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Onychomycosis

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Onychomycosis is a major cause of nail disease in developed countries. The condition is world-wide in occurrence and in Europe at least, has been met with increasing frequency during the present century [1]. The proportion of nail infections among superficial mycoses was reported as 30% in 1987 [2]. Estimates for the prevalence of this condition in the general population have been calculated from questionnaire surveys of over 9,000 subjects in the UK [3] and 10,007 in Spain [4] and these have indicated figures of 2.7% and 2.6% respectively. A later study from Finland, which included laboratory mycological data, reported a prevalence of 8.4% [5]. Since most toenail infections result from the spread of disease from the toe spaces, higher figures of onychomycosis would be expected in groups which are prone to tinea pedis such as coal miners, sportsmen, frequent swimmers etc. [5,6] the rate of infection being influenced by factors such as the provision of communal bathing facilities, the use of occlusive footwear and frequent trauma. The prevalence of mycotic nail conditions also increases with age, being rare in pre-pubertal children [4] and significantly higher in adults over the age of 55 years [3]. Finally, onychomycoses are included among the infections found in patients with impaired immunity and these frequently show variation in the clinical presentation and in the fungal species involved [7,8].

The agents of onychomycosis include three groups of fungi, the dermatophytes which are responsible for the majority of infections, non-dermatophyte moulds and yeasts. The latter two groups are usually secondary invaders whereas the dermatophytes can cause primary infections.

Although the advent of griseofulvin in 1958 provided an effective systemic remedy for many fungal nail infections, the development of the triazole and allylamine drugs in the 1980s has provided a wider choice of both systemic and topical therapy, an improved cure rate and an increased spectrum of antifungal activity [8,9]. This has generated a greater need for accurate diagnosis both to confirm the mycotic nature of the infections and to determine the identity of the infecting organism which may be relevant to the choice of therapy.

Clinical patterns. The different clinical appearances of fungal nail infection have been well documented in the literature [8,10,11]. Four major patterns are described: distal and lateral subungual onychomycosis, superficial white onychomycosis, proximal subungual onychomycosis and total dystrophic onychomycosis. The majority of cases of onychomycosis present with distal and lateral subungual onychomycosis, which is the typical appearance seen in most dermatophyte infections: the disease spreads from the hyponychium into the nail and then progresses proximally, frequently along the lateral margins to affect the whole nail. Superficial white onychomycosis can be caused by a dermatophyte, usually *Trichophyton mentagrophytes* var. *interdigitale* or by non-dermatophytic moulds. There is typically a white, crumbly patch on the surface of the nail. Proximal subungual onychomycosischomycosis, where the disease starts at the proximal region of the nail, is associated with immunocompromised patients such as those suffering from AIDS. Total dystrophic onychomycosis can be seen as the result of a long-standing dermatophyte infection but it is also a characteristic feature in patients with chronic mucocutaneous candidosis. There are other clinical patterns associated with *Candida* infection and the most frequent of these is chronic paronychia. Distal nail disease may also occur but the infections are usually secondary and the appearance will depend on the patient's underlying disease.

Laboratory diagnosis. Although the clinical features described for onychomycosis are characteristic for the condition, since systemic therapy is generally the first choice of treatment, confirmation of the diagnosis by laboratory tests should be considered an essential requirement. Both direct microscopy of the nail material and culture to identify the infecting organism are performed. The recognition of fungal elements in the nail allows the clinician to immediately commence antifungal therapy and culture subsequently confirms the initial positive findings and, where necessary, indicates a suitable choice of therapeutic agent, particularly in the case of non-dermatophyte and mixed infections. The absence of fungus in the nail alerts the clinician to consider some other etiology and the features seen in a series of conditions associated with nail dystrophy are listed by Denning [8].

The tests used to determine the presence of fungus in nail material are simple and, although some experience in the ability to recognise the features seen on microscopy is needed, they do not require any sophisticated expertise. The media employed for culture are readily available and, provided that sufficient material is generated by the laboratory's workload so that specimens are continually processed, the majority of nail infections can be confirmed by any standard laboratory. However, the distinction between dermatophyte and other mould infections and the identification of these non-dermatophyte fungi may require additional experience in mycology.

Sampling of nail material. The majority of mycotic nail infections present with distal and lateral subungual onychomycosis and specimens from these nails should be taken with nail clippers to include fragments from the area as close as possible to the advancing edge of the infections and also any subungual debris. Nails showing superficial white onychomycosis or proximal subungual onychomycosis can be sampled by scraping the affected area with a scalpel. Part of the specimen is then used for

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microscopy and the remainder reserved for culture. If the specimen is to be transferred to the laboratory, a folded paper packet, sealed with a clip or adhesive label, is the most suitable means for transport of the material. Paronychial folds are sampled by moistening the area with sterile saline, gently inserting a probe along the fold and collecting any exudate with a standard bacteriological swab.

Direct microscopy of nail samples. Nail material is digested in 20-30% potassium hydroxide (either aqueous solution or in 40% dimethyl sulphoxide) directly on a microscope slide. When using an aqueous solution, gentle heat over the pilot flame of a burner will hasten the procedure, or the material can be incubated at 37°C until it has softened completely. It is essential to have a uniformly flat specimen to ensure sufficient transparency throughout the specimen before viewing the slide. Brightfield illumination is satisfactory to scan the preparation for the presence of fungal elements but the use of phase contrast can increase the resolution. If fluorescence microscopy is available, the utilisation of a 0.1% aqueous solution of calcofluor white mixed in equal volumes with the potassium hydroxide can allow for earlier recognition of the fungus in tissue under ultra violet illumination (Figure 1) but the reagent and nail preparations must be protected from exposure to light as much as possible to prevent

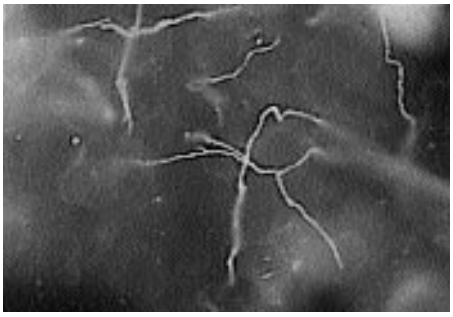

Figure 1. Nail preparation in equal parts 30% potassium hydroxide and 0.1% calcofluor showing dermatophyte filaments (X40 objective).

fading of the reaction. In the authors' laboratory both methods, brightfield and fluorescence, are applied to all nail specimens before reporting them as negative. If the option of fluorescence is not available, enhancement of the fungus, particularly for non-dermatophytic moulds, can be obtained by incorporating equal parts of Parker's Blue-Black Quink with the potassium hydroxide [12]. The features revealed on microscopy of infected nails are well described in the literature. Hyphae of regular width which may be divided into arthroconidia are characteristic of dermatophyte infections but the recognition of atypical features such as the thick walled conidia shown by *Scopulariopsis*, the sinuous hyphae with constrictions seen with *Scytalidium* species or fronding of the hyphae [13] would aid in interpreting the significance of cultures yielding other moulds (Figure 2).

The presence of yeasts with or without associated hyphae indicate infection with *Candida* species. These yeasts are commensals in humans and frequently recovered from abnormal nails as secondary invaders and therefore the observation of these characteristic features in the nail material would be a significant factor to consider when determining the role of *Candida* isolates in these situations.

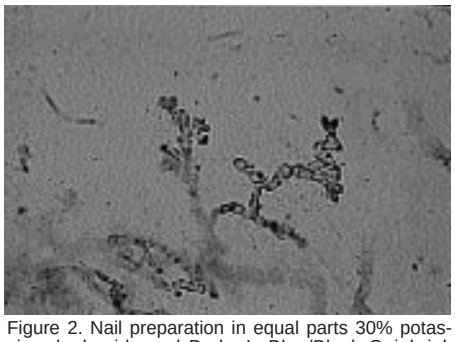

Figure 2. Nail preparation in equal parts 30% potassium hydroxide and Parker's Blue/Black Quink ink showing hyphae with fronds typical of a non-dermatophyte (X40 objective).

Culture of nail samples. The medium chosen by most laboratories for the culture of keratinous material is one adapted from the classical Sabouraud formula incorporating 1% peptone, 2-4% dextrose in 1.5% agar. A 4% malt extract in 1.5-2% agar is also widely used. Antibiotics must be included in the medium to provide a selective environment for fungi and a wide spectrum antibacterial such as chloramphenicol at 0.005% is suitable. In addition, cultures should be prepared with 0.04% cycloheximide which will inhibit non-dermatophyte moulds and some yeasts. This latter medium is necessary to ensure that dermatophytes, which are relatively slow growing, will develop relatively free from faster growing contaminants. Cultures are incubated at 26-28°C for a period of at least three weeks and inspected at weekly intervals, or more frequently if convenient, and particularly for the cultures with no cycloheximide

The failure to isolate a pathogen from a nail diagnosed as mycotic by microscopy is a frequent occurrence and failure rates of 30-50% are usual [8]. The figure has been constantly at 35-40% in the authors' laboratory [13-15] and during 1997 there were 42% of microscopically positive nails which failed to grow any fungus (Table 1). Several factors may be involved, but a major one must be that most of the material obtained would represent the distal part of the nail where the least viable fungal filaments would be present. However, attempts can be made to maximise the recovery of fungi from the nail. These include cutting the fragments as small as possible (1-3 mm in length), utilising subungual debris and inoculating the samples into the surface of the agar to make good contact with the medium. The agar plates must have sufficient medium (a minimum of 25ml) to support the incubation time of up to three weeks without excessive dehydration and, if necessary, measures should be taken to maintain a humid atmosphere in the incubator. With an incubation time of this length it is advisable when monitoring the cul-

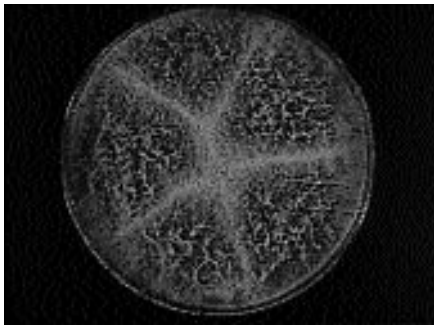

Figure 3. Dark coloured, fast growing colonies of *Scytalidium dimidiatum.*

Table 1. Fungal species isolated from nails with mycotic infections during the year 1997. Data from the Mycology Department of the St John's Institute of Dermatology.

Species	Toenails (% of culture positive nails)	Fingernails (% of culturepositive nails)	Unspecified nails (% of culture positive nails)	Total nails (% of culture positive nails)
Trichophyton rubrum	454 (73%)	44 (40%)	82 (70%)	580 (69%)
Trichophyton interdigitale	121 (20%)	0	14 (12%)	135 (16%)
Epidermophyton floccosum	2(0.3%)	0	0 2	
Trichophyton soudanense	0 0	1	1	
Trichophyton tonsurans	0 0	1	1	
Total with dermatophyte infection	577 (93%)	44 (40%)	98 (84%)	719 (85%)
Scytalidium spp	13 (2%)	5 (5%)	0	18 (2%)
Scopulariopsis brevicaulis	16 (3%)	0	3 (3%)	19 (2%)
Acremonium spp	5	0	0	5
Fusarium spp	5	0	0	5
Aspergillus terreus	1	0	0	1
Total with non-dermatophyte mould infection	40 (6%)	5 (5%)	3 (3%)	48 (6%)
Candida spp	1(0.2%)	61 (55%)	16 (14%)	78 (9%)
Total positive on culture	618	110	117	845
Negative on culture (% of total)	441 (42%)	63 (36%)	10 (47%)	609 (42%)
Total	1059	173	222	1454

tures to subculture atypically small colonies which are suspected as dermatophytes onto fresh plates to prevent them being overgrown by contaminants, or being inhibited by skin commensals and antifungal agents in the pathological material. Non-dermatophytes, particularly *Scytalidium* species, usually grow much faster than the dermatophytes and can be identified after 5 to 10 days incubation (Figure 3).

A further aid to confirm a dermatophyte infection is to recommend that a skin sample is always included in addition to the nail material. Tinea of the toe nails is usually accompanied by a toe space infection and similarly lesions on the hands may be present if finger nails are invaded. Skin specimens do not have as high a failure rate as nails and it is likely that a positive culture from the skin will indicate the species present in adjacent nails and so confirm the diagnosis. Even if skin material is not submitted, it is always helpful for the laboratory if clinical information such as the presence of associated skin lesions is provided along with the nail specimen, since this will help to interpret the mycological findings.

Species. As an example of the spectrum of species isolated from mycotic nail infections, the data from all nails processed during the year 1997 by the authors' laboratory in London, UK are given in Table 1. These results include nail specimens mailed to the laboratory from hospitals and general practice in the London area as well as specimens from patients referred from the dermatology department at St Thomas' Hospital. A total of 1454 nails were positive by direct examination and/or culture. Microscopically positive nails which failed to yield any fungi represented 42% of the total.

As in most published surveys, the majority of nail infections in this series were due to dermatophytes which infected 85% of all positive nails and 93% if toenails are considered separately (Table 1). *Trichophyton rubrum* was the predominant species and was responsible for 69% of the cultures with *T. mentagrophytes* var. *interdigitale* being isolated from 16%. There were very few isolates of other dermatophyte species, two of *Epidermophyton floccosum* and one each of *Trichophyton soudanense* and *Trichophyton tonsuran*s. Species, such as the latter two, which are frequent causes of tinea capitis, can occasionally infect nails particularly when associated with scalp infection [15], however the relevant information was not available for the two cases in the present series. Zoophilic species can also occasionally infect nails, often in association with skin lesions and examples in the literature are cases which have been reported due to *Trichophyton equinum* [16] and *Microsporum canis* [17]. Toenails were more frequently infected than fingernails and in Table 1, where the site had been specified, the number of toenails with a dermatophyte infection, 454, was much greater than the figure for fingernails which was only 44. Also, where the sex of the patient was known (only patients seen at the St Thomas' clinics), there were 58 males but 25 females. Again, these figures repeat earlier findings from this laboratory and are also compatible with those found in data from Finland [4] and Spain [18].

The occurrence of non-dermatophyte moulds in nail infections is being reported with increasing frequency [7,19,20] although they form a low proportion in temperate zones. In Table 1 these were recovered from only 6% of the culture positive nails and in fact, most surveys report figures of under 12 % [5,14,20]. *Scytalidium* species have been recognised as human pathogens since 1970 [21]. They cause infections of both toe and fingernails and will also invade the skin of the hands and feet. The organisms are known as plant pathogens in tropical and sub-tropical areas and the majority of cases of skin and/or nail infections originate in these zones [22]. Clinically, the signs resemble a dermatophyte infection, but *Scytalidium* may be suspected if the patient has lived in an endemic area, failed to respond to treatment with griseofulvin and failed to yield a dermatophyte from previous samples. With experience, the filaments seen in material infected with *Scytalidium* can be recognised by their irregular width and sinuous appearance. These species will not grow in the presence of cycloheximide but cultures should be prepared both with and without this agent as mixed infections with dermatophytes can occur. One case of *T. rubrum* and *Scytalidium dimidiatum* is included among the patients represented in the Table. *S. dimidiatum* (previously known as *Hendersonula toruloidea*) produces grey to black, fibrous colonies with conspicuous aerial hyphae which cover the area of a culture plate within a few days incubation (Figure 3), but slower growing variants have been

described [23]. *S. hyalinum* colonies are pale in colour and will also grow more rapidly than dermatophytes. Although *Scytalidium* infections represent a small percentage among cases in temperate parts of the world, they have been reported as major causes of foot infection, including the nails, in Thailand, Nigeria, Gabon and the West Indies [24-27].

The isolation of *Scytalidium* species is always considered as significant but the growth of other non-dermatophytes must be interpreted with care, as they are commonly found in the environment. *Scopulariopsis brevicaulis* is the most frequent species, usually isolated from a single toenail, frequently with a history of disease or trauma. Clinically these nails show a distal and lateral subungual onychomycosis but with a brown coloured, crumbly texture The diagnosis can often be made accurately on direct microscopy by recognition of the characteristic, thick-walled conidia in the nail [13] although cultures will develop comparatively rapidly on plates, omitting cycloheximide, to produce brown coloured, folded, powdery colonies within seven days. Examples of other fungi which are occasionally isolated from abnormal nails are *Acremonium, Fusarium* and *Aspergillus* species which are all represented in the data given in Table 1. These fungi can cause opportunistic infections in damaged or diseased nails, and in the aged they can be isolated more frequently than dermatophytes [28]. They typically cause a superficial white onychomycosis and the appearance in the nail of clumps of short filaments or of fronding hyphae will indicate the presence of a non-dermatophyte. To assess the significance of their recovery, several factors must be considered such as previous lack of response to treatment, failure to isolate a dermatophyte, repeated isolation of the same species, correlation with features seen on microscopy, absence of associated skin lesions, history of trauma, age of the patient and immunological state etc. The number of positive inocula has frequently been considered a deciding pointer in determining the role of an isolate, but a contaminated nail could yield as much growth as one where the fungus is invading the tissue, and the other factors mentioned above have more relevance. Nevertheless, even when a non-dermatophyte has been identified in a diseased nail, the removal of the organism with an antifungal agent need not necessarily lead to clinical cure and it may be more important to treat the underlying condition [11].

Another non-dermatophyte which has been reported from nails is *Onychocola canadensis*. Although the number of cases established so far is only 17, they are widely distributed geographically in Canada, New Zealand, France and Great Britain [29] and some of these showed apparent primary invasion.

Yeast infections of the nails caused by species of *Candida* almost invariably affect the fingernails where their frequency is comparable to that of the dermatophytes. In the data presented in Table 1, 55% of all the culture positive nails were due to yeasts and, where the sex was known, the majority were in females (9 cases from a total of 12). The most frequent clinical appearance with yeast infections is of paronychia either with or without lateral or proximal nail involvement. Distal onychomycosis is associated with underlying conditions such as vascular disease but total dystrophic onychomycosis can occur in patients with chronic mucocutaneous candidosis [30]. The presence of yeasts and filaments, or yeasts alone, seen in nail material and smears of paronychial exudates indicate an infection with *Candida* species. Cultures prepared on media without cycloheximide and incubated at 37°C for 2 to 3 days for exudates and up to 7 days for nail clippings will yield yeast colonies. The most frequent species from nails are *Candida albicans* and *Candida parapsilosis*. *Candida* species are commensals in the alimentary tract of humans and *C. parapsilosis* can be recovered from normal skin, therefore this must be taken into consideration when evaluating the laboratory findings, particularly as yeast infections are usually secondary conditions.

Management. The treatment of nail infections is not within the scope of this article but the current choices of antifungal agents are clearly defined in the review given by Denning *et al.* [8]. Their recommendations for proximal or extensive dermatophyte infections is oral terbinafine as first choice of therapy with itraconazole or griseofulvin as alternatives. For candidal and non-dermatophyte mould infections the choice is between topical therapy with amorolfine or azole preparations, or with systemic itraconazole. However, as expressed above, these latter infections are almost invariably secondary events, and treatment of the underlying condition would be of prime concern.

Summary. The accurate diagnosis of fungal nail infection is an essential component in the overall management of the disease. Systemic treatment is usually necessary and the demonstration of fungal elements in the nail will permit the immediate commencement of therapy whereas a negative result will indicate at the outset the presence of a non-fungal condition which can then be addressed. However, toenail infections may appear to be trivial complaints when considering a prolonged course of oral therapy, which may have cost implications, but the condition does not resolve spontaneously, and the arguments for treating this disease have been convincingly expressed by Roberts [11]. These include the likelihood of improved success of therapy if it is commenced while only distal areas of the nail are affected and before complications arise with onset of conditions associated with old age. These conditions predispose to advanced invasion with both dermatophytes and with other moulds which are known to be more difficult to eradicate. Infections of the finger nails can be debilitating as well as causing cosmetic problems and the clinician will have few problems in making a decision to treat these cases.

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