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Distribution of *Candida albicans* and non-*albicans Candida* species isolated in different clinical samples and their in vitro antifungal susceptibility profile in Ethiopia

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Abstract

Background: The spectrum of yeasts and their antifungal susceptibility profile are poorly known and treatment of fungal disease has remained empirical. The aim of this study is to determine the spectrum and antifungal susceptibility profile of yeasts particularly of *Candida* species.

Methods: A descriptive study on the composition of *Candida* species and antifungal susceptibility profile were conducted from January 2018 to September 2018. Clinical samples collected from different sites were cultured on Sabouraud dextrose agar and incubated for an appropriate time. Identification of yeast isolates and their antifungal susceptibility profile were determined by the VITEK 2 compact system. Descriptive statistics such as frequency and percentage of *Candida* species were calculated using SPSS version 20.

Results: Of 209 yeasts recovered, 104(49.8%), 90 (43.1%), 15(7.2%) were *C. albicans*, non *albicans Candida* species, and other yeasts, respectively. Among non *albicans Candida* species, *Candida krusei* was the commonest isolate. Of other yeast groups, 66.7% was represented by *Cryptococcus laurentii*. Regardless of *Candida* species identified, 85.6, 3.9, and 10.5% of the isolates were susceptible, intermediate, and resistant to fluconazole, respectively. *C. krusei* was 100% resistant to the drug. Voriconazole demonstrated the greatest antifungal activity against *Candida* isolates in which 99.4% of *Candida* isolates were susceptible. The susceptibility and the resistance rate of *Candida* isolate to both caspofungin and micafungin were the same being 96 and 4% respectively. However, micafungin was more potent than caspofungin. The susceptibility, resistant, and intermediate rates of yeasts against flucytosine were, 86.2, 6.6, and 7.2%, respectively.

Conclusions: The present study demonstrated the distribution of *Candida* species in different clinical specimens where the isolation rate of non-*albicans Candida* species was comparable to *Candida albicans*. The high resistance rate of *C. krusei* to fluconazole and flucytosine may demonstrate that the treatment of candidiasis empirically is questionable.

Keywords: *Candida albicans*, Non-*albicans Candida* species, Antifungal drugs, drug susceptibility profile of *Candida* species

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Background

Candida species are the most common cause of fungal infections, leading to a range of life-threatening invasive diseases such as blood stream candidiasis to non-life-threatening mucocutaneous candidiasis such as genitourinary candidiasis, vulvovaginal candidiasis, and oropharyngeal candidiasis [1]. They are also an important cause of superficial mycosis such as onychomycosis. Among fungal infections, invasive candidiasis is commonly associated with high morbidity and mortality rate. For example, *Candida* species are among the top ten pathogens causing bloodstream infections [1], resulting in increased death rate, patient hospitalization, and healthcare costs [2]. Mucocutaneous candidiasis is one of the indirect signs for cell-mediated immunodeficiency and estimated to have more than 90% positive predictive value for invasive candidiasis [3].

Until recently, *C. albicans* was recognized as the commonest species causing most of the cases of candidiasis. However, in the last few decades, several studies reported that there has been a progressive shift from a predominance of *C. albicans* to non-*albicans Candida* species (NAC) such as *C. tropicalis*, *C. glabrata* and *C. krusei* [4–6]. NAC species have been reported to be a major cause of fungal opportunistic infection [7, 8]. An increase in opportunistic fungal infections is the result of an increase in the number of immune-compromised patients. Excessive use of broad-spectrum antibiotics, metabolic disorders, and the emergence of AIDS are among the various contributing factors for an increase in opportunistic fungal infections [9–12].

Development of resistance to azoles, the treatment of choice for fungal infections, mainly by NAC species, differences in drug susceptibility profile among yeast isolates, and frequent isolation of emerging yeasts (i.e., NAC species) in clinical samples initiated the use of accurate species identification and in vitro susceptibility testing methods [13]. It has been recognized that many phenotypic automated and molecular yeast identification techniques as well as standardized methods for antifungal susceptibility testing are available in many laboratories. Unfortunately, in Ethiopia, even the simplest yeast diagnostic methods such as chromogenic medium and carbohydrate assimilation as well as simple antifungal susceptibility testing procedures are lacking. Consequently, the spectrum of yeasts and their antifungal susceptibility profile are poorly known and treatment of fungal disease has remained empirical. Against this background, the aim of this study is to determine the spectrum and antifungal susceptibility profile of yeasts particularly of *Candida* species recovered from different clinical samples from patients referred to Arsho Advanced Medical Laboratory for routine tests. Identification and sensitivity testing were performed by using the

VITEK 2 compact system. The VITEK 2 compact system (bioMérieux) is a fully automated system that is accurate in the identification of yeasts and evaluating their drug susceptibility profile simultaneously [14, 15].

Methods

Study population and period

A study on the composition of yeast species and antifungal susceptibility profile of yeasts recovered in culture from different clinical samples was carried out from January 2018 to September 2018 at Addis Ababa, Ethiopia.

Sample collection

A total of 776 clinical samples including throat swab (119), nail scrapings (154), sputum (142), corneal scrapings (146), and vaginal swabs (215) were collected from patients referred to Arsho advanced medical laboratory according to standard procedures related to each sample. One clinical sample per patient was collected. All clinical samples were inoculated on to Sabouraud dextrose agar (Oxoid, Basingstoke, UK) to which 50 µg/1 ml gentamicin is incorporated. Inoculated plates were incubated at a temperature of 37^o C for at least 72 h aerobically. Then yeast isolates were transferred to Brain heart infusion broth (Oxoid, Basingstoke, UK) containing 10% glycerol and transferred to the Department of Medical Laboratory Technology, College of health Sciences, Addis Ababa University and stored at minus 80^o deep freezer until use.

Identification and antifungal susceptibility testing

The viability of yeast cultures that have been stored at minus 80^o deep freezer was checked by inoculating them on to Sabouraud dextrose agar (Oxoid, Basingstoke, UK). Identification of yeast cultures and their in vitro antifungal susceptibility profile were determined by the automated VITEK 2 compact system (bioMérieux, France) using YST-21343 and AST-YS07 cards following the instruction of the manufacturer at the Ethiopian Public Health Institute situated in the same campus with the Department of Medical Laboratory Science. Briefly, inoculum suspensions for the VITEK 2 were prepared in sterile saline at turbidity equal to 2.0 McFarland standards, as measured using a DensiChek instrument (bioMérieux). The YST-21343 and AST-YS07 cards were automatically filled with the prepared culture suspension, sealed, and incubated by the VITEK 2 instrument. The cards were incubated at 35.5 °C for 18 h, and data were collected at 15-min intervals during the entire incubation period and final identification and break point minimum inhibitory concentration (MIC) values used to categories *Candida* species, susceptible, intermediate or resistant to each drug were investigated using version

7.0 software, an advanced expert system (AES) designed to evaluate the results produced by the VITEK 2 system. Identification of each isolate down to a species level assigned by the VITEK 2 compact system as excellent, very good, good, acceptable, or low- discrimination was taken as correct identification.

Quality assurance

Clinical samples were inoculated after the performance of culture media and their sterility was checked following standard procedure. A standard strain of *C. albicans* (ATCC 10231) was used to evaluate the performance of the VITEK machine.

Data analysis and interpretation

All data from the investigation were coded and analyzed using SPSS version 20. Descriptive statistics such as frequency and percentage of *Candida* species were calculated.

Ethical clearance

All ethical deliberations and responsibilities were appropriately addressed, and the study was conducted after the approval of the Internal Review Board (IRB) of the Department of Medical Laboratory Sciences (DRERC/323/18/MLS) and after permission letter was obtained from Ethiopian Public Health Institute and Arsho Advanced Medical Laboratory private limited company. Informed written consent was obtained from the study participant ahead of data collection. Each participant was given the right to refuse or to participate in the study and even to withdraw him/her from the study at any time during the course of the study. All information recorded from the study subjects were kept confidentially. An assent form was completed and signed by a family member and/or adult guardian for patients under the age of 16 years.

Results

A total of 209 yeasts were isolated from 776 different clinical samples (Table 1). Among the isolates, 84(40.2%)

Table 1 Distribution of yeast isolates per clinical samples

Clinical samples						
Species	Vaginal swab	Oropharyngeal	Nail	Eye Discharge	Sputum	Total (n, %)
<i>Candida albicans</i>	52	30	4	5	13	104
Sub-total						104(49.8)
Non-albicans Candida						
<i>C. krusei</i>	11	0	0	2	1	14(15.6)
<i>C. famata</i>	2	4	5	1	1	13(14.4)
<i>C. rugosa</i>	0	2	1	1	6	10(11.1)
<i>C. lusitaniae</i>	3	2	3	1	0	9(10)
<i>C. dubliniensis</i>	5	0	0	0	2	7(7.8)
<i>C. lipolytica</i>	2	4	0	0	0	6(6.7)
<i>C. parapsilosis</i>	2	4	0	0	0	6(6.7)
<i>C. ciferrii</i>	2	0	0	0	4	6(6.7)
<i>C. kefyr</i>	1	2	1	1	1	6(6.7)
<i>C. guilliermondii</i>	0	3	1	1	0	5(5.6)
<i>C. pelliculosa</i>	1	0	3	0	0	4(4.4)
<i>C. glabrata</i>	2	0	0	0	0	2(2.2)
<i>C. intermedia</i>	0	0	1	0	0	1(1.1)
<i>C. utilis</i>	0	1	0	0	0	1(1.1)
Sub total						90(43.1)
Other yeasts						
<i>Cryptococcus laurenti</i>	0	0		0	10	10(66.7)
<i>C. neoformans</i>		0		0	4	4(26.7)
<i>Trichosporon mucoides</i>	1	0	0	0	0	1(6.7)
Sub-total						15(7.2)
Grand Total (n, %)	84(40.2)	52(24.9)	19(9.1)	12(5.7)	42(20.1)	209

were recovered from vaginal swab while 52(24.9) were isolated from the oropharyngeal swab. The remaining 42(20.1%), 19(9.1%) and 12(5.7%) were isolated from sputum, corneal, and nail scrapings, respectively. The distribution of *Candida* with respect to clinical sample was variable where *C. albicans*, *C. famata*, and *C. kefyr* recovered in all clinical specimens. Of 209 yeasts recovered, 104(49.8%) were *C. albicans*, 90 (43.1%) were NAC species, and 15 (7.2%) were yeasts other than *Candida* species. Among NAC species, *C. krusei* 15.6%, *C. famata* 14.4%, *C. rugosa* 11.1%, and *C. lusitaniae* 10.0% were the commonest isolates. Out of yeasts other than *Candida* species *Cryptococcus laurentii* represented 66.7% of this group of yeasts.

The proportion of *C. albicans* to NAC species and the first three predominant *Candida* species in this study in comparison with earlier studies between and within countries are presented in Table 2. The three predominant *Candida* species in our study in their descending order were *C. albicans*, *C. krusei*, and *C. famata*.

The antifungal profile of *Candida* isolates

The in vitro antifungal susceptibility profile of *Candida* species to the five antifungal drugs expressed as MICs in µg/ml is shown in Table 3. Among 194 *C. albicans* and NAC isolates, the MIC values of *C. famata* to all drugs tested and *C. ciferrii* to echinocandins were not determined by the VITEK. Regardless of *Candida* species

identified, 85.6% were susceptible while 10% were resistant to fluconazole. The remaining 3.9% were intermediate to the agent. Hundred percent of *C. krusei*, 20% *C. rugosa*, 25% of *C. pelliculosa*, 17% *C. lipolytica* and 17% *C. ciferrii* demonstrated resistant to the agent. Furthermore, 2% *C. albicans*, 28.6% *C. dubliniensis*, 10% of *C. rugosa*, 17% *C. lipolytica*, and 17% *C. ciferrii* were intermediate to fluconazole. Voriconazole showed the greatest antifungal activity against *Candida* isolates in which all *Candida* isolates (99.4%) exhibiting susceptibility while one (0.6%) isolate of *C. ciferrii* was found out to be resistant. The susceptibility and the resistance rate of *Candida* isolates to both caspofungin and micafungin were the same being 96%). Four isolates of *C. albicans*, two isolates of *C. rugosa* and one isolate of *C. lipolytica* demonstrated resistance to the drugs. Similarly, 86.2% of yeast isolates were susceptible to flucytosine, 6.6% were resistant while 7.2% were intermediate to it. One isolate of *C. albicans* and 11 isolates of *C. krusei* were resistant to the drug.

Break points of different antifungal drugs against *Candida* Isolates are shown in Table 4. The MIC values of fluconazole, voriconazole, caspofungin, micafungin and flucytosine against *C. albicans* were variable. Relatively, higher fluconazole MICs were found in NAC species than *C. albicans*. All Isolates of *C. krusei* were resistant to fluconazole at a concentration of ≥ 16 µg/ml. The potency of micafungin was better than that of caspofungin.

Table 2 The proportion of *C. albicans* to NAC species and the first 3 dominant *Candida* species isolated in the current study compared to earlier studies between and with countries

No, isolates	<i>C. albicans</i> , n (%)	NAC n (%)	Clinical sample	Country	Dominate species	Reference
194*	104(49.8%)	90 (43.1%)	Various	Ethiopia	<i>C. albicans</i> , <i>C. krusei</i> , <i>C. fabata</i>	*
177	139(78.5)	38(21.5)	Oral (HIV patients)	Ethiopia	<i>C. albicans</i> , <i>C. glabrata</i> , <i>C. tropicalis</i>	Mulu et al, 2013 [16]
81	51(58.6)	30(41.4)	Vaginal swab	Ethiopia	<i>C. albicans</i> , <i>C. krusei</i> , <i>C. dubliniensis</i>	Bitew and Abebaw, 2018 [17]
63	38(60.3)	25(39.7)	Vaginal swab	Egypt	<i>C. albicans</i> , <i>C. glabrata</i> , <i>C. krusei</i>	Elfeky et al, 2016 [18]
111	44(39.6)	67(60.4)	Various	India	<i>C. albicans</i> , <i>C. krusei</i> , <i>C. tropicalis</i>	Mohandas & Balla 2011 [19]
90	33(36.7)	57(63.3)	Various	India	<i>C. albicans</i> , <i>C. tropicalis</i> , <i>C. parapsilosis</i>	Das et al 2016 [20]
102	37(36.3)	65(63.7)	Various	India	<i>C. tropicalis</i> , <i>C. albicans</i> , <i>C. guilliermondii</i>	Sida et al, 2017 [21]
250	154(61.6)	96(38.4)	Oral cavity	Thailand	<i>C. albicans</i> , <i>C. glabrata</i> , <i>C. tropicalis</i>	Muadcheingka&Tantivitayakul, 2015 [22]
108	61(56.5)	47(43.5)	Blood	Taiwan	<i>C. albicans</i> , <i>C. glabrata</i> , <i>C. tropicalis</i>	Chi et al,2011 [23]
90	33(36.7)	57(63.3)	Various	India	<i>C. tropicalis</i> , <i>C. albicans</i> , <i>C. glabrata</i>	Kaur et al, 2016 [24]
103	80(77.8)	23(22.3)	Oral (HIV patients)	Brazil	<i>C. albicans</i> , <i>C. tropicalis</i> , <i>C. parapsilosis</i>	Ribeiro, et al, 2015 [25]
1062	573(54)	489 (46)	Various	Germany & Austria	<i>C. albicans</i> , <i>C. glabrata</i> , <i>C. parapsilosis</i>	Schmalreck et al, 2012 [26]
580	420(72.4)	160(27.6)	Vaginal swab	America	<i>C. albicans</i> , <i>C. glabrata</i> , <i>C. parapsilosis</i>	Richter et al, 2005 [27]
428	273(63.8)	155(36.2)	Various	Iran	<i>C. albicans</i> , <i>C. tropicalis</i> , <i>C. parapsilosis</i>	Badiee et al, 2011 [28]
103	43(42)	60(58)	Blood	Latin America	<i>C. albicans</i> , <i>C. tropicalis</i> , <i>C. parapsilosis</i>	Godoy et al, 2003 [29]

* = current study

Table 3 Over all percentage antifungal drug profile of *Candida* species

Species N = 194	Fluconazole			Voriconazole			Caspofungin			Micafungin			FLu cytosine		
	S	R	I	S	R	I	S	R	I	S	R	I	S	R	I
<i>C. albicans</i> (104)	98 (102)	0	2(2)	100(104)	0	0	96.2(100)	3.8(4)	0	96.2(100)	3.8(4)	0	96(100)	1(1)	3(3)
<i>C. krusei</i> (14)	0	14	0	100 (14)	0	0	100(14)	0	0	100(14)	0	0	21.4 (3)	78.6 (11)	0
<i>C. famata</i> (13)	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*
<i>C. rugosa</i> (10)	70 (7)	20(2)	10(1)	100(10)	0	0	80(8)	20(2)	0	80(8)	20(2)	0	50 (5)	0	50(5)
<i>C. lusitanae</i> (9)	100(9)	0	0	100(9)	0	0	100 (9)	0	0	100(9)	0	0	100(9)	0	0
<i>C. dubliniensis</i> (7)	71.4(5)	0	28.6(2)	100(7)	0	0	100(7)	0	0	100(7)	0	0	71.4(5)	0	28.6(2)
<i>C. lipolytica</i> (6)	66(4)	17(1)	17(1)	100(6)	0	0	83.3(5)	16.7(1)	0	83.3(5)	16.7(1)	0	66.7 (4)	0	33.3(2)
<i>C. parapsilosis</i> (6)	100(6)	0	0	100(6)	0	0	100(6)	0	0	100(6)	0	0	100(6)	0	0
<i>C. ciferrii</i> (6)	66(4)	17(1)	17(1)	83(5)	17(1)	0	*	*	*	*	*	*	83.3(5)	0	16.7(1)
<i>C. kefyr</i> (6)	100 (6)	0	0	100(6)	0	0	100(6)	0	0	100(6)	0	0	100(6)	0	0
<i>C. guilliermondii</i> (5)	100(5)	0	0	100(5)	0	0	100(5)	0	0	100(5)	0	0	100(5)	0	0
<i>C. pelliculosa</i> (4)	75(3)	25(1)	0	100(4)	0	0	100(4)	0	0	100(4)	0	0	100(4)	0	0
<i>C. glabrata</i> (2)	100(2)	0	0	100(2)	0	0	100(2)	0	0	100(2)	0	0	100(2)	0	0
<i>C. intermedia</i> (1)	100(1)	0	0	100(1)	0	0	100(1)	0	0	100(1)	0	0	100(1)	0	0
<i>C. utilis</i> (1)	100(1)	0	0	100(1)	0	0	100(1)	0	0	100(1)	0	0	100(1)	0	0
Total yeast tested	155	19	7	180	1	0	168	7	0	168	7	0	156	12	13
%	85.6	10.5	3.9	99.4	0.6	0	96	4	0	96	4	0	86.2	6.6	7.2

* = MIC values were not provided by the machine

Most isolates were susceptible to micafungin at the lowest dilution drug tested (0.06 µg/ml).

Discussion

Rapid and accurate identification of *Candida* species down to the species level is of great importance for the selection of appropriate antifungal agents and for patient management. In the present study, 18 different species of yeasts comprising of 15 *Candida* species and three yeasts other than *Candida* species were identified. While *Candida albicans* was the most frequently isolated yeast in the present study, its prevalence was lower than a study conducted in Northwest Ethiopia [16] and higher than a study conducted in central Ethiopia [17]. *Candida albicans* as the most frequently isolated species was also reported by Sasso et al [30], Mnge et al [31], and Zeng et al [32]. *C. parapsilosis* as the most frequently isolated yeast was depicted in a study conducted by Sahal and Bilkay [33].

A preponderance of *C. albicans* compared with NAC species varies between and with countries. A preponderance of *C. albicans* was observed in our study. Our finding was consistent with previous studies conducted elsewhere [16–18, 21–23, 25–27]. However, a preponderance of NAC species were demonstrated by many earlier studies [19, 20, 24, 28, 29]. While *C. albicans* is still the predominant species in Ethiopian studies, the occurrence of NAC species in the current study was higher than those studies [16, 17] underlining the

shifting trend of *Candida* infections towards NAC species. A shift of *Candida albicans* towards non-*albicans Candida* species observed in the present study could be due to an improved detection rate of non-*albicans Candida* species or a true prevalence change. Our result is in line with a study conducted by Ghazi et al [34] that demonstrated a shift of *Candida albicans* towards non-*albicans Candida* species in the Middle East and North Africa. In the present study, *C. krusei* was the 2nd dominant species. Our result was in line with that of Bitew and Abebaw [17] and Mohndas and Ballal [19], but in contradiction with many other studies where *C. glabrata* or *C. tropicalis* was reported as a 2nd predominant NAC species [16, 18–20, 22, 23, 25–29]. A choice of fluconazole as a first line drug for the management of candidiasis in Ethiopia [35] may be a risk factor for a higher isolation rate of *C. krusei* in this study. Treatment of fungal infections by azole antifungals such as fluconazole was suggested to enhance the selection of resistant *Candida* species by shifting infection and/or colonization to more intrinsically resistant species chiefly of *C. krusei* or *C. glabrata* [36]. The lower isolation rate of *C. glabrata* compared to *C. krusei* which is also intrinsically resistant to fluconazole in the current study is unclear. The geographical difference can be a contributing factor for differences in the distribution of NAC species is suggested by Falags et al [37]. This can be demonstrated by the fact that the prevalence of *C. glabrata* is high in America, North and Central Europe while *C. tropicalis* is

Table 4 Break points of different antifungal drugs against *Candida* Isolates

Species	Antifungal drug	Break points µg/ml		
		Susceptible	Intermediate	Resistant
<i>C. albicans</i>	Fluconazole	1–4	16–32	0
	Voriconazole	0.12–0.5	–	–
	Caspofungin	0.12–1	–	4
	Micafungin	0.06–0.5	–	4
	Flucytosine	1	8	64
<i>C. krusei</i>	Fluconazole	0	–	16
	Voriconazole	0.12–0.25	–	–
	Caspofungin	0.12–0.5	–	–
	Micafungin	0.06–0.25	–	–
	Flucytosine	1	16	64
<i>C. famata</i>	Fluconazole	*	*	*
	Voriconazole	*	*	*
	Caspofungin	*	*	*
	Micafungin	*	*	*
	Flucytosine	*	*	*
<i>C. rugosa</i>	Fluconazole	1–8	32	64
	Voriconazole	0.12	–	–
	Caspofungin	0.25	–	4
	Micafungin	0.06–0.12	–	4
	Flucytosine	1	16	–
<i>C. lusitaniae</i>	Fluconazole	1–2	–	–
	Voriconazole	0.12–0.5	–	–
	Caspofungin	0.25–1	–	–
	Micafungin	0.06–1	–	–
	Flucytosine	1	–	–
<i>C. dubliniensis</i>	Fluconazole	1–8	32	–
	Voriconazole	0.12	–	–
	Caspofungin	0.12–0.25	–	–
	Micafungin	0.06–0.25	–	–
	Flucytosine	4	8	–
<i>C. lipolytica</i>	Fluconazole	1–8	16	64
	Voriconazole	0.12	–	–
	Caspofungin	0.12–0.25	–	≥ 4
	Micafungin	0.06–0.25	–	≥ 4
	Flucytosine	8	16	–
<i>C. parapsilosis</i>	Fluconazole	1	–	–
	Voriconazole	0.12	–	–
	Caspofungin	0.12–1	–	–
	Micafungin	0.25–2	–	–
	Flucytosine	1	–	–
	Fluconazole	1–8	16	64
	Voriconazole	0.12	–	32

Table 4 Break points of different antifungal drugs against *Candida* Isolates (Continued)

Species	Antifungal drug	Break points µg/ml		
		Susceptible	Intermediate	Resistant
<i>C. ciferrii</i>	Caspofungin	*	*	*
	Micafungin	*	*	*
	Flucytosine	2	8	–
	Fluconazole	1	–	–
	Voriconazole	0.12	–	–
<i>C. kefyr</i>	Caspofungin	.025–0.5	–	–
	Micafungin	0.06.0.12	–	–
	Flucytosine	1	–	–
	Fluconazole	1–4	16	–
	Voriconazole	0.12	–	–
<i>C. guilliermondii</i>	Caspofungin	0.25–1	–	–
	Micafungin	0.06–0.5	–	–
	Flucytosine	1	–	–
	Fluconazole	2	–	64
	Voriconazole	0.12–0.5	–	–
<i>C. pelliculosa</i>	Caspofungin	0.25	–	–
	Micafungin	0.06–0.12	–	–
	Flucytosine	1–4	–	–
	Fluconazole	4	–	–
	Voriconazole	0.12	–	–
<i>C. glabrata</i>	Caspofungin	0.25	–	–
	Micafungin	0.06	–	–
	Flucytosine	1	–	–
	Fluconazole	1	–	–
	Voriconazole	0.12	–	–
<i>C. intermedia</i>	Caspofungin	0.25	–	–
	Micafungin	0.12	–	–
	Flucytosine	1	–	–
	Fluconazole	2	–	–
	Voriconazole	0.12	–	–
<i>C. utilis</i>	Caspofungin	0.25	–	–
	Micafungin	0.12	–	–
	Flucytosine	1	–	–

* = No MIC values were provided by the machine, – No resistance and/or intermediate isolate

commonly isolated in South America and Asia [37]. *C. famata* was the third predominant *Candida* species in the present study. Similar results were obtained by many previous studies [38–40]. These studies have demonstrated that strains of *C. guilliermondii*, *C. lusitaniae*, *C. fermentati*, *C. intermedia*, and *C. palmiophila* have

been reported as *C. famata* by phenotypic methods such as the VITEK 2 compact system, and such drawback of the VITEK 2 could be a possible explanation for the higher isolation rate of *C. famata* in the current study. Therefore, *Candida* species identified as *C. famata* by phenotypic methods should be confirmed by molecular methods.

In the present study, the drug sensitivity patterns of all yeast isolates were tested against five antifungal drugs. The MIC for *C. famata* to all the drugs tested and *C. ciferrii* for echinocandins were not provided by the VITEK. This could probably be due to the fact that the MIC values of the drugs against the isolates are not present in the system database and/or the VITEK 2 system may take a longer incubation period to determine MIC endpoints of the drugs against the isolates.

In our study, 85.6, 10.5, and 3.9% of *Candida* species were susceptible, resistant and, intermediated to fluconazole, respectively. In view of the overall high level of susceptibility of *Candida* species to the antifungal demonstrated that the drug is still considered to be an active drug against *Candida* species, regardless of the indiscriminate use of the agent for therapy and prevention in Ethiopia [35]. However, *C. krusei* recognized to be a naturally non-susceptible to fluconazole [41] was found out to be 100% resistant to it. Our result was compatible with our previous study [17] and another study [42] but in disagreement with the result of Elfeky et al [18] who reported that as high as 60% of the species are susceptible to the agent. Based on our in vitro drug susceptibility testing result, we suggest a search for alternative agents is essential when treating fungal infections caused by *C. krusei*. Voriconazole was the most active drug against yeast isolates among all drugs tested. All yeast isolates were 100% susceptible to the antifungal except one species of *C. ciferrii* which was resistant to the agent giving the overall percentage susceptibility of 99.4%. While inhibition of ergosterol synthesis is a common mode of action to all azole antifungal drugs [42] susceptibility difference between fluconazole and voriconazole against *C. krusei* in our study is unclear. On the other hand, contrary to the notion that *C. glabrata* is intrinsically non-susceptible to fluconazole, all isolates in our study were sensitive to all azole drugs tested. More or less similar results were reported by many studies [17, 27, 43].

In our previous study [17], the in vitro susceptibility of all yeast isolates were 100% to both micafungin and caspofungin. However, in this study a resistance rate of 4% to each drug was noted. *C. rugosa* (20%), *C. lipolytica* (16.7%), and *C. albicans* (3.8%) were the isolates where resistance strains to the two drugs were observed. While the number of isolates categorized as susceptible, intermediate, and resistance to both drugs were the same, micafungin was more potent than caspofungin supporting the

work of Ostrosky-Zeichne et al [44]. Further, while the mode of action of the two antifungals is the same that is preventing fungal cell wall synthesis by inhibiting the enzyme that synthesizes β -glucan [36], a discrepancy in potency observed in our study is not clear.

Of 181 *Candida* isolates in which MIC values provided by the machine 86.2, 6.6% and, 7.1% were susceptible, resistant, and intermediate to flucytosine, respectively. Surprisingly, about 78.6% of *C. krusei* was resistant to the drug. Percentage drug resistance noted in this study was more than two fold than from that of our previous study [17]. Regarding the drug resistance profile of yeast other than *Candida* species no MIC values were obtained for both *C. laurentii* and *T. mucoid*. *C. neoformans* was 100% susceptible to fluconazole and flucytosine, but MIC values were not obtained against the remaining drugs tested.

Although attempts were made to collect demographic data and history of each patient from request forms filled out by physicians, unfortunately, the recording of these parameters in the test request form was not consistent. Consequently, demographic data and the clinical history of each patient were not documented for this study. The mechanisms responsible for the acquisition of antifungal resistance in our clinical isolates were not determined due to resource shortage and this can be considered as another limitation of this study.

Conclusions

The present study demonstrated the distribution of *Candida* species in different clinical specimens where the isolation rate of non-*albicans Candida* species was comparable to *Candida albicans*. The high resistance rate of *C. krusei* to fluconazole and flucytosine may demonstrate that the treatment of candidiasis empirically is questionable.

Abbreviation

AES: Advanced expert system; ATCC: American Type Culture collection; DRERC: Department Research Ethical Review Committee; IRB: Internal Review Board; MIC: Minimum Inhibitory Concentration; NAC: Non- albican candida; UK: United Kingdom

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Authors' contributions

ES, has participated in culture media preparation, identification of fungal pathogens, in drafting the manuscript, have given final approval of the version to be published; analysis and interpretation of data, and agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. AB has participated in design, analysis and interpretation of data, fungal identification, write up of the manuscript, agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. AM has participated in study design, analysis and interpretation of data, agree to be accountable for all aspects of the work in

ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. All authors read and approved the final manuscript.

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Availability of data and materials

The datasets during and/or analyzed during the current study are available from the corresponding author and can be obtained up on reasonable request.

Ethics approval and consent to participate

The study was conducted after it was ethically reviewed and approved by the Ethical Review board of The Department of Medical Laboratory Sciences (DRERC) and Internal Review Board (IRB) of Arsho Advanced Medical Laboratory private limited company. Written consents were also obtained from participants. Assent form was completed and signed by a family member and/or adult guardian for participants under the age of 16 years.

Consent for publication

Not applicable as details, images and/or videos related to study subjects were not recorded for this study.

Competing interests

The authors declare that they have no competing interests.

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