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STUDY OF FUNGAL INFECTIONS IN IMMUNOCOMPROMISED PATIENTS

Microbiology

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ABSTRACT

Fungal infections are common in immunocompromised. Although actual incidence has increased, its real frequency is often underestimated because of difficulty in diagnosis and obtaining proper biological samples. Prospective study was done on 63 cases of immunocompromised patients with fungal infections. The samples were processed, identified and AFST was performed according to CLSI guidelines. Candida species are predominant followed by Aspergillus and Trichosporon species. On AFST, Candida isolates showed high sensitivity to Ketoconazole, Miconazole followed by Clotrimoxazole and Fluconazole. The MICs of Amphotericin B and Itraconazole for all Aspergillus isolates are within the susceptible ranges. Candida species are common cause of fungal infections in immunocompromised patients followed by Aspergillus species and Trichosporon species. Reporting of antifungal susceptibility in terms of MICs for fungal isolates, helps in prescription of appropriate drug doses by clinician and for early recovery of the patient.

KEYWORDS

fungal infections, immunocompromised, AFST.

INTRODUCTION

- Fungal infections are becoming common and some of them are very serious and even fatal.[1] The epidemiology of fungal infections in immunocompromised patients has changed substantially in recent years.[2] Although the actual incidence of fungal infections has increased, its real frequency is often underestimated because of difficulty in diagnosis and obtaining proper biological samples.[1]

AIMS AND OBJECTIVES

- To isolate the fungi causing infections in the immunocompromised patients.
- To identify the species of fungi isolated.
- To determine the antifungal susceptibility pattern among the isolates according to CLSI guidelines.

MATERIALS AND METHODS[4,5,6,7,8]

The present study is prospective study conducted in 63 cases of immunocompromised patients with fungal infections. They include 42 cases - oral mucocutaneous fungal infections in head and neck cancer patients ,8 cases - vaginal infections in cervical cancer patients, 5 cases - superficial fungal infections in uterine, ovarian, Inguinal lymph node cancer patients on radiotherapy in oncology wards,8 cases - fungal pulmonary infections from patients in Respiratory Intensive Care Unit (RICU) at Government General Hospital.

Patients with Acquired Immune Deficiency Syndrome (AIDS), Paediatric age group patients were excluded.

All the relevant history like Age, Sex, Diagnosis, staging of Carcinoma, Duration of Radiotherapy, associated fungal infection, history of chronic usage of steroids, antibiotics and immune status of the patient were taken into consideration.

Samples from oral cavity, Vaginal swabs, Sputum are processed by Gram's staining for the presence of pus cells, yeast cells and Pseudohyphae .Skin scrapings were subjected to 10% Potassium hydroxide (KOH) wet preparation and observed for fungal elements. Following direct microscopic examination, irrespective of demonstration of fungal elements, the specimen was inoculated onto Sabouraud's Dextrose Agar (SDA) with 0.05% chloramphenicol. If growth was obtained on any test tube, they were identified by colony morphology. Yeast like colonies are processed by Gram's stain – gram positive budding yeast cells are seen. Germ tube test showed terminal chlamydoconidia for C.albicans. Chlamydospore formation on corn meal agar (Dalmau plate method) showed pine forest appearance for C.tropicalis, Cross match stick appearance for C.krusei, arthroconidia with Blastoconidia are seen for Trichosporon species.Candida species are also identified by inoculating onto Hichrome Candida agar. Further confirmation of Candida species were done by sugar fermentation and assimilation tests.

ANTIFUNGAL SUSCEPTIBILITY TESTING FOR YEASTS[9]

Disc diffusion method

Agar based disc diffusion susceptibility method was performed according to CLSI Document M44-A reference method for Antifungal disc diffusion susceptibility testing for yeasts using antifungal agents Fluconazole (25µ g/disk), Clotrimazole (10µg/disk), Ketoconazole(15 µg/disk), and Miconazole (10µg/disk).

ANTIFUNGAL SUSCEPTIBILITIES FOR ASPERGILLUS[9,10]

Broth microdilution method was followed to determine antifungal susceptibility testing for moulds against Amphotericin B and Itraconazole according to CLSI M38A reference method.

RESULTS AND DISCUSSION

Fungal infections are common in immunocompromised patients. This study was undertaken to study the etiology, common cause of fungal infections in immunocomprosied patients and their antifungal susceptibility patterns. Clinically suspected cases of fungal infections in immunocompromised patients admitted in Government General Hospital, Kakinada during the study period were included in the present study.

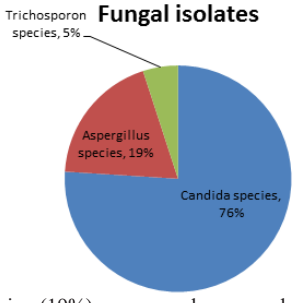
Among these fungal infections, majority were reported from Head and neck cancer patients (67%) followed by cervical cancer patients (12.5%) on radiotherapy, ventilator associated pneumonia's, chronic obstructive pulmonary disease patients (12.5%), uterine, ovarian, inguinal cancer patients (8%) on radiotherapy.

In total 63 cases, predominant samples are swabs from oral cavity (67%) followed by vaginal swabs (12%), skin scrapings (8%), Endotracheal Tube (ET) tips (8%), sputum (5%).

In present study out of 63 samples, 55.5% are from males and 45.5% are from females which correlates with Tzar MN et al (2013)[11] and

Nidhi Wardhe et al (2015)[12] 42 are fungal culture positive and 21 are negative. Among 42 positives, *Candida* species (76%) are common isolates, followed by *Aspergillus* species (19%) and *Trichosporon* species (5%)[Figure 1]. *Candida* species are the commonly isolated fungi in present study (76%) which correlates with Parmar et al[13] (55%) and Kashyap et al[13] (18.3%). *Cryptococcus* species (50%) is the common isolate only in Chen et al study[13].

Figure 1: Fungi isolated in present study



Aspergillus species (19%) are second commonly isolated fungi in present study which correlates with Parmar et al[13] (3%), Kashyap et al[13] (6.9%). Chen et al[13] study reported both *Candida* species (16.7%) and *Aspergillus* species (16.7%) as second common isolates .

Candida species generally constitute commensal flora of the gastrointestinal tract and genital tract, most common opportunistic pathogens that have the potential to cause numerous superficial and systemic infections.[2,14] Higher isolation of *Candida* species may be due to its flexibility in adaptation to several different habitats, and the formation of biofilms that increases its capacity to adhere to surfaces causing infection.

Among *Candida* species isolated from the patients with Head and neck cancer on Radiotherapy, *C.albicans* is the predominant isolate (40%). This correlates with Jayachandran et al[15] (58%), Bakki SR et al[16] (68.4%), Yogitha PVV et al[17] (60.5%), MS lone et al[18] (74.3%). Manish Jain et al[19] reported *C. tropicalis* (42.85%) as the most common isolate. Among *Non-albicans Candida*, *C.tropicalis* (24%) is common isolate in present study which correlates with Jayachandran et al[15] (20.9%), Yogitha PVV et al[17] (18.4%) and Bakki SR et al [16] (10.5%). *C.glabrata* (8.53%) and *C.stellatoidea* (28.57%) are common Non-albicans Candida isolated in MS lone et al[18] and Manish Jain et al[19] respectively.

TABLE I: Correlation between Hichrome agar and Biochemical tests for Candida speciation in various studies

Various studies	100% correlation	Misidentified by Hichrome agar
Metha et al (2016)	*C.albicans, C.tropicalis, C.krusei, C.glabrata.*	*C.gullermondi, C.parapsilosis, C.kefyr*
S Kaup et al (2016)	*C. albicans, C.tropicalis, C.krusei.*	*C.gullermondi, C.parapsilosis, C.glabrata.*
Veena et al (2016)	*C.albicans, C.tropicalis, C.krusei, C.dubeliensis.*	*C.gullermondi, C.parapsilosis, C.glabrata, C.kefyr.*
Present study	*C.albicans, C.tropicalis, C.glabrata.*	*C.krusei, C.kefyr.*

In present study, *C.albicans, C.tropicalis* and *C.glabrata* speciation by Hichrome agar correlated 100% with biochemical tests (sugar fermentation and assimilation) similar to Metha et al (2016)[20]. *C.kefyr* is misidentified in present study which correlates with Metha et al[20] and Veena et al[21] [Table I].

As appreciable correlation is observed between Hichrome *Candida* agar and biochemical tests, for preliminary identification of *Candida* species, Hichrome agar can be used which is cost effective and easy to process.

Aspergillus fumigatus (37.5%) is the most common among the *Aspergillus* species isolated in present study, which correlates with MA Milito et al[22] (39.7%), Jarhomi SB et al[23] (37.5%), and FS Taccone et al[24] (88%).

Following *A. fumigatus*, *A.flavus* (25%), *A.niger* (25%) and *A.terreus* (12.5%) are isolated in present study which correlates with Jahromi SB et al[23] (2013).

Aspergillus species is thought to be common mould infection in imunocompromised patients as it is ubiquitous and thermophilic in nature. *Aspergillus fumigatus* is the common isolate with its airborne conidia resulting in almost wide-ranging and constant exposure in almost all individuals[2].

C.albicans showed increased resistance to Fluconazole (42.8%) in present study which correlates with Njunda Anna L et al[25] (70.9%).High sensitivity against Fluconazole was reported by Mondal S et al[26] (84%), Birham Moges et al[27] (78%), Singh S et al[28] (80%) and Karuppayil et al[29] (78%).

Candida albicans isolates in present study showed high sensitivity to Miconazole and Ketoconazole which correlates with Birham Moges et al[27], increased resistance to Fluconazole which correlates with Njunda Anna L et al[25].

Among *Non albicans Candida* isolates in present study, increased resistance to fluconazole is reported which correlates with Singh S et al[28] and Mondal S et al.[26] Susceptibility to Ketoconazole is high in present study which correlates with Mondal S et al[26] and B Moges et al[27] studies.

In recent years, there is increased usage of Fluconazole for treatment of both local and systemic fungal infections in present study area. This may be the reason for high isolation of Fluconazole resistant strains among *C.albicans* and *NAC* in present study.

TABLE II: Antifungal susceptibilities for Aspergillus species in various studies

Various studies	MIC ranges (µg/ml)							
	A.fumigatus		*A.flavus*		*A.niger*		*A.terreus*	
	AMB	**ITR**	**AMB**	**ITR**	**AMB**	**ITR**	**AMB**	**ITR**
Kazemi et al (2013)	0.5-2	1-8	0.5-2	1-8	-	-	-	-
Safad Riyaz et al (2016)	0.5-4	1-16	0.5-2	1-8	0.5-4	1-4	-	-
Badiee et al (2012)	0.03-8	0.125-1	2-32	0.25-1	0.125-0.5	2-32	-	-
Present study	0.5-1	0.5-1	0.25-0.5	0.5-1	0.5-1	0.25-2	0.5-1	0.5-1

The MICs of Amphotericin B (<1µg/ml) and Itraconazole (< 2µg/ml) for all *Aspergillus* isolates are within the ranges given by CLSI guidelines. Safad Riyaz et al[30] (2016) reported high MICs for Itraconazole (32 µg/ml) against *A.fumigatus*. A study done by Badiee et al[31] (2012) reported high MICs for Amphotericin B against *A.fumigatus* (8 µg/ml), *A.flavus* (32 µg/ml), for Itraconazole against *A.niger* (32 µg/ml) [Table II].

Causative agents of fungal infections in immunocompromised patients were identified by available conventional methods in this study and their antifungal susceptibilities were determined. This aids the clinician in diagnosis of fungal infection and its susceptibility pattern benefits patient's treatment.

The analysis of our present study shows that *Candida* are the predominant etiological agents in immunocompromised.

Isolation of *Aspergillus* species is less in present study area and thus usage of AMB and ITR is less in this area. This may be the reason for susceptibility of all *Aspergillus* isolates to AMB, ITR. Larger samples, further studies are required to confirm the susceptible patterns of *Aspergillus* isolates.

Reporting of antifungal susceptibility in terms of MICs for fungal isolates, helps in prescription of appropriate drug doses by clinician and for early recovery of the patient.

Most of fungal infections in immunocompromised patients are invasive in nature and takes no time to spread thus requires early diagnosis and treatment. For such cases, rapid diagnosis using molecular methods like PCR, Serological diagnosis using Mannan, Anti-mannan, β D-glucan assay are of great use in resourceful settings.

CONCLUSION

Fungal infections in immunocompromised patients are common. Among the immunocompromised cases, HIV infections are predominant followed by oncology cases which includes Leukemia, Head and Neck cancers, Cervical cancers, etc on Radiotherapy, Organ and Bone marrow transplantations, Ventilator associated pneumonias and Chronic steroid / Antibiotic usage.

Head and neck cancer patients on Radiotherapy formed the major study group in present study as HIV cases and Paediatric age group as excluded.

Candida species are common cause of fungal infections in immunocompromised patients followed by *Aspergillus* species and *Trichosporon* species. Among the Candida species isolated, *C. albicans* is common followed by *C.tropicalis* in *Non-albicans candida.*

Hichrome agar can be substituted to conventional methods for speciation of *Candida* as appreciable correlation is observed between them.

Antifungal suscetibility testing for *Candida* showed high sensitivity to Ketoconazole, Miconazole and least susceptibility to Fluconazole. *Aspergillus* isolates showed susceptible MICs against Amphotericin B and Itraconazole.

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