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ISOLATION IDENTIFICATION AND ANTIFUNGAL SUSCEPTIBILITY TESTING OF CANDIDA SPECIES ISOLATED FROM VARIOUS CLINICAL SPECIMEN IN THE HOSPITAL.

Medical Microbiology

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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 species isolated from various clinical specimens in the multispecialty hospital.

MATERIAL AND METHODS: 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.

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, chlamydosporesformation 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 161 (52.00%) Candida albicans, 41 (13.00%) samples contain Candida Krusei, 21 samples (7.00%) has Candida Glabrata, 31 (10.00%) samples were contains Candia parasilopsis and Candia tropicalis (18.00%). In our study out of total candida species in 310 samples.Candida albicans more prevalent rather than other candida species in various clinical samples. Amphotericin-B shows 305 samples were sensitive (98.39%) and 5 (1.61%) samples were resistant for Amphotericin-B. fluconazole were shows 242 (78.06%) samples were sensitive and 68 (21.94%) samples was shows resistant. Variconazole were shows 242 (78.06%) samples were sensitive and 68 (21.94%) samples was shows resistant. Itraconazole were shows 280 (90.32%) samples were sensitive and 68 (9.68%) samples was shows resistant. Nystatin was shows 291 (93.87%) samples were sensitive and 68 (6.13%) samples was shows resistant.

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.

KEYWORDS

Candida Species, Nosocomial, Antifungal Resistance, infants.

INTRODUCTION: -

Candida species are important nosocomial pathogens in critically ill and Immunocompromised patients and there has been an important shift in the species causing invasive candidacies away from Candida albicans to more resistant non-albicans infections are often severe, rapidly progressive and difficult to diagnose and refractory to therapy [2,4,5].

Candidemia is associated with increased cost of treatment and Attributable mortality of 38%. As a result of introduction of fluconazole prophylaxis non-albicans candida has now emerged as a significant pathogen [4,9]. Candida species are components of normal microbial flora of human body inhabiting mouth, Intestines andvagina [1, 2].When immunological defense mechanisms are compromised it causes infection in the sites where it is colonized and also elsewhere in the body.

Candida is an opportunistic endogenous infection. The factors predisposing to opportunistic infections acteither by altering the balance of normal microbial flora of the body or by lowering the host resistance [1,2,3,4].The growing problem of mucosal and systemic candidiasis reflects an enormous increase in the pool of patients at risk and increased opportunity for candida spp. to invade tissue normally resistant to invasion. Technological advances are exploited to gain access to the circulation and deep tissue [3].

MATERIALAND 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.

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.

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.

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

Figure 1Candida species 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.

Antifungal Susceptibility Testing. Fungal susceptibility to routinely used drugs like amphotericin B (100 units), fluconazole (25 μg), voriconazole (1 μg),Itraconazole, 5 fluro-cysteine (5FC), Ketoconazole and Caspofungin 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 (106 CFU/ml) in a manner that is currently being used for testing antibacterial agents. 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 [11, 12].

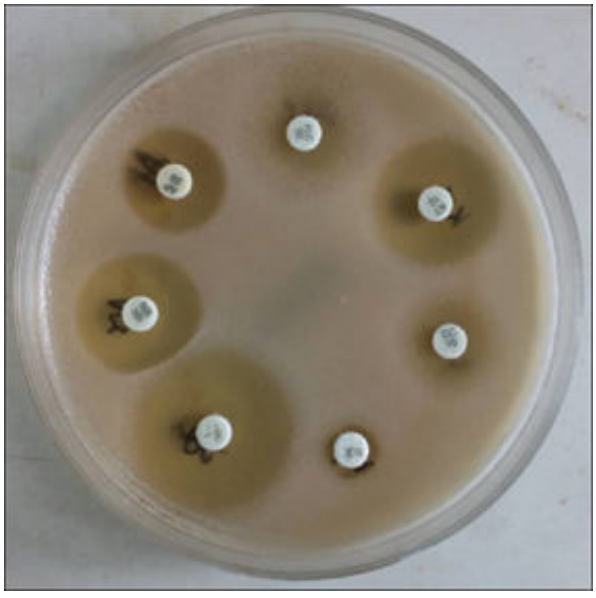


Figure:1 antifungal susceptibility testing (as per CLSI) method.

STATISTICALANALYSIS: -

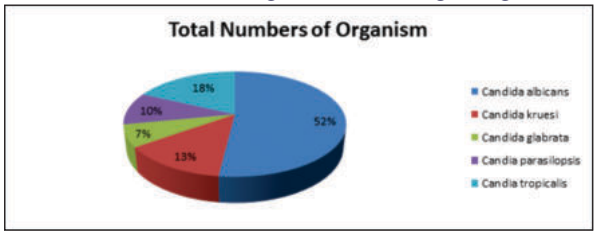
The data was statistically analyzed using the statistical package for Social science (SPSS)/ 21.0 (Copyright © SPSSInc.). Frequency of qualitative variables was calculatedand correlation was tested by Chi-square test. Statisticalsignificance was accepted at $p < 0.05$.4.

RESULTS

Out of 310 samples of candida contains 161 (52.00%) Candida albicans, 41 (13.00%) samples contain Candida Krusei, 21 samples (7.00%) has Candida Glabrata, 31 (10.00%) samples were contains Candia parasilopsis and Candia tropicalis (18.00%). In our study out of total candida species in 310 samples.Candida albicans more prevalent rather than other candida species in various clinical samples.

Name of Organism	Total Numbers	Total %
Candida albicans	161	52.00%
Candida kruesi	41	13.00%
Candida glabrata	21	7.00%
Candia parasilopsis	31	10.00%
Candia tropicalis	56	18.00%
Total	310	100.00%

Table 01:- Total Number of organism & Percentage of organism



Graph 01 :- Total Number of organism & Percentage of organism
Total Number of Candida albicans and Non albicans & Percentage of Candida albicans and Non albicans

Out of total 310 samples Candida albicans 161 (52%) samples and 149 (48%) candida non albicans samples are isolated.

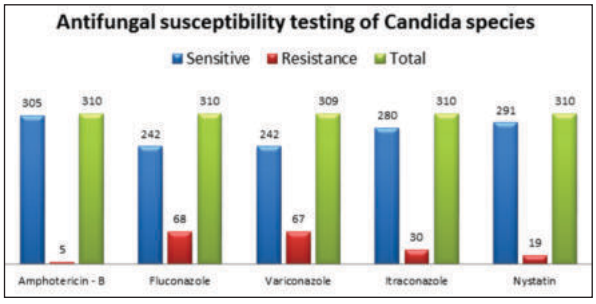
Gender wise Candida Species.

Gender wise candida species distribution was shows 143 (46.13%) males were affected from Candida infection and 167 (53.87%) females affected from Candida species. It was shows more prevalence in females for candida species.

Antifungal susceptibility testing of Candida species					
Antifungal	Sensitive	Resistance	Total	Sensitive	Resistance
Amphotericin - B	305	5	310	98.39%	1.61%
Fluconazole	242	68	310	78.06%	21.94%
Variconazole	242	68	310	78.06%	21.94%
Itraconazole	280	30	310	90.32%	9.68%
Nystatin	291	19	310	93.87%	6.13%

Table:-2 Total Number & Percentage of Antifungal susceptibility testing of Candida species.

Amphotericin-B shows 305 samples were sensitive (98.39%) and 5 (1.61%) samples were resistant for Amphotericin-B. fluconazole were shows 242 (78.06%) samples were sensitive and 68 (21.94%) samples was shows resistant. Variconazole were shows 242 (78.06%) samples were sensitive and 68 (21.94%) samples was shows resistant. Itraconazole were shows 280 (90.32%) samples were sensitive and 68 (9.68%) samples was shows resistant. Nystatin was shows 291 (93.87%) samples were sensitive and 68 (6.13%) samples was shows resistant.



Graph:-2 Total Number Sensitive and Resistance of Antifungal susceptibility testing of Candida species.

DISCUSSION:

All of our patients had history of administration of Broad spectrum antibiotics for variable periods, 92 % of our patients were having indwelling devices (intravenous cannulae, Central venous catheter, Urinary catheter, Ryle's Tube, Endotracheal Tube), 64% had history of prolonged hospitalization, half of the patients were in extremes of age, 26% were on steroids, 20% were diabetic, 12% were on mechanical ventilation, 8% were having Acute or chronic renal failure, 8% were on hemodialysis and 6% had undergone major surgery.

These findings are in accordance with various Indian studies conducted by Capoor MR et al, Chakrabarti et al, Saha et al, Sahni et al, Chown MN et al, Banerjee U et al, Goel N et al, Adhikari R et al, Rani R et al, Arora D et al and international studies like that of NurYapar et al, Benzamin DK et al and Dimopoulos G et al.

CONCLUSION

The present study reported a significant shift in incidence of invasive Candidiasis from C.albicans to Non albicans species.This shift is alarming as the infections caused by non-albicans species are often server, rapidly progressive, refractory to therapy and associated with higher mortality and significant morbidity. 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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