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Isolation and characterization of opportunistic fungi causing secondary infection in debilitated patients

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Abstract

An opportunistic infection is caused by variety of pathogens such as bacteria, virus, fungi or protozoans that usually do not cause disease in a healthy host. In order to accomplish the objectives of the present research work clinical samples were collected from debilitated patients. Out of 45 samples 27 were found to be positive for fungal infection. A total number of 76 mold form and ample number of *Candida* spp. clinical isolates were obtained. The common molds isolated were *Alternaria alternata*, *A. fumigatus*, *A. niger*, *A. terreus*, *A. nidulans, A. flavus*, *Rhizopus* spp. *Mucur* spp. and *Curvularia lunata*. In order to study the antifungal profile of the clinical isolates *in vitro* antifungal susceptibility test was performed by Kirby Bauer Method. Ketoconazole was found to be most effective azole against the clinical isolates followed by Clotrimazole, Itraconazole and Amphoterecin B.

Keywords: Opportunistic infection, Pathogens, Debilitated patients

INTRODUCTION

Mycosis (plural; Mycoses) is a condition in which fungi pass the resistance barriers of the human or animal body and establish infection. The incidence of invasive opportunistic mycoses has increased because of the expanding population of immunocompromised patients, including solid-organ transplant (SOT) and hematopoietic stem cell transplant (HSCT) recipients, patients with cancer, patients with AIDS, premature neonates, elderly patients, and patients recovering from major surgery [1, 2]. People are at risk of fungal infections when they are taking strong antibiotics for a long period. Opportunistic infections can occur all over the body and be relatively localized (affect only one part of the body) or systemic or disseminated (spread to other parts of the body and other body systems). Opportunistic mycoses show distinct regional incidence patterns throughout the world and may exhibit different epidemiologic features, depending on the geographic region; this may be particularly true for mycoses (such as mold infections) that are acquired from the environment [3].

With the increasing incidents of fungal infections in immunocompromised and debilitated patients, the use of antifungal therapy for clinical purpose is demanding. The present work deals with the isolation of common fungi in immunocompromised patient and the study of their antifungal profile. The study will help in clinical therapy. The use of spices and herbs alone and in combination with synthetic drugs can be suggested for clinical outcomes.

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METHODOLOGY

The present research work deals with collection clinical samples from debilitated patients. The samples were subjected to direct microscopic observation and culture. *In vitro* antimicrobial activities were determined by the agar disc diffusion method (Dugler & Gonuz, 1997; Dugler *et al.,* 2004). The disc diffusion method (antimicrobial susceptibility testing) is also known as Kirby – Bauer method being recommended by the NCCLS. The thermo tolerance test of the clinical fungal isolates was performed which is suggestive of their pathological nature.

A total number of 45 samples were collected from the diabetic patients from Jabalpur Hospital and Research Centre. The samples were collected in McCartney's bottle from pathology department of Jabalpur Hospital and Research Centre. Then the clinical samples were subjected to direct microscopic observation and culture.

RESULT AND DISCUSSION

Out of 45 samples 29 were found to be positive for fungal infection. The results are expressed in table no. 1 The samples were subjected to direct culture. The collected blood sample was spread on the SDA plates with Chloramphenicol @ 30mg/1000ml. The results obtained are shown in table no.2. The fungi (clinical isolates) were identified Macro morphological, Microscopic characteristics and biochemical tests.

The common *spp.* isolated from diabetic patients was common causal agents of opportunistic infections. From the table no. 2 it is evident that the common *spp.* which causes opportunistic infection are *Aspergillus, Alternaria, Candida, Curvularia, Rihzopus*, and *Mucur* spp.

Thermotolerance test of clinical isolates were done at the temperature 28°C, 37°C and 45°C. The results are expressed in table no. 3.From the results it is evident that almost all clinical isolates have the potential to grow at high temperature identifying

their pathological nature.

Antifungal susceptibility test was performed for different clinical isolates by disk diffusion method (Kirby Bauer). Kirby Bauer method was suitable for routine testing in clinical laboratory where large number of isolates is served for susceptibility to numerous antibiotics.

The antifungal susceptibility exhibited by the clinical isolates is expressed in table no. 4 from the table it was evident that the clinical isolates were susceptible to the azoles used in the present study.

Table 1. Case History of Diabetic Patients

Patient ID	Gender	Sugar Level	Presented Fungal Strain
1	F	158	+
2	M	256.6	-
3	F	209	+
4	M	132	+
5	F	220	+
6	F	140	-
7	M	226	+
8	F	192	-
9	F	197	+
10	F	189	+
11	M	156	+
12	F	123	+
13	F	189	-
14	M	156	-
15	F	119	+
16	M	125	+
17	M	145	+
18	M	189	+
19	F	178	-
20	M	214	+
21	M	128	+
22	F	148	+
23	M	169	-
24	F	186	-
25	F	174	+
26	F	210	+
27	M	184	-
28	M	178	-
29	F	160	-
30	M	182	+
31	F	248	+
32	M	212	+
33	F	149	-
34	M	145	+
35	F	186	+
36	M	125	-
37	M	149	-
38	F	210	+
39	M	156	-
40	F	181	+
41	F	170	+
42	M	198	+
43	M	158	-
44	F	221	+
45	F	171	+

Table 2. Results of direct culture: isolation and identification of Fungi

S.No	Patient id	No. of Colonies	Fungi Identified
1	1	6	*Alternaria alternata*
2	2	1	*A. flavus*
3	4	Many	*Candida* spp
4	6	8	*Rhizopus* spp.
5	7	5	*A.fumigatus*
6	9	7	*C. albicans*
7	11	6	*A.terreus*

8	13	3	*R.arrhyzus*
9	14	2	*Curvularia lunata*
10	17	3	*A.niger*
11	20	6	*A.flavus*
12	21	2	*Candida albicans*
13	22	2	*Curvularia lunata*
14	23	2	*Mucur* spp.
15	26	2	*A.umigatus*
16	27	2	*Alternaria alternata*
17	28	4	*A. terreus*
18	31	2	*A. niger*
19	32	3	*A.nidulans*
20	33	2	*Mucur* spp.
21	35	5	*Rhizopus* spp.
22	38	7	*Mucur* spp.
23	39	4	*A.nidulans*
24	40	3	*A.niger*
25	41	12	*C. albicans*
26	43	3	*A.niger*
27	44	7	*C. albicans*

Table 3. Thermotolerance Test of Clinical Fungal Isolates

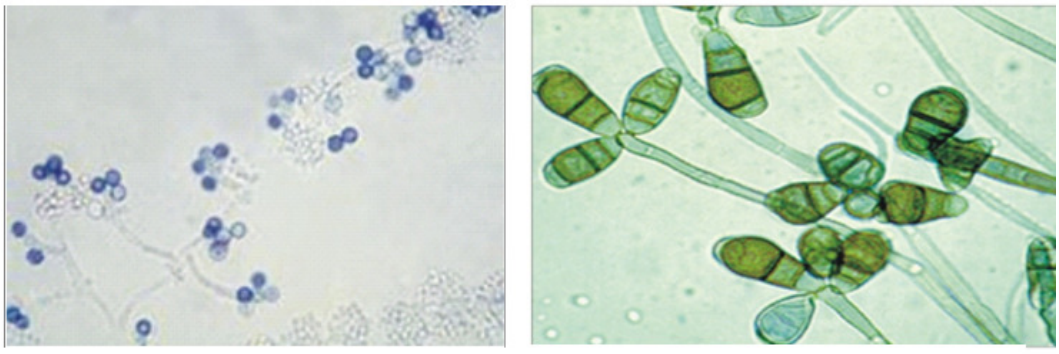
Fungal Isolates	Temperature		
	28°C	37°C	45°C
Alternaria alternata	++	+	++
Aspergillus fumigatus	++	++	++
A. niger	+++	+++	+++
A. terreus	++	++	++
A. nidulans	+++	++	+++
Candida albicans	++	++	++
Rhizopus spp.	+++	+++	+++
Rhizopus arrhyzus	+++	+++	+++
Mucur spp.	+++	+++	+++
Curvularia lunata	++	+	+++

+ : Less Growth, ++ : Moderate Growth, +++: Effluent Growth

Table 4. Antifungal Susceptibility Test of Different Fungal Isolates

Fungal Isolates	**Antibiotics**			
	CC	**KT**	**IT**	**Amp.B.**
Alternaria alternata	2 cm.	3 cm.	2 cm.	1 cm.
Aspergillus fumigatus	2 cm.	1.2 cm.	2 cm.	1 cm.
A.niger	0.3 cm.	1.5 cm.	0.3 cm.	0.2 cm.
A.terreus	1.4 cm.	1.9 cm.	1.5 cm.	1.5 cm.
A.nidulans	1.4 cm.	1.8 cm.	1.3 cm.	0.1 cm.
Aspergillus flavus	0.06 cm.	1.18 cm.	1.28 cm.	0.6 cm.
Candida albicans	1 cm.	1 cm.	1.5 cm.	1 cm.
Rhizopus spp.	1.6 cm.	1.5 cm.	1.3 cm.	1 cm.
Mucur spp.	1.5 cm.	0.7 cm.	1.1 cm.	Resistance
Curvularia lunata	0.2 cm.	0.4 cm.	0.4 cm.	1.2 cm.

CC =Clotrimazole, KT =Ketoconazole, IT =Itraconazole, AmpB =Amphotericin B



Direct culture of blood sample showing *Candida* sp. Microscopic observation of clinical isolate *Curvularia* sp.

Fig 1. Photograph of *Candida* sp. in blood sample and microscopic view of *Curvularia* sp.

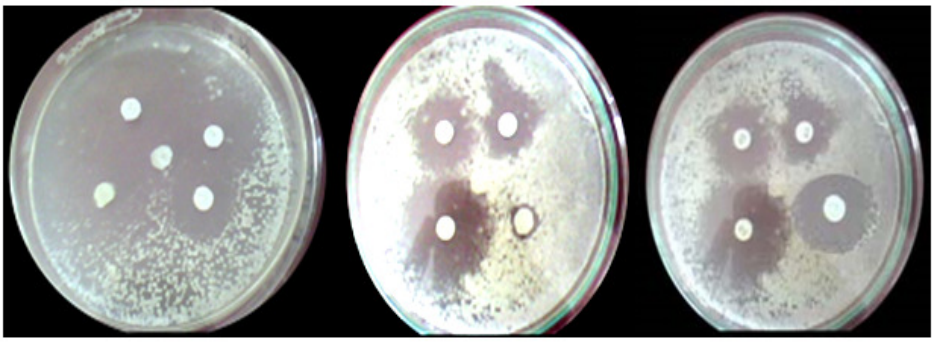


Fig 2. Photograph of antibiotic susceptibility test

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