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FUNGI IN TOE NAILS*

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ABSTRACT

One hundred and eighty three abnormal toe nails were cultured for dermatophytes, molds, and yeasts. Incidence figures for all above mentioned organisms were obtained. In approximately 25% of the abnormal toe nails, dermatophytes were isolated. *Trichophyton rubrum* and *T. mentagrophytes* were equally isolated. In approximately 25% of abnormal nails, no organisms were isolated. Non-dermatophytic fungi were commonly associated with abnormal nails (50%). Among these were: *Arthroderma quadrificum; Aspergillus sydowii; A. nidulans; Cephalosporium acremonium; Curvularia lunata; Fusarium oxysporum; Hormodendrum cladosporidides; Penicillium citrinum; Scopulariopsis brevicalis.*

Yeasts were also isolated from 25% of nails (in combination with other organisms). *Candida parapsilosis* was by far the most commonly yeast isolated. Approximately one half of dermatophytes were recovered by cultural methods when compared to the number of KOH positive nails.

The present study records the incidence of fungi and yeasts recovered from abnormal toe nails in 183 patients.

Nail samples were obtained from in-patients of the orthopedic and gynecological services, and dermatologic out-patients in Jackson Memorial Hospital, Miami, Florida. Most patients experienced no symptoms from their abnormal nails.

The number of times that fungal elements are seen in direct microscopic examination of nail samples has always been greater than the times the fungus has been recovered from identical nail samples by cultural methods. This failure to recover fungi from abnormal nails has been a mystery and has led investigators and clinicians to culture multiple nail samples in an effort to achieve fungi recovery (1–4). The usual method is to plant nail pieces or subungual debris on the surface of tubed media. In this study special technics are used to prepare the nail samples and various media are used to encourage a better yield of yeasts, fungi, and bacteria found in the sample.

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MATERIALS AND METHODS

Nail samples were obtained from 183 hospitalized patients and out-patients seeking medical attention for reasons other than their abnormal nails. Sources of toe nail samples were recorded as well as other associated skin diseases. Direct microscopic examination using an improved clearing preparation containing 20% potassium hydroxide and dimethylsulfoxide was made on each nail sample (5).

Nail sample. Where possible, the friable, soft subungual debris was used in preference to the hard nail plate. Multiple samples from each toe nail were obtained. *Samples were planted directly onto tubed media as well as being gently homogenized in an all glass Tembrock homogenizer.* The homogenate was then streaked on petri dishes containing various media devised for selective recovery of bacteria, yeasts, dermatophytes, and molds.

If no dermatophytes were isolated from a nail sample, the nail was recultured.

Media

A) Pieces of subungual debris were planted directly into bottled dermatophyte test media (DTM) (6) containing peptone, glucose, and an agar base; antibacterial antibiotics (chlortetracycline and gentamycin); and a mold inhibitor, cycloheximide. This medium was devised for the isolation and growth of various species of dermatophyte fungi belonging to the genera Trichophyton, Microsporum, Epidermophyton, and some Candida species.

B) Homogenized nail sample: one large nail sample was homogenized with a small quantity of Eugon Broth (BBL) and equal portions of the homogenate planted and streaked on the following media in petri dishes:

1) Sabouraud dextrose agar (Difco) with 0.5% yeast extract to allow growth of all fungi and yeasts.

TABLE I

Recovery of fungi from 183 abnormal toe nails (not psoriasis) expressed as percentages

	Dermatophyte only	Dermatophyte and molds and/or yeasts	Molds only	Molds and yeasts	Yeast only	Non-growth
Positive KOH 41%	8.8	9.3	8.8	5.4	2.7	6.7
Negative KOH 58.6%	2.2	2.4	17.2	9.8	5.4	21.3
Total	11.0	11.7*	26.0	15.2	8.1	28.0

In many instances more than one fungus was isolated from each nail sample.
* Yeasts were isolated in 2.7% of cases.

2) DTM to allow for dermatophytic fungi growth only.
3) DTM without the cycloheximide to allow the growth of molds and dermatophytic fungi but not bacteria.
4) Sheep blood agar for bacterial growth.
5) Brain heart agar infusion agar (Difco) for bacterial growth.

RESULTS

None of the patients studied had psoriasis or other known dermatologic clinical entities other than possibly onychomycosis. The microbiologic flora in psoriatic nails is reported elsewhere (7).

In Table I a summary of results is presented. In spite of the fact that all toe nails were abnormal, i.e. subungual keratosis, only 41% were found to have mycelial elements by direct microscopy. A greater number of abnormal toe nails (58%) were microscopically negative.

Dermatophytes. As would be expected, the recovery of dermatophytic fungi was much greater from KOH positive nails, 18.1% (total recovery = dermatophyte only, plus dermatophytes, molds, and yeasts), than from the KOH negative nails, 4.6%.

It is interesting to note that the total percentage of dermatophytes recovered by cultural methods was 22.7%. This is approximately half of the number of positive KOH specimens. Table II lists dermatophytes recovered.

Non-dermatophytic fungi. There is a large population of non-dermatophytic fungi associated with abnormal nails. The recovery from KOH negative nails was approximately twice as high as from KOH positive nails.

It appears that there are two populations: one commonly associated with abnormal nails (Table III), and the other infrequently isolated, as follows: *Acrothecium apicale; Aspergillus versicolor; A. foetidus* (parasitized by A. parasiticus); *A. fumigatus; A. glaucus; A. jovus; A. niger; A proliferans; A. tamarii; A. thomei; A. unguis; A. wentii; Basidiobotrys species; Botryotrichium atrogriseum; Brachysporum bloxani; Cephalosporium species; C. asperum; C. humicola; C. maculans; Chaetomium glodosum; Curvularia geniculata; Didymobotryum cookei; Fusarium chlamydosporum; F. linii; F. moniliforme; Gliocladium roseum; G. penicilloides; Harpographium fasciculatum; Hormodendrum pallidum; H. hordei;*

TABLE II

Dermatophytes recovered from 183 abnormal toe nails

Trichophyton rubrum	20
Trichophyton mentagrophytes	20
Epidermophyton floccosum	2
	42

TABLE III

Recovery of fungus other than dermatophytes from 183 abnormal toe nails (common isolates)

	Number of times isolated from nails
Arthroderma quadrificum	6
Aspergillus sydowii	11
A. nidulans	9
A. species	4
A. terricola	3
Cephalosporium acremonium	5
Curvularia lunata	8
Fusarium oxysporum	4
Hormodendrum cladosporidides	5
H. nigrescens	4
Penicillium citrinum	10
P. lilacium	7
P. oxalicum	6
P. chrysogenum	4
Scopulariopsis brevicaulis	8

TABLE IV

Yeasts recovered from 183 abnormal toe nails

Candida albicans	4
C. guillermondii	3
C. intermedia	1
C. parapsilosis	43
C. robusta	1
C. tropicalis	3
Torulopsis candida	4
T. glabrata	1
	—
	60

Nigrospora sphaerica; Penicillium furiculosum; P. purpuragen; P. urticae; Phaeoscopulopsis allii; Phialophora jeanselmii; Scopulariopsis constantini; Spicaria violacea; Thielariopsis basicola; Verticillium sulphurellum.

Yeasts. Yeasts were recovered from approximately 26% of the total of abnormal toe nails (total yeasts = 8.1% yeasts only, plus 15.2% molds and yeasts, plus 2.7% of yeasts associated with dermatophytes). Table IV lists the yeasts isolated. It can be seen that *Candida parapsilosis* is the most commonly isolated yeast.

Culture negative. Twenty eight per cent of all abnormal nails were negative culturally for fungus, yeasts, or dermatophytes (see Table I). There was, however, bacterial isolation. This is being reported elsewhere.

In KOH negative nails there were three times as many negative cultures as in the KOH positive nail group.

DISCUSSION

It has long puzzled mycologists and clinicians why many seemingly diseased nails have failed to show evidence of fungal infection, especially when the supporting evidence of the direct examination would support this diagnosis.

Evidence from this paper would suggest that many fungi besides dermatophytes are present in abnormal nails. Whether or not these may be incriminated as pathogens remains to be seen, but the consistent findings of non-dermatophytes would lead one to suspect that some so called "contaminants" could attain a pathogenic role.

This paper again confirms the results of previous workers, i.e. that in KOH positive samples there will be a large recovery of dermatophytes. However, it is of interest to note the large number of yeasts and molds recovered from KOH negative samples. How is it that such large numbers escape microscopic detection?

This study shows that *Candida parapsilosis* is recovered in far greater numbers from abnormal toe nails that is *C. albicans*.

Keeping in mind the fact that all of the nails used in this study were abnormal and clinically showed signs of infection, we feel that perhaps there exists a relationship between two different types of fungi that together form a pathogenic entity. This, of course, needs much investigation and experiments along these lines are being conducted in this laboratory now.

To attain more knowledge and a deeper comprehension of nail disease, we feel that the total fungal ecology of the diseased and normal nail should be fully established.

To this end we hope that this paper has contributed to the fungal ecology. There still remain, however, the bacterial and possibly viral ecology to be established.

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