

A MICROBIOLOGICAL STUDY OF FUNGAL ETIOLOGY IN VARIOUS CLINICAL SPECIMENS OF PATIENTS ATTENDING THE TERTIARY CARE HOSPITAL

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ABSTRACT

Objectives: To detect fungal elements from various clinical specimens. To show effectiveness of the methods used in this study for detection of fungi from clinical specimens and thereby help in choosing the antifungal treatments

Material and methods: The study was carried out from various clinical specimens of patients attended tertiary care hospital. All clinical specimens were subjected to KOH (Potassium hydroxide) examination and positive samples were cultured on SDA (Sabouraud's Dextrose Agar) medium. This was followed by identification of fungi with LCB (Lacto phenol Cotton Blue) mount and fungal slide culture methods.

Results: Out of total 508 specimens, 32 (6.30%) specimens showed presence of fungi. Fungi were isolated from clinical specimens like sputum (305), stool (61), urine (91), body fluid (09), Skin scrapping (07), nail (02) etc. The most predominant species was *Candida albicans* 14 (43.75%) and other *Non albicans Candida* 11 (34.37%) species, in which we could identify one species as a *C. glabrata*. Among molds, *Aspergillus spp.* 04 (12.50%), *Penicillium spp.* 02 (6.25%), and *Alternaria spp.* 01 (3.125%) were isolated and they were from the nail and skin scrapings.

Conclusion: From this study, we found that the risk of fungal infection is increased in hospitalised patients and immune-compromised individuals as well as healthy individuals. Early detection of fungus by microscopic examination (KOH examination) helps clinician in cessation of antibacterial drugs and in initiating empirical antifungal therapy earlier, which serves patient for good betterment.

Keywords: *Candida albicans*, *Aspergillus spp.*, *Alternaria spp.*, *Penicillium spp.*, Fungal slide culture

INTRODUCTION

The epidemiology of invasive fungal infections has changed during the past 25 years. The incidence has increased, and the populations at risk have expanded. Furthermore, the aetiology of these infections has changed. In the 1980s, yeasts (particularly *Candida albicans*) were the most common causative agents of invasive mycoses. In recent years, moulds (especially *Aspergillus spp.*) have become frequent in certain groups of patients, such as patients with solid organ transplantations, cancer or prolonged neutropenia. Fungal infections have emerged as a world-wide healthcare problem in recent years [1], owing to the extensive use of broad-spectrum antibiotics [2], long-term use of immune-suppressive agents, increasing use of hyper-alimentation and indwelling devices [3] and the increasing population of terminally ill, debilitated and immune-compromised patients [4]. Fungal infection is a very serious cosmetic problem, one such infection is Dandruff. Medical plants may offer valuable sources for antidandruff molecules. *Vernonia cinerea* L. plant leaves extract was used to study as antifungal activity against *Candida albicans*, *C. parapsilosis* and *C. tropicalis*. The extract has shown very good antifungal activity [5]. Various compounds are used to study antifungal activity, e.g., a series of mannich bases of benzimidazole derivatives were synthesized from o-phenylenediamine in two steps via benzimidazole intermediates. All synthesized compounds showed significant inhibitory activity against the microorganisms with 100µg/mL which produce 100% inhibition against the microorganism and the antifungal activity was determined using standard Ketoconazole [6].

MATERIALS AND METHODS [7, 8, 9, 10]

The study was conducted at Department of Microbiology, GMERS Medical Collage & Hospital, Sola, Ahmedabad. This one is tertiary care & multi-speciality hospital affiliated with Medical Collage. The patients for study were selected on the basis of random sampling method. Various clinical specimens like skin scraping, sputum, urine, pus, stool, Wound swab, etc. were collected from patients reporting to the microbiology laboratory. The total 508 clinical specimens were included in this study. All specimens were tested for fungal presence by Slide KOH wet mount method and positive specimens for fungi were processed for cultural examination by their growth on SDA (Sabouraud's Dextrose Agar) medium. All clinical specimens

were collected under appropriate clinical guidelines by well-trained technicians and proper criteria were maintained during the transportation of specimens. All fungal species isolated were processed for identification based on various methods including LCB (Lacto-phenol Cotton Blue) wet mount, fungal slide culture, Gram's staining, germ tube test. Cornmeal Agar (CMA) Medium was used for fungal slide culture method.

RESULTS AND DISCUSSION

In this study, a total 508 specimens were involved, among which 32 (6.30%) clinical specimens were positive for the presence of fungi while remaining 476 specimens were negative for the presence of fungi. In these 476 specimens, 88 specimens indicated no growth of organisms while remaining 388 specimens showed the growth of pathogenic bacteria. In these 388 specimens, 85 isolates were Gram positive bacteria and 303 isolates were Gram negative bacteria. The Gram positive bacterial species isolated and identified were *Staphylococcus spp.*, *Enterococcus spp.* and *Streptococcus spp.* The Gram negative bacteria isolated and identified were *Pseudomonas spp.*, *Escherichia spp.*, *Klebsiella spp.*, *Morgenalla spp.* and *Salmonella spp.* Out of these 32 fungal positive patients, 16 were from Outdoor patient & remaining 16 were from various wards (Indoor department). Report of KOH examination was immediately dispatched to clinicians, so they can start empirical antifungal treatment to these patients. Fungal culture of clinical specimen were performed in all 16 patients from indoor department, while fungal culture did not perform in all 16 patients of OPD because of unavailability of these patients for further follow up.

KOH examination and fungal culture are good tools for the detection of fungal elements from the clinical samples; therefore these techniques were selected as primary tools in this study. Clinical specimens from 165 patients were subjected to KOH examination and culture examination in which all 165 specimens were KOH positive and 110/165 specimens were culture positive [11]. The study indicated that the fungi to be isolated were *Candida spp.* from the sputum, nail, stool, urine and body fluid. The most predominant species was *Candida albicans* 14/32 (43.75%), followed by *Non albicans Candida* species 11/32 (34.37%). We could identify one *Non albicans Candida* species as a *C. glabrata*. In other study, examination of clinical specimens indicated that 12.2% *Candida spp.* were

present. The majority of *Candida spp.* among *Candida* isolates were *C. albicans* (70%) followed by *C. tropicalis* (13.33%), *C. krusei* (10%), *C. parapsilosis* (3.33%) and *C. stellatoidea* (3.33%) [12]. Among the

molds, *Aspergillus spp.* 04/32 (12.50%), *Penicillium spp.* 02/32 (6.25%) and *Alternaria spp.* 01/32 (3.125%) were isolated and they were from the nail and skin scrapings.

Table 1: Prevalence of fungi in various clinical specimens

S. No.	Clinical Specimen	Positive Specimen	Negative Specimen	Total Specimen
1	Sputum	14 (4.59%)	291	305
2	Urine	04 (4.30%)	87	91
3	Stool	04 (6.78%)	57	61
4	Pus	00 (0.00%)	18	18
5	Other specimens	10 (30.3%)	23	33
	Total	32 (6.30%)	476	508

Out of 508 clinical specimens, 305 were sputum followed by urine (90), stool (61), pus (18) and other specimens (33). Positivity for fungus were 14/305 (4.59%) in sputum, 4/91 (4.30%) in urine, 4/61 (6.78%) in stool, 0/18 (0.0%) in Pus, 10/33 (30.3%) in other clinical specimens.

As skin & nail are known as common sites for Dermatophytic infection and Onychomycosis, maximum prevalence of fungi (30.3%) were from skin scrapping and nails. In all pus, we could not able to

find fungus (0.0%). Because these pus were mainly collected from post-operative patients, which were mainly infected with pyogenic bacterial infection.

Gender-wise distributions of various clinical specimens for the presence of fungal elements

All clinical specimens were undergone analysis and then they were characterized on the basis of gender of patients.

Table 2: Gender-wise distributions of clinical specimens

S. No.	Samples	Male	Female		
		Positive for fungus	Total	Positive for fungus	Total
11	Sputum	07 (3.54%)	198	07 (6.54%)	107
22	Urine	04 (8.33%)	48	00 (0.00%)	43
33	Stool	01 (3.33%)	30	03 (9.68%)	31
44	Pus	00 (0.00%)	08	00 (0.00%)	10
55	Other specimens	04 (26.67%)	15	06 (33.33%)	18
	Total	16 (5.35%)	299	16 (7.66%)	209

The results indicated that in male 07/198 (3.54%) sputum were positive for fungi while in female 07/107 (6.54%) sputum were positive for fungi, 04/48 (8.33%) urine specimens were positive while in female, there was none of (00/43, 0.00%) specimens showed fungi, 01/30 (3.33%) stool specimens were positive while in female, 03/31 (9.68%) specimens were positive for fungi, in pus specimens no fungi (0/8, 0%) in male and (0/10, 0%) in female, 04/15 (26.67%) other specimens of male were

positive for fungi and 06/18 (33.33%) specimens of female were positive for fungi.

Age-wise distributions of various clinical specimens for the presence of fungal elements

All the collected specimens were analysed for the presence of fungal elements and then they were distributed on the basis of age of the patients.

Table 3: Age-wise distributions of clinical specimens

S. No.	Age (years)	Positive specimens		
		Sputum	Urine	Stool
1	≤ 10	00/05 (0.00%)	01/39 (2.56%)	02/17 (11.77%)
2	11-30	04/98 (5.10%)	01/27 (3.70%)	00/23 (0.00%)
3	31-50	04/105 (3.81%)	00/13 (0.00%)	01/11 (9.09%)
4	51-70	06/82 (7.32%)	02/12 (16.67%)	01/10 (10.0%)
5	71-90	00/15 (0.00%)	00/00 (0.00%)	00/00 (0.00%)
	Total	14/305 (4.59%)	04/91 (4.30%)	04/61 (6.78%)

From above results, it can be indicated that 06/82 (7.32%) sputum specimens were positive for fungal presence in the age of 51-70 years, 02/12 (16.67%) urine specimens were positive for fungal presence in the age of 51-70 years and 02/17 (11.77%) stool specimens were positive for fungal presence in the age below 10 years.

There was no positive indication of fungi in pus specimens (0.0%). Because these pus were mainly collected from post-operative

patients, which were mainly infected with pyogenic bacterial infection.

Prevalence of Fungi in Other Clinical Specimens

A total 33 other clinical specimens were studied, among which there were 02 nail, 07 skin scraping, 15 wound swab and 09 body fluid.

Table 4: Prevalence of fungi in other clinical specimens

S. No.	Specimens	Positive specimens	Total specimens
1	Nail	02 (100%)	02
2	Skin scraping	07 (100%)	07
2	Wound Swab	00 (0.00%)	15
4	Body fluid	01 (11.11%)	09
	Total	10 (30.3%)	33

Among these 33 specimens, we found 10 (30.30%) positive specimens that showed the presence of fungi. We found that 02/02 (100%) nail, 07/07 (100%) skin scraping, 01/09 (11.11%) body fluid showed the presence of fungi. There were no fungi reported in wound swab during this study. As these all swabs were from infected wounds of post-operative patients. In all swabs pyogenic bacteria were isolated. In all nail & skin specimens, fungi were isolated, as these sites are common sites of fungal infection.

In our study, the presence of fungi in nail was 100% and the fungi to be isolated were *Non Candida albicans* (100%) and one isolate was identified as a *Candida glabrata* (50%). Similar findings were obtained in other studies in which the predominant pathogenic fungi to be isolated were yeast (64.7%) [13]. A 2228 clinical specimens from various infections suspected of fungal etiology were received in which nail was the most frequent and dermatophytes were found to be the most frequent fungal isolates [14]. All skin scrapings showed positive results in culture examination (100%). While in other study, the presence of fungi by culture examination was 60.8% [15]. There were no fungi to be isolated from wound swab in our study. Among various body fluids, the presence of fungi was 01/09 (11.11%). Gastric aspirate was positive for fungus among all body fluid.

CONCLUSION

As fungal infections not notifiable infections like viral, bacterial or parasitic diseases. Hence these are not given much attention and usually diagnosis is established very late. Most of the serious types of fungal infections are more common in developed countries due to underlying immune-compromised situations as compared to developing countries where majority of diseases are due to low hygienic standards and the environment.

KOH examination and fungal culture are good tools for the detection of fungal elements from the clinical samples, that's why they were selected as primary tools in this study. From this study, we found that the risk of fungal infection is increased in hospitalized patients & immune-compromised individuals as well as healthy individuals. Early detection of fungus by microscopic examination (KOH examination) helps clinician in cessation of antibacterial drugs & in initiating empirical antifungal therapy earlier, which serves patient for good betterment.

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