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Original Research Article

Speciation and antifungal susceptibility pattern of *Candida* isolated from blood and body fluids

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ABSTRACT

Background: *Candidia* infections are increasing now a day due to various factors.
Aims: The present study was carried out to study the speciation and antifungal susceptibility pattern of *Candida* isolated from blood and body fluids.
Settings and Design: Blood and body fluids received in the mycology laboratory of Microbiology Department, AIMSR, over a period of six months were included. After microscopy, culture was done. The *Candida* spp. were diagnosed on the basis of colony characteristics, Gram staining and Germ tube testing and identification by Automated Vitek 2 system. Antifungal susceptibility testing was done by using YST08 cards.
Results and Conclusions: A total of 735 samples received, 53 showed Candida spp. Maximum *Candida* spp. isolated from blood 23 (43.39%), followed by pleural fluid 14 (26.4%). *C. albicans* were 24(45.3%) followed by *C. tropicalis* 11(20.75%), *C. glabrata* 9(16.98%) *C. parapsilosis* 7(13.20%). *Candida* spp. were sensitive to Amphotericin B and resistant to Fluconazole.

1. Introduction

Candidiasis is the commonest fungal disease found in humans affecting mucosa, skin, nails and internal organs. Systemic candidiasis (candidemia) is only seen in severely immunocompromised patients. *Candida* infection has been associated with many risk factors like long-term hospitalization, increasing use of antibiotic-therapy, use of intravascular catheters and underlying diseases like diabetes, malignancy, AIDS, neutropenia, total parentral nutrition and increased number of patients receiving immunosuppressive therapy for transplantation. Recovery of yeasts from normally sterile body fluids (blood, cerebrospinal fluid, etc), recovery from patients whose defenses were compromised from chronic diseases and repeated recovery from multiple specimens certainly indicates infection with the yeasts.[1]

Candida albicans is the single most common fungal species causing nosocomial infections. However, *non-albicans Candida* species, including fluconazole less susceptible *Candida glabrata*, have become more common pathogens. Main species cassociated with human infections are *Candida albicans, Candida glabrata, Candida tropicalis, Candida parapsilosis, Candida krusei, Candida dubliniensis* and *Candida kefyr*.[2]

The ability of the highly adaptable fungal species to transition from commensal to pathogen is due to repertoire of virulence factors that include biofilms, adhesions, enzymes, toxins, Complement receptors and phenotypic switching.[3]

Various clinical samples that can be collected from patients of candidiasis depend upon the site of infection like oral swab from whitish plaques present on mucous membrane of mouth in AIDS patients, vaginal swabs

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from patients of vulvovaginitis, skin scraping and nail clipping in patients of cutaneous infections, urine from diabetic and catherised patients, sputum from patients of pulmonary candidiasis, blood from septicemic neonates etc.[4] As drug resistance in *Candida* species is now very common, therefore antifungal susceptibility testing should be performed by determining the MICs. This can play major role in management of resistant candidal infections, particularly caused by *Non-albicans Candida* species.

2. Aim & Objectives

1. Isolation and identification of *Candida* species of blood and body fluids.
2. Antifungal susceptibility testing of *Candida* isolates.

3. Materials and Methods

The present study was conducted in Mycology section of Microbiology laboratory under Department of Microbiology for period of six months after getting approval from Institutional Research committee and ethics committee.

All blood, CSF, Peritoneal, pleural, Synovial, pericardial fluid and BAL samples received in Microbiology Department for culture were included in the study. Clinical data of patients was collected from patient's files. The blood and body fluid samples received in the bacteriology laboratory was inoculated into the Bact/Alert standard aerobic bottles and incubated in this system while the other samples were directly inoculated on Sabouraud dextrose agar (SDA) and blood agar and put in glucose broth for further subculture. The inoculated bottles loaded into the Bact/Alert were incubated for a maximum period of 5 days. Bottles flagging as positive by the Bact/Alert instrument were Subcultured on SDA media slope and blood agar and incubated at 37°C. Cultures were examined after overnight incubation and every day in first week and than twice a week for evidence of growth. Culture were hold for up to 4 weeks before being declared as negative. Identification was done on the basis of colony characters, LCB preparation and gram staining. *C. albicans* produced medium sized 1.5mm diameter, circular smooth and grayish white colored colonies, *C, tropicalis* produced large grey mycelia fringe colonies, *C. krusei* produced 0.2-1.0mm diameter, small, round or irregular, flat or heaped colonies. Gram positive budding yeast cells with pseudohyphae were seen on Gram staining and in LCB preparation showed 4-7um budding yeast cells with pseudohyphae.[4] Germ tube test is used to differentiate between *albican and non-albican Candida*.[4]

The Vitek 2 compact system was used for final identification of *non-albican Candida* using yeast identification card. Antifungal susceptibility was done by using YST08 cards in Vitek 2 Compact which uses spectrophotometric readings to determine an MIC value for clinically relevant *Candida* species. The cards contain wells with dried concentrations of antifungal drugs in medium. After the isolate was adjusted to a standardized concentration in saline, it was used to rehydrate the drug wells and the card was placed into a Vitek 2 card reader/incubator, The growth within each well was monitored up to 36 hrs (an average of 12 to 14 hour) by an optical scanner and a report containing an MIC value along with the interpretive category for each antifungal on the card was generated. The drugs tested for antifungal susceptibility by using YST08 cards include Amphotericin B, Fluconazole, Voriconazole Micafungin Caspofungin and Flucytosine.

4. Results

A total of 735 clinical samples were received. Out of which 53 (7.21%) *Candida* species were isolated from various clinical samples like: Blood, Pericardial fluid, peritoneal fluid, Cerebrospinal fluid, Pleural fluid and BAL in bacteriology laboratory of Microbiology department over a period of six months. Out of 53 patients with *Candida* species, female patients were 24 (45.29%) and male patients were 29(54.71%).Table 1 shows that the maximum number of patients belonged to age group >60 years, followed by 41-60years age group among both males and females. Out of 53 isolates, 17 were from intensive care Unit (ICU), 14 were from department of medicine, 9 were from Cardiac care Units (CCU), 6 from department of pediatrics, 4 were from department of surgery, and 3 were from the department of Pulmonary medicine. Table 2 shows that the maximum number of species were obtained from blood 23 (43.39%), followed by pleural fluid 14 (26.4%), pericardial fluid 6 (11.32%), bronchial fluid 5 (9.43%), peritoneal fluid 3(5.66%) and cerebrospinal fluid 2(3.8%). Among 53 isolates 24 were *C. albicans* and 29 were *non albican Candida* spp. Table 2 shows *C. albicans* 24(45.3%) was obtained as most common species followed by *C. tropicalis* 11(20.75%), *C. glabrata* 9(16.98%), *C. parapsilosis* 7(13.20%) and *C. krusei* 2(3.77%). Antifungal susceptibility pattern of *Candida* spp. (Table 3) indicates that *C. albicans* maximum sensitivity to Amphotericin B (91.67%) followed by micafungin (79.17%), caspofungin (75%), flucytosine (70.83%), voriconazole (66.67%) and fluconazole (62.5%). Antifungal susceptibility pattern of *C. tropicalis, C. glabrata and C. parapsilosis* was as shown in Table 3.

5. Discussion

The *Candida* species which were considered as normal flora component is now responsible for the endogenous infections and various systemic infections. It is found mainly as secondary cause of infection in individuals with some underlying immunocompromised conditions and very rarely

Table 1: Age wise distribution of patients with *Candida* species

Age group (years)	Female no. (%)	Male no. (%)
0-10	3 (5.7%)	2 (3.77%)
11-20	2 (3.77%)	2 (3.77%)
21-40	6 (11.32%)	4 (7.54%)
41-60	6 (11.32%)	10 (18.86%)
>60	7 (13.20%)	11. (20.75%)
Total	24 (45.31%)	29 (54.69%)

Table 2: Species wise distribution of *Candida* species

Species	No. of isolates	Percentage
C. albicans	24	45.3%
C. tropicalis	11	20.75%
C. glabrata	9	16.98%
C. parapsilosis	7	13.20%
C. krusei	2	3.77%
Total	53	100%

Table 3: Antifungal susceptibility pattern of albicans and non-albicans *Candida*

Antifungal agents	*C. albicans* (n=24)		*C. tropicalis* (n=11)		C. glabrata (n=9)		C. parapsilosis (n=7)	
	Sen	Resis	Sen	Resis	Sen	Resis	Sen	Resis
Amphotericin B	22 (91.67%)	2 (8.33%)	10 (90.9%)	1 (9.1%)	9 (100%)	0 (0%)	7 (100%)	0 (0%)
Fluconazole	15 (62.5%)	9 (37.5%)	4 (36.4%)	7 (63.6%)	0 (0%)	9 (100%)	1 (14.3%)	6 (85.7%)
Voriconazole	16 (66.67%)	8 (33.33%)	7 (63.6%)	4 (36.4%)	6 (66.7%)	3 (33.3%)	6 (85.7%)	1 (14.3%)
Micafungin	19 (79.17%)	5 (20.83%)	8 (72.7%)	3 (27.3%)	3 (33.3%)	6 (66.7%)	4 (57.1%)	3 (42.9%)
Caspofungin	18 (75%)	6 (25%)	9 (81.8%)	2 (18.2%)	4 (44.4%)	5 (55.6%)	3 (42.9%)	4 (57.1%)
Flucytosine	17 (70.83%)	7 (29.17%)	8 (72.7%)	3 (27.3%)	7 (77.8%)	2 (22.2%)	5 (71.4%)	2 (28.6%)

as primary pathogen.

In the present study the higher prevalence of *Candida* species was seen in males, which coincides well with the Gupta et al[5] where as much higher prevalence shown by Xess et al[6] and lower prevalence shown by Singh and Abraham.[7] It is concluded that males are more prone to *Candida* infection because of poor hygiene, diabetes and prolonged use of antibiotics and the yeast like fungus form normal commensal of oral, mucosal, gastrointestinal and genitourinary tract of human. In the present study, the age of patients ranged from 0-79 years. Among them >60 years age group was most common age group affected followed by 41-60 years and then 21-40 years (Table 1). Deorukhar and Saini (2012) and Shaik et al. (2016) reported greater number of patients with >60 year age group.[8,9] It is concluded that high incidence of systemic candidiasis is among old age as compared to children and adults. The aging population is especially susceptible to fungal infection due to underlying medical conditions and increase in the rates of hospitalization. From the above study, it is concluded that *Candida* is most commonly isolated from blood sample as compared to other samples because *Candida* species is fourth most common nosocomial pathogen causing 11% of all bloodstream infections. The result of the present study (43.39%) are nearby Palwa et al (24.5%) where as lower prevalence was shown by Gandhi and Patel (7.14%), Arora et al. (9.36%).[10–12] In the present study, *C. albicans* (45.3%) showed higher prevalence followed by *C. tropicalis*, (20.75%), *C. glabrata* (16.98%), *C. parapsilosis* (13.20%) and *C. krusei* (3.77%)(Table 2).

Dharvad and Dominic (2011) showed higher prevalence by *C. albicans* (47%) followed by *C. tropicalis* (30%), *C.krusei* (14%) and *C. glabrata* (9%).[8,13]

Deorukhkar and Saini (2012) and Gupta et al. (2015) showed higher prevalence by *C. albicans*.[5] Chander et al. (2013) and Kaur et al (2014) showed higher prevalence by *C. tropicalis*.[8,14] showed higher prevalence by *C. albicans* (50%) followed by *C. glabrata* (25%), *C. krusei* (17%) and *C. tropicalis* (8%).[5] In the present study, *C. albicans* showed maximum sensitivity to Amphotericin B (91.67%) followed by micafungin (79.17%), caspofungin (75%), flucytosine (70.83%), voriconazole (66.67%) and fluconazole (62.5%).(Table 3)

C. albicans showed maximum resistance to fluconazole (37.5%) followed by voriconazole (33.33%) flucytosine (29.17%), caspofungin (25%), micafungin (20.83%) and amohotericin B (8.33%).(Table 3). Chander J. et al. (2013) in their study on 27 *Candida* species obtained higher sensitivity to Amphoterici (100%) and higher resistance to fluconazole (62%) as compared to our study among *c. albicans* species.[15] Kaur et al (2014) in their study on 103 *Candida* species obtained higher resistance to fluconazole (68%) and low resistance to Amphotericin (5%) as compared to our study among *C. albicans* species.[14] Gupta et al. (2915) in their study on 12 *Candida* species obtained higher resistance to Fluconazole (33%), voriconazole (17%) and less resistance Amphotericin B as compared to our study among *C. albicans*.[5]

The resistance to fluconazole is of great concern because it is most common azole used for treatment of disseminated candidiasis including candidemia. Increasing use of fluconazole had led to predominance of Non-*albicans Candida* species over *C. albicans*. Resistance to voriconazole, micafungin, caspofungin and flucytosine was also seen in our study due to decreased susceptibility to fluconazole and cross resistance to other azoles.

6. Conclusion

Systemic candidiasis includes a spectrum of yeast infections caused by different species of *Candida.* The most common form of this invasive infection is Candidemia though it can affect heart, lungs, brain, eyes, bones and other body part. Systemic candidiasis is usually treated with oral or intravenous (IV) antifungal medications like Caspofungin, Fluconazole and Amphotericin B and early diagnosis and antifungal susceptibility testing is necessary for starting appropriate treatment timely.

7. Source of Funding

None.

8. Conflict of Interest

None.

References

1. Martins N, Ferreira I, Barros L, Silva S, Henriques M. Candidiasis: Predisposing factors, prevention, diagnosis and alternative treatment. *Mycopathologia*. 2014;177(5-6):223–40.
2. Pfaller MA, Moet GJ, Messer SA, Jones RN, Castanheira M. Candida bloodstream infections; comparison of species distributions and antifungal resistance patterns in community-onset and nosocomial isolates in the SENTRY Antimicrobial Surveillance Program. *Antimicrob Agents Chemother*. 2008;55(2):561–6.
3. Sardi JCO, Scorzoni L, Bernardi T, Fusco-Almeida AM, Giannini M. Candida species: current epidemiology, pathogenicity, biofilm formation, natural antifungal products and new therapeutic option. *J Med Microbiol*. 2013;62(Pt 1):10–24.
4. Chander J. Characteristic of fungi In: A text book of Medical Mycology. 3rd ed. New Delhi: Mehta Publishers; 1995. p. 14–22.
5. Gupta P, Prateek S, Chatterjee B, Kotwal A, Singh AK, Mittal G. Prevalence of candidemia in ICU in a tertiary care hospital in North India. *Int J Curr Microbiol Appl Sci*. 2015;6:566–75.
6. Xess I, Jain N, Hasan F. Epidemiology of candidaemia in a tertiary care centre of North India: 5 year study. *Infection*. 2007;35:256–265.
7. Singh N, Abrahim J. Incidence of Candida in patients admitted to ICU. *Adv Appl Sci Res*. 2013;4(5):282–6.
8. Deorukhakar C, Saini S. Non albicans candida species: a rewiew of epidemiology, pathogenicity and antifungal resistance. *Pravara Med Rev*. 2015;7(3):15.
9. Shaik N, Penmetcha U, Myneni RB, Yarlagadda P, Singamsetty S. The study of identification and antifungal susceptibility pattern of candida species isolated from various clinical specimens in a tertiary care hospital, Chinakakani, Gyntur, AP, South India. *Int J Curr Microbiol and Appl Sci*. 2016;5(7):71–91.
10. Pahwa N, Kumar R, Nirkhiwala S, Bandi A. Species distribution and drug susceptibility of Candida in clinical isolates from a tertiary care-centre at Indore. *Indian J Med Microbiol*. 2014;32(1):44–8.
11. Gandhi V, Patel M. Prevalance of Candida species and its antimicrobial susceptibility isolated from blood culture at a tertiary care hospital. *Int J Current Microbiol Appl Sci*. 2017;6(6):884–92.
12. Arora S, Dhuria N, Jindal N. Speciation, biofilm formation and Antifungal susceptibility of Candida isolates. *Int J Res Dev Pharm Life Sci*. 2017;6(2):2517–21.
13. Dharwad S, Dominic RM. Species identification of Candida isolates in various clinical specimen with their antifungal susceptibility pattern. *J Clin Diagn Res*. 2011;5(6):1177–81.
14. Kaur R, Goyal R, Dhakad MS, Bhalla P, Kumar R. Epidemiology and virulence determinants including biofilm profile of Candida infections in an ICU in a Tertiary Hospital in India. *J Mycol*. 2014;doi:10.1155/2014/303491.
15. Chander J, Single N, Sidhu SK, Gombar S. Epidemiology of Candida blood stream infections: experience of a tertiary care centre in North India. *J Infect Dev Ctries*. 2013;7(9):670–5.

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