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NEWER CANDIDA SPECIES IN INTENSIVE CARE UNITS OF A TERTIARY CARE TEACHING HOSPITAL AND THEIR MINIMUM INHIBITORY CONCENTRATION

Microbiology

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ABSTRACT

Background: Newer candida species like *C. auris*, *C. duobushaemulonii, C. hemolinni, C. pelliculosa, C. famata, C. lipolytica, C. rugosa and C. utilis* are being reported and are known to demonstrate reduced susceptibility to antifungal agents which may either be inherent or acquired. **Objective:** To determine the isolation of newer Candida species from ICU patients with their AFST. **Materials and Methods:** A prospective study was conducted for 1 year. 63 non-albicans Candida which were isolated from blood cultures of ICU patients, were speciated using conventional microbiological methods and automated Vitek 2® system. AFST was done on these isolates using broth microdilution method as per CLSI M27-A3 and 4th edition. **Results:** *C. pelliculosa* (10) was the most common, followed by *C. auris* (8). All the isolates of *C. auris* had an MIC of >64 µg/ml for fluconazole, 5 of them had an MIC of 0.125 µg/ml for flucytosine, 3 had an MIC of 0.25 µg/ml and 0.125 µg/ml for caspofungin and voriconazole respectively, 6 of the isolates had an MIC of 0.0313 µg/ml for posaconazole. The isolates had higher MICs of 8 µg/ml and 16 µg/ml for itraconazole. 4 and 6 *C. pelliculosa* isolates had an MIC of 0.25 µg/ml for fluconazole and flucytosine respectively, MIC of amphotericin B was 0.0625 µg/ml in 4 of those isolates, MICs of 0.015 µg/ml, 0.0313 µg/ml and 0.0625 µg/ml were present in 3 isolates each for caspofungin, 6 and 5 isolates had MIC of 0.0313 µg/ml each for posaconazole and voriconazole. 8 µg/ml was the observed MIC in 3 isolates for itraconazole. **Conclusion:** In case of Candida species where MIC break points have not been standardized by CLSI, the epidemiological cut offs can be used and treatment can be started.

KEYWORDS

Newer Candida species, MIC, Candida auris

INTRODUCTION

Although, non-albicans Candida species namely *C. tropicalis*, *C. parapsilosis*, *C. krusei* and *C. glabrata* have been increasingly isolated from cases of candidemia, newer candida species like *C. auris*, *C. duobushaemulonii, C. hemolinni, C. pelliculosa, C. famata, C. lipolytica, C. rugosa and C. utilis* are also being reported. These newer candida species have a greater propensity for being associated with healthcare associated infections (HAIs) and are multi drug resistant (MDR) (1).

They are known to demonstrate reduced susceptibility to various classes of antifungal agents which may either be inherent or acquired (2). The need of the hour is to identify these newer Candida species in clinical settings and know their antifungal susceptibility pattern to ensure appropriate therapy.

In the last 10 years, *C. auris* has emerged as a notorious healthcare associated yeast causing invasive infections with high rates of clinical treatment failure. This yeast also has a predilection for epidemic spread and is difficult to treat since it is resistant to fluconazole and shows variable susceptibility to other azoles, amphotericin B and echinocandins. Being a notifiable pathogen, it is important to contain its spread and prevent horizontal transmission in hospitals (3).

Cases of *C. pelliculosa* have been mainly observed in infants, children and immunocompromised patients, admitted in nurseries, pediatric intensive care units, hematologic and surgical units respectively (4).

C. lipolytica, is being recognized to cause sporadic cases and nosocomial clusters of human infections, especially catheter-related suppurative thrombophlebitis and fungemia associated with biofilm formation in immunocompromised or critically ill patients who are hospitalized for a long time and may have required long dwelling intravascular catheterization and broad-spectrum antibiotics (5).

C. famata has been known to cause catheter-related bloodstream infections, peritonitis, retinopathy, and mediastinitis (6). *C. haemulonii* complex, have been described to cause peritonitis, neonatal candidemia and osteitis (7).

In view of this, the study was designed to determine the isolation of these newer Candida species from ICU patients with their antifungal susceptibility testing (AFST).

MATERIALS AND METHODS

After Institutional ethics committee (IEC) approval, a prospective study was carried out during a period of 1 year, 63 non-albicans Candida (NAC) which were isolated from blood cultures of ICU patients were speciated using conventional microbiological methods and automated Vitek 2® system.

AFST was done on these isolates using broth microdilution method as per Clinical Laboratory Standard Institute (CLSI) M27-A3 (8) and 4th edition (9).

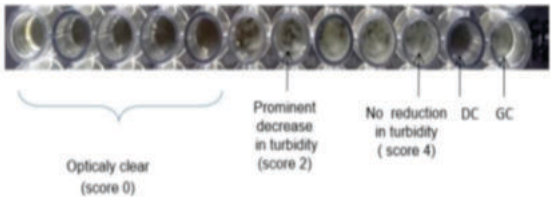

Image 1: Reading of MIC results

RESULTS

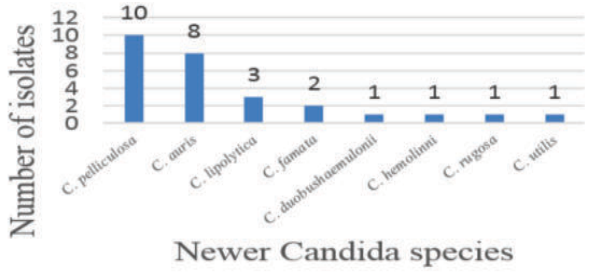

Figure 1: Distribution of newer candida species (n=27)

A total of 63 non-albicans Candida species were isolated. Out of which, 27 (42.85%) were newer candida species.

The distribution of newer candida species is as per figure 1 with *C. pelliculosa* being the most common, followed by *C. auris*.

Table I: MIC distribution of *C. auris* (n=8)

	Drug concentration (µg/ml)											
	0.015	0.0313	0.0625	0.125	0.25	0.5	1	2	4	8	16	64
Fluconazole	-	-	-	-	-	-	-	-	-	-	-	8
Flucytosine	-	-	-	5	2	-	-	-	-	-	-	1
Amphotericin B	-	-	-	-	3	1	1	1	1	1	-	-
Caspofungin	2	1	-	1	4	-	-	-	-	-	-	-
Posaconazole	-	6	1	1	-	-	-	-	-	-	-	-
Voriconazole	-	2	2	4	-	-	-	-	-	-	-	-
Itraconazole	-	-	-	-	-	-	-	-	-	4	4	-

All the isolates of *C. auris* had an MIC of >64 µg/ml for fluconazole. 5 of them had an MIC of 0.125 µg/ml for flucytosine. 3 had an MIC of 0.25 µg/ml and 0.125 µg/ml for caspofungin and voriconazole respectively. 6 of the isolates had an MIC of 0.0313 µg/ml for posaconazole. The isolates had higher MICs of 8 µg/ml and 16 µg/ml (4 each) for itraconazole (Table I).

Table II: MIC distribution of *C. pelliculosa* (n=10)

	Drug concentration (µg/ml)									
	0.015	0.0313	0.0625	0.125	0.25	0.5	1	8	16	64
Fluconazole	-	-	-	2	4	1	-	-	-	3
Flucytosine	-	-	-	2	6	1	-	-	-	1
Amphotericin B	-	-	4	2	1	2	1	-	-	-
Caspofungin	3	3	3	-	1	-	-	-	-	-
Posaconazole	-	6	2	1	-	-	1	-	-	-
Voriconazole	-	5	4	1	-	-	-	-	-	-
Itraconazole	-	-	-	1	2	2	1	3	1	-

4 and 6 *C. pelliculosa* isolates had an MIC of 0.25 µg/ml for fluconazole and flucytosine respectively. The MIC of amphotericin B was 0.0625 µg/ml in 4 of those isolates. MICs of 0.015 µg/ml, 0.0313 µg/ml and 0.0625 µg/ml were present in 3 isolates each for caspofungin. 6 and 5 isolates had MIC of 0.0313 µg/ml each for posaconazole and voriconazole. 8 µg/ml was the observed MIC in 3 isolates for itraconazole (Table II).

Table III: MIC distribution of other infrequently isolated newer Candida isolates (n=9)

Drug	C. duobushaemulonii (n=1)	C. famata (n=2)	C. haemulonii (n=1)	C. lipolytica (n=3)	C. rugosa (n=1)	C. utilis (n=1)
Fluconazole	>64	32 (1) >64 (1)	32	0.125 (1) 0.0625 (2)	16	0.125
Flucytosine	0.25	0.25 (2)	0.5	0.25 (1) >63 (2)	0.25	0.25
Amphotericin B	4	0.5 (2)	4	0.5 (3)	1	0.5
Caspofungin	4	0.25 (2)	4	0.25 (10) 0.5 (2)	0.25	0.0313
Posaconazole	0.0625	0.0313 (1) 0.0625 (1)	0.0313	0.0313 (3)	0.0313	0.0625
Voriconazole	2	0.125 (2)	0.125	0.125 (1) 0.0625 (2)	0.125	0.0625
Itraconazole	8	16 (1)	16	0.0625 (1)	16	0.125

Table III shows MIC values of infrequently isolated Candida species for which MIC breakpoints have not been standardised by CLSI.

DISCUSSION

Change in epidemiology of Candida species from albicans to non-albicans in majority of hospital settings warrants the need to know the prevalent Candida species in any setup. Isolation of newer Candida species to the increasing list of non-albicans Candida which has been made possible due to automated identification system and their varied susceptibility pattern with a propensity for greater resistance queers the pitch for management. Although CLSI has standardized methods for performing and interpreting AFST for majority of Candida species. MIC breakpoints are not standardised for some of the newer Candida species. This necessitates the need for speciating and knowing the epidemiological cut-off of the newer Candida species in clinical setup.

Vitek 2 has certain limitations as it misidentifies certain species. The gold standard for identification remains DNA sequencing of the D1/D2 region. But in resource constrained settings Vitek 2 is the preferable option due to a low turnaround time (10).

Concern about the international emergence and spread of *C. auris* as a cause of invasive infection in hospitals stems from 3 characteristics of this opportunistic pathogen- resistance to various antifungal drugs, horizontal transmission among hospitalised patients causing nosocomial outbreaks and increased (11).

In the current study, there were 8 isolates of *C. auris*. The MICs of fluconazole and itraconazole were elevated. For amphotericin B the MIC ranged between 0.25 to 8 µg/ml. The MIC of flucytosine ranged between 0.125 to 64 µg/ml and for caspofungin, it ranged between 0.015 to 0.25 µg/ml. The *C. auris* isolates had MICs ranging between 0.0313 to 0.125 µg/ml for posaconazole and voriconazole.

Shivaprakash *et al.* (12) in their study observed a prevalence rate of 6.4% for *C. auris* among ICU acquired candidemia. AFST revealed MICs ranging from >64 µg/ml to 0.12 µg/ml for fluconazole among the isolates with 30 out of 74 isolates having MICs >64 µg/ml. The isolates had MICs ranging from 8 µg/ml to 0.06 µg/ml for amphotericin B, and from 4 µg/ml to 0.12 µg/ml for caspofungin. The MICs of the isolates varied from 4 µg/ml to 0.03 µg/ml for voriconazole, and for posaconazole the range was between 16 µg/ml to 0.03 µg/ml. The *C. auris* isolates showed an MIC range between 2 µg/ml to 0.03 µg/ml for itraconazole.

Anuradha *et al.* (13) conducted a multicentre study of AFST of *C. auris*. The MICs varied between >64 µg/ml to 1 µg/ml and between 16 µg/ml to 0.032 µg/ml for fluconazole and itraconazole respectively. For voriconazole the MICs ranged between 16 µg/ml to 0.032 µg/ml. The *C. auris* isolates had MICs between 8 µg/ml to 0.016 µg/ml for Posaconazole. The MICs of the isolates for flucytosine ranged from 0.125 µg/ml to >64 µg/ml. The MIC distribution of the isolates for amphotericin B and caspofungin was between 8 µg/ml to 0.125 µg/ml and 16 µg/ml to 0.125 µg/ml respectively. 170 *C. auris* isolates had an MIC of 1 µg/ml for amphotericin B and 175 of the isolates had an MIC of 1 µg/ml for caspofungin.

The study done by Ben-Ami *et al.* (14) revealed MICs of >8 µg/ml for fluconazole among all the *6 C. auris* isolates with MICs ranging between 16-64 µg/ml. MICs of the other azoles were also elevated. Amphotericin B MIC ranged from 1 to 2 µg/ml and caspofungin MIC was 0.5 µg/ml for the isolates.

In the present study, we had 10 isolates of *C. pelliculosa*. The MICs for fluconazole ranged between 64 to 0.125 µg/ml, whereas for itraconazole they ranged from 16 to 0.125 µg/ml, for Posaconazole and voriconazole the MIC range was between 1 to 0.0313 µg/ml and 0.125 to 0.0313 µg/ml respectively. The ranges for caspofungin, flucytosine and amphotericin B were 0.25 to 0.015 µg/ml, 0.5 to 0.125 µg/ml, and 1 to 0.0625 µg/ml respectively.

Jung *et al.* (4) had isolated 30 strains of *C. pelliculosa*, AFST was performed on these isolates. 28 of the strains had an MIC for amphotericin B ≤0.25 µg/ml while 2 of them had an MIC of 0.5 µg/ml. All the isolates had an MIC of ≤0.12 µg/ml for voriconazole and ≤0.25µg/ml for caspofungin. 18 of the isolates had MIC of 2 µg/ml for fluconazole and 2 had MIC of ≤0.25 µg/ml for caspofungin. 28 of the isolates had an MIC of 2 µg/ml for fluconazole and 2 had MIC ≤1 µg/ml for fluconazole.

Lin et al. (15) had 8 isolates of *C. pelliculosa* from the neonatal ICU. MICs for amphotericin B in 6 isolates were 1 µg/ml whereas 2 isolates had an MIC of 2 µg/ml. Fluconazole and voriconazole showed good antifungal activity with MICs between 2 to 4 µg/ml and 0.125 µg/ml respectively. Low susceptibility to itraconazole with an MIC of 2 µg/ml was observed.

A study on genotypic variation and AFST of 46 *C. pelliculosa* clinical isolates was done by Barchiesi *et al.* (16). The MICs of fluconazole ranged between 4 to 64 µg/ml whereas for itraconazole the MICs ranged between 0.25 to 2 µg/ml. For Posaconazole, flucytosine and amphotericin B the MICs were between 1-8 µg/ml, 0.06-64 µg/ml and 0.06-1 µg/ml.

In the present study there were 3 isolates of *C. lipolytica*. For fluconazole, 2 isolates had an MIC of 0.0625 µg/ml and 1 isolate had an MIC of 0.125 µg/ml. all the 3 isolates had MICs of 0.5 µg/ml and 0.0313 µg/ml for amphotericin B and posaconazole respectively. 2 isolates had an MIC of 0.5 µg/ml for caspofungin whereas 1 had an MIC of 0.25 µg/ml. For flucytosine, 2 isolates had an MIC of >64 µg/ml, whereas 1 had an MIC of 0.25 µg/ml. MICs of 0.0625 µg/ml, 0.125 µg/ml and 0.25 µg/ml were found in the 3 isolates for itraconazole.

In the study done by Zhao *et al.* (5), 14 cases of *C. lipolytica* were identified. The MICs for fluconazole, itraconazole, posaconazole and voriconazole ranged between 0.50->256 µg/ml, 0.06->16 µg/ml, 0.03->8 µg/ml and 0.03-2 µg/ml respectively. The MICs for caspofungin were 0.02-1 µg/ml and for flucytosine it was 0.12-16 µg/ml. The isolates had 0.25-2 µg/ml MIC for amphotericin B.

In the present study, there were 2 isolates of *C. famata*. The MICs for flucytosine, amphotericin B and caspofungin were 0.25 µg/ml, 0.5 µg/ml, and 0.25 µg/ml respectively. Both the isolates have MICs of 0.125 µg/ml for voriconazole. One of the isolates, had an MIC of 32 µg/ml and the other had had MIC of >64 µg/ml for fluconazole. 16 µg/ml and 8 µg/ml were the MICs for itraconazole.

In the study done by Beyda *et al.* (6), they found MICs ranging from 4-128 µg/ml, 0.5-16 µg/ml, 0.5-2 µg/ml, and 0.12-4 µg/ml for fluconazole, itraconazole, posaconazole and voriconazole respectively. Similarly, the MICs for flucytosine, amphotericin B and caspofungin were ≤0.06 µg/ml, ≤0.12-1 µg/ml and 0.5-1 µg/ml respectively.

The current study showed high MICs for fluconazole for each of the isolates of *C. haemulonii* and *C. duobushaemulonii* had an MIC of 0.5 µg/ml and 0.25 µg/ml for flucytosine, 16 µg/ml and 8 µg/ml for itraconazole, 0.0313 µg/ml and 0.0625 µg/ml for posaconazole and 0.125 µg/ml and 2 µg/ml for voriconazole respectively. This was similar to a study done by Hou et al. (7), they performed AFST on 26 isolates of *C. haemulonii* and 5 isolates of *C. duobushaemulonii*. All 31 *C. haemulonii* complex isolates showed low MICs for echinocandins (≤5 µg/ml) and high MICs for amphotericin B (range, 2 to >8 µg/ml), and fluconazole (>128 µg/ml).

Moreover, 96.2% of *C. haemulonii* and 80% of *C. duobushaemulonii* isolates were resistant to voriconazole (MIC>8 µg/ml). For itraconazole, 96.2% of *C. haemulonii* and all 100% of *C. duobushaemulonii* isolates were resistant (MIC≥1 µg/ml). a total of 69.2% of *C. haemulonii* and 40% of *C. duobushaemulonii* isolates had MICs of ≥2 µg/ml to posaconazole. A total of 26.9% of C. haemulonii and 20% of *C. duobushaemulonii* isolates were resistant to 5-flucytosine (MIC >64 µg/ml).

CONCLUSION

Isolation of newer Candida species carries a risk for HAIs. These highly resistant strains were associated with increased morbidity and mortality. Strict infection control measures and judicious use of antifungals for prophylaxis and treatment especially in ICUs should be mandatory. In case of Candida species where MIC break points have not been standardized by CLSI, the epidemiological cut offs can be used and treatment can be started accordingly.

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