ORIGINAL ARTICLE

Speciation and Antifungal Susceptibility of Clinical Non-albicans *Candida* isolated from Patients in a Tertiary Care Centre in Egypt

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ABSTRACT

Background: *There has been a significant rise in morbidity and mortality due to infections caused by Candida spp. with an upsurge in the incidence of non-albicans Candida (NAC), where the clinical outcome is affected by their decreased susceptibility to azoles. Accurate identification is an important step that leads to selecting a suitable antifungal agent.* ***Objectives:*** *This study aimed to identify the main NAC clinical spp. in the hospital setting and to determine the antifungal susceptibility.* ***Methodology:*** *133 isolates of Candida were identified by culture on Sabouraud Dextrose Agar, Gram stain, and germ tube test. Isolates were then subcultured on HiCrome chromogenic agar medium, and identification was confirmed using Vitek 2 automated compact system. NAC isolates antifungal susceptibility was detected using disk diffusion method and Vitek 2 compact system.* ***Results:*** *The majority of the Candida isolates (75%) were identified as NAC spp. with C. glabrata predominance (96%). HiCrome showed a sensitivity of 97% compared to Vitek 2 system. Disk diffusion showed high sensitivity when tested with amphotericin B, fluconazole, voriconazole and caspofungin of 100%, 95.2%, 100%, and 92.86% respectively compared to Vitek 2.* ***Conclusion:*** *This study confirms the substantial shift in Candida spp. from albicans to NAC. HiCrome chromogenic agar medium could be used as a rapid method for identification of the common NAC spp. Disk diffusion represents a reliable alternative method for routine antifungal susceptibly testing in the absence of automated systems.*

INTRODUCTION

The incidence and burden of fungal infections are increasing worldwide. They are of great concern to clinicians as they are associated with high morbidity and mortality, mainly in critically ill and immunocompromised patients. Invasive Candida infections are usually acquired in hospitals. The predominant *Candida spp.* isolated from patients with invasive candidiasis worldwide was *C. albicans*[1]. However, new threats have emerged in recent decades leading to the increased incidence of non-albicans *Candida* spp. (*NAC*) infections. The most commonly reported *NAC* spp. include *C. glabrata, C. tropicalis, C. parapsilosis and C. krusei*[2]. These species together with *C. albicans*, are responsible for more than 90% of cases of invasive candidiasis[3]. The frequency of each type was influenced by geographic differences between countries, regional hospital epidemiology, different units within the same hospital, underlying patient characteristics, and antimicrobial agents used[4].

The importance of *NAC* spp. clinically is due to their antifungal resistance potentiality, which may cause failure of treatment and deterioration in patients' outcomes. Commonly used antifungal agents differ in susceptibility patterns according to *Candida* spp. Drug resistance has risen in recent decades because of the increased usage of empirical antifungal agents. Therefore, *Candida* spp. isolation, identification, characterization, and antifungal susceptibility testing have become crucial for the treatment of fungal infections. Chromogenic agar medium is a simple, rapid and reliable tool for isolating and identifying *Candida* spp. overcoming limitations of conventional methods[5].

Broth microdilution is the standard approved reference assay for determining antifungal susceptibility. Nevertheless, this method is not practical in all clinical laboratories due to the high cost and increased turnaround time. Disk diffusion (DD) antifungal susceptibility test is an alternative, simple, rapid, inexpensive, and accurate method[6]. Echinocandin, a class of antifungals with milder side effects compared to polyenes and azoles, are fungicidal against many *Candida* spp. They are recommended for the initial management of invasive infections caused by *Candida* spp. according to the current clinical guidelines[7].

This study aimed to generate data on the frequency of various *NAC* spp. from various clinical samples, characterizing them to the species level, and determining their antifungal susceptibility.

METHODOLOGY

This prospective case-control study was conducted from October 2020 to April 2022. A total number of 133 clinical isolates including 100 *NAC* isolates were gathered for the study from Theodor Bilharz Research Institute's (TBRI) Microbiology Department. According to microbiological guidelines, various clinical specimens were taken from patients admitted to TBRI outpatient clinics or those hospitalized in TBRI hospital from various departments, including urology, nephrology, internal medicine, surgery, and intensive care units (ICUs). These specimens were rapidly delivered to the Microbiology laboratory of TBRI for further processing. Identification and speciation of isolates were done by Vitek-2 system, which was taken as the gold standard method.

This Research was approved by the Ethics Committee of the Institutional Review Board (Code: MS-72-2021), Faculty of Medicine, Cairo University and Theodor Bilharz Institute (Code FWA 00010609).

Isolation and identification of *Candida* spp.:

Sabouraud dextrose agar (SDA) (Oxoid, UK) was used for inoculation of clinical specimens and then incubated at 37 °C for 24-72 h. The colonies were examined microscopically after Gram staining. Identified *Candida* isolates were further categorized to the species level by the standard protocol that includes germ tube test (GTT) and Vitek 2 compact system.

Primary identification of isolates to species level:

GTT was used to categorize *Candida* isolates into *C. albicans* and *NAC* spp. It is positive for *C. albicans* and *C. dubliniensis* and negative for other species[8].

Primary identification by chromogenic agar medium:

Further species identification to *C. albicans*, *C. tropicalis*, *C. krusei*, and other species was done using chromogenic media HiCrome™ *Candida* Differential agar (HiMedia, Mumbai, India) and incubated aerobically at 30 °C for 48 hours. The colony color was recorded and interpreted following the manufacturer's instructions.

Confirmation by Vitek-2 system:

All Isolates were further tested by the automated Vitek 2 compact system (bioMerieux, France) for their identification and testing of their antifungal susceptibility.

Antifungal susceptibility test:

Identified *NAC* isolates were tested for their antifungal susceptibility by disc diffusion (DD) method against 4 antifungal agents namely: fluconazole, voriconazole, amphotericin B and caspofungin. Disk diffusion was not applied to *C. glabrata* as there were no available reference breakpoints for it in CLSI documents. The Vitek-2 system was used to determine the minimal inhibitory concentration (MIC) for clinically relevant *Candida* spp. using the following antifungal agents; amphotericin B, fluconazole, flucytosine, micafungin, caspofungin and voriconazole[9].

RESULTS

Out of the 100 *NAC* clinical isolates, 59 % were from females and 41% from male patients. The majority of the specimens (43%) were from the ICUs, followed by outpatients (24%), internal medicine (15%), nephrology (9%), surgery (5%) and urology (4%) departments as shown in figure (1).

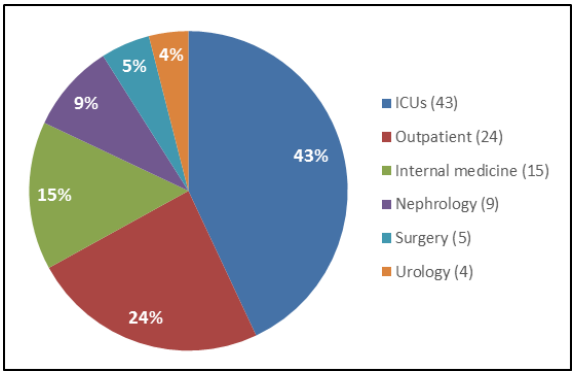

Fig. 1: Different Departments as sources of clinical specimens with *NAC* spp.

Urine was the predominant specimen from all departments displaying *NAC* spp.

Speciation of *Candida* isolates

GTT was done for all 133 isolates, 41 were germ tube positive and 92 were germ tube negative.

Confirmation of species identification was done using Vitek 2 compact system and revealed that *C. glabrata* was the main isolated species. From the 33 *C. albicans* isolates identified by the HiCrome differential agar medium and Vitek 2 system, 29 (87.88%) were positive by GTT, while four isolates (12.12%) showed false negative GTT results. Whereas 88 (88%) of the 100 *NAC* isolates identified by Vitek 2 showed negative GTT result, while 12 (12%) showed false positive results. Thus, GTT showed an overall sensitivity of 87.88%, specificity of 88%, PPV of 70.73%, NPV of 95.65% and accuracy of 87.97% as shown in table (1).

Table 1: Statistical analysis of the performance of the germ tube test in comparison to Vitek 2 system results

		Vitek 2 system		Sensitivity	Specificity	PPV	NPV	Accuracy
Germ tube		***Positive (C. albicans) (n= 33)***	***Negative (C. non- albicans) (n= 100)***	87.88%	88.0%	70.73%	95.65%	87.97%
	Positive	29 (87.88%)	12 (12%)					
	Negative	4 (12.12%)	88 (88%)					

Data are expressed as number (%)

Subculturing on HiCrome agar showed different colors with respect to the diverse species of *Candida* as shown in Figure (2). All the isolates that didn't show filamentous growth and yielded different colours other than *C. albicans* (green colour) were considered *NAC* spp.

For *C. glabrata*, 65 isolates (95.59%) produced the white creamy expected color while 3 (4.4%) isolates produced pale purple color. For the three *C. parapsilosis* isolates, two isolates produced the white expected colour (66.7%) while 1 isolate (33.3%) produced a pale purple colour. All 18 *C. tropicalis* isolates (100%) and 11 *C. krusei* isolates (100%) showed the expected reference colour (Blue for *C. tropicalis* and purple for *C. krusei*). Thus, HiCrome differential agar medium showed an overall sensitivity of 97% in case of *NAC* spp. Figure (2)

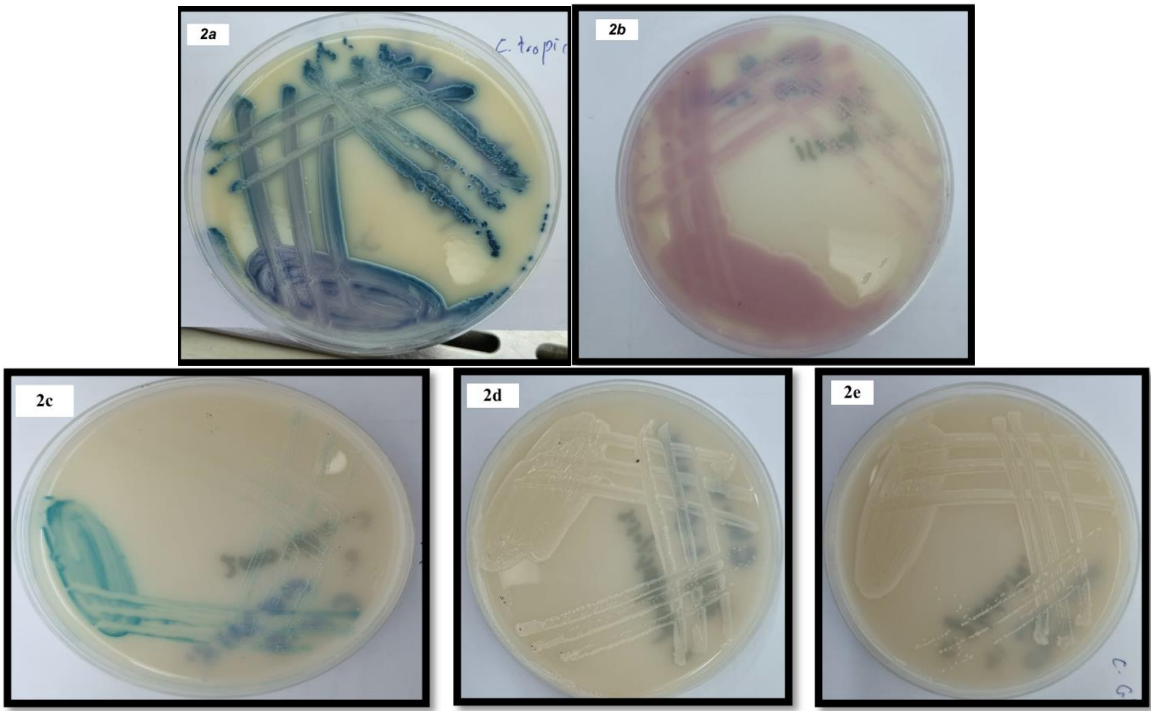

Fig. 2a: *C. tropicalis* showing blue to purple colonies, **b:** *C. krusei* showing fuzzy purple colonies, **c:** *C. albicans* showing green colonies, **e:** *C. parapsilosis* or *C. glabrata* showing white colonies on HiCrome agar

Only *NAC* spp. were included in further antifungal susceptibility using Vitek 2 compact system to detect the MIC of six antifungal agents; amphotericin B, micafungin, fluconazole, voriconazole, caspofungin, and flucytosine. The interpretive reference breakpoints of MIC were according to recent CLSI guidelines[10].

Among the six antifungal agents tested by Vitek 2, all *NAC* spp. isolates were susceptible to amphotericin B, voriconazole and micafungin. Both *C. krusei* and *C. glabrata* show intrinsic resistance to fluconazole[11]. The highest susceptibility was shown to voriconazole, amphotericin and micafungin, where all *NAC* isolates were sensitive. Among the *NAC* spp., 20% were susceptible to fluconazole and resistance in 1%. Whereas 79% of the isolates (including *C. glabrata* and *C. krusei*) had intrinsic resistance to fluconazole. In caspofungin, more resistance was shown in *C. glabrata* (69.1%) and in *C. parapsilosis* (33.3%); whereas no resistance was observed for *C. tropicalis* and *C. krusei*. For flucytosine, 88% were sensitive, while 12% were resistant as shown in Table (2).

Table 2: Overall resistance rate against the tested antifungal agents among different *NAC* isolates included in the study by Vitek 2 system

Species of *NAC*(N)	Fluconazole N (%)	Voriconazole N (%)	Caspofungin N (%)	AmphotericinB N (%)	Micafungin N (%)	Flucytosine N (%)
C. glabrata, *N*=68	Intrinsic resistance	0 (0%)	47 (69.1%)	0 (0%)	0 (0%)	0 (0%)
C. tropicalis, *N*=18	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	1 (5.6%)
C. krusei, *N=11*	Intrinsic resistance	0 (0%)	0 (0%)	0 (0%)	0 (0%)	11 (100%)
C. parapsilosis, *N*=3	1 (33.3%)	0 (0%)	1 (33.3%)	0 (0%)	0 (0%)	0 (0%)

Disk diffusion was done using four antifungal drugs; amphotericin B, flucytosine, voriconazole and caspofungin following the reference breakpoints of CLSI[10]. All of the 11 collected *C. krusei* isolates were susceptible to amphotericin B and voriconazole while six isolates were susceptible to caspofungin and five isolates showed intermediate resistance. Fluconazole was not tested on *C. krusei* as it is intrinsically resistant to it as shown in Figure (3).

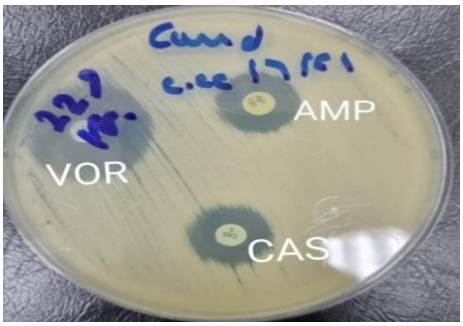

Fig. 3: *C. krusei* showing sensitivity to voriconazole (VOR) and amphotericin B (AMP) and intermediate sensitivity results to caspofungin (CAS).

All of the 18 *C. tropicalis* isolates were sensitive to amphotericin B, fluconazole, voriconazole and caspofungin as shown in Figure (4).

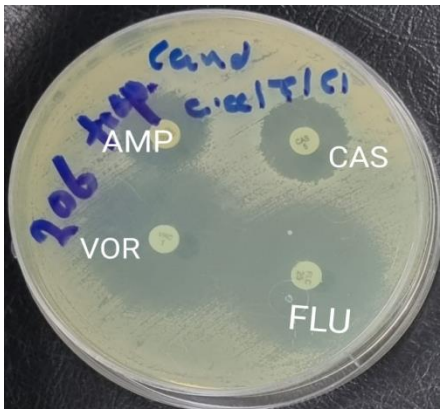

Fig. 4: *C. tropicalis* showing sensitivity to voriconazole (VOR) and amphotericin B (AMP), caspofungin (CAS) and fluconazole (FLU)

All three isolates of *C. parapsilosis* isolates were sensitive to amphotericin, flucytosine, voriconazole and caspofungin as shown in Figure (5).

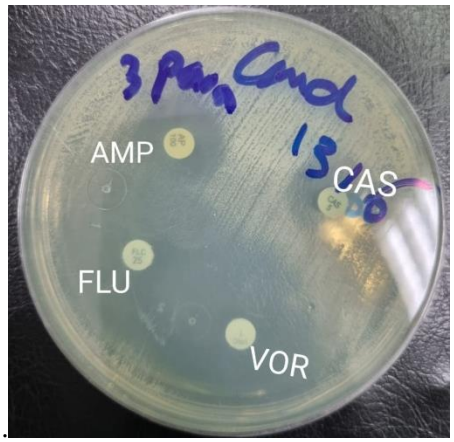

Fig. 5: *C. parapsilosis* showing sensitivity to voriconazole (VOR) and amphotericin B (AMP), caspofungin (CAS) and fluconazole (FLU)

All the 32 *NAC* isolates tested by DD and Vitek 2 compact system for susceptibility to Amphotericin B and voriconazole showed 100% sensitivity and specificity. Regarding different tested *NAC* spp. susceptibility to fluconazole, all of the *C. tropicalis* results were sensitive by DD to fluconazole and by Vitek 2 with sensitivity of 100%. However, one isolate out of the 3 *C. parapsilosis* isolates was sensitive to fluconazole by DD and was resistant by Vitek 2, the other 2 isolates were sensitive by DD which is consistent with Vitek 2 results with sensitivity of 75%.

Disk diffusion result of caspofungin for *C.krusei* showed that of the 11 tested isolates, 6 were sensitive and 3 were non-susceptible which is consistent with the Vitek 2 results. While 2 isolates were non-susceptible by DD and susceptible by the Vitek 2.

For *C. tropicalis* all the 18 isolates were sensitive by DD as with the Vitek 2 system. For *C. parapsilosis*, 2 of the 3 collected isolates were sensitive by disc diffusion and by the Vitek 2 results, while one isolate was sensitive by disc diffusion but resistant by the Vitek 2.

Among 32 isolates sensitive to caspofungin by disc diffusion; 26 of them were also sensitive by Vitek 2 system, one isolate was resistant by disc diffusion but sensitive by Vitek 2 system, and 2 were non-susceptible by disc diffusion but sensitive by Vitek 2. Three isolates were non-susceptible by both DD and Vitek 2. These results showed that disc diffusion testing for caspofungin has sensitivity of 92.86%, specificity of 75%, PPV of 96.3 %, NPV of 60% and accuracy of 90.63%. The measurement of agreement (Kappa) is 0.613 showing substantial agreement.

DISCUSSION

Over the past years, the frequency of mycotic infections has gradually increased. *Candida* spp. is among the most prevalent fungal pathogens[12]. Recently, the shift from *C. albicans* dominance to non-albicans together with the worldwide reports of increased resistance to antifungals as fluconazole and echinocandins especially with *NAC* are alarming and call for prompt antifungal stewardship. However, in countries with low income and limited resources, laboratories usually limit the diagnosis to *C. albicans* or *NAC* according to results of GTT to reduce financial burden[11].

The present study aimed to identify and determine the antifungal susceptibility profiles of different *NAC* from clinical infections.

In this study, *NAC* species (n=100) were the predominant representing 75.2 % compared to *C. albicans* (n=33) (24.8%). *NAC* isolates were isolated mainly from female patients (59%), whereas only (41%) were from male patients. This was similar to a study done by *Amar et al.* [13], who isolated *Candida* species more from females (60.2%) than male patients (39.8%). Also, *Istalingam et al.,*[14] found that *Candida* infection was more prevalent in females (59%) than males (41%). The reason for higher *Candida* isolates in females could be due to vaginal yeast infection, pregnancy, and prolonged antibiotic therapy.

In our work, 133 *Candida* isolates were isolated from different patients admitted to TBRI inpatient and outpatient clinics. *NAC* candiduria prevalence was high where nearly half of the isolates were isolated from urine (53%). Our results were consistent with other studies that reported that *NAC* spp. has now replaced *C. albicans* as the predominant pathogen in nosocomial UTIs, suggesting a better adaptation to the urinary tract environment[15].

The *NAC* frequency in our study (75.2%) is in line with other studies in Egypt; as in *El-Kholy et al.,*[16] where *NAC* (66.7 %), were predominant compared to *C. albicans* (33.3%). Also, *Mokhtar et al.,*[17] found that among all *Candida* spp. recovered from females with frequent vulvovaginal candidiasis, *NAC* accounted for 56%.

However, our finding contradicted the earlier report of *El-Ganiny et al.,*[18] from Egypt who stated that *C. albicans* was the main prevalent spp. (57.4%). Such discrepancy may be because of geographical variations and different antifungal regimens usage among different countries and different regional hospitals.

In our study, *C. glabrata* ranked first before *C. albicans* with a prevalence rate of 51.1%, *C. albicans* (24.8%), *C. tropicalis* (13.5%), *C. krusei* (8.2%) and *C. parapsilosis* (2.25%) showing a high incidence of *C. glabrata* in comparison to other species.

Our observation is as that of *Okungbowa et al.,*[19] who reported *C. glabrata* as the most common *Candida* spp. from symptomatic individuals in Nigerian cities. Whereas in a former Egyptian study by *Abass et al.,*[20] *C. krusei* was the most common isolate (18.5%) from ICU patients, followed by *C. parapsilosis* (20%), *C. tropicalis* (16%), and *C. glabrata* (10%). Another study done in Egypt stated that *C. krusei* was the second after *C. albicans* with a prevalence of about 20%, followed by *C. tropicalis* and *C. parapsilosis*[18].

The change in spp. distribution is due to different factors such as different geographical regions, hospital factors, specimen sources, and antifungal therapy[7].

The GTT is the most used conventional technique for *Candida* spp. identification. On comparing the GTT results to the results of Vitek 2 compact system as a gold standard; Our results showed a sensitivity of 87.88%, specificity of 88%, PPV of 70.73%, NPV of 95.65% and Accuracy of 87.97%, it also showed measurement of agreement (Kappa) showing a substantial agreement with the Vitek 2 results. This was in line with *Bhaskaran et al.,*[21] who reported sensitivity and specificity of 88% and 100% respectively. However, germ tube positive result was not adequate for all *C. albicans* identification as 5-10% of *C. albicans* do not form germ tubes. In addition, there are reports indicating that other species of *NAC*, such as *C. tropicalis* and *C. parapsilosis* may give false positive results[22]. GTT is easy and inexpensive, and it is a suitable rapid diagnostic test for routine activity. However, GTT is subjective and is prone to personal errors[23].

HiCrome agar is a readily accessible chromogen-based culture medium that has been evaluated in this work. Although colours of some *Candida* strains were doubtful and some spp. couldn't be identified, this did not detract from its efficiency in the differentiation of *Candida* spp. where it showed 97% concordance in comparison to Vitek 2 system. The agreement between the HiCrome differential agar medium and the Vitek 2 system was 95.5 for *C. glabrata*, 100% for *C. tropicalis*, 100% for *C. krusei* and 66.6% for C. *parapsilosis*.

This was similar to a study in India that confirmed the respectable performance of chromogenic

"HiCrome" agar[14]. A study done by *Bhaskaran* and his colleagues has shown a high degree of precision of HiCrome agar in the discrimination of diverse *Candida* spp. where the sensitivity and specificity of HiCrome agar in comparison with the molecular method was 91.75% and 100% respectively[21]. Moreover, a prior study from Egypt agreed with our results and stated that using Chromogenic media and Vitek 2 compact system demonstrated similar results in identifying NAC spp[16].

Swati and Baveja,[24] stated that HiCrome *Candida* differential agar presents a convenient and rapid method for the identification of *Candida* spp.

In our study, the sensitivity of *NAC* was tested against six antifungal drugs by Vitek-2. *NAC* spp. were found 100 % susceptible to amphotericin B, voriconazole and micafungin, followed by 88% susceptibility to flucytosine. In addition, 40% of isolates were susceptible to caspofungin, whereas 20 % were susceptible to fluconazole. Exposure to azoles continuously, along with infection with *Candida* strains which are intrinsically resistant to azole drugs such as *C. krusei*, has an important role in the acquisition of antifungal resistance. Voriconazole and fluconazole were the azole group drugs used in this study. Voriconazole showed excellent sensitivity of 100 % which was similar to findings in Turkey[25] and Egypt[17]. However, about half of the isolates (56%) were non-susceptible to voriconazole in India[26].

Regarding NAC sensitivity to fluconazole, similar findings were published in a study from India[27]. The increased resistance to fluconazole is of serious concern as it is the most used azole for superficial and deep candidiasis[28].

The antifungal drug, Amphotericin B, has been available for over 40 years and remains the most effective drug for the treatment of most systemic and visceral fungal infections[29]. Similar to our results, a study from Turkey found that all of the strains were sensitive to amphotericin B except for one *C. parapsilosis*[30]. Also, *Yüksekkaya et al.,*[31] reported that no amphotericin B-resistant strain in 56 *Candida* spp.

Alternatives as echinocandins including caspofungin, micafungin, and anidulafungin were used because of the increased incidence of infusion-related toxicity and nephrotoxicity with amphotericin B and the fluconazole-resistant strains emergence[29]. *Demir et al.,*[32] performed a systematic review and meta-analysis of the randomized controlled trials evaluating antifungal drugs and found that echinocandins are the most effective choice with sensitivity of 98%. In our study micafungin; which was licensed as a first-line treatment for invasive candidiasis; showed 100% sensitivity for *NAC* which was similar to a study done in Italy[33].

In our study, *NAC* spp. exhibited 48 % resistance to caspofungin, and in *C.glabrata* only 17.6% were sensitive and 13% were intermediately resistant and 69% were resistant to caspofungin. This is in line with *Spreghini et al.,*[34] from Italy, which showed that micafungin activity exceeds that of caspofungin in terms of MICs in the treatment of *C. glabrata*. Also, *Montagna et al.,*[33] from Italy stated that *C. glabrata* showed high resistance of 44% to caspofungin.

Our study showed 88 % sensitivity to flucytosine, which was similar to 88.5% by *Vinodkumar et al.,* from India[35]. Also, this was similar to *Seyoum et al.,* from Ethiopia[36], who observed that 86.2% of the tested *Candida* isolates showed susceptibility to flucytosine by Vitek 2 system.

Concerning disk diffusion results, *C. tropicalis* showed no resistance to amphotericin B or micafungin. That was consistent with previous studies from Turkey, in which the updated profile of antifungal susceptibility of *C. tropicalis* indicated the absence of echinocandin resistance[37]. Variations in the rate of azole resistance could be due to differences in intervention strategies, including the abuse of azoles and infection control practices.

Voriconazole DD results showed excellent activity against all *Candida* spp. (100% sensitivity) which is consistent with studies from Turkey[38] and Egypt[17]. So, the use of voriconazole DD testing appears to be reliable for detecting *Candida* spp susceptibility.

On comparing the antifungal susceptibility testing results by the DD method to the Vitek 2 system results, amphotericin B and voriconazole showed a very high accuracy with a sensitivity of 100%. While the sensitivity of the DD method for fluconazole was shown to be 95.2 %. The DD for caspofungin has a sensitivity of 92.86%, specificity of 75%, PPV of 96.3 %, NPV of 60% and accuracy of 90.63% and a measurement of agreement (Kappa) showing a substantial agreement between the two methods. In our study, DD showed no errors for *C. krusei* and C. *tropicalis* testing for amphotericin B, voriconazole, fluconazole and caspofungin. There were minor errors compared to Vitek 2 results regarding the *C. parapsilosis*. Additionally, there was a strong categorical agreement between the disc DD and the Vitek 2 compact system for tested antifungal agents.

Other studies compared the DD method to other standardized methods. *Lee et al., 2009* compared the results of the DD test for voriconazole and fluconazole with the reference antifungal macrodilution susceptibility test and found that the DD can be performed quickly, simply, and cost-effectively, and is a practicable assay for the initial testing of *Candida* spp. susceptibility to these agents[39].

Also, *Negri et al.,*[40] found 71% agreement between the DD and broth microdilution methods for itraconazole and 67% for fluconazole. Thus, the DD method highly correlates with the microdilution method, making it a viable alternative to microdilution for routine susceptibility testing.

CONCLUSION

Our study confirms the predominance of *NAC* compared to *C. albicans* and ensures the inappropriate empirical usage of antifungal agents especially in invasive infections. Accurate identification of *Candida* spp. is mandatory in critical infections using the Vitek 2 compact system or HiCrome agar media, along with antifungal susceptibility testing using Vitek 2 or disc diffusion method allowing more appropriate use of antifungal agents and better clinical outcomes.

Recommendations

A larger epidemiological study should be done for *NAC* spp. detection from different regions in Egypt. The identification of *Candida* to the species level, especially in invasive candidiasis, is of great medical importance. Also, the development of antifungal stewardship programs is highly encouraged.

Conflict of interest

This manuscript has not been previously published and is not under consideration in the same or substantially similar form in any other reviewed media. I have contributed sufficiently to the project to be included as an author. To the best of my knowledge, no conflict of interest, financial or others exist. All authors have participated in the concept and design, analysis, and interpretation of data, drafting and revising of the manuscript, and they have approved the manuscript as submitted.

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Contributions:

Amr Alkholy: Supervision, Validation, Project Administration. Doaa Gamal: Supervision, Validation, Project Administration. Rania Yahia Shash: Supervision, Validation, Writing – Review & Editing. Heba M. Dahroug: Resources, Formal Analysis, Writing – Review & Editing. Basma Tarek: Conceptualization, Methodology, Investigation, Writing – Original Draft Preparation. Hoda Helmy: Resources, Formal Analysis, Review & Editing. Dalia Salem: Resources, Formal Analysis, Review & Editing. Inas El-Defrawy: Resources, Formal Analysis, Review & Editing

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