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ISOLATION IDENTIFICATION AND IN-VITRO ANTIFUNGAL SUSCEPTIBILITY OF CANDIDA ALBICANS ISOLATED FROM VARIOUS CLINICAL SPECIMENS

Medical Microbiology

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ABSTRACT

Objective: The aim of this study was to investigate different species of Candida in-vitro antifungal susceptibility testing. Candida is an opportunistic endogenous infection. The factors predisposing to opportunistic infections act either by altering the balance of normal microbial flora of the body or by lowering the host resistance

Material and methods: 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.

Results: Candida albicans shows antifungal susceptibility against total 322 samples of Candida albicans. Amphotericin-B shows 316 (98.14%) samples were sensitive and 6 (1.86%) were resistant for it. Fluconazole shows 240 (74.53%) samples were sensitive and 82 (25.47%) samples were resistant. Variconazole shows 216 (67.08%) samples were sensitive and 106 (32.92%) samples were resistant for it. Itraconazole shows 274 (85.09%) sensitive out of total 322 samples of Candida albicans and 48 (14.91%) samples were resistant for Itraconazole. Nystatin was sensitive for 294 (91.30%) samples and 28 (8.70%) samples were resistance Candida albicans ($p < 0.001$).

Conclusion: 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, hemodialysis, mechanical-ventilation, major surgeries and extremes of age.

KEYWORDS

biofilm, Candida, Diabetes-mellitus, hemodialysis, liner.

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 and vagina (38).When immunological defence 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 (39).

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 (39).

The dramatic surge in incidence of candida spp. is due to increasing population of terminally ill, debilitated, immuno compromised patients, increasing use of advanced therapeutic modalities for advanced life support ; indwelling devices ; widespread use of broad spectrum antibiotics ; long term use of immunosuppressive agents; increasing incidence of HIV infection ; high prevalence of Candida hand carriage in health care workers and ability of candida sp. to survive on environmental surfaces [40,41,42,43,44].

Numerous factors operate collectively to make Candida, the 4th most common primary blood stream organism and sixth most common nosocomial pathogen [39,41,44,45,46].

Candida species are important nosocomial pathogens in critically ill and immunocompromised patients and there has been an important shift in the species causing invasive candidiasis away from Candida albicans to more resistant non-albicans infections are often severe, rapidly progressive and difficult to diagnose and refractory to therapy [40,42,45].

Candidaemia 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 [42,47].

Candida species is part of the normal flora of healthy individuals and are consideredopportunistic pathogens as they colonize different tissues and cause systemic mycosis when the immune system of the host is depressed.1 Over the last few years, the incidence of fungal infections has progressively raised and it has been a primary cause of morbidity and mortality in immune-compromised and severely ill patients and hence is rightly called the “disease of diseased”.2 Vulvovaginal candidiasis (VVC) affects75% of all women at least once in their lifetime, most commonly during childbearing age.3 *Candida* species is the fourth most common cause of nosocomial bloodstream infections (BSIs), with a significantly high attributable mortality (49%–70%). The use of central venous catheters has been found to be responsible for >70% of bloodstream and deep-tissue infections.

MATERIALAND METHODS

Sample Size:- A clinical samples will be submitted to Dept. of Microbiology Pacific Medical College and Hospital Udaipur Rajasthan, for routine diagnostic workups were assessed. Candida isolates selection from 620 cases will be fulfilled the diagnostic criteria for Candidiasis will included in the study and will further process for identification of Candida Albicans, 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 620 samples 322 samples were isolated as Candida albicans.

- **Mention date of research start**

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

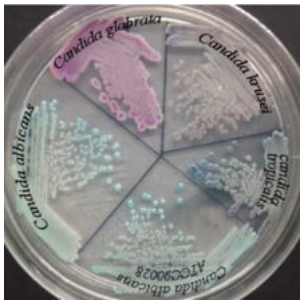

Figure: 1. Candida species identification on chrome agar.

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. Germ tube test were done with other mycological examination for confirmation of Candida albicans as per protocol.

Figure: 2. Germ tube test for identification of Candida albicans (40X)

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].

Statistical Analysis:-

The data was statistically analyzed using the statistical package for Social science (SPSS)/ 21.0 (Copyright © SPSS Inc.). Frequency of qualitative variables was calculated and correlation was tested by Chi-square test. Statistical significance was accepted at $p < 0.05.4$.

RESULT AND OBSERVATION

Table 02:- Total Number Of Candida Albicans And Non Albicans & Percentage Of Candida Albicans And Non Albicans.

Name of Organism	Total Numbers	Total %
Candida albicans	322	52%
Candida non albicans	298	48%
Total	620	100%

Out of total 620 samples Candida albicans 322 (52%) samples and 298 (48%) candida non albicans samples are isolated.

Candida albicans shows antifungal susceptibility against total 322 samples of Candida albicans. Amphotericin-B shows 316 (98.14%) samples were sensitive and 6 (1.86%) were resistant for it. Fluconazole shows 240 (74.53%) samples were sensitive and 82 (25.47%) samples were resistant. Variconazole shows 216 (67.08%) samples were sensitive and 106 (32.92%) samples were resistant for it. Itraconazole shows 274 (85.09%) sensitive out of total 322 samples of Candida albicans and 48 (14.91%) samples were resistant for Itraconazole. Nystatin was sensitive for 294 (91.30%) samples and 28 (8.70%) samples were resistance Candida albicans ($p < 0.001$).

DISCUSSION AND CONCLUSION

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 Nur Yapar et al, Benzamin DK et al and Dimopoulos G et al.

Infection of Candida albicans leads to prolonged hospitalization, increased costs of treatment and delays recovery requiring extra resources for investigations, management and nursing care. It also more prominently associated with young females which more prone towards developing urinary tract infection.

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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