Asian Journal of Dermatology, 2010
ISSN 1996-3424

Isolation of Opportunistic Fungi from Dermatophytic Samples

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Abstract: This study was carried out for three years during November 2004 to November 2007. The aim of this study was to isolate the fungal flora from skin and nails samples which are usually present in the household environment. This study excludes the recoganized dermotophytes like *Trichophyton*, *Microsporum* and *Epidermatophyton*. *Candida* species were not included. Out of a total five hundred samples of skin and nail received during this study period, yielded 19 fungal species from skin and nails belonging to fungal genera *Alternaria*, *Aspergillus*, *Bipolaris*, *Cladosporium*, *Exophiala*, *Fusarium*, *Graphium*, *Malassezia*, *Prototheca*, *Rhizopus*, *Rhodotorula*, *Trichosporon* and *Ulocladium*. *Aspergillus* was the leading genus represented by six species. Previously well known methodology for isolation of dermatophytes was followed here except that Sabouraud dextrose agar with three antibiotics viz., Ciprofloxacin, vancomycin and gentamycin sulphate (0.05 g L^{-1} each) was used here to suppressed the growth of bacteria hence enhanced the isolation of molds. Only KOH positive cases are included here. Although, these fungi may not cause infections as true dermatophytes but these fungi should also be considered for tropical antifungal treatment to avoid further spreading of these fungi as a precaution.

Key words: Opportunistic fungi, infections, skin, nail, household environment

INTRODUCTION

Molds or non-dermatophytic filamentous fungi (generally belong to Hyphomycetes or Fungi Imperfectii) are quite often suspected as pathogen by public than by the medical care community but during past few decades these molds getting increasing attention by medical mycologist due to increased in number of skin and nail infections in humans and animals by these so called environmental fungi or laboratory contaminant (Evans and Richardson, 1989) because these fungi produced lesions that are similar to those caused by dermatophytes (Kwon-Chung and Bennet, 1992). Like dermatophytes, these fungi also have the capability of utilizing keratin *in-vitro* and produce proteolytic enzymes including keratinase. The outbreak of cutaneous infection is triggered by increasing in number of immunodeficient population (Vennewald and Wlollina, 2005).

The problem of increased fungal infection could also be attributed to modern living conditions with closed house environment, use of wall to wall carpets which provide a very suitable environment to these molds for growth. All these molds which causes cutaneous, sub-cutaneous, allergic or even systemic mycoses are common inhabitant of household environment (Bokhary and Parvez, 1995; Bakhali and Parvez, 1999).

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Alternaria species have been reported to cause cutaneous infections in both immunocompetent and immunocompromised patients (Sood *et al*., 2007; Robertshaw and Higgins, 2005; Romano *et al*., 2005; Mayser *et al*., 2004). *Aspergillus* species are well recognized as a causal organisms of pulmonary aspergillosis in immunocompromised patients but cutaneus aspergillosis occurs less frequent and thus remain poorly described as primary or secondary infections although there are some reports of *Aspergillus* causing cutaneous infections in immunocompromised hosts (Romano and Miracco, 2003) but numerous reports have described primary or secondary cutaneous aspergillosis in an array of non-HIV infected immunodeficient patients, including burn victims, neonatus, individual with the cancer, bone marrow and solid organ transplant patients (Riddel *et al*., 2004; Talbot *et al*., 2002). Although, in healthy hosts cutaneous aspergillosis can also develop in surgical wounds by traumatic inoculation or by exposure to high spore counts (Romano and Miracco, 2003). Initial lesions of cutaneous aspergillosis appears as macules, nodules, plaques or popules. In the case of neonatus, lesions occurs with prulent discharge or pustules (Fleming *et al*., 2002; Restrepo *et al*., 2004). In the case of catheter, infection begins from arm board or occlusive tape and resulting in hemorrhage bulla type infection. Infection in the case of intravenous catheter puncture, typically begins with erythma and induration at the skin puncture site and progress to necrosis that extends radially from initial focus. High fever, a change in the character of the wound surface, swelling induration and tenderness are the symptoms of primary cutaneous aspergillosis arising in a wound. Erythematous macules or papules that evolved to hemorrhage bullae or ulceration nodules are the symptoms for secondary cutaneous aspergillosis, secondary lesion can resemble ecthyma gangrenosum, traditionally caused by Pseudomonas aeruginosa (Warnok, 2006; Groll and Walsh, 2001).

Cutaneous infections caused by *Bipolaris* species and *Cladosporium* species (*Cladophialophora*) are mainly restricted to wound infections in the case of immunocompromised patients but could also cause infections in immunocompetant hosts (Fleming *et al*., 2002). Exophiala could cause cutaneous or sub-cutaneous infections while *Graphium* (*Pseudoallescheria boydii* or *Scedesporium apiospermum*) could also cause deep mycoses (Riddel *et al*., 2004; Talbot *et al*., 2002). *Prototheca* although not a fungus but an alga which lacks chlorophyll is well known to cause cutaneous mycoses but respond well to antifungals (Kantrow and Boyd, 2003; Zaitz *et al*., 2006). *Rhizopus* and *Ulocladium* could cause skin infections specially in immunocompromised patients and children (Oh and Notrica, 2002).

Trichosporan is a yeast fungus which is well known to cause infections in humans and animals, starting from hairs to skin to deep mycoses like endocarditis and meningitis (Kwon-Chung and Bennet, 1992; Bassetti *et al*., 2004; Pini *et al*., 2005; Ramos *et al*., 2004). *Fusarium* species now well recognized as causal organism of skin and nails (Kwon-Chung and Bennett, 1992).

The aim of this study to focus on isolation of non-dermatophytic (molds) flora from dermatophytic samples (skin and nails), which are usually inhabitant of household environment, laboratory contaminants or so called environmental fungi but these fungi are also the causal organisms of superficial mycoses to deep mycoses and could not be ignored just as an environmental fungi.

MATERIALS AND METHODS

Samples of skin scrapings and nail clippings were collected from Dermatology and Out-patient clinics. A total number of 500 samples (400 skin scraping and 100 nails scraping) were collected during the study period between Nov. 2004 to Nov. 2007.

Isolation of fungi was carried out according to Standard Operating Procedure for Medical Mycology (Parvez, 2010; Evans and Richardson, 1989).

Medium for Isolation

Sabouraud Dextrose Agar and Dermatophyte Medium (Oxoid Ltd. London) were use for isolation of these fungi. Three antibiotics, ciprofloxacin, vancomycin and gentamycin sulphate (0.05 g L^{-1} each) were added to Sabouraud dextrose agar. Potato dextrose agar (Oxoid Ltd. London) was also used but without the addition of antibiotics.

Isolation of Fungi from Nails

Samples of nails clippings was at first dipped into sterile water into a sterile petri dish for 10-15 min for softening of nail. Then small pieces were cut by using sterile scalpel and 5-10 pieces were put on the surface of medium plates. These plates were then incubated at 30°C for further study.

Isolation of Fungi from Scraping

Scarping samples were directly cultured by putting scraping pieces over the agar plates. These plates then incubated at 30°C for further study.

Microscopic Examination of Samples

Microscopic examination was done by using KOH (20% KOH + 15% glycerol) mount. Parker blue-black ink was also added when required.

Identification of Fungi

Identification of isolated fungi was carried out according to the following literature: (Evans and Richardson, 1989; Ellis, 1971, 1976; Raper and Fennell, 1965; Zycha *et al*., 1969; Howard, 1983; Nelson *et al*., 1983), while the identification of *Rhodotorula muciliginosa* and *Prototheca wickerhamii* were done by API 20C system (BioMerieux Vitek, Inc., France).

Isolated fungi were sub-cultured and maintained on Sabouraud Dextrose Agar slants in tissue culture bottles.

RESULTS AND DISCUSSION

Non dermatophytic fungi isolated form dermatophytic samples during the period between November 2004 and November 2007 are summarized in Table 1. True dermatophytes like *Trichophyton*, *Microsporum*, *Epidermatophyton* and also *Candida* species which were isolated during the study period but not included here. Three yeast fungi *Malassezia furfur*, *Rhodatorula muciliginosa* and *Trichosporon beigellii*, are included here. *Prototheca wickerhamii* which is not a fungus but an alga devoid of chlorophyll is also included in the Table 1. Out of 400 skin scraping samples 30 samples (7.5%) were found positive and out of 100 nails samples, 9 samples (9%) were found positive. A total number of 19 fungal species were isolated from scraping and nail samples. Seventeen fungal species were isolated from scraping while only 5 species could be isolated from nail samples. *Aspergillus* was the predominant genus and represented by 5 species followed by *Alternaria* (3 species). All other fungi were represented by one species each.

Alternaria alternata, *Fusarium solani* and *Exophiala jeanselmei* were the only fungi which were isolated from both type of samples. *Aspergillus fumigatus* and *A. niger* were represented by highest number of isolates (4 each) followed by *A. flavus* (3 isolates), *Alternaria alternata*, *Fusarium solani*, *Prototheca wickerhamii* and *Ulocladium*

chartarum (2 isolates each). All other species was represented by one isolate each in the case of skin scraping. While in the case of nails, *Fusarium solani* represented by highest no. of isolates (4 isolates) followed by *Alternaria* (2 isolates), *Cladosporium, Exophiala* and *Rhodotorula* represented by one isolates each.

Molds that isolated from different type of skin samples are summarized in Table 2, highest number of isolates were yielded from scalp (11 isolates) followed by underarm, neck, chest region (7 isolates), leg (5 isolates) and thigh (4 isolates). Scraping samples from palm and face yielded two isolates each.

Table 3 gave the details of positive cases according to age group and male and female ratio of positive cases. The highest number of positive cases were found among the age group of 50 years and older persons in both type of samples.

In the case of skin scraping, the age group of 11-30 years yielded lowest number of isolates while age group 31-50 was the second highest in the number of isolates. In the case of female, the ratio of the number of positive cases were almost double as compared to male.

Table 1: Isolation of fungi from skin and nail samples (n = 400 from skin and n = 100 for nail)

Fungi	Skin	Nail
Alternaria alternata	2	2
A. chlamydospora	1	-
A. chartarum	1	-
Aspergillus candidus	1	-
A. flavus	3	0
A. fumigatus	4	0
A. glaucus	1	0
A. niger	4	0
Bipolaris australiensis	1	0
Cladosporium cladosporioides	0	1
Exophiala jeanselmei	2	1
Fusarium solani	2	4
Graphium (*Scedosporium apiospermum*)	1	0
Malassezia furfur	1	0
Prototheca wickerhamii	2	0
Rhizopus arrhizus	1	0
Rhodotorula muciliginosa	0	1
Trichosporon beigellii	1	0
Uloclaedium chartarum	2	0
Total number of isolates	30	12

Table 2: Isolation of molds from different type of skin samples

	Type of scraping					
Fungi	Palm	Scalp	Underarm, neck, chest	Thigh	Face	Leg
Alternaria alternata	-	-	-	1	-	1
A. chlamydospora	-	-	-	-	-	1
A. chartarum	-	-	-	1	-	1
Aspergillus candidus	1	-	-	-	-	-
A. flavus	-	2	1	-	-	-
A. fumigatus	-	2	1	-	1	-
A. glaucus	-	1	-	-	-	-
A. niger	-	2	1	-	1	-
Bipolaris australiensis	-	-	2	-	-	-
Exophiala jeanselmei	-	1	-	1	-	-
Fusarium solani	1	-	-	-	-	1
Graphium (*Scedosporium apiospermum*)	-	-	1	-	-	-
Malassezia furfur	-	-	1	-	-	-
Prototheca wickerhamii	-	1	-	-	-	-
Rhizopus arrhizus	-	1	-	-	-	-
Trichosporon beigellii	-	1	-	-	-	-
Uloclaedium chartarum	-	-	-	1	-	1
Total number of positive cases	2	11	7	4	2	5

Table 3: Number of positive cases according to age group and also male and female ratio

Age group	Skin scraping	Nail
0-10	6	0
11-30	3	2
31-50	8	3
50-older	13	4
Male:Female ratio	10:20	3:6

Dermatophytes like *Trichophyton*, *Microsporum* and *Epidermatophyton* which are traditionally only accepted causal organism of superficial cutaneous mycoses among medical community but the mold flora which has the ability to utilize keratin and therefore could cause superficial cutaneous infection have usually been ignored. The mold flora isolated here like *Alternaria*, *Aspergillus*, *Bipolaris*, *Cladosporium*, *Exophiala*, *Fusarium*, *Rhizopus* and *Ulocladium* are usually common inhabitant of household environment (Niedoszytko *et al*., 2007; Bokhary and Parvez, 1995; Bakhali and Parvez, 1999). But the serious problem is that, unlike true dermatophytes, the pathogenicity of these mold flora are not restricted to skin or nail infections (Niedoszytko *et al*., 2007). *Alternaria* species could cause sub-cutaneous phaeohyphomycosis both in immunodeficient and immunocompetent patients (Sood *et al*., 2007; Robertshaw and Higgins, 2005).

Aspergillus species could pose more problems as not only causing cutaneous infection but also sub-cutaneous nodules, posing problem of infections in neonate, cancer patients, patients on catheter, transplant patients and a leading agent of fungal sinusitis (Romano and Miracco, 2003; Warnok, 2006; Restrepo *et al*., 2004). The fungal species reported here as causative agent of cutaneous mycoses but they are also reported to cause serious and fatal deep mycoses (Warnok, 2006; Groll and Walsh, 2001).

The isolation of mold from cutaneous sample should not be overlooked as they cause almost similar lesion that are produced by true dermatophytes (Kwon-Chung and Bennet, 1992). Secondly, they could infect other parts of the body especially in the case of any sort of immunodeficiency or wound or trauma.

CONCLUSIONS

Isolation of such a number of opportunistic mold from dermatophytic samples which could cause fatal deep mycoses and difficult to cure. Therefore, it is suggested that along with dermatophytic samples, a brief clinical history of patient should also be noted like patient having some sort of immunodeficiency symptoms, diabetes, trauma, wound, use of long term antibiotics, any kind of transplant etc. These will help in co-relating fungal isolation and infection.

Although, these fungi may not cause infections as true dermatophytes but these fungi could grow initially on dead skin cells on scalp and other parts of the body which could cause allergenic type itching or rashes. Hence resulting in scraping of the site by the person which may leads to a deeper infection or spreading of these fungi to other parts of the body. Therefore, we recommend that if these fungi isolated from dermatophytic samples, these fungi should also be considered for tropical antifungal treatment to avoid further spreading of these fungi as a precaution.

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