8(89%)	1
C. parapsilosis	2(67%)	1	3(100%)	0
C. dubliniensis	`0(0%)	1	1(100%)	0
C. guilliermondii	1(100%)	0	1(100%)	0
Total	**64 (88.9%)**	**8 (11.1%)**	**69 (94%)**	**3 (4.2%)**

Table 5: Antifungal susceptibility profile of common Candida species

Antifungal / species	*Disc diffusion*			*Vitek 2*		
	S	**SDD**	**R**	**S**	**I**	**R**
fluconazole (67)	**43**	**2**	**22(33%)**	**43**	**1**	**23(34%)**
C. albicans (29)	27	2	0(0%)	26	1	2(7%)
C. tropicalis(16)	11	0	5(31%)	12	0	4(25%)
C. glabrata (13)	5	0	8(62%)	5	0	8(62%)
C. krusei (9)	0	0	9(100%)	0	0	9(100%)
voriconazole (67)	**61**	**0**	**6(9%)**	**58**	**0**	**9(13%)**
C. albicans (29)	29	0	0(0%)	29	0	0(0%)
C.tropicalis(16)	15	0	1(6%)	14	0	2(12%)
C. glabrata (13)	10	0	3(23%)	10	0	3(23%)
C. krusei (9)	7	0	2(22%)	5	0	4(44%)
amphotericin B(67)	**64**	**1**	**2(3%)**	**60**	**3**	**4(6%)**
C. albicans (29)	29	0	0(0%)	29	0	0(0%)
C.tropicalis(16)	15	0	1(6%)	15	0	1(6%)
C. glabrata (13)	11	1	1(8%)	8	3	2(15%)
C. krusei (9)	9	0	0(0%)	8	0	1(11%)

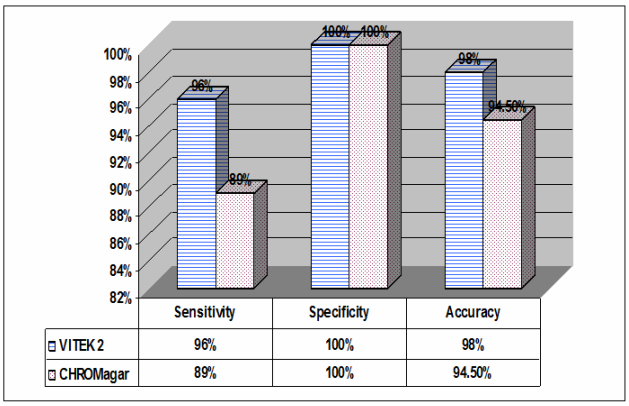

Fig. 4: Performance data of the Vitek 2 and CHROMagar Candida agar

The in vitro antifungal susceptibilities of 67 isolates of common Candida species as determined by the disc diffusion according to CLSI document M44-A (Figure 3b) and Vitek 2 Yeast susceptibility cards results expressed as MICs in ug/mL provided by the manufacturer were compared. In general, the overall fluconazole resistance was 22/67 (33%) by disc diffusion and 23/67(34%) by Vitek 2 system with most of the resistance present in *C. krusei* (100%), *C. glabrata* (62%) and *C. tropicalis* (31% and 25%). As regard voriconazole, overall resistance was minimal among common Candida species, 6/67(9%) by disc diffusion and 9/67 (13%) by Vitek 2 with no resistance in *C. albicans*. Also common Candida species showed minimal resistance for amphotericin B, 2/67 (3%) by disc diffusion and 4/67(6%) by Vitek 2 system. (Table 5)

DISCUSSION

The incidence of opportunistic fungal infections such as Candida infections has considerably increased recently[17]. Accurate identification of Candida isolates from clinical specimens is vital for the establishment of etiological diagnosis, selection of appropriate antifungal therapy. Molecular methods such as PCR-RFLP which is more accurate than traditional phenotypic methods are used for genetic identification of Candida species [13]. In this study, we identified different Candida species by PCR-RFLP method using 2 universal primers, ITS1 and ITS4, and the restriction enzyme MspI. [17], other investigators as *Isogai et al.* [18] and *Williams et al.* [19] used the restriction enzymes HaeIII and HaeIII, DdeI, and BfaI, respectively, after amplification of the ITS1–ITS4 regions.

Our study showed that the distribution of Candida species using PCR-RFLP method was *C. albicans* (40.3%), *C. tropicalis* (22.2%), *C. glabrata* (18%) , *C. krusei* (12.5%), *C. parapsilosis* (4.2%), *C. dubluniensis* (1.4%), and *C. guilliermondii* (1.4%). Although *Candida albicans* (40.3 %) was the predominant species in this study, non-albicans Candida species (NAC) (59.7%) have been isolated with increasing frequency. *Candida albicans* to non-albicans ratio was 29:43 (1:1.48), this is in agreement with a previous study in which the ratio was 47:73(1:1.55) [20]

Similar results were observed by *Hung et al* in a Taiwanese population [21] and by *Katiraee* [22] and *Shokohi* [16] in Iranian populations. The results of our study were different from those shown by Enweani et al., Okungbowa et al. and Clark et al. [23, 24, 25]. In a study on incidence of candidiasis in 103 asymptomatic females, *Enweani et al.*[23] reported that *C. guilliermondii* was the most commonly isolated pathogen in women who used contraceptive drugs. Okungbowa et al. [24] reported that the predominant species isolated in the genitourinary tract was *C. glabrata* (33.7%), whereas Clark et al. [25] reported that the predominant species in cases of bloodstream infection was *C. parapsilosis* (57.8 %).

Kocoglu et al. [26] investigated various clinical specimens in a one year period. The most common one was *C. albicans* (56.8%), secondly *C. tropicalis* (7.7%) and thirdly *C. sake* (6.8%). *C. parapsilosis* was the second most common isolated strain in blood culture specimens. Motta et al. [27] investigated the distribution and the antifungal susceptibility of yeast like fungi isolated from blood cultures at a tertiary education hospital in Brazil in 2006. The isolated yeast like fungi was *C. albicans* (52.2%), *C. parapsilosis* (22.1%), *C. tropicalis* (14.8%) and *C. glabrata* (6.6%), respectively. Also, *Badiee et al.*[28] investigated the mucosal Candida colonization in 273 HIV seropositive patients, among these 50% *C. albicans* was isolated and this was followed by *C. glabrata* (21.4%), *C. dubliniensis* (13.3%), *C. krusei* (9.8%), *C. kefyr* (3.1%), *C. parapsilosis* (1.6%) and *C. tropicalis* (0.8%).

In this study , the Vitek 2 system identifies most clinically important Candida species reliably within 15 hours and appears to be an excellent identification method, the overall agreement between the Vitek 2 and PCR- RFLP on initial testing was 67/72 (93%) with 2/72 (2.8 %) were low-discrimination results, after confirmatory tests the Vitek 2 identified 69/72 (95.8%) Candida isolates correctly and 1/72 (1.4%) incorrectly, and 2/72 (2.8%) were unidentified, with regard to correct identification of *Candida albicans*, CHROMagar Candida agar also performed very well, its sensitivity and specificity for *C. albicans* was 93% and 100 % respectively.

Similar results were found in previous studies, Willinger et al. [29] reported 98.8% sensitivity and 100% specificity for *C. albicans*, also Yucesoy et al.[30] studied 270 yeast strains identified by germ tube test and cornmeal agar, Vitek 2 and API 20 C AUX and reported 99.4% sensitivity and 100% specificity.

Rapid identification of *C. glabrata* has a special importance because *C. glabrata* is less sensitive than other species to ketoconazole and fluconazole. A sensitivity of 92% and specificity of 100 % were reported in this study, in agreement with our results Willinger et al.[29] reported 98% sensitivity and 95.7% specificity for *C. glabrata* on Chromagar, Peng et al.[31] reported sensitivity and specificity values of Chromeagar for *C. glabrata* as 90.2% and 95.4% respectively and *Yucesoy et al.* reported 90.9% sensitivity and 100% specificity of CHROMagar Candida agar for *C. glabrata.*[30]

15 of 16 strains of *C. tropicalis* in this study produced blue color on CHROMagar Candida agar with 94% sensitivity and 100% specificity. Other studies with nearby results done by Willinger and Manafi[33] showed sensitivity of 66.7% and specificity of 99.8% on chrome agar, Peng et al. [31] showed 100% sensitivity and 78.8%

specificity for *C. tropicalis*, while Yucesoy et al.[30] showed 97% sensitivity and 100% specificity on chrome agar.

The present study had one isolate of *C. dubliniensis* showed green colonies similar to *C. albicans* and misidentified with it. Thus for *C. dubliniensis*, CHROMagar Candida agar showed low sensitivity and specificity. It has been suggested that dark green coloration might be taken as an indication of the presence of *C. dubliniensis* but could not be used as criteria for identification. *Pfaller et al.* and Jabra et al. suggested the same color for *C. dubliniensis* [34, 35]. As the number of isolates of *C. dubliniensis* studied is low, it is difficult to conclude that dark green color on CHROMagar Candida agar can be used as a marker for its identification. The restriction enzyme MspI does not distinguish between C. albicans and C. dubliniensis that produce similar sizes of DNA fragments.[8]

We observed that for those strains which could not be resolved by CHROMagar Candida agar medium alone, sensitivity increased when we used both CHROMagar Candida agar and Vitek 2 from 93 % to 97% for *C. albicans* and up to 100% for some of Candida species Thus Vitek 2 could be an additional help in those situations where CHROMagar Candida agar could not give correct identification. [36] However there were certain limitations in our study such as there was only one isolate of *Candida dublinensis* which is difficult to distinguish from *Candida albicans* on CHROMagar Candida agar and few isolates of *C. krusei, C. parapsilosis* and *C. guilliermondii* and no isolate of *C.lusitaniae ,C. kefyr, C. rugosa* and *C. famata* for comparison.

In vitro susceptibility data of 67 Candida isolates against amphotericin B, fluconazole and voriconazole was detemined by the disc diffusion and Vitek 2 Yeast susceptibility cards. All Candida isolates were susceptible to amphotericin B by vitek 2, except for 4 isolates, 1 *C. tropicalis*, 2 *C. glabrata* and 1 *C. Krusei*. Susceptibility to fluconazole was similar to that seen in other major surveillance studies reported from Europe and the USA[7,37]. In our isolates, with the exception of *C. krusei* which are resistant to fluconazole (100%), the other Candida species showed an increase in resistance especially with *C. glabrata* (62%) and *C. tropicalis* (25%), Ostrosky-Zeichner et al. [37] found that 34% of *C. krusei* bloodstream isolates were resistant to fluconazole, which is different to what we observed in our study but this may be due to low number of *C. krusei* isolates, Interestingly, most of Candida species isolates exhibiting resistance to fluconazole were not resistant to voriconazole which showed overall resistance about 13% . Hence voriconazole, due to its wider species coverage, could be used in treatment of cases caused by fluconazole-resistant strains.

CONCLUSION

CHROMagar Candida agar supported by Vitek 2 system is a valuable method for identification of common Candida species, these methods are easy to interpret and give rapid results in comparison with the expensive PCR-RFLP method. Although amphotericin B and fluconazole are widely used in clinical practice, there was no evidence of enhanced resistance. Moreover, voriconazole could be used in treatment of fluconazole-resistant Candida species

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