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DIFFUSION OF CANDIDA SPECIES IN DIFFERENT CLINICAL SAMPLES AND THEIR SPECIATION AND ANTIFUNGAL SUSCEPTIBILITY PROFILE AT TWO TERTIARY CARE CENTERS, JAIPUR

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ABSTRACT

Introduction: Among fungal infections, invasive candidiasis is often associated with increased number of morbidity and death rate. Candida is an yeast like fungus that causes so many infections, range from non fatal mucocutaneous infections to fatal blood stream infections so the aim of this study is speciation of Candida species and evaluation of their antifungal susceptibility to avoid unnecessarily consumption of antifungal drugs. **Material & Methods:** A Prospective study which was carried out in the department of Microbiology, RUHS College of Medical Sciences, Jaipur. Clinical samples collected from concerned sites were cultured on SDA agar and incubated. Identification of candida species was done through HiCrome^{TM} Candida Differential Agar and antifungal susceptibility was evaluated by VITEK-2 (Biomerieux) system and results were calculated through statistical analysis. **Results:** Out of 64 Candida isolates, 34.38% were candida albicans, followed by Candida tropicalis (31.25%) Candida krusei (20.31%), Candida kefyr (12.50%) and C. glabrata (1.56%). The most common Candida species isolated from urine were Candida albicans (34.24%) followed by Candida tropicalis (31.25%). Voriconazole (92.19%) and Amphotericin B (89.06%) were found the most sensitive drugs against the Candida isolates followed by Flucytosine (79.69%), Caspofungin (76.56%), Micafungin (70.31%) and Fluconazole (62.50%). All the candida Kefyr isolates were susceptible to Voriconazole, Amphotericin B and Flucytosine and resistant to Fluconazole. all Candida glabrata isolates were susceptible to all the 4 drugs except Micafungin and Caspofungin. **Conclusion:** The present study showed the Distribution of various candida species in various clinical samples and also revealed that Non-albicans Candida species are emerging as predominant species and increased resistance of Candida isolates towards common antifungal drugs which is a concern around all over the world.

KEYWORDS: Candida albicans, Non-albicans Candida, Speciation, Antifungal Susceptibility.

INTRODUCTION

The primary cause of the elevated rate of mortality and morbidity is fungus infections in the patients who are immunocompromised and in ICU patients. Among fungal infections, Candida is the commonest pathogenic organism causing invasive infections resulting in increased hospitalization and life threatening conditions.[1]

Candida is an yeast like fungus that produces pseudo-hyphae. Candida species which are common residents of skin, gut and genitals but occasionally these organisms causes so many infections, range from non fatal mucocutaneous infections to invasive blood stream infections in immunocompromised patients. From several studies of last few years, it is found out that non-albicans Candida species have now become predominant.[2]

There are about 20 distinct species of the heterogeneous genus Candida that are known to cause infections in humans.[3] Invasive Candida (IC) infections are broadly reported in critical patients, admitted in the ICU. The key risk factors for invasive Candidiasis included organ transplants, over consumption of broad-spectrum antimicrobial agents, prolonged Hospitalization, surgery, advanced life support, and pugnacious chemotherapy, older age (over 60 years), chronic renal failure and diabetes mellitus, gastrointestinal or cutaneous colonization.[4] In recent years, the epidemiology of invasive candidiasis (IC) has gradually changed all over the world.[5] The non-Albicans Candida species emphasized the significance of identification of the

Candida isolate's infecting species for commencement of prompt and efficient therapy, particularly when antifungal susceptibility results are not readily available.[6]

By starting the appropriate antifungal medication early enough, based on the susceptibility profile of the infecting Candida species, the therapy for invasive fungal infections can be improved.[7] in these medical conditions, the commensal Candida may convert into opportunistic pathogenic microorganisms causing candidiasis in host.[8] The potential clinical significance of speciation has been acknowledged as Candida species shows dissimilarity in the expression of putative virulence factors and antifungal susceptibility.[9]

Surveillance of fungal ecology and the antifungal resistance either within patients in ICU or within Candida species is necessary for the superintendence of invasive fungal infections.[10]

MATERIAL AND METHODS

Study design: Prospective study, carried out by the Department of Microbiology, RUHS College of Medical Sciences, Jaipur.

Study place: The study was carried out in the Department of Microbiology, R.U.H.S. College of Medical Sciences, Jaipur. The samples were received at Govt. R. D. B. P. Jaipuria Hospital and Attached Hospital of RUHS College of medical Sciences, Jaipur in the Department of Microbiology.

Study universe: All those patients who attended R.D.B.P. Jaipuria Hospital and RUHS-CMS Hospital are advised for culture and sensitivity testing by clinicians, who fulfilled both inclusion & Exclusion criteria.

Study duration: 8 months

This is a prospective study which was carried out in the department of Microbiology, RUHS College of Medical Sciences, Jaipur. Total of 978 patients were tested for Candida infection. Patient's clinical samples including urine sample from mid-stream urine, nasal swab, Endotracheal tube, Stool, Central line tip, Pus, Pleural fluid, Throat swab or sputum sample, skin scrapings from the infected part, blood samples and vaginal swabs etc. were collected as per standard procedure, from the patients suspected for fungal infections, referred to RUHS-CMS hospital and Govt. R. D. B. P. Jaipuria Hospital. Participants in the study were the patients who met the inclusion and exclusion criteria. The samples were followed as per the laboratory SOP (standard operative procedures).[2] The samples were cultured on Nutrient agar, Blood agar and MacConkey agar for the primary inoculation. After incubation of 24 – 72 hours, the colonies grown on culture stained with gram stains and microscopy is done. If the mix growth is obtained than the colonies were separated by subculture on saboraud's dextrose agar with antibacterial antibiotics incubated at 25° C and 37° C. Colonies are appeared in 1-3 days. Identification was done on the basis of colony morphology (On Sabouraud's Dextrose agar colonies were creamy white, smooth and with a yeasty odour) and microscopy. In microscopy purple budding yeast cells are seen. Further the identification of Candida species was done with various methods. The method of Candida species identification was HiCrome^{TM} Candida Differential Agar. Inoculation was done for differentiating the candida species based on the colour production on HiCrome^{TM} Candida Differential Agar.

Antifungal susceptibility testing by automated method VITEK-2 –

We used the VITEK-2 to check the susceptibility of fungal agents against different fungal drugs. 3 ml of sterile normal saline was taken in polystyrene tubes, picked a colony from culture of Candida isolates and it was mixed in the saline. It was mixed properly. The Densichek plus instrument provided by the BioMerieux was used to check the density of prepared inoculums upto McFarland standard 2.0 for (VITEK-2 YST cards). Now the 280 ml of prepared 2.0 MacFarland suspension was picked with pipettes and mixed it with another tube with 3 ml of saline and mixed it properly by pipetting. VITEK-2 AST cards were placed into tubes. Tubes were placed with VITEK-2 AST cards in the cassettes and after inserting the cassettes into the VITEK-2 device, the corresponding yeast AST cards were loaded, incubated, and automatically read by the device. The growth rate in the drug-free control well determined the length of incubation, which ranged from 18 to 20 hours. The outcomes were reported as MICs, or micrograms per millilitre. The density of the inoculums was checked by the Densichek plus instrument was used to check the density of prepared inoculums.

RESULT AND DISCUSSION

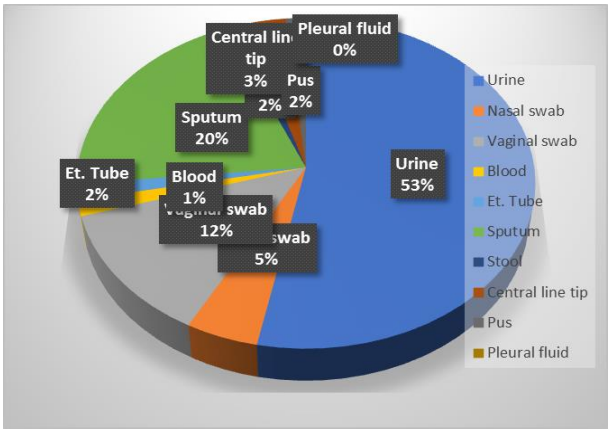

Figure 1: Distribution of candida species according to sample type.

The most common sample from which Candida were isolated was urine (53.12%). Then other samples were sputum (20.30%) and vaginal swab (12.50%). Percentage of Candida species isolated from nasal swab were

(4.68%), central line tip (3.12%), blood (1.57%), Et. Tube (1.57%), stool (1.57%) and pus (1.57%).

Table 1: Candida species distribution based on sample type.

Sr. No.	C. Species	C. Albicans	C. Tropicalis	C. Krusei	C. Kefyr	C. Glabrata
1	Urine	13 (38.24%)	11 (32.35%)	8 (23.53%)	1 (2.94%)	1 (2.94%)
2	Nasal swab	-	-	-	3 (100%)	-
3	Vaginal swab	4 (50%)	-	3 (37.5%)	1 (12.5%)	-
4	Blood	-	-	1 (100%)	-	-
5	Et. Tube	-	1 (100%)	-	-	-
6	Sputum	5 (38.46%)	5 (38.46%)	1 (7.69%)	2 15.38%)	-
7	Stool	-	1 (100%)	-	-	-
8	Central line tip	-	2 (100%)	-	-	-
9	Pus	-	-	-	1 (100%)	-
10	Pleural fluid	-	-	-	-	-
Total		22 (34.38%)	20 (31.25%)	13 (20.31%)	8 (12.50%)	1 (1.56%)

Table 1 shows that the most common Candida species isolated were Candida albicans (34.38%), followed by Candida tropicalis (31.25%). Other Candida species isolated were Candida krusei (20.31%), Candida kefyr (12.50%) and C. glabrata (1.56%). It shows the most common Candida species isolated were Candida albicans (34.28%) from urine (38.24%) followed by sputum (38.46%). The 2nd most common isolated Candida species were Candida tropicalis (31.25%) from urine (32.35%) followed by sputum (38.46%). The other species isolated were Candida krusei most commonly from Urine (23.53%) followed by Vaginal swab (37.5%). Candida kefyr (12.50%) were isolated most commonly from nasal swabs (100%). Candida glabrata were isolated from urine (2.94%). According to results the non-albicans candida species are predominant in all the clinical samples.

Table 2: Antifungal susceptibility pattern of Candida isolates by Automated method (VITEK-2).

Drugs	Sensitive	Intermeduate	Resistance	TRM
Fluconazole	40 (62.50%)	0	24 (37.5%)	0
Flucytosine	51 (79.69%)	0	10 (15.63%)	3 (4.69%)
Voriconazole	59 (92.19%)	0	0	5 (7.81%)
Amphotericin B	57 (89.06%)	0	0	7 (10.94%)
Micafungin	45 (70.31%)	0	0	19 (29.69%)
Caspofungin	49 (76.56%)	3 (4.69%)	0	12 (18.75%)

Table 2 shows the total sensitivity pattern by Automated Method and it revealed that Voriconazole (92.19%) and Amphotericin B (89.06%) were found the most sensitive drugs against the Candida isolates. The antifungal sensitivity patterns showed by other drugs were Flucytosine (79.69%), Caspofungin (76.56%), Micafungin (70.31%) and Fluconazole (62.50%). 4.69% isolates show intermediate sensitivity for Caspofungin. Some drugs Micafungin in 29.69% isolates and Caspofungin in 18.75%, Amphotericin B in 10.94%, Voriconazole in 7.81% and Flucytosine in 4.69% isolates were terminated by Machine.

Table 3: Antifungal susceptibility pattern in various Candida species by Automated method VITEK-2.

Drugs	Candida albicans		Candida tropicalis		Candida krusei		Candida kefyr		Candida glabrata	
	Sensitive	I/R/TRM	Sensitive	I/R/TRM	Sensitive	I/R/TRM	Sensitive	I/R/TRM	Sensitive	I/R/TRM
Fluconazole	17 (77.27%)	5 R (22.73%)	19 (95%)	1 R (5%)	2 (18.18%)	11 R (81.82%)	0	8R (100%)	1 (100%)	0
Flucytosine	20 (90.91%)	2 TRM (9.09%)	20 (100%)	0	3 (23.08%)	10 R (76.92%)	8 (100%)	0	1 100%)	0
Voriconazole	17 (77.27%)	5 TRM (22.73%)	20 (100%)	0	13 (100%)	0	8 (100%)	0	1 100%)	0
Amphotericin-b	20 (90.91%)	2 TRM (9.09%)	17 (85%)	3 TRM (15%)	12 (92.31%)	1 TRM (7.69%)	8 (100%)	0	1 100%)	0
Micafungin	20 (90.91%)	2 TRM (9.09%)	20 (100%)	0	6 (46.15%)	7 TRM (53.85%)	0	8 TRM (100%)	0	1 TRM (100%)
Caspofungin	20 (90.91%)	2 TRM (9.09%)	20 (100%)	0	9 (69.23%)	3I/1TRM	0	8 TRM (100%)	0	1 R (100%)

Table 3 demonstrates the pattern of antifungal susceptibility in various Candida species using an automated approach (VITEK-2). According to the pattern, Candida albicans was most responsive to fluconazole and voriconazole (77.27%), followed by flucytosine, amphotericin B, micafungin, and capsofungin (90.91%). Candida tropicalis was most sensitive to Flucytosine, Voriconazole, Micafungin, Caspofungin (100%) Followed by Fluconazole (95%) and Amphotericin B (85%). Candida krusei was most sensitive to Voriconazole (100%) and Amphotericin B (92.31%), followed by Caspofungin (69.23%) and Micafungin (46.15%). Candida kefyr showed complete sensitivity (100%) against Flucytosine, Voriconazole, and Amphotericin B. Candida glabrata showed complete sensitivity (100%) against Fluconazole, Flucytosine, Voriconazole and Amphotericin B.

DISCUSSION

Fungal infections are among the most prevalent causes of morbidity and mortality in the globe. Among them Candida is also a major concern worldwide due to its increasing incidence and also the increased resistance towards drugs. Rapid and accurate identification of Candida species and evaluating their antifungal susceptibility pattern are of great importance for the selection of appropriate antifungal agent and for patient management.

The most common Candida species isolated were Candida albicans (34.28%) from urine (38.24%) followed by sputum (38.46%) shows similarity with the results of A. Rengaraj. Et al[3] study in 2019.

The present study reported that the burden of Candida albicans species and non-albicans species to be 34.38% and 65.62% respectively. In our study the Non-albicans Candida species showed dominance on Candida albicans species. Which is similar with Reshma Bhaskaran et al[2] study in 2020, which also showed predominance of non-albicans Candida species over Candida albicans species.

In many previous studies Candida albicans was still the most predominant isolated species as showing in some recent studies also (Seyoum E. et al[1] study in 2020) comparatively to non albicans candida species which shows quite differences from our study and the reason behind differences occurred in the studies or this shifting trend of species of Candida could be the geographical area, age, different hospital settings, different departments, environmental conditions, comorbidities, immunosuppression, underlying diseases and antibiotic therapies etc.

In our study Vitek-2 is used to evaluate the antifungal susceptibility of all candida isolates and it revealed that In Our study all the Candida isolates showed high susceptibility towards Voriconazole (92.19%) which is co-relatable with studies Singh R et al,[14] Sundaram M. et al[16] and Seyoum et al.[1]

Amphotericin B (89.06%) were found the second most sensitive drugs against the Candida isolates and results of our study co-relate with Kaur R et al.[14] and Sundaram M. et al.[16]

For Micafungin and Caspofungin results are correlated well with the study Sundaram M. et al.[16]

In our study Fluconazole shows less sensitivity against candida isolates (62.50%) which showed difference with the result in Elias Seyoum et al[1] study in 2020, in which 85.6% of Candida species were susceptible to Fluconazole.

Flucytosine (79.69%), Caspofungin (76.56%), and Micafungin (70.31%) also showed good sensitivity against all the candida isolates.

The shifting pattern of Candida species' distribution among isolates and altered antifungal susceptibility patterns by the time is the major concern worldwide that's why to prevent these alterations, a diagnosis of the species of candida and an assessment of the susceptibility to antifungals are therefore essential.

CONCLUSION

The current study demonstrated the distribution of different Candida species in a range of clinical samples. It also showed that non-albicans Candida species are becoming more common and that isolates of Candida are becoming more resistant to common antifungal medications, which is a global concern.

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