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FUNGAL INFECTIONS AMONG DIABETIC FOOT ULCER- PATIENTS ATTENDING DIABETIC CLINIC IN KENYATTA NATIONAL HOSPITAL, KENYA

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# FUNGAL INFECTIONS AMONG DIABETIC FOOT ULCER- PATIENTS ATTENDING DIABETIC CLINIC IN KENYATTA NATIONAL HOSPITAL, KENYA

A. M. GITAU, Z. W. NG'ANG'A, W. SIGILAI, C. BII and M. MWANGI

## ABSTRACT

***Objective*: To isolate and identify fungal pathogens associated with dermatophytoses in diabetic patients and identify the spectrum of yeasts colonising diabetic foot ulcers at Kenyatta National Hospital.**
***Design:* A cross sectional Laboratory based study.**
***Setting*: The Kenyatta National Hospital diabetic clinic.**
***Subjects*: Sixty one patients with diabetic foot ulcers from August to November 2009.**
***Results*: The five most occurring pathogens were *Biopolaris hawaiiensis* (5.5%), *Trichophyton schoenleinii* (3.7%), *Aspergillus niger* (3.0%), *Trichophyton rubrum* (3.0%), *Fusarium oxysporum* (3.0%). Other moulds accounted for less than 3.0%. One suspected case (0.6%) of *Penicilium marneffei* was isolated although it could not be ascertained due to its high containment requirement. Among the dermatophytes, the most occurring mould was *Trichophyton schoenleinii* (3.7%) while in non-dermatophyte was *Biopolaris hawaiiensis* (5.5%). Eight pathogenic yeasts were identified with *C. parapsilosis* (6.1 %) being the most common followed by *C. famata* (3.0%). Fungal infestation was highest in callus formation (78.6%).**
***Conclusion*: Fungal aetiological agents are significant cause of diabetic wound infection and may require antifungal intervention for successful management of diabetic foot ulcers.**

## INTRODUCTION

Diabetes mellitus (DM) is a clinical syndrome of chronic and degenerative condition caused by a disorder in insulin secretion and/or action which results in metabolic changes, especially high blood glucose (1). Individuals with diabetes are more susceptible to infections. This is because high blood sugar levels weaken the patient's immune system. Studies have shown that diabetic patient (even those who have minimal elevated blood sugar levels) are susceptible to infections (2). Diabetes is an important predisposing factor for fungal infections and causes significant morbidity and mortality. Fungal pathogens may colonise pre-existing lesions by traumatic abrasive implantation resulting in severe clinical condition (3,4). Diabetes foot infections occur more frequently because the disease causes nervous system changes and poor circulation. Since the nerves that control sweating no longer function, the skin of the feet can become very dry and cracked, and calluses tend to occur more frequently and build up faster (5). If not trimmed regularly, these calluses can turn into open sores or ulcers. Diabetic nerve damage can cause loss of sensation (neuropathy), if the feet are not regularly inspected; an ulcer can quickly become infected (6).

## MATERIALS AND METHODS

This cross sectional laboratory based study was carried out on 61 patients with diabetic foot ulcers for a period of four months, from August to November 2009 at Kenyatta National Hospital diabetic clinic. Patients presenting with foot ulcer(s) and those admitted to the surgical wards with diabetes were recruited. Those without visible foot ulcer and/or superficial lesions or declined consent to participate

were excluded from the study.

A questionnaire was administered to collect demographic characteristics of the patients. Samples were collected from the locations of ulcers during dressing of the diabetic foot. Particularly attention was over the dorsal portion of the toes and on the plantar aspect of the metatarsal heads and the heel. The skin scrapings from the active border areas of lesions were taken with a sterile scalpel and placed in sterile Petri dishes. Scrapings of infected nails or clippings of nails were collected by clipping nails after cleaning the nails with 70% ethanol. Purulent wounds samples were collected using sterilised swabs and tissue biopsy was obtained from the depth of the wound after taking the necessary aseptic precautions and was then transported in sterile solution of normal saline and sterile test tube respectively. These specimens were immediately transported to the mycology laboratory for analysis. Direct microscopic examination of skin scrapings, hairs and nails for detection of fungal spores or hyphae and yeast was carried out using 10% Potassium hydroxide (KOH). Fungal culture was carried out using Sabouraud's Dextrose Agar for isolation of both moulds and yeasts. Lactophenol cotton blue stain was used for morphological identification of filamentous fungi. ChroMagar Candida and API 20 C aux Yeast Identification Systems were used for confirmation of yeast.

Data were analysed as mean ± standard deviation (SD). Qualitative variables expressed as percentages were compared using Chi-square test. A probability (P) of less than 0.05 was taken as statistically significant.

## RESULTS

A total of 164 samples from 61 individuals with mean age 59.5 ± 10.1 ranging between 38 and 90 years were analysed. The ratio of males to females was 1:2.8, a proportion distribution of 16/61(26.2%) and 45/61(73.8%) respectively. Among the study participants, 14.8% were less than 50 years. Majority of respondents (69.2%) were 50 to 69 years, while 18.0% were 70 years and above (Table 1).

Among 164 samples that were analysed, majority of them were obtained from nail clippings 52/164(31.7%), followed by skin scrapings 43/164(26.2%), biopsy 38/164(23.2%), and pus swabs 31/164(18.9%). The sources of samples by gender indicate that 15/43(31.9%) of the skin scrapings were taken from male compared to 23.9% from female and 21.8% of the pus samples were taken from male compared to 21.4% from female. There was no significant difference in source of samples by gender (P=0.501). The sources of samples by age shows that 36.4% of skin scrapings were from patient aged 40-49 years being the upper most compared to 35.7% of nail samples from clippings among the 50-59 years old. Similarly, there was no significant difference in source of samples analysed by age among the patients (P=0.925) (Table 2).

Among those positive on gram stain, (31.1%) were Gram -ve rods, (15.2%) Gram +ve cocci, (8.5%) yeast and (4.9%) had fungal hyphae. The majority of the samples (54.9%) were negative for mould and yeast using KOH preparation. However, (30.5%) were positive for moulds and (14.6%) for yeast. In culture (34.1%) were positive for moulds and (11.6%) for yeast. There was high occurrence of moulds in skin samples (48.8%) compared to nail (40.4%), pus (32.3%) and biopsy (10.5%) (Figure 1).

Distribution of specific species of moulds identified using LCB by source of sample shows that out of 85 moulds, 31/85 (36.5%) were isolated from nail and 30/85 (35.3%) in skin samples. Only 13/85(15.3%) were isolated from pus while 12/85 (14.1%) were from biopsy samples. Out of 85 moulds, 36 specific species of moulds were identified. Two organisms were unidentified while (61.6%) of the samples were negative for moulds. The five most occurring moulds were *Biopolaris hawaiiensis* 9/85(5.5%), *Trichophyton schoenleinii* 6/85(3.7%), *Aspergillus niger* 5/85(3.0%), *Trichophyton rubrum* 5/85(3.0%), *Fusarium oxysporum* 5/85(3.0%) (Fig 2-9). There was one suspected *Penicilium marneffei* 1/85(0.6 %) (Fig 10-13). The isolate exhibited morphologic dimorphism at 30°C and 37°C. However due to level three containment requirements it could not be confirmed.

The isolated moulds could be broadly categorised into dermatophytes and non-dermatophytes. There was 33/85(20.1 %) occurrence of dermatophytes compared to 50/85(30.5%) of non-dermatophytes. Among the dermatophytes, the most occurring mould was *Trichophyton schoenleinii* 6/85(3.7%) while in non-dermatophytes it was *Biopolaris hawaiiensis* 9/85 (5.5%) (Fig 6 and 7) (Table 3).

The most common types of yeast were *C. parapsilosis* 10/25(6.1%), followed by *C. famata* 5/25(3.0%). The occurrence of the other yeasts was less than 3.0%. Table 4 shows distribution of the specific species of yeast. There was no significant relationship between occurrence of yeast and gender (P=0.347). Similarly, there was no significant association between occurrence of yeast and age of patient (P=0.854) (Table 5).

Analysis of clinical conditions was done to establish whether there exists a relationship between the risk factors and occurrence of Fungi. Three most occurring risk factors were Neuropathy (28.0%), Poor glucose control (22.0%) and Poor circulation (20.7%). Occurrence of fungi was highest in callus formation (78.6%), compared to (65.2%) in Neuropathy (Table 6).

**Table 1**
*Selected demographic characteristics of the study participants*

| Characteristic | n=61 | % |
|---|---|---|
| Gender: | | |
| Male | 16 | 26.2 |
| Female | 45 | 73.8 |
| Age in years: | | |
| <40 | 2 | 3.3 |
| 40 - 49 | 7 | 11.5 |
| 50 - 59 | 23 | 37.7 |
| 60 - 69 | 18 | 29.5 |
| 70 and above | 11 | 18.0 |

**Table 2**
*Sources of samples analysed by selected demographic characteristics*

| Variables | Skin | | Pus | | Nail | | Biopsy | | |
|---|---|---|---|---|---|---|---|---|---|
| Categories | n | % | n | % | n | % | n | % | P-value |
| Gender: | | | | | | | | | |
| Male | 15 | 31.9 | 6 | 12.8 | 14 | 29.8 | 12 | 25.5 | 0.501 |
| Female | 28 | 23.9 | 25 | 21.4 | 38 | 32.5 | 26 | 22.2 | |
| Age in years: | | | | | | | | | |
| <40 | 2 | 33.3 | 0 | 0.0 | 2 | 33.3 | 2 | 33.3 | |
| 40 - 49 | 8 | 36.4 | 4 | 18.2 | 6 | 27.3 | 4 | 18.2 | |
| 50 - 59 | 11 | 19.6 | 13 | 23.2 | 20 | 35.7 | 12 | 21.4 | 0.925 |
| 60 - 69 | 16 | 30.8 | 9 | 17.3 | 15 | 28.8 | 12 | 23.1 | |
| 70 and above | 6 | 21.4 | 5 | 17.9 | 9 | 32.1 | 8 | 28.6 | |

**Table 3**
*Distribution of different moulds in different samples as identified using characteristic mycelia and fruiting structures*

| | Categories of samples | | | | Total | |
|---|---|---|---|---|---|---|
| Moulds Identified using LCB | Skin | Pus | Nail | Biopsy | n | % |
| Dermatophytes | | | | | | |
| *Trichophyton rubrum* | 2 | 0 | 2 | 1 | 5 | 3 |
| *Epidermopyton floccosum* | 0 | 0 | 2 | 0 | 2 | 1.2 |
| *Microsporu f/errugineum* | 1 | 1 | 1 | 0 | 3 | 1.8 |
| *Microsporum audouinii* | 1 | 0 | 1 | 1 | 3 | 1.8 |
| *Micosporum gypsium* | 0 | 0 | 1 | 0 | 1 | 0.6 |
| *Trichophyton tonsurans* | 0 | 0 | 1 | 0 | 1 | 0.6 |
| *Trichophyton interdigitale* | 0 | 0 | 1 | 0 | 1 | 0.6 |
| *Trichophyton metagrophyte* | 2 | 1 | 0 | 0 | 3 | 1.8 |
| *Trichophyton schoenleinii* | 2 | 2 | 1 | 1 | 6 | 3.7 |
| *Trichophyton soudanase* | 1 | 0 | 0 | 1 | 2 | 1.2 |
| *Trichophyton tonsurans* | 1 | 0 | 1 | 0 | 2 | 1.2 |
| *Trichophyton verrucosum* | 2 | 1 | 0 | 1 | 4 | 2.4 |
| Non-dermatophytes | | | | | | |
| *Aspergillus candidus* | 0 | 0 | 3 | 0 | 3 | 1.8 |
| *Aspergillus flavipes* | 0 | 0 | 1 | 0 | 1 | 0.6 |
| *Aspergillus flavus* | 0 | 0 | 1 | 0 | 1 | 0.6 |
| *Aspergillus fumigatus* | 1 | 1 | 1 | 1 | 3 | 1.8 |
| *Aspergillus nidulans* | 0 | 1 | 0 | 0 | I | 0.6 |
| *Aspergillus niger* | 3 | 0 | 2 | 0 | 5 | 3 |
| *Aspergillus terreus* | 2 | 0 | 0 | 0 | 2 | 1.2 |
| *Aspergillus versicola* | 1 | 0 | 1 | I | 3 | 1.8 |
| *Biopolaris hawaiiensis* | 2 | 1 | 3 | 3 | 9 | 5.5 |

| | | | | | | |
|---|---|---|---|---|---|---|
| *Paecilomyces lilacinus* | 1 | 0 | 0 | 0 | 1 | 0.6 |
| *Fusarium solani* | 0 | 0 | 2 | 0 | 2 | 1.2 |
| *Penicilium marneffti** | 0 | 0 | 0 | 1 | 1 | 0.6 |
| *Cylindrocorpon lichencola* | 1 | 0 | 0 | 0 | 1 | 0.6 |
| *Fusarium oxysporum* | 3 | 2 | 0 | 0 | 5 | 3 |
| *Mucor circinelloides* | 0 | 0 | 1 | 0 | 1 | 0.6 |
| *Mucor hiemalis* | 1 | 0 | 0 | 0 | I | 0.6 |
| *Onychocola canadensis* | 0 | 1 | 1 | 0 | 2 | 1.2 |
| *Penicilium spp* | 0 | 0 | 1 | 0 | 1 | 0.6 |
| *Rhizopus arrhizus* | 0 | 0 | 1 | 0 | I | 0.6 |
| *Scedosporium apiospermum* | 0 | 0 | 1 | 0 | 1 | 0.6 |
| *Scopulariopsis brevicaulis* | 1 | 0 | 0 | 0 | 1 | 0.6 |
| *Scytalidium dimidictum* | 0 | 0 | 1 | 0 | 1 | 0.6 |
| *Syncephalastrum racemosum* | 1 | 0 | 0 | 0 | 1 | 0.6 |
| *Alternaria alternaria* | 1 | 0 | 0 | 0 | I | 0.6 |
| Unidentified organism | 0 | 1 | 0 | 1 | 2 | 1.2 |
| Negative | 43 | 31 | 52 | 33 | 101 | 61.6 |

* Suspected not confirmed

**Table 4**

*Yeasts as identified using Analytical Profile Index (API) 20 C AUX*

| Types of yeasts | n | % |
|---|---|---|
| *Candida boidiii* | 1 | 0.6 |
| *C.famata* | 5 | 3.0. |
| *C. guilliermondii* | 3 | 1.8 |
| *C. parapsilosis* | 10 | 6.1 |
| *C. tropicalis* | 1 | 0.6 |
| *C. albicans* | 3 | 1.8 |
| *Pichia ohmeri* | 2 | 1.2 |
| *Rhodotorula muciloginosa* | 1 | 0.6 |
| None | 139 | 84.8 |

**Table 5**

*Yeasts identified in relation to the demographic characteristics*

| | Positive for Yeast (n=25) | | Negative for yeast (n=139) | | |
|---|---|---|---|---|---|
| Characteristic | n | % | n | % | P value |
| Gender | | | | | |
| Male | 5 | 10.6 | 42 | 89.4 | 0.347 |
| Female | 20 | 17.1 | 97 | 82.9 | |
| Age | | | | | |
| <40 | 0 | 0.0 | 6 | 100.0 | |
| 40 - 49 | 3 | 13.6 | 19 | 86.4 | |
| 50 - 59 | 9 | 16.1 | 47 | 83.9 | 0.854 |
| 60 - 69 | 9 | 17.3 | 43 | 82.7 | |
| 70 and above | 4 | 14.3 | 24 | 85.7 | |

**Table 6**
*The relationship of fungi infection and Risk factors*

| Risk factor | Positive for Fungi (n=82) n | % | Negative for Fungi (n=82) n | % |
|---|---|---|---|---|
| Callus formation | 11 | 78.6 | 3 | 21.4 |
| Neuropathy | 30 | 65.2 | 16 | 34.8 |
| Structural Deformity / Char cot's joint | 8 | 53.3 | 7 | 46.7 |
| Poor circulation | 14 | 41.2 | 20 | 58.8 |
| Ischaemia | 2 | 40.0 | 3 | 60.0 |
| Poor glycemic control | 13 | 36.1 | 23 | 63.9 |
| Amputation | 2 | 33.3 | 4 | 66.7 |
| Corns and callus | 2 | 25.0 | 6 | 75.0 |
| Total | 82 | 50.0 | 82 | 50.0 |

**Figure 1**
*Proportions of Fungi by categories of samples in SDA Culture*

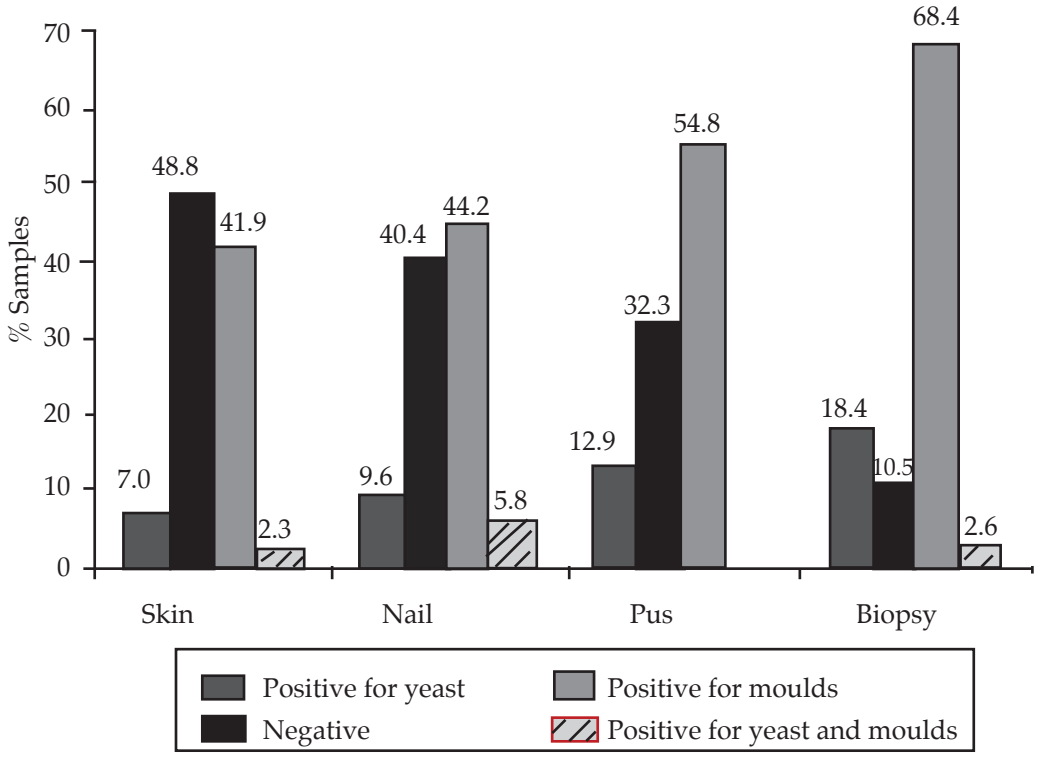



**Figure 2**
Culture plate of *Trychophyton rubrum* on SDA+ at 28$^0$C showing domed downy to floccose white colony

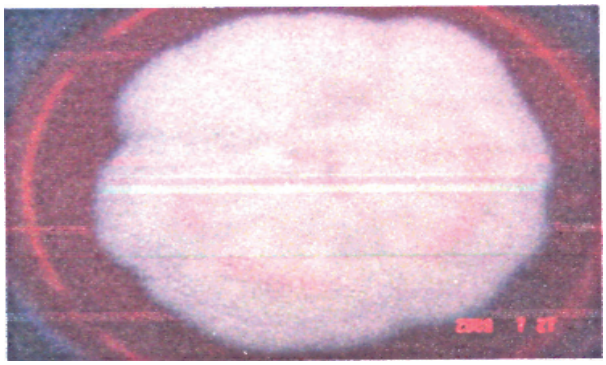


**Figure 3**
Culture plate of *Trychophyton rubrum* on SDA+ at 28$^0$C showing colony with dark red-brown pigmentation

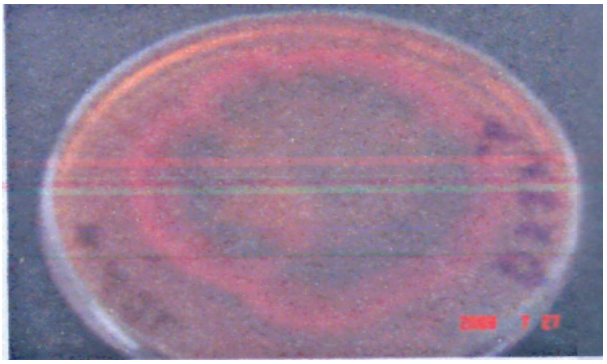


**Figure 4**
Culture plate of *fusarium oxysporium* on SDA+ at 28$^0$C showing flat floccose white to pale apricot, with purple tinge colony

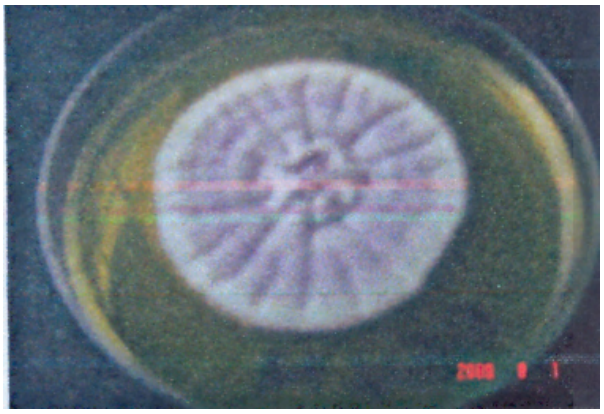


**Figure 5**
Culture plate of *fusarium oxysporium* (reverse) on SDA+ at 28$^0$C showing purple colony with cream margin

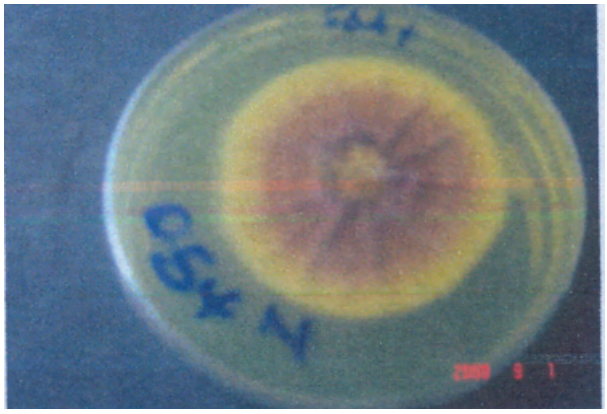


**Figure 6**
Culture plate of *Bipolaris hawaiiensis* (green on SDA+ at 28$^0$C showing flat floccose white to pale apricot, with purple tinge colony

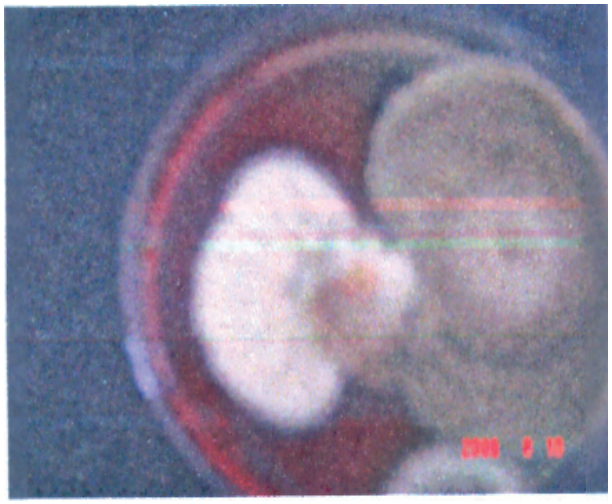


**Figure 7**
Colonies of *Aspergillus niger* yellow covered by dark-brown conidial heads. on SDA+ at 28$^0$C

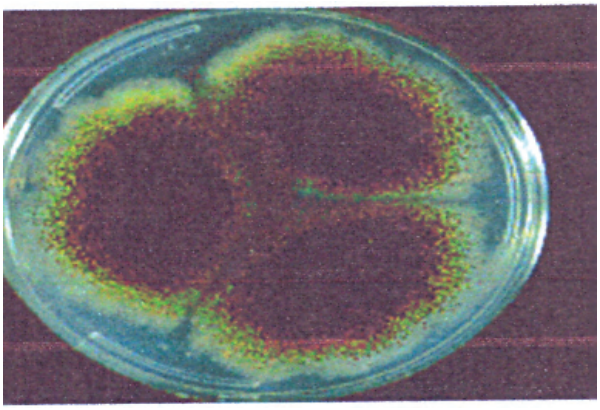


**Figure 8**
Colonies of *Trichophyton schoenleinii* waxy with a deeply folded honeycomb-like thallus (Cream coloured to yellow)on SDA+ at 28$^0$C

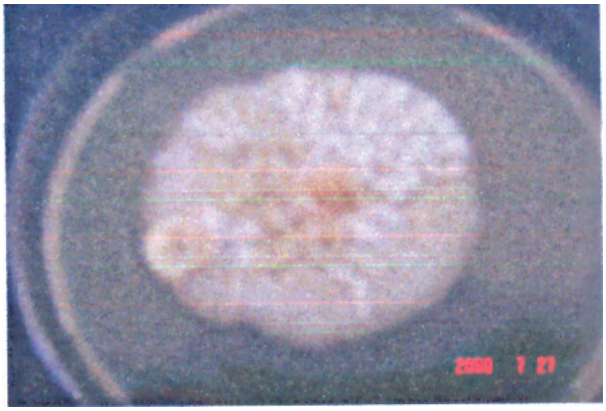


**Figure 9**
Colonies of *Trichophyton schoenleinii* cream coloured to yellow to orange brown with no reverse pigmentation is present on SDA+ at at 28$^0$C

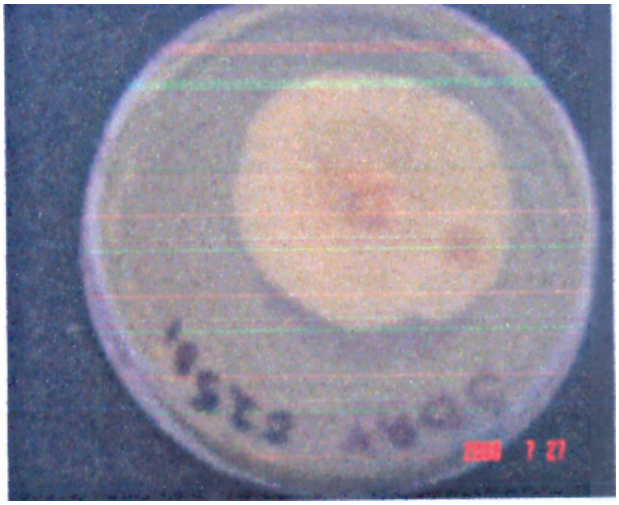


**Figure 10**
Thermaly dimorphic *Penicillum marneffei* (hazard group 3 pathogen) isolated on SDA+ at 28$^0$C

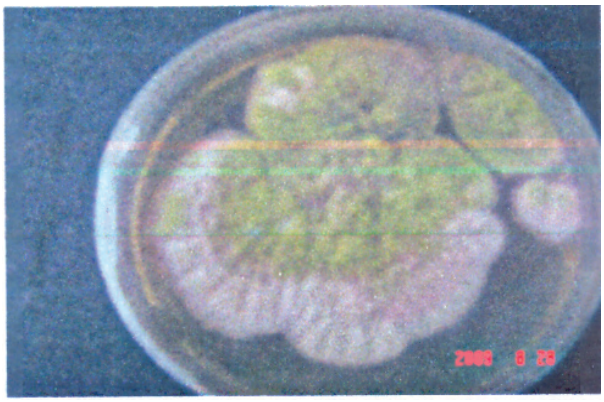


**Figure 11**
*Penicillum marneffei* plates reverse on SDA+ at 28$^0$C with red diffusing pigment

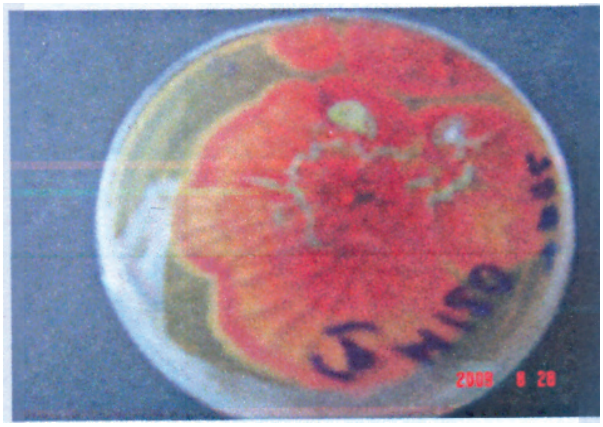


**Figure 12**
*Penicillum marneffei* on BHI at 37$^0$C, growing as yeast colonies

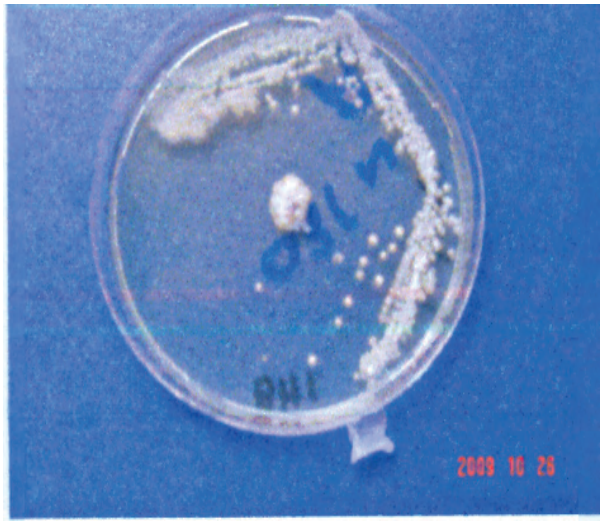
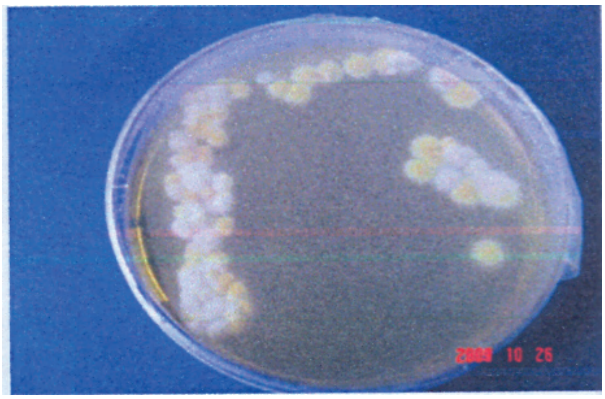


**Figure 13**
*Penicillum marneffei* on BHI at 28$^0$C growing like mould



## DISCUSSION

There was high occurrence of moulds in skin scrapping (44.2%), these may be attributed to skin function, as barrier to inner tissues and thus it is prone to colonisation by moulds and eventually infected preceding other tissues. It is also notable that dermatophytes are inhibited by serum factor in the subcutaneous skin. (7).

Positive fungal culture results were slightly higher than those found using KOH preparation with 34.1 % positive for moulds and 11.6% for yeast. Five samples (3.0%) were positive for both moulds and yeast. Culture is regarded as the gold standard for confirming a clinical diagnosis of fungal infection with specificity of 100% but variable sensitivity. However the sensitivity of the test is determined by sample collection technique, storage and transport conditions (8).

Lactophenol Cotton Blue mount (Microscopy) in conjunction with culture technique SDA 28°C were able to identify 36 specific species of moulds, two organisms were unidentified. Among the dermatophytes (20.1%), *Trichophyton schoenleinii* (3.7%) was the most predominant. This was low compared to study by Eckhard *et al.*, (9), which showed the dermatophytes as the most commonly identified etiology accounting for 69.2% of fungal isolates. Most of the dermatophtes were isolated from skin. High occurrence of *Trichophyton rub* rum and *Epidermopyton floccosum* were isolated from the nail samples. There was low occurrence of dermatophytes in pus and biopsy. This was comparable to study by Manzano *et al*., (10) which showed *Trichophyton rubrum* being the common species (37.1 %) in *onychomycosis*.

Among non-dermatophytes (30.5%), *Biopolaris hawaiiensis* (5.5%) was the most predominant. *Fusarium oxysporum* was 3.0%. Non-dermatophytes should be considered potenitial pathogen rather than colonisers. *Fusarium spp*. is usually considered

as environmental contaminant. However, study by Saad *et al*. (8) reported a case of a 68-year-old patient with a diabetic foot infection that developed into a gangrenous necrosis as a result of *Fusarium acutatum* infection.

Fungi are seldom pathogenic in normal host but occur most often in the immunocompromised host with or without an underlying pathology. In this study, there was no significant relationship between occurrence of moulds and gender (p=0.595). Similarly, there was no significant association between occurrence of moulds and age (P=0.383). Similar results were obtained by Gupta *et al*., (11) but differed with study by Bradford *et al*., (12) which showed that mycotic foot ulcer infection correlate with age, gender and duration of diabetes disease.

One case of suspected *Penicilium marneffei* (0.6 %) was isolated as characterised by its dimorphism at different temperatures. *Penicillium marneffei* is rare and has not been reported in Kenya. The fungus is geographically restricted in distribution and all natural human infections have occurred in individuals who resided in or visited the endemic regions. Penicilliosis is a treatable condition, but is often fatal if left untreated. The organism may pose a serious threat to laboratory workers handling live cultures (13). *Penicilium marneffei* infections have been documented in HIV-infected individuals from endemic regions of South East Asia (14). There is no documented case *of P. marneffei* in Kenya. This may be associated to deficient in routine mycological investigations. The paucity of published work in mycology may also be attributed to no cases reported.

Among the moulds isolated two organisms were not conclusively identified using culture technique and lactophenol cotton blue stain. This was because the features did not match any description available in the identification pathogenic fungi key. However further study should be done using molecular technique to establish possibility of emergent of new organism. Most of the identification keys are non conclusive probably because they were generated from fungi outside our geographical region.

The results of this study and other reports suggest that Candida species are the most common fungal isolates from diabetic foot ulcer, with *C. parapsilosis* (6.1 %) being the most common causative agent of fungal and mixed infection. However, this was low compared to the study by Saunte *et al*., (15) which showed that, *C. parapsilosis* accounted for 61.5% of infection among the *Candida spp* among diabetics' patients.

There was no significant relationship between occurrence of yeast and gender (P=0.347). Majority of the females (17.1%) were positive for yeast compared to males (10.6%). Similarly, there was no significant association between occurrence of yeast and age (P=0.854). There was high occurrence of yeast in biopsy samples (21.1%). A possible explanation for ulcer yeast infection is long wrapping of the foot and application of antibiotics during treatment. From literature, covering the skin with dressing material (which stimulate sweating and increase local temperature of the skin), selective antibacterial and immunodulating action of antibiotic favours the growth and replication of yeasts (16-18). Nevertheless, this investigation showed fungal isolates, originating not only from a primarily sterile ulcer sample (biopsy specimen) but also from foot ulcer swabs to be the causative agents of the foot ulcer infection.

The pathogenic effect of fungi in foot ulcer is indicated by the severity of clinical finding, chronic course of infection and infection progression despite antibiotic therapy (19). There was a positive significant relationship between fungal infection and the risk factors (P=0.029). Occurrence of Fungi was highest in callus formation (78.6%), compared to 65.2% in Neuropathy. This may be attributed to nutritional requirement of fungi. Since the nerves that control sweating no longer work in diabetic patients, the skin of the feet can become very dry and cracked, and calluses tend to occur more frequently and build up faster. Callus are typically dead skin which are composed of keratin material which are source of nutritional requirement to most of the fungi especially dermatophytes (20).

In conclusion, fungi are opportunistic organism which normally colonise and infect immune suppressed individuals with diabetes and other immune-suppressive conditions. Fungal infections in diabetic patients if not treated the results can be fatal. Therefore, patients with diabetes seem to have diagnostic, therapeutic and preventive needs with regard to mycotic infections that have hitherto been underestimated.

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