Section: Microbiology

Original Research Article

SPECIATION AND ANTIFUNGAL SUSCEPTIBILITY TESTING OF CANDIDA FROM CLINICAL SPECIMENS AT A TERTIARY CARE HOSPITAL, GUJARAT

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Abstract

Background: Candida species are prevalent in the human body as commensals. They can induce a variety of infections in immunocompromised and hospitalized patients, ranging from superficial to deep seated infections, and are therefore capable of causing morbidity and mortality. The objective of the present study was to speciate the isolates of Candida and to determine their antifungal susceptibility from various clinical specimens received at a tertiary care hospital, Gujarat, India. **Materials and Methods:** The present study is an observational study conducted in the Department of Microbiology at the Parul Sevashram Hospital. A total of 224 specimens of Candida isolates from various clinical 5242 specimens of patients were included in the study. Sputum, urine, blood, sterile body fluids, pus, tissue, vaginal swab were among the samples received. The results were expressed as percentage analysis. **Result:** Candida albicans was the most prevalent isolate from urine, sputum, pus, Broncho alveolar lavage fluid and other bodily fluids. Out of 224 Candida isolates, 180 were sensitive to all antifungal drugs, whereas the remaining 44 were resistant to one or more antifungal drugs. Amphotericin B was effective against all Candida albicans isolates, whereas Voriconazole had the lowest susceptibility (85%). Fluconazole showed the greatest susceptibility in Candida tropicalis isolates, whereas Voriconazole showed the least susceptibility (91.4%). **Conclusion:** The knowledge of the incidence, resistance and emergence of different Candida species might guide clinicians to select an appropriate antifungal therapy and plan effective strategies to control invasive and systemic Candida infections.

INTRODUCTION

Candida species is a natural commensal flora of the human body, occupying the skin, mucous membranes, and gastrointestinal tract, but it has been associated with superficial and deep-seated fungal infections. The transition of Candida species from commensal to pathogenic is assisted by virulence factors such as adhesion to host tissues, medical devices, biofilm formation,and production of extracellular hydrolytic enzymes. Furthermore, in recent years, non-albicans Candida (NAC) species have been identified as significant pathogens causing serious infections in humans.[1,2]

Candida albicans was the most prevalent isolate in the present study followed by Candida tropicalis and Candida parapsilosis as significant isolates, which agrees with the findings of Kaur R et al. Candida albicans has been identified as the most isolated species from patients in a number of different studies conducted in Asian, European, and American nations.[3,4] Candida tropicalis was the most prevalent non-albicans Candida species isolated in our study which is similar with the findings of Chakrabarti A et al and Lakshmy JA et al.[5,6]

Candida species are prevalent in the human body as commensals. They can induce a variety of infections in immunocompromised and hospitalized patients, ranging from superficial to deep seated infections, and are therefore capable of causing morbidity and mortality. The genus has a diverse range of organisms, with 20 distinct Candida species known to cause human infections.[7,8]

Recovery of yeasts from sterile bodily fluids (blood, cerebrospinal fluid, etc.), recovery from people whose defenses were reduced by chronic disorders, and recurrent recovery from several specimens all

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suggest yeast infection. Thus, identification of yeasts isolated from clinical specimens up to species level, as well as antifungal susceptibility testing has become more essential and can aid in the treatment of these infections.[9] The objective of the present study was to speciate the isolates of Candida and determine their antifungal susceptibility from various clinical specimens of patients received at a tertiary care hospital.[10-12]

MATERIALSANDMETHODS

The present study is an observational study conducted in the Department of Microbiology at the Parul Sevashram Hospital, Gujarat, India. The study data collected over a period of 2 years. The study comprised both male and female patients of various ages. Prior to the start of the study, the Institutional ethics committee was notified, and Institutional Ethical clearance certificate was received for the study. A total of 224 specimens of Candida isolates from various clinical 5242 specimens of patients were included in the study.

Sample Processing: A retrospective analysis was carried out to understand the distribution of Candida species in different clinical samples and their drug susceptibility pattern from the Department of Microbiology Vitek 2 database of 750 bedded tertiary care hospital at Parul Sevashram Hospital for 2 years. The database included patient identifiers, hospital. Wards/departments, sample collection date, specimen type, and results of speciation and AST pattern. The samples were processed as per the standard microbiological procedures. Microbiological methods: Two smears from each sample were prepared and examined microscopically in 10% potassium hydroxide (KOH) and gram staining. The specimens were inoculated on Sabouraud Dextrose Agar (SDA) media supplemented with chloramphenicol and incubated under aerobic conditions at 37°C for 24-48 hours. Growth on SDA was evaluated for colonial morphology and the yeast identified by conventional methods including germ tube test, microscopic morphology on KOH and gram staining. Further identification including speciation was done using VITEK-2 System (YST card). The isolates were identified to the species level, and AST patterns were determined using the Vitek 2 system (BioMerieux). Antifungal susceptibility test was done using AST-YS08 cards.

The AST panel included the antifungal such as Amphotericin B, Flucytosine, Caspofungin, Micafungin, Fluconazole and Voriconazole. The susceptibility results were concordant with CLSI and EUCAST Methodologies and were interpreted as sensitive (S), intermediate (I) and resistant (R).[13,14]

Statistical Analysis

The results were expressed as percentage analysis. The data was analyzed statistically using SPSS statistics version 19.0 (Chicago, IL, USA) and values of $P < 0.05$ were considered statistically significant.

RESULTS

A total of 224 Candida isolates were examined. Candida albicans was the most frequent species among the 224 Candida isolates, accounting for 21.42% of all isolates. Candida tropicalis was the most common species among non-albicans Candida (41.51%). [Table 1]

Table 1: Specimen distribution of Candida Species

	C.albicans	C.glabrata	C.tropicalis	C.krusei	C.auris	C.ciferrii	C.duobushaemulonii	C.famata	C.guilliermondii	C.lustaniae	C.parapsilosis	C.pelliculosa	C.rugosa
Blood	9	1	20	2	1	1	2	1	2	0	9	0	0
Urine	24	8	62	2	1	1	1	1	21	1	20	1	1
Sputum	4	0	4	0	0	0	0	1	2	0	0	0	0
Pus,Tissue	2	0	1	0	0	0	0	0	0	0	0	0	0
BAL fluid	1	0	0	0	0	0	0	0	0	0	0	0	0
Vaginal swab	2	0	0	0	0	0	0	0	1	0	0	0	0
ET	3	0	3	0	0	0	0	0	1	0	0	0	0
TT	0	0	0	1	0	0	0	0	0	0	0	0	0
Others	3	0	3	0	0	0	0	0	0	0	0	0	0

Table 2: Antifungal susceptibility test by Vitek 2

		Fluconazole			Flucytocine			Micafungin			Voriconazole			Amphotericn B			Caspofungin		
	Total no. of isolates	Sensitive	Intermediate	Resistance	Sensitive	Intermediate	Resistance	Sensitive	Intermediate	Resistance	Sensitive	Intermediate	Resistance	Sensitive	Intermediate	Resistance	Sensitive	Intermediate	Resistance
C.albicans	48	36	2	10	39	1	8	39	0	9	36	2	10	38	0	10	40	0	8
C.glabrata	9				7	0	2	6	0	3	8	1	0	7	0	2	4	0	5
C.tropicalis	93	67	4	22	77	2	14	79	2	12	69	3	21	78	0	15	80	2	11
C.krusei	5	3	0	2	3	0	2	4	0	1	2	1	2	3	0	2	4	0	1
C.auris	2																		
C.ciferrii	2										2	0	0	1	0	1			
C.duob	3	0	0	3	2	0	1				1	0	2	0	0	3			

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ushaemulonii																			
C.famata	3																		
C.guilliermondii	27	18	0	9	18	1	8	19	4	4	21	3	3	19	0	8	20	0	7
C.lusitaniae	1	1	0	0	1	0	0				1	0	0	1	0	0			
C.parapsilosis	29	19	0	10	23	0	6	27	1	1	19	4	6	22	0	7	27	0	2
C.pelliculosa	1	0	0	1	1	0	0				0	0	1	0	0	1			
C.rugosa	1	1	0	0	1	0	0							0	0	1	1	0	0

Table 3: Prevalence of Candida spp. in patients samples (n-5242)

Sample	Total no. screened	Total no. of isolates.
Blood	1280	47
Urine	1917	143
Sputum	331	11
Pus, Tissue	1034	3
BAL fluid	16	1
Vaginal swab	11	3
ET	321	8
TT	149	1
Others	183	7
Total	5242	224

Amphotericin B was effective against all Candida albicans isolates, whereas Voriconazole had the lowest susceptibility (85%). Fluconazole showed the greatest susceptibility in Candida tropicalis isolates, whereas Voriconazole showed the least susceptibility (91.4%). Other non-albicans Candida species susceptibility results included: Two isolates (50%) of Candida ciferrii were sensitive to all antifungal drugs, one isolate (33.3%) was resistant to both Amphotericin B and Voriconazole, and one isolate (16.7%) was resistant to both Amphotericin B and Voriconazole. Three isolates (80%) of Candida lusitaniae were sensitive to all antifungal drugs, whereas one isolate (20%) was resistant to Voriconazole. [Table 2]

Out of 224 Candida isolates, 180 were sensitive to all antifungal drugs, whereas the remaining 44 were resistant to one or more antifungal drugs. Voriconazole resistance was highest among Candida isolates, followed by Flucytosine, Amphotericin B, Caspofungin, Micafungin, and Fluconazole.

Candida albicans was the most prevalent isolate from urine, sputum, pus, broncho alveolar lavage fluid and other bodily fluids. Of the Candida albicans isolates, 143 were from urine and 11 were from sputum. Candida tropicalis was the most frequent isolate from urine and blood, whereas Candida albicans was the most common isolate from sputum and Endo-Tracheal secretions. [Table 3]

DISCUSSION

Candida spp. has emerged as a leading pathogen of opportunistic infections in hospital settings. As a result, early isolation, speciation and antifungal susceptibility testing are critical for clinicians in selecting the optimum treatment strategy for patients in order to limit morbidity and mortality. The majority of the isolates in this study were from Blood and urine. [Table 3].

Our study found an increase in antifungal drugs resistance, particularly for the azole group of drugs in both C. albicans and non-albicans Candida species, which is consistent with studies by Guo-Shi Xiang et al,[11] and Kamiar Zomorodianet al.[12] In our study, Candida albicans isolates were 85% and 97% resistant to Voriconazole and Fluconazole, respectively. Candida tropicalis was susceptible to Voriconazole in 93.7% of cases.

Resistance to Echinocandins (Caspofungin and Micafungin) in Candida species is unusual; in our study, it was shown to be (2.4%), which was consistent with a study by Mariana Castanheira et al. Resistance to Amphotericin B among Candida isolates is restricted, which was consistent with our study as only (2.4%) of the isolates were resistant to Amphotericin B; nevertheless, three out of four Candida isolates resistant to Amphotericin B were Candida ciferrii. Primary resistance to Flucytosine or 5-fluorocytosine (5-FC) is frequent in yeasts, and this agent is susceptible to subsequent resistance in patients on Flucytosine monotherapy. Flucytosine has so been utilized exclusively in conjunction with Amphotericin B.

CONCLUSION

Candida infections in hospitalized patients are a major concern across the world. Resistance among Candida species is rising. To summarize, the current study found that there is a shifting trend of rising incidence of Non albicans Candida in our Study region. Candida non albicans outnumbered Candida albicans. An increase in the predisposing factors in recent years has resulted in an increase in the incidence of Candida infections.

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REFERENCES

1. Talapko, J.; Juzbašić, M.; Matijević, T.; Pustijanac, E.; Bekić, S.; Kotris, I.; Škrlec, I. J. J. o. F. Candida albicans—the virulence factors and clinical manifestations of infection. 2021, 7, 79.
2. Mayer, F. L.; Wilson, D.; Hube, B. J. V. Candida albicans pathogenicity mechanisms. 2013, 4, 119-128.
3. Bhattacharjee, P. J. C. m. m. Epidemiology and antifungal susceptibility of Candida species in a tertiary care hospital, Kolkata, India. 2016, 2, 20.
4. Gharanfoli, A.; Mahmoudi, E.; Torabizadeh, R.; Katiraee, F.; Faraji, S. J. C. M. M. Isolation, characterization, and molecular identification of Candida species from urinary tract infections. 2019, 5, 33.
5. Chakrabarti A, Sood P, Rudramurthy SM, Chen S, Kaur H, Capoor M, Chhina D, Rao R, Eshwara VK, Xess I, Kindo AJ, Umabala P, Savio J, Patel A, Ray U, Mohan S, Iyer R, Chander J, Arora A, Sardana R, Roy I, Appalaraju B, Sharma A, Shetty A, Khanna N, Marak R, Biswas S, Das S, Harish BN, Joshi S, Mendiratta D. Incidence, characteristics and outcome of ICU-acquired candidemia in India. Intensive Care Med. 2014;41:285–295.
6. J. Abirami Lakshmy, Radhika Katragadda, J. Balaj. Speciation and antifungal susceptibility of esophageal candidiasis in cancer patients in a tertiary care hospital in South India. J M e d A l l i e d S c i 2 0 1 6 ; 6 (1) : 2 9 - 3 4
7. Spampinato, C.; Leonardi, D. J. B. r. i. Candida infections, causes, targets, and resistance mechanisms: traditional and alternative antifungal agents. 2013, 2013.
8. Dalal, R. J. J. o. A. M.; Research, D. S. Unraveling Eagle's Syndrome: Comprehensive Case Report. 2023, 11, 22-24.
9. Sandven, P.; Lassen, J. J. J. o. c. m. Importance of selective media for recovery of yeasts from clinical specimens. 1999, 37, 3731-3732.
10. Saha, D.; Sharma, A.; Borah, N.; Saikia, D. J. C. The Spectrum of Pathogenic Yeast Infection in a Tertiary Care Hospital in Assam, India. 2022, 14.
11. Xiang, G.-S.; Zeng, L.-B.; Zhang, J.-Y.; Ying, Y.; Deng, X.-R.; Hu, X.-F.; Yan, Y.; Yu, K.-H.; Zou, W.-W.; Huang, X.-T. J. J. J. o. M. In Vitro Antidrug Susceptibility Testing of Candida Species Isolated from Aseptic Body Fluids. 2018, 11.
12. Zomorodian, K.; Bandegani, A.; Mirhendi, H.; Pakshir, K.; Alinejhad, N.; Fard, A. P. J. J. j. o. m. In vitro susceptibility and trailing growth effect of clinical isolates of Candida species to azole drugs. 2016, 9.
13. M27-A3 reference method for broth dilution antifungal susceptibility testing of yeasts; approved standard-third edition (2008) (cited 2022 Dec 6). Available from: www.clsi.org.
14. Wayne PA. Clinical and laboratory standards institute (CLSI): reference method for broth dilution antifungal susceptibility testing of filamentous fungi (2008) 28(16). Available at: https://clsi.org/media/1897/m27ed4_sample.pdf 20. Perlroth J, Choi B, Spellberg B. Nosocomial fungal infections: epidemiology.

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