Candida Species Isolation, Identification and Biofilm Detection at a Tertiary Care Hospital

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ABSTRACT

Introduction: Early diagnosis and accurate treatment of candida infected patients helps to reduce the risk of infection and improves patient outcome. Candida isolation, speciation and its invasiveness can be determined by culture, antigen and antibody estimation, glucan estimation and PCR. The present study aimed at candida isolation, speciation and detection of biofilm production among various clinical samples.

Material and Methods: Various clinical samples such as urine, pus, blood, cerebrospinal fluid, body fluids, tissue, oral and ear swabs etc. were collected from patients in a sterile container and transported immediately to Microbiology laboratory and processed according to standard protocols.

Results: Out of 64 candida isolates from various clinical isolates, Majority were Candida albicans (37.5%), followed by candida tropicalis (32.8%), candida krusei (20.3%), Candida parapsilosis (6.2%) and Candida glabrata (3.1%).

Conclusion: Early diagnosis and accurate treatment of candida infected patients helps to reduce the risk of infection and improves patient outcome. Assessing biofilm production of candida isolates helps us to plan treatment and identify the niche for production of biofilms.

Keywords: Candida, Clinical Samples, Sensitivity Pattern, Biofilm.

INTRODUCTION

Candida species are usually commensals which colonize the skin and mucosal surfaces of Humans. Candida can be isolated primarily from gastrointestinal tract, the skin, the vagina and the oropharynx.[1] The incidence of candidemia can range from 0.39/1000 to 14.2/1000discharges or admissions or 0.026/1000 to 4.2/1000 patient days among hospitalized patients.[2]

The predisposing factors for invasive Candida infections include immunosuppressive conditions such as diabetes, HIV, patients on chemotherapy, the use of broad spectrum antibiotics or steroids, presence of indwelling devices. The implantation of foreign bodies like vascular catheters, cardiac pacemakers, neurosurgical shunts, prosthetic heart valves, and orthopedic devices also add to the list of risk factors.[3,4]

C. albicans the predominant cause of invasive fungal infections which poses a serious public health threat, with increasing medical and economic importance due to the high mortality rates and increased costs of care and duration of hospitalization. Even though C. albicansis the most predominant species involved in invasive fungal infections, the incidence of infections due to non-albicans species is steadily increasing. The change in epidemiology can be associated with severe immunosuppression or disease, prematurity, exposure to broad-spectrum antibiotics or elderly patients.[5,6]

Candidiasis also called moniliasis, can cause a wide spectrum of clinical syndromes including Cutaneous candidiasis syndromes, Chronic mucocutaneous candidiasis, Gastrointestinal tract candidiasis, Respiratory tract candidiasis, Genitourinary tract candidiasis, Hepatosplenic candidiasis, Systemic candidiasis, Disseminated candidiasis, Candida endophthalmitis, Renal candidiasis, CNS infections due to Candida species, Candida arthritis, osteomyelitis, costochondritis, and myositis, Myocarditis-pericarditis, Candida peritonitis.

Virulence traits specialized and established by Candida species are invariably related to the factors that determine infection i.e. adhesion and invasion of host tissues, biofilm formation and evasion of the immune system. C. albicans produces highly structured biofilms composed of multiple cell type sencased in an extracellular matrix. Candida biofilms are especially widespread and have been observed in most, if not all, medical devices in current use, such asstents, shunts, implants, endotracheal tubes, pacemakers, and various types of catheters.[7] Candida species have innately highly variable antifungal susceptibilities.[8]

Candida isolation, speciation and its invasiveness can be determined by culture, antigen and antibody estimation, glucan estimation and PCR.

The present study aimed at candida isolation, speciation and detection of biofilm production among various clinical samples

MATERIAL AND METHODS

This prospective study on Candida speciation, and biofilm detection was conducted on 64 isolates of Candida species isolated from various clinical samples in the Department

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Age group in years	Males	Percentage	Females	Percentage	Total	Percentage
0-<1	22	56.4%	17	43.5%	39	60.9%
1-20	2	40%	3	60%	5	7.8%
21-40	3	37.5%	5	62.5%	8	12.5%
41-60	4	80%	1	20%	5	7.8%
>60	4	57.1%	3	42.8%	7	10.9%
Total	35	54.6%	29	45.3%	64	100%

Table-1: Age and sex distribution of patients with candidal infections

Antifungal drug	Sensitive	Resistant
Amphotericin B (100µg)	64 (100%)	0
Nystatin (100 µg)	64 (100%)	0
Fluconazole (10 µg)*	17 (60.7%)	11 (39.2%)
Itraconazole (30 µg)	56 (87.5%)	8 (12.5%)
Clotrimazole (10 µg)	52 (81.2%)	12 (18.7%)
Ketoconazole (30 µg)	54 (84.3%)	10 (15.6%)
*Candida krusei isolates were excluded, as they are inherently resistant to flucanozole.		

Table-2: Antibiotic sensitivity pattern of candida isolates

Organism	Biofilm Non producers	Biofilm producers
Candida albicans (n=24)	7 (29.1%)	17 (70.8%)
Candida krusei (n=13)	2 (15.3%)	11 (84.6%)
Candida tropicalis (n=21)	7 (33.3%)	14 (66.6%)
Candida parapsilosis (n=4)	1 (25%)	3 (75%)
Candida glabrata (n=2)	2 (100%)	0
Total (n=64)	17 (26.5%)	47 (73.4%)

Table-3: Assessment of Bio film production among Candida isolates

of Microbiology, Government Medical College, Kurnool. Study conducted from July 2018 to December 2018, Single isolate per patient was included in the study.

Various clinical samples such as urine, pus, blood, cerebrospinal fluid, body fluids, tissue, oral and ear swabs etc. were collected from patients in a sterile container and transported immediately to Microbiology laboratory and processed according to standard protocols.

All the clinical samples were inoculated on MacConkey agar, 5% sheep blood agar and two sets of Sabouraud Dextrose Agar (SDA) slopes and incubated for 48 hours. Blood samples were first inoculated in Brain Heart Infusion broth (BHI), incubated at 37°C for 48 hours and then sub cultured onto SDA, 5% sheep blood agar and MacConkey agar. Along with culture, samples were processed for KOH mount and Gram stain.

Candida identification and speciation was done by colony morphology, gram stain, germ tube test, characterization of spores using corn meal agar, carbohydrate fermentation and assimilation tests.

Biofilm production was detected by Percentage Transmission method or percent transmittance (%T) by measuring optical density (%T) in microtitre plate with ELISA reader.

Antifungal susceptibility testing was assessed by disk diffusion method on Mueller Hinton agar (MHA) + 2%

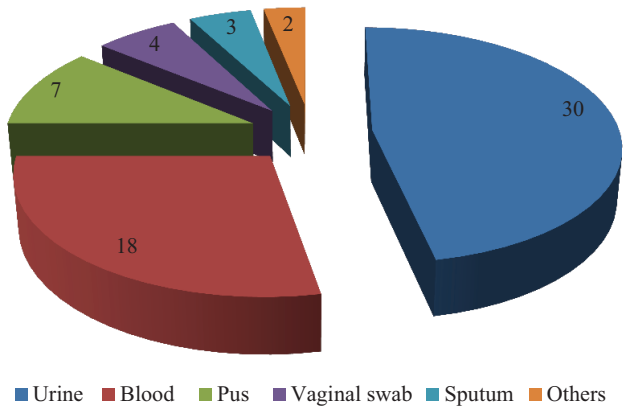

Figure-1: Percentage of candida isolated from various clinical samples

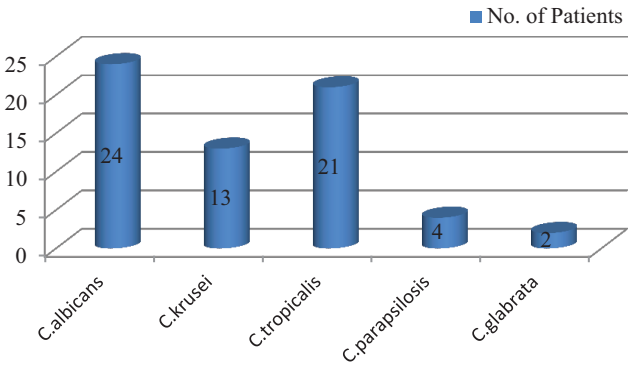

Figure-2: Different species of candida isolated

Glucose and 0.5 µg/mL Methylene Blue Dye(GMB) Medium by using Amphotericin B 100 µg, Fluconazole 10 µg, Ketoconazole 30 µg, Itraconazole 30 µg, Nystatin 100 µg andClotrimazole 10 µg. The plates were inverted and placed in an incubator set to 35 °C (± 2 °C) for 20 to 24hours. The interpretation was done according to CLSI M44A guidelines.

RESULTS

Candida isolated most commonly in the age group of 0- <1 year (60.9%), followed by 21-40 years (12.5%), ≥ 60 years (10.9%), 1-20 years (7.8%) and 41-60 years (7.8%).

Out of 64 Candida isolates, 29 (45.3%) were females and 35 (54.6%) were males (Table 1).

Most of the candida were isolated from urine (46.8%) followed by blood(28.1%), pus (10.9%), vaginal swab (6.2%), sputum (4.6%) and other samples including oral swab, ear swab, sterile fluids, tissue bits of about 3.1% (Fig 1).

Out of 64 candida isolates from various clinical isolates, Majority were Candida albicans (37.5%), followed by

candida tropicalis (32.8%), candida krusei (20.3%), Candidaparapsilosis (6.2%) and Candida glabrata (3.1%) (Fig 2)

On assessment of disk diffusion test by CLSI guidelines, all 64 isolates of Candida species were sensitive to Amphotericin B and nystatin. 62.7% of candida species other than Candida krusei shown sensitivity to fluanozole. Candida species were shown 87.5% sensitivity to itraconazole, 81.2% sensitivity to clotrimazole and 84.3% sensitivity to ketoconazole. (Table 2).

Among 24 Candida albicansisolates, 17(70.8%) were biofilm producers. 11 (84.6%) out of 13 isolates of C.kruseiwere biofilm producers. Among 21C.tropicalisisolates, 14(66.6%) were biofilm producers.75% C.parapsilosis isolates were biofilm producers. All the C. glabrata isolates, were biofilm non-producers (Table 3).

DISCUSSION

Invasive fungal infections (IFIs) have become an emerging cause of morbidity and mortality

inneutropenic patients with hematological malignancies and recipients of hematopoietic stem

cell transplants (HSCT).[9]

The ability of C. albicans to infect diverse host niches is reinforced by an extensive range ofvirulence factors and fitness characteristics. Some of the virulence factors include themorphological evolution between yeast and hyphal forms, the expression of adhesins andinvasins on the cell surface, thigmotropism, the biofilm formation, phenotypic switching andthe secretion of hydrolytic enzymes. Moreover, fitness attributes include robust stress response machineries, rapid adaptation to fluctuations in environmental pH, powerful nutrient acquisition systems and metabolic flexibility.[10]

Verma et al. in their study reported incidence of candidiasis as 1.61/1000 admissions in a tertiary care hospital, India.[11]

Systemic antifungal agents shownto be effective for the treatment of invasive candidiasis comprise 4 major categories: thepolyenes (amphotericin B [AmB] deoxycholate, liposomal AmB, AmB lipid complex [ABLC] and amphotericin B colloidal dispersion), the triazoles (fluconazole, itraconazole,* voriconazole, and posaconazole), the echinocandins (caspofungin, anidulafungin, andmicafungin), and flucytosine. Recently, biofilm production by Candida species and inadequate antifungal therapy havebeen described as independent mortality predictors for patients with candidemia.[12]

Candida isolated most commonly in the age group of 0- <1 year (60.9%), followed by 21-40 years (12.5%), ≥ 60 years (10.9%), 1-20 years (7.8%) and 41-60 years (7.8%).Out of 64 Candida isolates, 29 (45.3%) were females and 35 (54.6%) were males in the present study. In similar to this study Chi et al [166] reported male preponderance (68.9%) and Laupland et al[13] observed candida was most commonly isolated from >75 years and <1 year of age, while in contrast Loster et al[14] reported female preponderance of 62.2%.

In this study most of the candida species were isolated from blood (46.8%) and pus (28.1%) which is supportedby many other studies.[15,16]

Out of 64 candida isolates from various clinical isolates, Majority were Candida albicans (37.5%), followed by candida tropicalis (32.8%), candida krusei (20.3%), Candida parapsilosis (6.2%) and Candida glabrata (3.1%) as per this study. Kaur et al[17] and sundaram et al[15] also observed Candida albicans and Candida tropicalis were most common pathogens with 36.7% and 41.1%, 54% and 18% respectively. On assessment of disk diffusion test by CLSI guidelines, all 64 isolates of Candida species were sensitive to Amphotericin B and nystatin. 62.7% of candida species other than Candida krusei shown sensitivity to fluanozole. Candida species were shown 87.5% sensitivity to itraconazole, 81.2% sensitivity to clotrimazole and 84.3% sensitivity to ketoconazole. This study supported by Gandhi et al[18], observed among candida isolates 98.3% sensitive to amphotericin B, 100% sensitive to nystatin, 79% sensitive to fluconazole, 79.5% sensitive to itraconazole, 52% sensitive to itraconazole and 76% sensitive to ketoconazole. Mishra et al[19] reported lower sensitivity to nystatin (69.6%).

Among 24 Candida albicans isolates, 17(70.8%) were biofilm producers. 11 (84.6%) out of 13 isolates of C.krusei were biofilm producers. Among 21 C.tropicalis isolates, 14(66.6%)were biofilm producers. 75% C.parapsilosis isolates were biofilm producers. All the C. glabrata isolates, were biofilm non-producers as per this study.

In a study by Agwan et al[20], observed biofilm producers in 40% candida albicans, 67.5% candida tropicalis, 22.7% candida parapsilosis, 25% candida glabrata. Shin et al[21] reported percentage of biofilm producers in candida albicans as 8%, candida tropicalis – 80%, candida parapsilosis – 73%, candida glabrata – 28%.

CONCLUSION

Fungal infections are neglected clinical problem and diagnosis and sensitivity testing of fungi is also routinely done only in few hospitals. Early diagnosis and accurate treatment of candida infected patients helps to reduce the risk of infection and improves patient outcome. Assessing biofilm production of candida isolates helps us to plan treatment and identify the niche for production of biofilms.

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