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Multidrug resistance of Candida Krusei isolated from various clinical isolates to some antifungal agents

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Abstract

Introduction: Antifungal susceptibility testing is a subject of interest in the field of medical mycology which deals with effectiveness of antifungal drugs. The aim of the present study was the identification, distributions and antifungal susceptibility patterns of various Candida Krusei isolated from various clinical specimens in the multispecialty hospital.

Material and methods: A clinical samples will be submitted to **Dept. of Microbiology L.N. Medical College Bhopal** for routine diagnostic workups were assessed. of 310 Candida samples 41 samples have Candida Krusei so 41 sample that have Candida krusei were used for further study for antifungal susceptibility testing by a battery of microbiological investigations aimed at detecting, isolating, identifying and characterizing the Candida sp. so as to determine the spectrum of Candidiasis.Samples were processed by Gram staining, KOH mount and culture on SDA and BHI agar. Isolated yeasts were identified and speciated by germ tube test, chlamydospores formation on corn meal agar, color production on CHROM agar, sugar fermentation test and sugar assimilation test. Antifungal susceptibility testing of isolates was performed as per CLSI guidelines.

Results: Out of 310 samples of candida contains 41 (13.00%) samples contain Candida Krusei. In our study out of total candida species in 310 samples.Candida albicans more prevalent rather than other candida species in various clinical samples.Out of total 41 samples of Candida Krusei 41 (100.00%) samples were found susceptible for Amphotericin-B and 0 (0.00%) samples were resistant for Amphotericin –B. Fluconazole were shows 35 samples that was 85.37% of total samples were found susceptible and 6 samples were resistant that was make 14.63% of total samples. Variconazole was show

susceptibility for 38 samples that was making 92.68% of total 61 samples and resistant for 3 samples that was form 7.32% of total samples. Itraconazole was show susceptibility for 38 samples that was making 92.68% of total 61 samples and resistant for 3 samples that was form 7.32% of total samples. . Nystatin was show susceptibility for 38 samples that was making 92.68% of total 61 samples and resistant for 3 samples that was form 7.32% of total samples.

Conclusion: The present study reported a significant shift in incidence of invasive Candidiasis from C.albicans to Non albicans species. Non - albicans Candida has emerged as an important opportunistic pathogen. Non-albicans species are assuming an increasing role in nosocomial infections particularly in infants. The incidence of Candidaemia caused by non-albicans species is frequent and increasing significantly.

Key words: *Candida* Species, Nosocomial, Antifungal Resistance, infants.

Introduction: -Over last two decades there has been a phenomenal rise in occurrence of fungal lung infections. Specific diagnosis of fungal pneumonia assumes importance in view of different therapeutic strategies involved and higher mortality associated with acute invasive fungal infections [(1,2)]. Since patients with chronic lung pathology provide a suitable nidus for fungal colonization of respiratory tract[(3,4)], the screening for the same would enable us to identify the individuals requiring close monitoring for development of possible complications like acute invasive fungal infection or dissemination via hematogenous spread [(4,5)].

Candida urinary tract infections are an increasingly prevalent nosocomial problem and constitute an important subgroup of nosocomial UTI (10-15%) [(6,7,8)].

Mucosal candidiasis including oropharyngeal, esophageal and vaginal candidiasis is most common among HIV infected patients. In particular oropharyngeal candidiasis occurs in upto 90% of patients during thecourse of HIV infection [(9)].

Vulvovaginal candidiasis remains a common cause of morbidity with 3/4th of women affected during their lifetime. Knowledge of patterns of genital candida sp. level identificationis important for management asspecies other than Candida albicans often fail first line treatment. Definite microbiological diagnosis is made for women with recurrent symptoms or those failing initial treatment to guide appropriate therapy. Early detection

and initiation of appropriate therapy may alter the course of these infections and improved the prognosis [12,13,14].

In the light of above facts present study was undertaken to characterize species of candida isolatesfrom various clinical specimens from DGH to facilitate early detection and reducing in morbidity and mortality in such cases. This study intends to determine the incidence of candida infection among patients of Pacific medical college and hospital and to detect any shift in incidence from C.albicans to non-albicans[15,16] .

In the light of above facts present study was undertaken to characterize species of candida isolates from various clinical specimens from DGH to facilitate early detection and reducing in morbidity and mortality in such cases[17,18]. This study intends to determine the incidence of candida infection among patients of Pacific medical college and hospital and to detect any shift in incidence from C.albicans to non-albicans in which such as C. Krusei[19].

Nosocomial infections due to *Candida* species have increased significantly during the past three decades[20]. Use of a wide range of biomaterial instruments (urinary and in- dwelling vascular catheters, denture appliances, orthopedic prostheses and heart valves) in clinical practice accelerates infection by *Candida* species[In addition, Nosocomial *Candida* infections are more prevalent among immune compromised individuals and those with a history of diabetes, malignancy, neutropenia, cancer chemotherapy, organ transplantation, hemodialysis, use of broad-spectrum antimicrobial agents and prolonged hospitalization.·Candiduria, vulvovaginal candidiasis and oral candidiasis are the most important forms of the disease [21-25].

Candida Krusei still considered as the major etiologic agent in candidiasis and several factors are associated with its pathogenesis. The ability of *C.* Krusei to form biofilms and adhere to host tissues and biomaterial surfaces is an important pathogenesis factor·Biofilm formation can act as a reservoir of agents, allow co-infection with other pathogens, promote persistence of infection and increase mortality [26-30].

Material and Methods

Sample Size: - A clinical samples will be submitted to Dept. of Microbiology for routine diagnostic workups were assessed. Candida isolates selection from 310 cases will be fulfilled the diagnostic criteria for Candidiasis will included in the study and will further process by a battery of microbiological investigations aimed at detecting, isolating, identifying and characterizing the Candida sp. so as to determine the spectrum of

Candidiasis. A detailed review of Clinical History & clinical examination findings will be taken. Out of 310 Candida samples 41 samples have Candida Krusei so 41 sample that have Candida krusei were used for further study for antifungal susceptibility testing.

Inclusion criteria:

1. Clinical manifestations of invasive Candidiasis.
2. Patients associated with immunocompromised diseases.
3. Presence of associated predisposing conditions and risk factors.
4. Patients under the chemotherapy and steroids.
5. Patients treated with broad spectrum antimicrobial agents for longer duration[43-47].

Exclusion criteria:

1. Candida found as a commensals on many sites such as skin, mouth, small intestine, large intestine and colon so we excluded these sites from the study except we don't find any lesion at particular site. Candida always has competition to many bacteria on naturally occurring sites so it never overgrow and cause disease [39-42].

Note: In this study we are concerned only with Candida infection or invasive Candidiasis and not with Candida colonization. The isolates from cases not fulfilling the inclusion criteria will be considered as Candida colonization and to be excluded from the study.

- Identification on chrome agar

Incubation will be at 35°-37° C and 25° C in BOD incubator examined regularly for growth of Candida species. Morphological identification will be done on chrome agar by the development of different types of color according to their species. Slants will incubate for 1 week and discarded if no growth occurred by them [31-34].

Antifungal Susceptibility Testing: -Fungal susceptibility to routinely used drugs like amphotericin B (100 units), fluconazole (25 μg), voriconazole (1 μg),Itraconazole and Nystatin was done by the disk diffusion method, using Mueller-Hinton agar supplemented with 0.5 mg/ml methylene blue. Agar plates were inoculated with a suspension of yeast cells whose turbidity was adjusted to 0.5 McFarland standards (10^6 CFU/ml) in a manner that is currently being used for testing antibacterial agents (35-38). Antifungal disks were placed on the inoculated plates and incubated at 27°C for 24–48 hours) diameter of the zone of inhibition was measured. Results were interpreted as per CLSI guidelines [10,12].

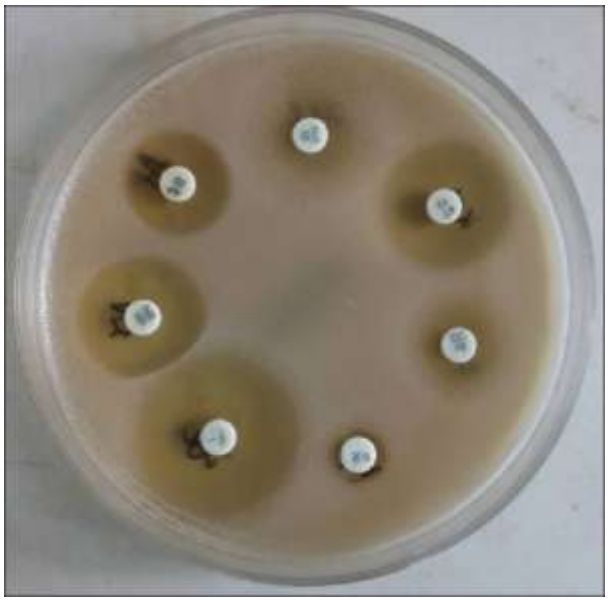

Figure: antifungal susceptibility testing (as per CLSI) method.

Statistical analysis: - The data was statistically analyzed using the statistical package for Social science (SPSS)/ 21.0 (Copyright © SPSSInc.). Frequency of qualitative variables was calculatedand correlation was tested by Chi-square test. Statisticalsignificance was accepted at p <0.05.4.

RESULTS:-

Antifungal susceptibility testing of Candida Kruesi					
	Sensitive	**Resistance**	**Total**	**Sensitive**	**Resistance**
Amphotericin - B	41	0	41	100.00%	0.00%
Fluconazole	35	6	41	85.37%	14.63%
Variconazole	38	3	41	92.68%	7.32%
Itraconazole	38	3	41	92.68%	7.32%
Nystatin	38	3	41	92.68%	7.32%

Table 1:- Total Number & Percentage of Antifungal susceptibility testing of Candida Krusei.

Out of total 41 samples of Candida Krusei 41 (100.00%) samples were found susceptible for Amphotericin-B and 0 (0.00%) samples were resistant for Amphotericin –B. Fluconazole were shows 35 samples that was 85.37% of total samples were found susceptible and 6 samples were resistant that was make 14.63% of total samples (40-42). Variconazole was show susceptibility for 38 samples that was making 92.68% of total 61

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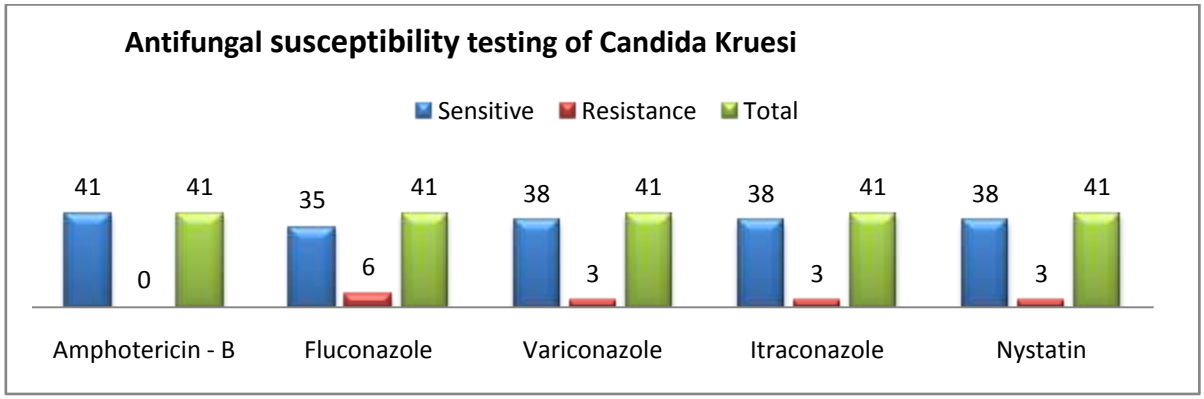

Graph 1: Total Number of Antifungal susceptibility testing of Candida Krusei.

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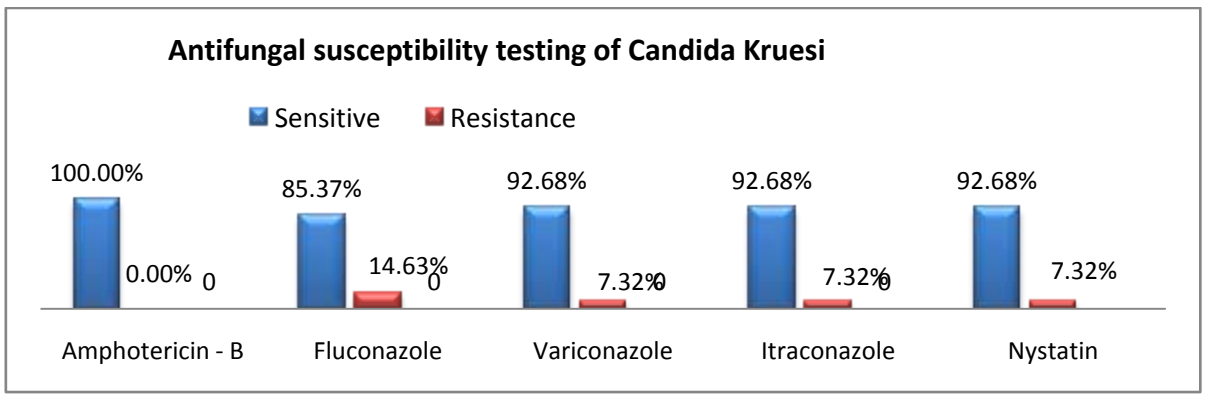

Graph 2: Total Percentage of Antifungal susceptibility testing of Candida Krusei.

Conclusion

The present study reported a significant shift in incidence of invasive Candidiasis from C.albicans to Non albicans species such as Candida krusei.This shift is alarming as the infections caused by Candida krusei are often server, rapidly progressive, refractory to therapy and associated with higher mortality and significant morbidity and also develop resistance against major antifungal agents.

Candida Krusei responsible for Multi drug resistance in recent clinical samples which commonly responsible for spread of multi drug resistance candida infection in clinical setup. The innate and intrinsic resistances of Non-albicans sp. to the commonly used antifungal agents creates problems and complicates the management of the patients. This leads to prolonged hospitalization, increased costs of treatment and delays recovery requiring extra resources for investigations, management and nursing care.

Early detection of the pathogen and institution of appropriate timely therapy alters the course of infection and improves the prognosis thus benefitting the patient.

In our study the important associated predisposing factors detected were persistent use of broad spectrum antibiotics, indwelling devices, prolonged- hospitalization, steroid-therapy, Diabetes-mellitus, Renal-failure, haemodialysis, mechanical-ventilation, major surgeries and extremes of age.

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