Candida Prevalence and Its Antifungal Susceptibility in Various Clinical Specimens in Tertiary Care Centre.

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Abstracts: Background: The purpose of this study was to isolate the *Candida spp.* & examine their susceptibility to antifungal drugs from various clinical specimens. One hundred fifty isolates of *Candida spp.* were included in this study. Clinical history revealed that all patients were on systemic broad spectrum antibacterial drugs. Materials and Methods: *Candida spp.* was differentiated by germ tube test, culture characteristics on special media for fungus, sugar fermentation, sugar assimilation and growth on corn meal agar. Antifungal drug susceptibility testing against Fluconazole, Ketoconazole, Itraconazole, Voriconazole, Nystatin and Amphotericin B were done on basis of CLSI guidelines on Methylene blue containing Mueller Hinton Agar by disk diffusion method. Result: We found 52% and 48%, *C.albicans* & *Non albicans candida spp*., respectively. There were no resistance to Nystatin and Amphotericin B. *C.albicans* was more susceptible than *Non albicans candida*. Nystatin & Amphotericin B were susceptible to all isolated *Candida spp*. In present scenario, Fluconazole is most commonly used empirical antifungal drug, which is more effective to *C.albicans* than *Non albicans Candida*. Conclusion: Due to emergence of resistance in Azole group of antifungal among *Non albicans candida*, it should be mandatory to use antifungal drugs as per the susceptibility testing. [Patel Bh et al NJIRM 2012; 3(4): 118-120]

Key Words: *Candida albicans, Non albicans candida*, Methylene blue containing Muller Hinton agar, Antifungal drug susceptibility.

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Introduction: Candida infection is increased in frequency in various age groups, particularly in extreme of age group like neonate, children and in old aged persons; prolonged hospital stay, patients on long term antibiotics therapy, long term indwelling catheters, on immunosuppressive drugs, immunocompromised conditions, chronic debilitating diseases etc. In the era of HIV & AIDS, though opportunistic pathogen are detected frequently in HIV positive patients, but it's prevalence is also very high in HIV negative patient which is reflected in majority hospitalized patients of tertiary care centre across the India. Prompt treatment with antifungal is required for these patients.[1] The emergence of resistance to Fluconazole among Candida albicans and isolation of Non albicans candida species such *C. tropicalis, C. parasilopsis* and *C. krusei* were detected in undertaken this study.

Materials and Methods: From One hundred fifty patients; various clinical specimens (e.g. Urine, sputum, swab, pleural fluid, peritoneal fluid, CSF, blood) collected by maintaining proper aseptic precaution & under stringent sample collection criteria for microbiological specimen, during December-2010 to November-2011, from tertiary care hospital affiliated with Medical College in western India. Detailed clinical history was taken. These specimens were inoculated over Nutrient agar, Mac-Conkey, Blood agar or Chocalate agar and incubated at 37^0C for 24 hrs. Blood sample were taken in Hartley's digest broth incubated at 37^0C & on 1^{st},3^{rd},5^{th} & 7^{th} day subculture done over Nutrient agar, Mac-Conkey agar, Blood agar or Chocalate agar.[1,2]

Candida spp. suspected from their colony characteristics (dry, pin point, opaque & fluffy; growth on all above mentioned media). Suspected growths of candida were confirmed by wet mount preparation & Gram stain. Isolated candidas were further subcultured on Saboraud's Dextrose Agar (SDA). These were further identified into species level by Germ

Tube Test (GTT), GTT positive isolates were *Candida albicans* and *Candida dubliniensis*, GTT negative were *Non albicans candida spp*. They were further differentiated by (a) growth on corn meal agar, (b) sugar fermentation (2% sugar), (c) carbohydrate assimilation tests. [1, 2,, 8]

Antifungal susceptibility to Fluconazole (30 mcg), Ketoconazole (50 mcg), Itraconazole(30 mcg), Voriconazole (1 mcg), Nystatin (50 mcg), Amphotericin B (50 mcg) were tested by Disk diffusion method, as recommended by CLSI document M - 44 A, in Muller Hinton agar containing Glucose (2%) & Methylene blue (50 mcg/ml) and was incubated at 37^0C for overnight. If there was no confluent growth, it was further incubated for another 24 hrs.[3] Amphotericin B also tested for minimum inhibitory concentration (MIC) by E-test (AB Biodisk, Sweden). The isolates were categorized into susceptible(S), dose dependent susceptible (SDD) or intermediate (I) and resistant (R) on basis of measured zone of Inhibition. [2, 3]

Result: Table-1 enumerates the predisposing factors for candidal infection. All patients had received broad spectrum antibiotics & none of them were seropositive to HIV I & II. Out of 150, 18 patients were neonates and 26 patients were children (< 12 years) age. Sixteen patients had respiratory complaints and 82 patients suffered from sepsis.

Out of 150, 132 were admitted in intensive care unit and out of them 62 were on ventilator. The 150 isolates belonged to four species, namely *C. albicans* (78/150), *C. tropicalis* (61/150), *C. parasilopsis* (6/150) and *C. krusei* (5/150). Table 2 shows the antifungal susceptibility pattern to various antifungal drugs. Out of 78 *C. albicans*, 48(15.38%) were resistant to Fluconazole. All isolates of *C. krusei* showed resistance to Fluconazole. Out of 61 C. tropicalis, 12(19.67%) were resistance to Fluconazole. Out of 06 *C. parapsilopsis*, 02 (33.33%) were resistance to Fluconazole. All the isolates were susceptible to Amphotericin B as the MICs were less than 16 mcg/ml.

Table 1: Predisposing Factors candidal infection.

Underlying predisposing factor	n (%)
Prolonged Antibiotic therapy	150 (100%)
Pt. in NICU	18 (12%)
Below 12 years of age	26 (17.33%)
Respiratory system complaints	16 (10.67%)
Sepsis	82 (54.67%)
G.I. system complaints	05 (3.33%)
Neurological symptoms	21 (14%)
Admitted in ICU	132 (88%)
Pts. on ventilator	62 (41.33%)

Among azole group of antifungal drugs, Voriconazole is the best performing dugs followed by Fluconazole, Ketoconazole & Itraconazole. There were no resistance to Amphotericin B and Nystatin in *C. albicans* and *Non albicans candida spp.*

Table 2: Antifungal susceptibility of 150 isolates.

Drug	*C.albicans* (n = 78)		*Non albicans Candida* (n = 72)					
			C. tropicalis (n = 61)		*C. parapsilosis* (n= 06)		*C. krusei* (n = 05)	
	S	R	S	R	S	R	S	R
Fluconazole	66	12	49	12	4	2	-	5
Ketocanazole	63	15	45	16	3	3	3	2
Itracanazole	59	19	42	19	2	4	2	3
Voriconazole	76	02	58	03	6	-	4	1
Amphotericin B	78	-	61	-	6	-	5	-
Nystatin	78	-	61	-	6	-	5	-

Discussion: Candidiasis is flare up in the presence of predisposing factors such as broad spectrum & prolonged antibiotics therapy, low weight, Intravenous hyper alimentation, ventilator therapy, etc. [4,7]

Earlier studies have found *C. albicans* found as the most common isolate followed by *C. parapsilosis* and *C. tropicalis*. In our study, *C. albicans* was found the most common isolate followed by C. tropicalis and C. parapsilosis.[9] A striking feature of our study was the isolation of *C. krusei*, a species known for its innate resistance to Fluconazole.[5] This could be a result of non judicious use of Fluconazole for the treatment of candidiasis, which might have led to selection of such innately resistant species. Drug resistance seen in our study but we do not have any previous data for internal comparison, as this is the first study in our hospital. *Candida spp*. is important pathogens in various clinical infections. Their speciation as well antifungal susceptibility patterns need to be studied especially in light of increasing drug resistance.[6] Alternatives to Fluconazole such as 5-FU and Amphotericin B needed to be evaluated for therapy. Antifungal susceptibility of each Candida isolated from clinical specimen should be performed in view of its being emerging as hospital acquired pathogen. *Non albicans candida is* now emerging hospital acquired pathogens & they are more resistant to Azoles group of antifungal drugs.[5]

Conclusion: Now a days, *Candida spp*. infection increased in hospital setting, particularly *Non albicans candida* are now emerge as hospital acquired pathogen. Because of this, it is advisable to do Candida speciation and antifungal susceptibility testing. Antifungal susceptibility testing is easy & user's friendly method, possible to perform in any Microbiology laboratory by disk diffusion method. Physician should changes antifungal therapy of patients and shift patients on susceptible antifungal drug therapy from initial empirical antifungal therapy.

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