Research & Reviews

ISSN: 2320-3528

Research and Reviews: Journal of Microbiology and Biotechnology

In - vitro Sensitivity of Otomycotic Agents against Syenthetic Antifungals and Natural Herbs

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Article

Received: 20/03/2013
Revised: 04/04/2013
Accepted: 09/04/2013

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Keywords: *In vitro* sensitivity, antifungals, otomycosis.

ABSTRACT

In vitro sensitivity methods against various antifungals were performed for otomycotic agents isolated from 10 patients. The agents were isolated on SDA supplemented with chloramphenicol @ 30µg/100ml. The common fungi isolated were *Aspergillus fumigatus, A. flavus, A. terreus, A.alternata, Paecilomyces,* and *Candida albicans.* These clinical isolates were subjected to in vitro sensitivity methods against synthetic antifungals and natural herbs and spices. The in vitro sensitivity methods applied were disc diffusion method, E test and broth dilution method. The most effective antifungal was itraconazole and garlic was the most effective amongst the natural products tested.

Abbreviations: A.- Aspergillus; SDA - Saboraud's Dextrose Agar Media;

INTRODUCTION

Otomycosis is a superficial infection of the outer ear canal and is also known as mycotic otitis externa. It is more common in hot climates and in those who indulge in aquatic sports. Factors predisposing otitis externa include absence of cerumen, high humidity, increased temperature, and local trauma, usually from use of cotton swabs or hearing aids. Other predisposing conditions include eczema, allergic rhinitis and asthma. It is easy enough to make a diagnosis of fungal infection when inspection of the ear canal reveals a forest of waving conidiophores, as in cases of sporing aspergilli. However, yeasts, even in their mycelial form, do not make closely woven masses of hyphae like molds do, and they are therefore more likely to be missed [9]. Thus, overlooking the fungal etiologic agent might lead to an unnecessary or excessive use of toxic broad spectrum antibiotics and potent steroids for prolonged periods, which might aid in the alteration of the local flora of the ear and lead to increased morbidity. Several filamentous and yeast forms like *Aspergillus terreus, A. flavus, A. fumigatus*, *Alternaria alternata*, *Penicillium spp., Rhizopus spp. Candida spp* have been reported as otomycotic agents [5,10,14,17]. Many antifungals have been recommended for treating otomycosis [1,4,15], but no antifungal has been widely accepted. Thus, in vitro effectivity of different antifungals and natural antifungals has been screened to reveal the antifungal profile of the clinical isolates. The effectivity of an antifungal would help in clinical prevalence and thus in the treatment of the infection.

MATERIALS AND METHODS

The clinical samples were collected from otomycotic patients admitted in Harshey's Clinic, Jabalpur. The clinical samples were subjected to direct observation and direct culture. For direct culture SDA plates with chloramphenicol were used. The isolated cultures were subjected to identification on the basis of microscopic and macroscopic features; slide culture techniques, cultural characteristics and literature available. The isolated cultures were purified and maintained on SDA slants. The clinical isolates were subjected to in vitro susceptibility tests against various antifungals viz. Amphotericin B, Ketoconazole, Itraconazole, Fluconazole, 5 Fluocytosine and also to natural herbal extracts of Garlic, ginger, turmeric, tulsi and neem, by various methods like disc diffusion method (Kirby Bauer). The sensitivity was measured as zone of inhibition in centimeters in disc diffusion method.

ISSN: 2320-3528

RESULT AND DISCUSSION

Amongst the 10 clinical samples studied seven were positive for fungal growth. The common fungi isolated were *Aspergillus flavus, A. fumigatus, A. terreus, Alternaria alternata*, *Candida albicans, Paeciliomyces spp*. The results obtained by disc diffusion method against the clinical isolates are expressed in table no. 1.

Table no 1: Results of in vitro sensitivity tests expressed as zone of inhibition in cms.

S.No	Fungal isolate	Amp B	Ket	Itra	Flu	5FC
1	*Candida albicans*	0.5	2.3	1.6	1.5	1.2
2	*Alternaria alternata*	0.8	–	–	–	0.3
3	*Aspergillus flavus*	–	2.0	1.3	–	0.3
4	*Aspergillus fumigatus*	–	2.1	1.5	1.1	1.2
5	*Aspergillus terreus*	1.2	1.8	1.3	1.0	0.8
6	*Paecilomyces spp*	2.5	2.3	1.0	2.0	1.8

From the table it is evident that ketoconazole is the most effective antifungal against all the clinical isolates. Amphotericin B is the most effective antifungal against *Paeciliomyces spp.*followed by *A.terreus*, *A. alternata* and *Candida albicans* and ineffective to *A. flavus* and *A. fumigatus*. Ketoconazole is equally effective against *Candida albicans* and *Paeciliomyces spp.* followed by *A. fumigatus, A. flavus* and *A. niger.*fluconazole is effective against *Paeciliomyces spp.* followed by Candida albicans, *A. terreus* and *A. fumigatus. A. flavus* was found to be resistant against fluconazole. 5FC was effective against *Paeciliomyces spp* followed by Candida albicans and *A. fumigatus. A. flavus* , *A. niger* and *A. alternata* was found to be slightly sensitive to 5FC.

The susceptibility of the clinical isolates is expressed as zone of inhibition in cms in Table no. 2.The extent of zone of inhibition of the fungal growth indicates the sensitivity of the fungi to the plant extract studied. On comparison of the results tabulated in table no. 2 it can be suggested that the herbs are not as effective as the spices. It is evident from the results that garlic is most effective antifungal agent as the zone of inhibition is largest with the garlic disc followed by onion, ginger, neem, tulsi, turmeric. The present results are quite similar to those obtained by Ali et. al. The fungal species differed in their resistance to the spices and herbs. There is significant difference between the different treatments against one species.

Table no 2: In vitro susceptibility of fungal isolates to plant extracts by disc diffusion method (Kirby Bauer) expressed as zone of inhibition in cms.

S.No	Fungal isolate	Neem	Tulsi	Turmeric	Garlic	Ginger	Onion
1	*Aspergillus fumigatus*	0.5	0.4	–	2.0	0.8	1.0
2	*Aspergillus flavus*	0.4	0.4	–	1.8	1.0	1.2
3	*Aspergillus tereus*	–	–	–	2.0	1.0	1.0
5	*Candida albicans*	–	–	–	0.4	1.1	0.5
6	*Paecilomyces spp.*	1.5	1.9	–	1.6	–	0.8
8	*Alternariaalternata*	1.0	0.8	0.5	0.8	–	0.6

It is clearly illustrated from the table that the common spices used in our daily life and the extracts of natural herbs effects the fungal growth adversely and therefore might be considered to control fungal diseases. The bioactive compounds in these plant extracts must be analyzed, purified and then used as drug for controlling fungal pathogens without the development of drug resistance in pathogens and also without any side effects[2,3,6,7,8,11,12,13,16].

ACKNOWLEDGEMENT

The authors are thankful to UGC, CRO, and Bhopal for providing financial assistance and to the Principal, Mata Gujri College, Jabalpur for providing laboratory facilities.

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ISSN: 2320-3528

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