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Identification & characterization of fungi causing superficial mycoses

Swati Dekate[1], Sanchali Padhye[1] and Atul Gautam[2*]
1, Department of Microbiology, Mata Gujri College, Jabalpur (M.P.) - India
2, Nimar Institute of Pharmacy, Dhamnod Dist- Dha,r (M.P.) - India

Abstract

Diabetic patients are highly susceptible to various kinds of skin infections or mycoses. As compared to healthy population, diabetics seen to suffer from certain types of mycoses more frequently. During the study identification & isolation of micro-organism that causes superficial mycoses was carried out. Thus the present study was purposed to investigate the presence of unreported skin lesions in diabetic patients. The present study were undertaken to evaluate incidence of pathogen in diabetic & non-diabetic patients.Wet mount method was used to identification of fungal isolates. Topical therapies were successful in less than 20% of patients. Essentials oils have been shown to possess antibacterial, antifungal & antioxidant properties. In antifungal sensitivity test, antifungal agent (ketaconazole, nystatin, itraconazole, chloramphenicol, Amphotericin-B, fluconazole) & herbs like garlic, eucalyptus, neem, tulsi, turmeric were used. This method was done by disc diffusion method (kirbey bauer method).

Key-Words: Antifungal, Antibiotics, Itraconazole, Diabetics, Fluconazole, Chloramphenicol.

Introduction

Diabetic patients are more susceptible to skin infections.[6] Although the over all incidence of skin mycoses in diabetics is not higher as compared with healthy population.[16] Diabetic suffers from certain types of mycoses more frequently.The cutaneous signs of diabetes are the manifestations of multiple factors. Skin of diabetic patient's increases the capillary fragility & blood vessels show decreased circulation & abnormal carbohydrate metabolism etc.[5] Mycoses cause a wide range of disease in humans. Opportunistic fungi are normally saprobic fungi which suddenly become pathogenic. Fungal infection may be classified according to the site of infection, route of acquisition & type of virulence. Superficial fungal infections of the skins are some of the most common dermatologic conditions seen in clinical practice. When classified according to the sites of infection are designed as-

Superficial, Cutaneous infections of the outer layer of skin. Sub-cutaneous infection where fungi have infected into the tissue and Deep infection where the fungus becomes established in host.

*** Corresponding Author:**
E-mail: atul.gautam.pharma@gmail.com
Mob.: 8085443672

Superficial infection or mycoses are limited to the stratum corneum & essentially elicit no inflammation. Superficial fungal infections of the skin are some of the most common dermatologic condition seen in clinical practice. So recognition is important for physicians. Superficial fungal infections can be divided into 3 broad categories-

1. Dermatophytic infections
2. Tinea versicolor
3. Cutaneous candidiasis- is skin infection caused by C. albicans & other species.[10]

Tinea versicolor is common in adult. It is found in region of the body that has sebaceous glands. Such as the upper trunk, neck & arms. The characteristic finding is skin depigmentation. Systemic therapy often is needed in treatment of moccasin-type Tinea pedis.[3]

Cutaneous infection involves the integument & its appendages including hair & nails. Infections may involve the stratum corneum or deeper layers of the epidermis. Cutaneous mycoses may be classified as dermatophytoses or dermatomycoses.

Sub-cutaneous mycoses include a range of different infections characterized by infection of the sub-cutaneous tissues usually at the point of traumatic inoculation.

Deep mycoses involve the lungs, abdominal viscera bone & central nervous system. The most common

portals of entry are the respiratory tract, gastrointestinal tract & blood vessels.

Antifungal susceptibility testing includes the introduction of standardized method of antifungal susceptibility tests for both yeast & molds given by NCCLS.[15] Some oils & herbs have been used against mycosis, cancer & preservation. Clove oils had show antibacterial & antifungal activity.[4, 17] Itraconazole, Fluconazole, Amphotericin-B, shows the activity against Candida sp. Dermatophytes & many systemic mycoses.[19, 9, 20, 1] Fluconazole is a triazole with activity against Candida species, Dermatophytes & many systemic mycoses. [21] Ketoconazole is effective against Dermatophytes, yeasts, some systemic mycoses & superficial cutaneous fungal infection.[3, 17] Polyenes are useful in topical treatment of candidiasis. Amphotericin-B is an older polyenes used in superficial fungal infection. [10]

Material and Methods

- The study protocol was approved by Annamali The location of the infection site considerably helps in the diagnosis of a mycotic infection.
- The collections of the sample for the study of mycotic infection in diabetic & non-diabetic patients were done at the dermatological ward of the central railway hospital & Netaji Subhash Chandra Bosh medical college Jabalpur.
- The infected nails, hair & skin were treated with 10% KOH to destroy tissue elements & were examined microscopically.
- Fungal pathogens were isolated on sabouroud dextrose agar (SDA) at 26°c-28°c for 3-5 days. Antibacterial was added in SDA media for inhibiting bacterial growth.
- These fungal isolates were subjected to various tests like biochemical (casein & albumin hydrolysis & phospholipase activity) & thermo tolerance tests.

Results and Conclusion

All fungal isolates were subjected in different test method. In biochemical test, 3 tests were performed on 15 fungal isolates from diabetic patients. The results showed that all the fungi from diabetic patients have the degrading capacity for casein, albumin & phospholipase. Thermo tolerance test were performed on all 21 fungal isolates. Aspergillus brevipes & Aspergillus fumigatus were able to grow at all the temperature whereas Helminthosporium didn't grow in any temperature that means 2 species of Aspergillus are virulent in nature because they were able to grow at 50°c.

During the study isolation & identification of micro-organisms that causes the superficial infection in diabetic patients was carried out.

The spread of drug resistance pathogens is one of the most serious threats to successful treatment of microbial diseases.

The mortality rate of acute mycotic infection is also very high even in non-immuno-compromised patients.

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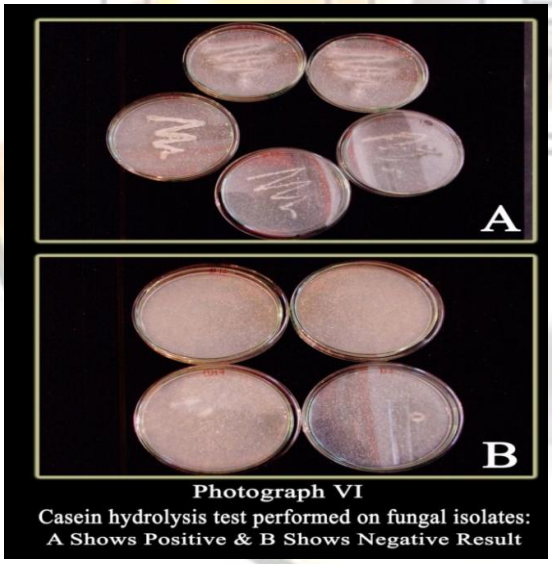
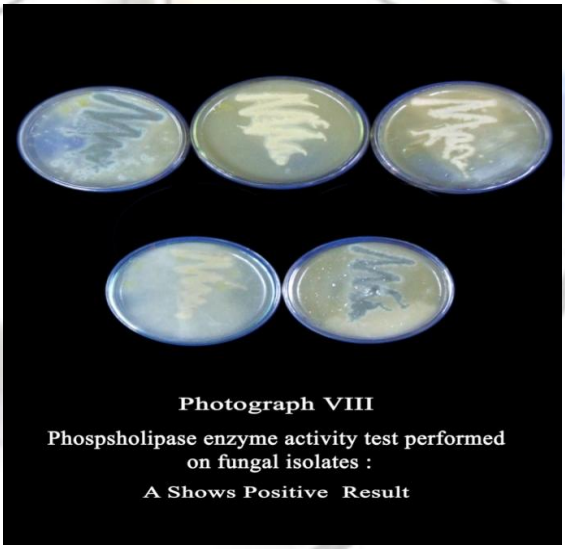
Table 1: Biochemical test diabetic fungal isolates

No. of Isolates	Casein	Phospholipase	Albumin
A. brevipes	++	+	+
A. fumigatus	++	+	+
A. niger	++	+	+
Aspergillus sp.	++	+	+
Rhizopus sp.	++	+	+
Alternaria sp.	+	±	-
Candida sp.	++	+	+
Pythium sp.	++	+/-	-
Cladosporium sp.	++	+	+
Yeast	+/-	+	-
Absidia sp.	++	+	-
P. nigricans	+	+	+
Fusarium sp.	+/-	+	-
Helminthosporium sp.	+/-	-	-
Unidentified	++	+/-	+

Abbr.: - = Negative hydrolysis, += Positive hydrolysis , ++= Excess hydrolysis, ±= Partial hydrolysis



Photograph VI
Casein hydrolysis test performed on fungal isolates:
A Shows Positive & B Shows Negative Result



Photograph VIII
Phospsholipase enzyme activity test performed on fungal isolates :
A Shows Positive Result

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Table 2: Thermotolerance test diabetic fungal isolates

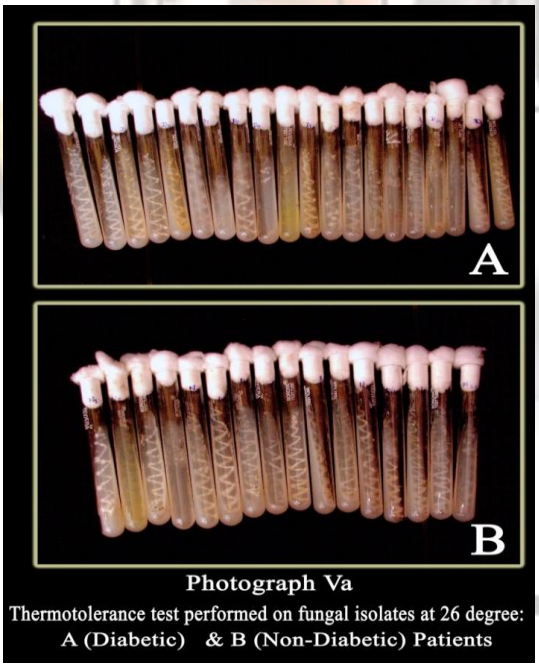
No. of Isolates	26°C	30°C	35°C	40°C	45°C	50°C
A. brevipes	++	++	++	++	++	+
A. fumigatus	++	++	++	++	++	+
A. niger	++	++	++	++	±	-
Aspergillus sp.	+	+	+	+	-	-
Rhizopus sp.	++	++	++	±	-	-
Alternaria sp.	+	±	±	-	-	-
Candida sp.	++	++	++	+	±	+
Pythium sp.	+	+	+	+	+	-
Cladosporium sp.	++	++	+	+	-	-
Yeast	+	+	+/-	+/-	+/-	-
Absidia sp.	++	++	++	++	++	-
P. nigricans	++	++	++	+	+	-
Fusarium sp.	+	+	+	-	-	-
Helminthosporium sp.	±	±	±	-	-	-
Unidentified	++	++	++	++	±	-

Abbr.: - =No growth, +=Growth, ++=Excess growth, ±=Partial growth

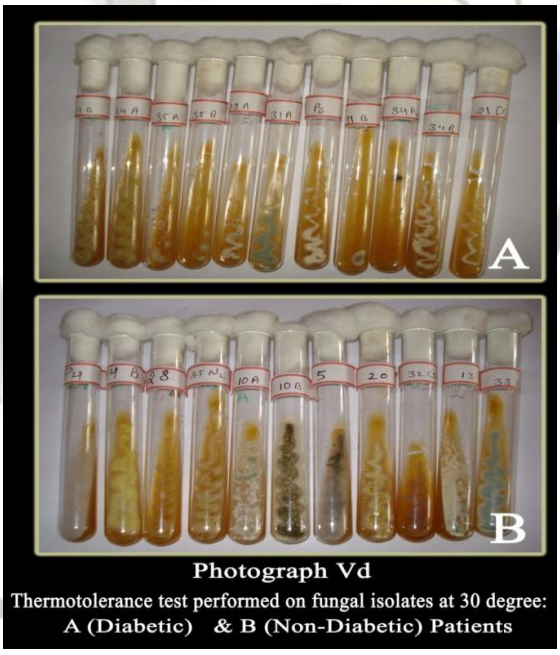
Table 3: Thermotolerance test non-diabetic fungal isolates

No. of Isolates	26°C	30°C	35°C	40°C	45°C	50°C
Curvularia sp.	+	+	+	-	-	-
P. nigricans	+	+	+	-	-	-
P. resticulosum	+	+	+	+/-	-	-
Mucor	++	++	++	++	+	-
A. funigatus	++	++	++	++	++	+
Aspergillus sp.	++	++	++	+	+	-

Abbr. : - =No growth, +=Growth, ++=Excess growth, ±=Partial growth



Photograph Va
Thermotolerance test performed on fungal isolates at 26 degree: A (Diabetic) & B (Non-Diabetic) Patients



Photograph Vd
Thermotolerance test performed on fungal isolates at 30 degree: A (Diabetic) & B (Non-Diabetic) Patients

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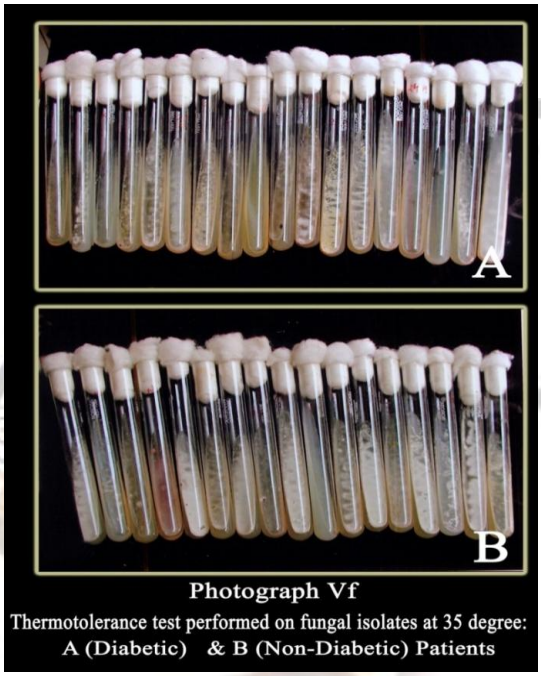
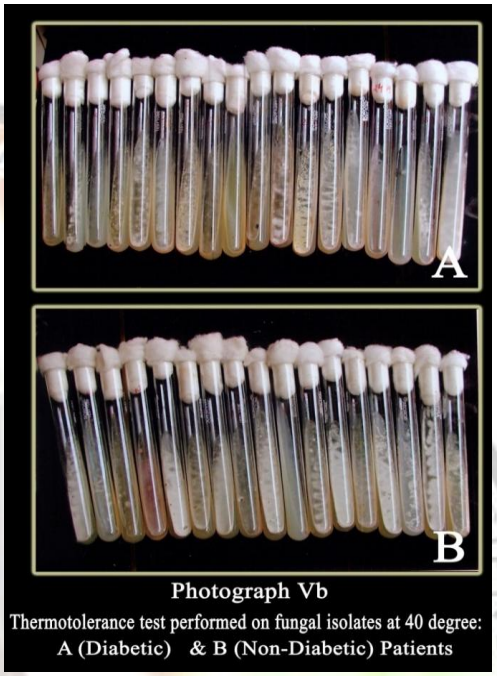


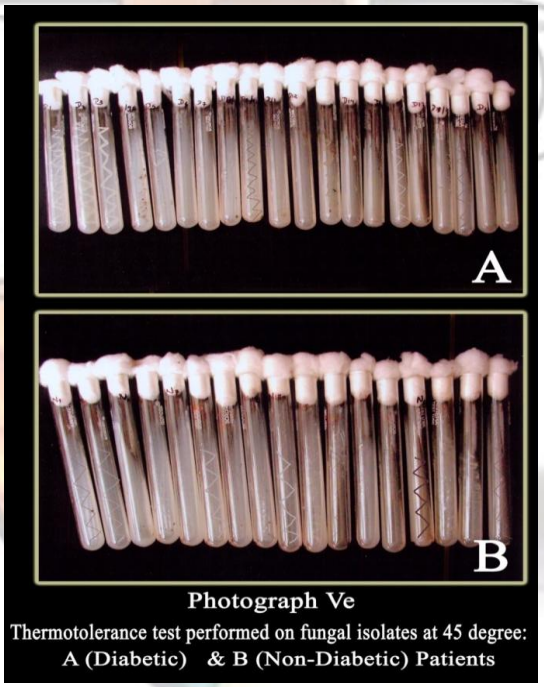
Photograph Vf

Thermotolerance test performed on fungal isolates at 35 degree: A (Diabetic) & B (Non-Diabetic) Patients



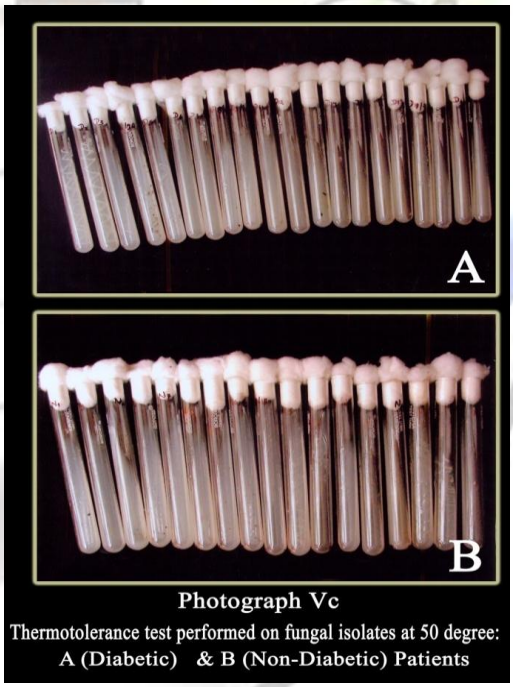
Photograph Vb

Thermotolerance test performed on fungal isolates at 40 degree: A (Diabetic) & B (Non-Diabetic) Patients



Photograph Ve

Thermotolerance test performed on fungal isolates at 45 degree: A (Diabetic) & B (Non-Diabetic) Patients



Photograph Vc

Thermotolerance test performed on fungal isolates at 50 degree: A (Diabetic) & B (Non-Diabetic) Patients