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Prevalence and Antifungal Susceptibility Pattern of *Yeast* Species Isolated from the Diverse Samples at *Dr Lal Path Labs*, Delhi, India

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Research Article

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Abstract

Objective: This study aimed at determining the prevalence and antifungal susceptibility pattern of *Candida albicans* and Non-*albicans Candida* species from various clinical specimens tested in microbiology department of *Dr Lal Path Labs,* Delhi.

Materials and Methods: This retrospective study conducted on 2240 samples, tested between January2019 to July 2019, performed at Microbiology department of *Dr. Lal Path Labs.* Yeast species were cultured by Conventional (culture) and identified by MALDI-TOF which followed by antifungal susceptibility testing was done using Vitek 2 YST YS07 card.

Results: 737 ***C****andida isolates* of 16 diverse species were isolated from 2240 various clinical specimens. *Candida albicans* (54.9%) was predominant followed by *C. tropicalis* (14.7%), *C. glabrata (*11.7%), *C. parapsilosis* (4.3%), *and C krusei* (3.5%). Uncommon Candida species such as *Candida auris, Rhodoturula mucilaginosa, Trichosporon asahii, Malassezia pachydermatitis, Kodameae ohmeri* were also isolated. The most predominant age group infected with yeast isolates were elderly adults >=60 years of age which constituted 45.5% of the fungal culture positive cases. Alarming antifungal resistance noticed in Non-albicans *Candida species* as compared to *Candida albicans.* In the present study, *Candida albicans* showed high resistance to newest generation Azole, Voriconazole (42.2%) whereas the burden of Echinocandin resistance is still alarming in Non-albicans *Candida* infections.

Conclusion: The fungal pathogens has changed over a period with new species emerging as well as old species increasing become more virulent and resistant to primary antifungal drugs. We should be concern about the national emergence of resistance among varying Candida species obtained in diverse clinical situations.

Keywords: BAL (Broncho Alveolar Lavage); Echinocandins; Polyenes; Azoles; Non-albicans *Candida*

Introduction

Candida as is incidence has increased over the last decades, which includes the fungal infections in human beings by various *Candida species* causing superficial infections involving skin, hair and nail and systemic infections among the uncompromised and severe immune compromised patients such as cancers patients, undergoing organ transplants, HIV infected individuals. Although *Candida albicans* remains the most common *Candida* species and predominantly reported,

whereas *Candida tropicalis, Candida glabrata*, *Candida parapsilosis* and *Candida krusei* is emerging yeast among Non *albicans Candida species* and approximately 90% of invasive infections are caused by these few species. The morbidity and mortality caused by Non-albicans *Candida* (NAC) species are increasing [1,2].

Studies regarding the drug susceptibilities profile vary among *Candida albicans,* Non-albicans carried out worldwide and apart from their reduced susceptibility to Azole and Echinocandins here; we briefly discussed our findings among pathogenic species of yeast and their antifungal susceptibility patterns [3-5].

Materials and Methods

This retrospective study conducted on 2240 samples, tested between January to July 2019, performed at Microbiology department of *Dr Lal Path Labs.*

The samples subjected to direct microscopy-using KOH wet mount, and India ink preparations depending on the type of specimen. Fungal culture done on SDA agar, with chloramphenicol (16µg/mL) and with cycloheximide (0.3µg/mL) plus chloramphenicol (16µg/mL), Specimens were cultured in duplicate; one set of inoculated slants incubated at 25°C and the other incubated at 37°C, and they were examined every day for growth up to 4 weeks before discarding as negative. Fungal growth as identified by colony morphology and use of more advanced and standardized methods, such as MALDI TOF-MS (Bruker, Daltonics) were included in this study. VITEK-2 (Biomerieux) system used for the antifungal susceptibility testing of isolates from the pure culture of isolated colonies of the Candida species on CHROM agar Candida, and antifungal susceptibility was carried out on YS07 cards respectively. The results interpreted according to CLSI criteria M60-1edition 2017 [6].

Statistical analysis: For the evaluation of the Data analysis, Myla statistical program (bioMerieux, India, Pvt. Ltd) used.

Results

During the period of January 2019 to July 2019, A total number of 737(32.9%) *Candida species* isolated from 2240 various clinical specimens like BAL (42.9%), sputum (25.1%) endotracheal secretion (3.7%), urine (6.9%), pus (4.2%), nails (4.1%), tissue (3.1%), high vaginal swab (2.8%), sterile body fluids (2.8%), blood (2.3%), skin (1.2%), stool (0.2%) respectively (Table 1). Overall, 737 positive isolates of *Candida species* were collected from 426 males and 311 females and the age range of our study participants was 0 to 95 years**.** Ninety-two per cent of the study population belonged to the age group of 21–>70 years which is the major population group (Figure 1).

Specimen	Sputum	BAL	Endotracheal tip	Pus	Tissue	Sterile Body fluids	Nail	Skin	Urine	Stool	Genital Vaginal	Blood
Yeast spp. n=737	185	31.6	27	31	23	21	30	9	51	2	21	17
% of yeast spp.	25.1	42.9	3.7	4.2	3.1	2.8	4.1	1.2	6.9	0.2	2.8	2.3

Table 1: Prevalence of total fungal (Yeast species) isolates in different samples during January to July 2019.

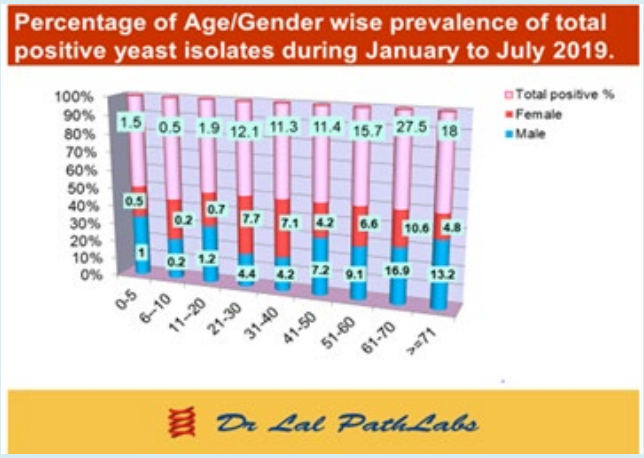

Figure 1: Age and gender wise prevalence of positive fungal (yeast species) isolates from different type of samples.

Most of our specimens collected from Delhi and the neighbouring states of North India. Total 16 *Candida* species identified from these 12 different clinical samples. Among them, *Candida albicans* (54.9%), *C. tropicalis* (14.7%), *C. glabrata (*11.7%), *C. parapsilosis* (4.3%), *and C. krusei* (3.5%), *C. kefyr* (2.3%) were the six predominant candida species. Numerous uncommon Candida species such as *Candida auris, Candida cantenulata, Candida metapsilosis, Candida lusitaniae, Candida haemulonii, Rhodoturula mucilaginosa, Trichosporon asahii, Malassezia pachydermatitis, Cryptococcus magnus, Kodameae ohmeri* were also isolated in this study (Figure 2).

Over the 6 month period, *Candida albicans* 405/737 (54.9%) and Non-albicans *Candida* constituted 332/737 (45.1%) of the total isolates. In this study, the *Candida* species isolated from the BAL specimen were predominant (Table 1, 3). 45.1% of Non-*albicans candida* species including *C. tropicalis, C. glabrata*, *C. parapsilosis, C. krusei* and *C. kefyr* accounted 36.5% whereas the other ten non-albicans *Candida* accounted for 8.6% (Figure 2).

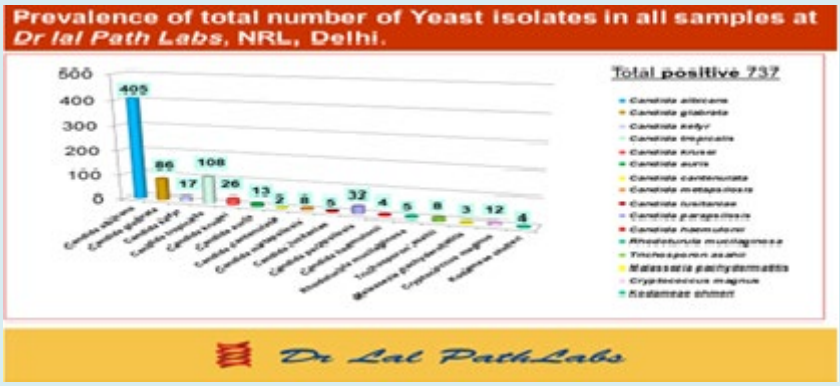

Figure 2: Total number and Percentage wise prevalence of positive fungal (yeast species) isolates from different type of samples.

In this study *Candida albicans* were most frequently isolated from BAL, Sputum, Endotracheal tip (83.4%) followed by Urine, Pus, Nail specimen respectively (Table 2).

Among the most dominant Non-albicans *Candida species, Candida tropicalis* accounted for (66.7%) *Candida glabrata* (55.8%) and *C. parapsilosis* (56.3%) of isolates obtained from respiratory tract samples and rest *Candida species* obtained from other specimens. Of the later other uncommon non-albicans *C. metapsilosis species* (87.5%), *Trichosporon asahii* (55.5%), *and Cryptococcus magnus* (66.7%), obtained from other specimens whereas *Rhodoturula mucilaginosa and Malassezia pachydermatitis* isolated only from skin and tissue (Table 2). Of the 737 Candida cases reported earlier 13 (1.8%) were due to *C. auris.* The majority of these *C. auris* cases were isolated from elder adults 10(>60yrs) and three were from (0-2yrs) age. In Delhi *Candida auris* is also, an emerging infection reported and were isolated from BAL, sputum respectively. In this study four isolates of *Kodameae ohmeri* (0.5%) infections were isolated from sputum, BAL, urine in patients of elder (>60) age groups (Table 2).

Yeast Species	Sputum N (%)	Bal N (%)	Blood N (%)	Body fluids N (%)	Pus N (%)	Tissue N (%)	Nail & Skin	Urine N (%)	Genital Vaginal	Endotracheal N (%)
Candida albicans (n=405)	126 (31.1)	201 (49.6)	5 (1.2)	7 (1.7)	13 (3.2)	6 (1.5)	9 (2.3)	19 (4.7)	8 (1.9)	11 (2.7)
Candida glabrata (n=86)	18 (20.9)	26 (30.2)	2 (2.3)	5 (5.8)	3 (3.5)	2 (2.3)	9 (10.5	11 (12.8)	6 (6.9)	4 (4.7)
Candida kefyr (n=17)	4 (23.5)	7 (41.2)	1(5.9)	--	---	---	---	4 (23.5)	---	1 (5.9)
Candida tropicalis (n=108)	22 (20.4)	45 (41.7)	5 (4.6)	8 (7.4)	5 (4.6)	1 (0.9)	4 (3.7)	9 (8.3)	4 (3.7)	5 (4.6)

Candida krusei (n=26)	5 (19.2)	11 (42.3)	2 (7.7)	--	2 (7.7)	---	2 (7.7)	1 (3.8)	1 (3.8)	2 (7.7)
Candida auris (n=13)	3 (23.1)	7 (53.8)	---	1 (7.7)	1 (7.7)	---	---	---	---	1 (7.7)
Candida cantenulata (n=2)	1(50)	--	----	--	1 (50)	---	---	---	---	---
Candida metapsilosis (n=8)	---	1 (12.5)	1 -12.5	1 -12.5	1 (12.5)	2 (25)	---	2 (25)	---	---
Candida lusitaniae (n=5)	1 (20)	3(60)	----	---	---	----	---	1(20)	---	-----
Candida parapsilosis (n=32)	2 (6.3)	8 (25)	1 (3.1)	---	5 (15.6)	6 (18.8)	---	1 (3.1)	1 (3.1)	8 (25)
Candida haemulonii (n=4)	---	2 (50)	---	1 (25)	1 (25)	----	---	----	-----	-------
Rhodoturula mucilaginosa (n=5)	---	---	---	---	---	3 (60)	2 (40)	---	---	----
Trichosporon asahii (n=9)	2 (22.2)	2 (22.2)	---	----	----	1 (11.1)	2 (22.2)	2 (22.2)	----	----
Malassezia pachydermatitis (n=3)	---	---	---	----	----	1 (33.3)	2 (66.7)	---	---	----
Cryptococcus magnus (n=12)	---	4 (33.3)	---	---	--	2 (16.7)	2 (16.7)	1 (8.3)	1 (8.3)	2 (16.7)
Kodameae ohmeri (n=4)	1(25)	2 (50)	---	---	---	---	---	1 (25)	---	------

Table 2: Positive percentage of different type of yeast species in different type of samples during January to July 2019.

This study has been noted that Micafungin, Flucytosine and Amphotericin B were recorded highly sensitive to most of the yeast isolates while Azole antifungal have long provided effective treatment for *Candida* species so development of high level Azole resistance is a problem of critical importance in clinical settings in North India (Figure 3).

Figure 3: Total percentage of Cumulative interpretation of Antifungal Sensitivity/Resistant patterns of all *Candida* species during January to July 2019.

In addition, this study describes for the cumulative MIC interpretation of antifungal sensitivity patterns among most dominant *Candida* species with help of Myla statistical analysis (Biomerieux, India) which causes complicated invasive infection such as *Candida albicans* shown highly resistant to Voriconazole (42.2%) and Fluconazole (32.8%) respectively. Voriconazole activity ($MIC_{50/90\ <=0.12/4}$) against *Candida albicans* demonstrated that 50% of isolate were within 0.12µg/ml MIC and 90% isolates were within 4µg/ml. Total 224 (55.5%) *Candida albicans* isolates were tested against all antifungal, 98.9% *Candida albicans* sensitive to Caspofungin was having MIC $_{50/90}$ (<=0.12/0.25µg/ml) and 98.1% of Flucytosine sensitive isolates having MIC $_{50/90}$ (<=1/2µg/ml) were noted (Table 3). Out of 86 tested isolates of *Candida tropicalis* all antifungal drugs having $_{50/90}$ (<=0.12to1µg/ml) only 3% isolates resistant to Voriconazole, Caspofungin and Micafungin. *Candida krusei* were recorded decreased susceptibilities to Flucytosine (MIC at which 50% and 90% of isolates were inhibited (MIC_{50} & MIC_{90}), 8µg/ml) and Fluconazole activity ($MIC_{50/90}$ 8/8µg/ml) were demonstrated that all isolates were resistant to these drugs (Table 3). Second highest resistance recorded (25%) of all drugs except Flucytosine in *Candida parapsilosis*. Most species of non-albicans *Candida* recorded resistant to Caspofungin except *Candida kefyr*. Nearly all the isolates of *Candida species* had 100% sensitive for Flucytosine, the notable exception being *Candida krusei* where 100% isolates demonstrated resistant in Delhi. The distribution of Antifungal drugs MIC values against sensitivity patterns of *Candida species* followed in (Table 3).

Antifungal	MIC(µg/ml)/ Cumulative%	Yeast Species					
		***Candida albicans* (n=224)**	***Candida Tropicalis* (n=86)**	***Candida Glabrata* (n=60)**	***Candida Krusei* (n=25)**	***Candida Kefyr* (n=16)**	***Candida Parapsilosis* (n=31)**
Fluconazole	MIC50 /MIC90	8-Jan	2-Jan	----	0	0.5/2	<=0.5/8
	%S	67.2	90	----	0	100	75
Voriconazole	MIC50 /MIC90	<=0.12/4	<=0.12/ 0.12	----	<=0.12/ <=0.12	0.125	<=0.12/8
	%S	57.8	97	-----	100	100	75
Caspofungin	MIC50 /MIC90	<=0.12/0.25	<=0.12/ 0.25	<=0.12/ 0.25	0.5/0.5	0.125	0.5/8
	%S	98.9	97	50	42.9	100	75
Micafungin	MIC50 /MIC90	<=0.06/0.12	<=0.06/ 0.06	<=0.06/ 0.06	<=0.12/ <=0.12	0.125	8-Jan
	%S	97.2	97	93.8	100	100	75
Amphotericin B	MIC50 /MIC90	0.5/4	<=0.25/0.5	<=0.25/0.5	0.5/1	0.25	0.5/2
	%S	89.5	100	100	92.9	100	75
Flucytosine	MIC50 /MIC90	<=1/2	<=1/8	0.5	<=8/8	<=1/2	1
	%S	98.1	100	100	0	100	100

Table 3: Percentage of Cumulative MIC interpretation and antifungal activity against most predominant yeast species from all age groups during January to July 2019.

Discussion

Among the Candida species, *Candida albicans* ranks first in incidence in our study followed by Non-albicans strains such as *Candida tropicalis, Candida glabrata* our findings were agreement with these western studies [2,7-9]. Whilst in the Indian settings which showed that *Candida tropicalis* was the most commonly isolated agent causing 30-60% of the cases [1,10,11].

It is notable that geographically distribution of C*andida* species in Asian and Western countries were not similar [1,9,12,13]. Various studies conducted at different part of India have found a higher incidence of Candidiasis due to Non-albicans *Candida*, with the isolation rate ranging from 40-70%, Non-albicans *Candida* caused about 45% cases in our study. *Candida tropicalis and Candida glabrata* has become predominant Non-albicans *Candida* species causing infection in our findings and it prevalence varies across geographic region and other previous studies by worldwide were concords with this study [1,2,7,12,14,15].

Several classes of compounds that is Polyenes, Azoles, Echinocandins and Nucleoside analog are use with sensitivity

of *Candida* species. The most common prescribed antifungal Azole have long provided effective treatment for *Candida* species while development of high level Azole resistance is a problem of critical importance in clinical settings, therefore this research has focused on investigating its virulence. Fluconazole resistance increasing is worrisome about the therapy for C*andida albicans* and Non-albicans *Candida* infection [16].

In this study, *Candida albicans* were shown unusual high level of resistant to newest generation azole Voriconazole (42.2%) in comparision to first line antifungal drugs Fluconazole (32.8%) similar observation was also reported by previous study [7,12]. However other antifungal drugs reported sensitive our results are in agreement with the previously published study [11].

Of the interest in emergence of drug resistant *Candida glabrata* in many countries which has been associated with longer hospital stay and high attributable death have been associated with delayed initiation of appropriate antifungal treatment [3,13,17].

Echinocandin are usually consider the first line treatment for *C. glabrata* infections but the burden of Echinocandin (Caspofungin) resistance is still worry some in patients with Candida infections due to *C. glabrata.* In our study whereas Amphotericin B considered the gold standard treatment for fungal infection and all isolates of *C. glabrata* shown susceptible to Amphotericin B and Flucytosine this finding were concord with other findings [1,9]**.**

However certain Non *albicans Candida* species in the Asia Pacific region Fluconazole resistance in *Candida tropicalis* reported ranges from 0 to as high as 83% [4,5] while in our findings the *Candida tropicalis* were 10% resistant to Fluconazole, this is similar with previous finding [1].

All *Candida kefyr* isolates found to be susceptible to tested antifungal drugs therefore the use of routine antifungal agents like Fluconazole, Amphotericin B and all antifungal drugs that are available in this region suggested for treatment of these *Candida species*.

Of the increasing concern, the number of multidrug resistant isolates of *Candida parapsilosis* ranges 25% of all antifungals except Flucytosine. Previous studies by worldwide are in agreement with this study [1-3,10,16,18].

Candida krusei is well known recognized as a potentially MDR fungal pathogen, due to its intrinsically resistant to Fluconazole. We find out all isolates of *Candida krusei* were 100% sensitive to Voriconazole, and decreased susceptibilities noted to Amphotericin B. In addition this species clearly exhibits, 100% decreased susceptibility to Flucytosine (Table 4) were in agreement with previous study by Plaffer MA, et al. [19].

Whereas we find out more than 50% of isolates were resistant to Caspofungin an Echinocandin which is new agents to treat serious *Candida* infections which targets the fungal cell wall and it retains activity against isolates with resistant to azoles or polyenes. The number of resistance increasing is worrying about the therapy for *Candida krusei* infection and were very similar to other studies [3,9,13,16,17,19].

This study highlighted only Voriconazole and Micafungin were suitable for *Candida krusei* treatment in this region.

Candida auris is an emerging opportunistic pathogen, first reported in 2009 as an isolate from an external ear of a patient in Japan. It identified as cause of nosocomial infection in numerous countries in East Asia, the Middle East, Africa, and Europe [15,20,21]. *C. auris*, said that almost 90 per cent of the fungal isolates found to be resistant to Fluconazole, the standard antifungal drug of choice in many countries [10,20,21]. According to Chowdhary, the first case of *Candida auris* reported in India was from 2011. Besides, the pathogen is capable of surviving on hospital paraphernalia such as mattresses, bedrails, and windowsills for long, making it possible to spread among patients [14].

In Delhi *Kodameae ohmeri* is also an emerging infection have been reported and were isolated from bal, sputum, urine respectively this is an agreement with previous study [6,22].

Kodameae ohmeri and *Candida auris* species are ubiquitous fungi causing severe disease in immunocompromised individuals and hampered by absence of accurate species identification this yeasts commonly misidentified as *Candida haemulonii* and *Rhodotorula glutinis* by routine laboratory using conventional methods. Yet correct identification up to species level characterized by Matrix-assisted laser desorption ionization–time of flight mass spectrometry (MALDI-TOF MS). Hence, it considered a more rapid diagnostic technique for identification of this rare yeast isolates *(Kodameae ohmeri* and *Candida auris)* and may help to switch effective treatment and reduce the duration of hospital stay.

Over the past two decades, several studies reported noscomial sepsis by the uncommon fungal species such *Trichosporon asahii.* This fungal species occasionally are part of the gastrointestinal and oral cavity and can transiently colonize the respiratory tract and skin. In our study 1.1% of *Trichosporon asahii* isolated from respitatory and other specimens. And this fungus was found to be sensitive to

Voriconazole and Micafungin but resistance of Amphotericin B, Caspofungin and Fluconazole were alarming, this is similar with previous finding [23-25].

In this study, we have demonstrated that the incidence of fungemia caused by *Rhodotorula mucilaginosa* was five (0.6%) that is isolated from tissue and skin. However, currently saprophytic yeasts like *Rhodotorula* spp. are emerging pathogens and few epidemiological studies have described *Rhodotorula* as the aetiological agent in 0.5-2.3% cases of fungemia are in agreement with this study [26,27].

Conclusion

The fungal pathogens have changed over a period with new species emerging as well as old species increasingly become more virulent, resistant to primary antifungal drugs and important to see the difference in antifungal susceptibility in different yeast species. Emergence of increasing resistance of antifungal drugs has become the concern for policy makers and urgent need of strict antifungal prescription policy in our country. Judicious selection of antifungal drugs as per yeast species recommendation by CLSI M-60 is the need of hour. We should be concern about the national emergence and awareness of this rare fungus (*Kodameae ohmeri, Candida auris*) as a cause of invasive infection and severe sepsis.

Ethical Approval

It is not applicable.

Conflicts of interest

There are no conflicts of interest.

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