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Original Research Article

Isolation, identification and antifungal susceptibility of *Candida* in patients with fungal sepsis

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ABSTRACT

Background: In this study, our aim was to identify and isolate Candida species from patients admitted in ICU,s of our hospital and to determine their susceptibilities to various antifungal agents so as to find the local resistance pattern and guide for empirical treatment.
Methods: In our study 37 strains of candida were isolated (4 *Candida albicans*, 33 *Non-albicans Candida* strains). Candida species were identified by conventional, biochemical and molecular methods. Antifungal susceptibility tests for amphotericin B, fluconazole, itraconazole, ketoconazole and voriconazole were performed with broth microdilution method and E- tests as described by National Committee for Clinical Laboratory Standards (NCCLS).
Results: Out of 37 Candida strains, the most prevalent species were *C. tropicalis* (43.2%), *C. parapsilosis* (24.3%), *C. krusei* (16.2%), *C. albicans* (10.8%), and *C. glabrata* (2.7%). Among all strains four strains (10.8 %) were resistant, two *Candida albicans* where found resistant to fluconazole one *Candida krusei* and one *Candida parapsilosis* were found to be resistant to all azoles.
Conclusions: Candidemia continues to be associated with substantial morbidity and mortality and non albicans Candida species are the commonly isolated pathogen from those patients admitted in tertiary care hospitals in Indian scenario. Thus, it is imperative to perform antifungal susceptibility to select appropriate and effective antifungal therapy.

Keywords: Anti-fungal susceptibility, Broth microdilution method, Candida, E-test

INTRODUCTION

Candidemia is a life threatening infection and is associated with high attributable mortality rate. During past few decades nosocomial *Candida* infection have been increasingly reported in immunocompromised patients and severely ill like burn patients. The incidence of *Candidemia* has increased tremendously in ICU Patients.

The growing incidence of *Candida* is in part related to the widespread use of immunosuppressive chemotherapy.[1,2]

Isolation and identification of infecting strains of *Candida* is important as isolates of *Candida* species differ widely, both in their ability to cause infection and also in their susceptibility to antifungal agents.[3,4]

Epidemiological studies have identified intravascular catheters, broad spectrum antibiotic therapy, mucosal colonization, and neutropenic patients as most possible predisposing factors for candidiasis. Although *Candida albicans* remains the most frequent cause of fungemia in abroad, however studies in India reported non-albicans emergence from last few decades.

The incidence and mortality rate due to candidemia have remained grossly unaltered in the last two decades. Despite major advances in the field of antifungal therapy however a temporal change in species distribution has been observed during this period with the emergence of many *Non-albicans Candida* species.[5] *Candida glabrata* has emerged second to *Candida albicans* as a cause of blood stream infection in virtually every Survey based on US Hospitals. In Contrast in other Countries *Candida parapsilosis*, *Candida tropicalis* and *Candida krusei* are main agents among *non-albicans Candida* species causing blood stream infection in Indian Scenario.[6]

The rise in frequency of Infection of *Non-albicans Candida* species has been also observed in tertiary care hospital in India with isolation rate from 5% to 96% showing dramatic increase in *candidemia* due to non-albicans species with increase as 20% in 1991 and 90% in 1997. However predominant isolation of *Candida glabrata* or *Candida parapsilosis* in all age groups in Indian Scenario is unique. Thus, there is greater need for reproducible susceptibility testing method as a guide to selecting and monitoring antifungal therapy. Two most common susceptibility testing methods include E-test and broth micro dilution method. The E-test is a relative novel susceptibility testing method. The E-tests and broth microdilution method are used to determine the MIC of drugs. The purpose of this study is also to compare the results of E-test and broth microdilution methods.[7]

METHODS

The present study was conducted in a tertiary care hospital of North India over a period of one year and near about 37 strains of *Candida* species were isolated. Samples were taken from blood, CSF, nails, skin tissues, sputum and oral scrapping in patients who were admitted in ICU,s of our hospital. Sampling was done to determine their fungal spectrum and antifungal susceptibility. Repeated samples were taken whenever required. Control strains taken in our study for testing were *Candida parapsilosis*, (ATCC 22019) *Candida krusei* (ATCC 6258) *Candida albicans* (ATCC 90028). Identification of different species of *Candida* was done by direct examination, fungal culture (Sabouraud Dextrose Agar) gram staining and biochemical tests.[8-10]

Susceptibility testing was done with broth-microdilution and E test. The broth microdilution method was used as the reference method as described by National Committee for Clinical Laboratory Standards (NCCLS)

RESULTS

The present study was conducted in a tertiary care hospital of North India over a period of one year and near about 37 strains of *Candida* species were isolated. The most common strain isolated was of *C. tropicalis* .A total of 37 samples were taken from 18 males, 12 females and 7 children. The strains of *Candida* isolated from clinical samples and their distribution in categories of *C. tropicalis, C. parapsilosis C. krusei C. albicans* and *C. glabrata* represented in Figure 1.

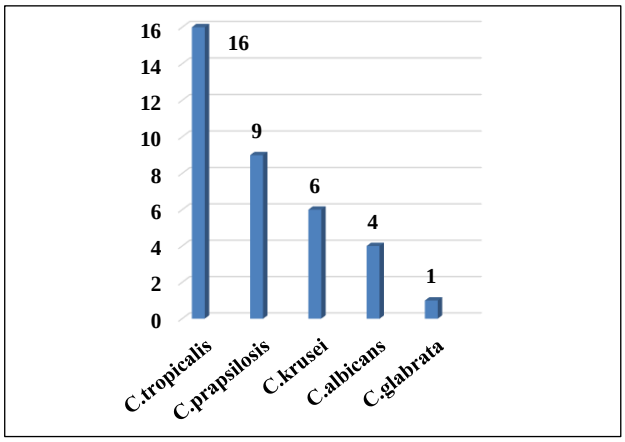

Figure 1: Total number of strains of *Candida* isolated from clinical samples and their distribution in categories of *C. tropicalis, C. parapsilosis, C. krusei, C. albicans and C. glabrata*.

Susceptibility testing by broth microdilution (BMD) and E-test of all 37 isolates of *Candida* strains with three antifungal agents of Amphotericin B, Fluconazole and Itraconazole are compiled in tables (Table 1, 2, 3, 4 and 5).

Amphotericin B

For amphotericin B the MIC for *Candida species* isolates are tightly clustered between 0.25 and 1µgram/ml when isolated that appear resistant to amphotericin B in animal models are tested by M27 methods, MIC values greater than 1µgram/ml may be obtained. Unfortunately, M27 methodology does not consistently permits detection such isolates and all that can at present be concluded is that if an amphotericin B MIC of >1 µgram/ml is obtained from *Candida species*. Isolates then that isolate is resistant to amphotericin B .Current work suggest that with antibiotic medium supplemented with 2% glucose permits more reliable detection of resistant isolates.

Fluconazole

Based on large data package presented by fluconazole manufacturer interpretive breakpoints for *Candida species* and fluconazole susceptibility has been established as susceptible dose <8, dependent 16-32, resistant>64. These data are principally drawn from

studies from oropharyngeal candidiasis and of invasive infections due to *Candida* species in non-neutropenic patients and their clinical relevance. In addition, these interpretive break points are not applicable to *Candida krusei* and this identification to the species level is required in addition to MIC determination.

Itraconazole

Based on large data package presented by Itraconazole manufacturer interpretive break points for *Candida species* and Intraconozole have been established as susceptible dose <0.25, dependent 0.25-0.5, resistant >1.

Table 1: *Candida tropicalis.*

E strips	Amphotericin B	Fluconazole		Itraconazole
	E strips	E strips	BMD	E strips
C. tropicalis	1	1.5	0.5	0.19
C. tropicalis	1	0.5	0.5	0.13
C. tropicalis	0.25	1.5	1	0.75
C. tropicalis	0.25	2	2	0.08
C. tropicalis	0.125	1	1	0.19
C. tropicalis	1	24	16	0.95
C. tropicalis	1	2-3	2-3	0.2
C. tropicalis	3	1-5	1-3	0.5
C. tropicalis	1	0.125	1	0.25
C. tropicalis	0.125	2	1	0.125
C. tropicalis	0.75	16	8	0.78
C. tropicalis	0.032	2	1-2	0.63
C. tropicalis	1	1.5	1	0.19
C. tropicalis	1	0.06	0.5	0.24
C. tropicalis	0.016	0.75	0.25	0.9
C. tropicalis	0.25	16	8	0.28

Table 2: *Candida parapsilosis.*

E strips	Amphotericin B	Fluconazole		Itraconazole
	E strips	E strips	BMD	E strips
C. parapsilosis	2	R	R	R
C. parapsilosis	0.75	12-16	8	0.29
C. parapsilosis	0.75	1	1	0.15
C. parapsilosis	0.125	1	1	0.87
C. parapsilosis	0.5	0.06	0.06	0.31
C. parapsilosis	0.25	2	0.5-1	0.41
C. parapsilosis	0.65	0.06	0.06	0.012
C. parapsilosis	0.125	10	12	0.97
C. parapsilosis	1-2	8	4	0.83

Candida Tropicalis remained the most frequent strain in this study. A total of sixteen *Candida tropicalis* strains were isolated and tested by using E test for Amphotericin B and Itraconazole, while for Fluconazole both BMD and E test were used. All strains were uniformly sensitive to all antifungal agents. In this setup authors also found *Candida tropicalis* as least resistant species.

Nine strains of *Candida parapsilosis* which were tested by using E test and BMD methods for three antifungal agents viz Amphotericin B, Fluconazole and Itraconazole. Both BMD and E Test were applied only on Fluconazole. One strain was found resistant to both Itraconazole and Fluconazole but was sensitive to Amphotericin B. The resistant strain was isolated from a patient who suffering from immunodeficiency and who died while on treatment.

Candida krusei showed a high level of resistance to Fluconazole and Itraconazole but for Amphotericin B were sensitive but with comparatively high drug concentrations. One strain was found resistant to both

Azoles Itraconazole and Fluconazole but was sensitive to Amphotericin. Most of *Candida krusei* were isolated from blood and urine specimen.

Table 3. *Candida krusei.*

E strips	Amphotericin B	Fluconazole		Itraconazole
	E strips	E strips	BMD	E strips
C. krusei	2	4	32	0.19
C. krusei	1	2	4	0.05
C. krusei	4	48	32	0.9
C. krusei	1.67	R	R	R
C. krusei	0.032	1	1	0.032
C. krusei	0.031	0.5	1	0.023

Table 4: *Candida albicans.*

E strips	Amphotericin- B	Fluconazole		Itraconazole
	E strips	E strips	BMD	E strips
C. albicans	0.064	0.52-1	5	0.5
C. albicans	0.05	51	21	0.53
C. albicans	0.032	R	R	0.5
C. albicans	0.05	R	R	0.61

Table 5: *Candida glabrata.*

E strips	Amphotericin B	Fluconazole		Itraconazole
	E strips	E strips	BMD	E strips
C. glabrata	0.5	0.5	1	0.032

There is worldwide concern of multidrug resistant *Candida albicans* same was true in this study, authors found high level of resistance by albicans group but the only fortunate thing which came out of our study was the frequency of albicans infection was less. In this study authors could only isolate 4 strains of *Candida albicans*, and among them two were resistant to fluconazole and all were sensitive to amphotericin B and itraconazole. The only uncommon species authors could isolate was *Candida glabrata* , which was isolated from urine sample of patient on immune suppressive drugs the strain however was susceptible to all drugs tested.

Out of all 37 strains of *Candida* isolated in our study four strains were resistant which included two of *Candida albicans*, 1 of *Candida krusei* and 1 of *Candida parapsilosis*.

DISCUSSION

The spectrum of candidemia has changed to *Candida* non *albicans* during the past few decades, but still *Candida albicans* is the leading cause of candidemia in most centers. However, in India, *Non-albicans Candida species* is the major cause of *candidemia* in tertiary care hospitals.[11,12] The emergence of such high rate of non albicans *Candida* species may indicate inadequate hospital care practices, as a majority of these are exogenous in origin. Aggressive use of intravascular devices and carriage of the organism on the hands of health care workers are probable reasons for nosocomial. Use of broad spectrum antibiotics in a constituting combination therapy, use of central catheters and stay in ICU or nursery were among other predisposing factors. The predominance of non albicans *Candida species* like *Candida tropicalis* was notable followed by *Candida parapsilosis*, *Candida krusei*, *Candida albicans* This finding is in agreement with several published reports from India and abroad some authors have attributed the emergence of *Candida krusei* and *Candida glabrata* to the use of fluconazole in transplant units and ICU,s whereas reports have failed to clear epidemiological association. It has been observed that the use of fluconazole for treatment of *candidemia* will lead to resistant non albicans *Candida* species.[13,14] In this study among 37 isolates of *Candida*, *Candida tropicalis* was predominant followed by *Candida parapsilosis*, *Candida krusei*, *Candida albicans* and *Candida glabrata.* In this study two *Candida albicans* where found resistant to fluconazole one *Candida krusei* and one *Candida parapsilosis* were found to be resistant to all azoles but they were sensitive to Amphotericin B. From this study it

was found that both E test and broth microdilution were found to be similar when tested with fluconazole. In conclusion E test is still a golden antifungal susceptibility test and is technically easier method than microdilution.

CONCLUSION

In conclusion, fungemia or *Candidemia* continues to be associated with substantial morbidity and mortality and *Non-albicans candida* species are the commonly isolated pathogen from those patients admitted in tertiary care hospitals in Indian scenario. Thus, there is urgent need to improve health care practices to fight this threat. Despite *Candida tropicalis* is the common isolate pathogen of *Non-candida albicans* species. *Candida krusei* is becoming the most common resistant to azoles. The emergence of azoles resistance at tertiary care centers is also matter of concern further studies are needed to find out the possible correlation between in vitro antifungal susceptibility testing and outcome to therapy, and to confirm the new finding of association between severe renal failure and fungemia. Unfortunately, in this country antifungal susceptibility testing is not widely used, moreover the emergence of non albicans *Candida* species and their resistance to antifungal agents especially in immunocompromised hosts is matter of concern.

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