Isolation of Dermatophytes (and Other Fungi) from Human Nail and Skin Dust Produced by Podiatric Medical Treatments in Australia

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Background: Podiatric physicians routinely use electric drills for the treatment of nail and skin conditions. The grinding process produces human nail and skin dust that is generally vacuumed into bags in the grinding unit. Many of the nails are thought to be mycotic, particularly because they are obtained from patients with symptoms of dermatophyte infections. Currently, there is limited information available on the detection of fungi from nail dust samples. Herein, we attempt to address this situation and outline some of the difficulties that pathology laboratories face in isolating and identifying dermatophytes from nail samples.

Methods: Fifty nail dust bags from podiatric medical clinics across all of the states and territories of Australia were collected and analyzed. Samples from the bags were inoculated onto primary isolation media. Fungal colonies that grew were then inoculated onto potato dextrose agar for identification using standard morphological (macroscopic and microscopic) features.

Results: One hundred fifty-one colonies of dermatophytes were identified from 43 of the 50 samples. In addition 471 nondermatophyte molds were isolated, along with some yeasts and bacteria.

Conclusions: The most common dermatophytes isolated were from the *Trichophyton mentagrophytes/interdigitale* complexes. *Trichophyton rubrum, Trichophyton tonsurans, Trichophyton soudanense,* and *Epidermophyton floccosum* were also isolated. An unidentified group of dermatophytes was also present. The three most common genera of nondermatophyte molds were *Aspergillus, Penicillium,* and *Scopulariopsis*, all of which have been implicated in onychomycosis and more general disease. The presence of viable fungal pathogens in the dust could potentially pose a health problem to podiatric physicians. (J Am Podiatr Med Assoc 105(2): 111-120, 2015)

Podiatric physicians routinely use electric drills in the treatment of dystrophic nail and skin conditions. A large proportion of nails ground by podiatric physicians show clinical signs of onychomycosis, such as discoloration and crumbling of the nail plate. The grinding process produces fine human nail and skin dust,[1-3] which is generally vacuum extracted into bags in the unit. A few studies have indicated that this dust contains viable fungi, including dermatophytes.[1,4-13] Concerns about the possibility of direct infections and hypersensitivity reactions have been raised in the literature for several decades.[1-3,7,9,14,15] In this research, specialized growth and isolation media were used to specifically target dermatophytes to investigate whether they were present and remained viable after undergoing the desiccating grinding process. To our knowledge, this is the first such study conducted in Australia.

Data from pathology laboratories over many years have shown that not only dermatophytes but also a large range of nondermatophyte molds (NDMs) and yeasts have regularly been found in clinical nail samples that have been supplied to

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them for diagnosis.[16-21] The limited number of research studies that have been performed on nail dust also indicate this to be the case. The reasons for the presence of NDMs and some yeasts in nail tissue have been vigorously debated, with a gradual acceptance that many are not simply nail contaminants but also may play a role in the pathogenesis of onychomycosis.[22] Many of the NDMs that have been isolated can also have serious medical consequences,[23,24] and this study confirms their presence in the dust.

To date, the largest study of human nail and skin dust was performed in the United States by Abramson and Wilton in 1985[1,15] using 70 dust bags. It showed that some mycologic and bacterial organisms were present, were viable, and could be isolated from these samples. All of the previous studies and this present one conducted their research using traditional laboratory methods. The traditional methods for identifying dermatophytes take into consideration the site of the infection, the macroscopic features of the colony, the microscopic morphological features of the hyphae/conidia/chlamydospores, and, on occasion supplementary biochemical test results.[25,26] Molecular methods, although giving more certainty in the identification of genus, species, and strains than traditional methods,[26-28] would not have been able, at this point in time, to indicate the viability of the organisms unless taken from a culture grown from the sample.

Materials and Methods

The Charles Sturt University ethics committee approved this study. Eighty nail dust bag samples from across Australia were collected. Each bag contained dust from the nails and skin of hundreds of podiatric medical patients. From these, a pilot study of 30 samples was conducted yielding 11 dermatophytes along with some unidentified NDMs, yeasts, and bacteria. The other 50 samples were used for the data analysis. Approximately 0.76% of each sample was inoculated onto one plate of Sabouraud dextrose agar containing chloramphenicol and gentamicin and three biplates of enhanced sporulation agar (ESA) and dermatophyte test medium (DTM). It was not possible to use the entire sample. The amount of sample processed was appropriate for the plates used (standard petri dishes used in a pathology laboratory) and was in keeping with standard practice in a pathology laboratory in Australia. These samples were then incubated aerobically at 30°C for up to 30 days. Colonies of interest were subcultured onto potato dextrose agar and were incubated at 30°C for up to 30 days.

The macroscopic features of each colony were examined and recorded. Sticky tape wet preparations of lactofuchsin or lactophenol cotton blue stains were used to observe and record the microscopic structures used for identification or categorization of the fungi. For dermatophytes, when no species-level identification was possible using these criteria, as in the case of nonsporulation or crossover features, an identification of genus or possible dermatophyte was recorded. Only dermatophytes that had classic macroscopic and microscopic morphological presentations[23,24] were identified to the species level.

For this study, the complexes *Trichophyton mentagrophytes* and *Trichophyton interdigitale* were combined. Differentiation between the *T mentagrophytes* complex and the *T interdigitale* complex can be very problematic with morphological and biochemical techniques.[29] The new genotyping of dermatophytes has resulted in revised species identification and classification of many *Trichophytons*, and this does not always agree with the traditional morphological typing of them.[28,30] Although much work is being undertaken to integrate the traditional methods of identification with the more recent molecular methods, the discipline is still in a state of flux and development.[29]

Hence, the following basic categories of identification were used in this study (based on morphological characteristics): *Trichophyton rubrum*, *T mentagrophytes/interdigitale* complexes (Figs. 1 and 2), *Trichophyton tonsurans*, *Trichophyton*

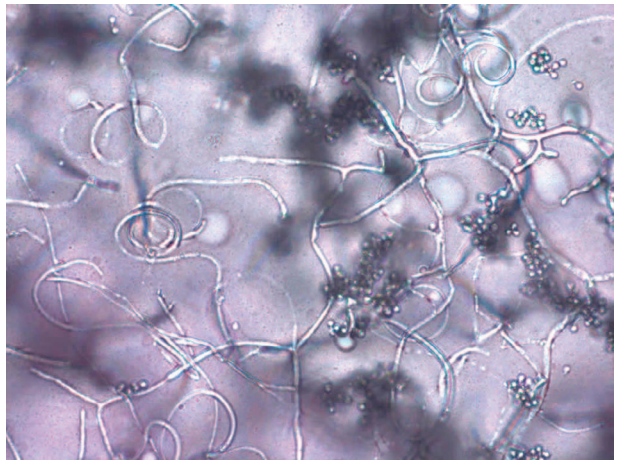


Figure 1. Spiral hyphae and microconidia from *Trichophyton mentagrophytes/interdigitale*.

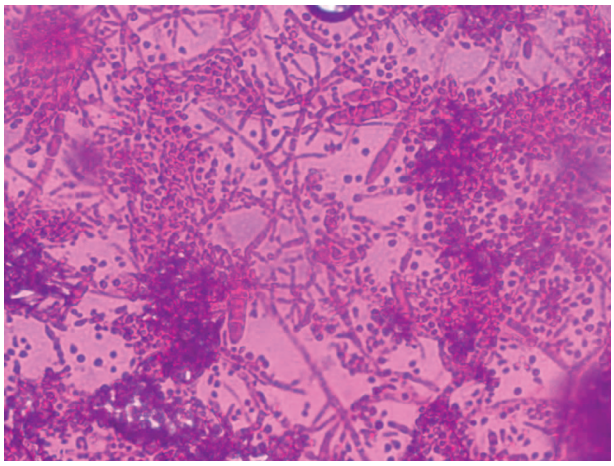


Figure 2. *Trichophyton mentagrophytes/interdigitale* showing microconidia and macroconidia.

soudanense, *Trichophyton* genera, *Epidermophyton floccosum*, and possible dermatophytes.

Results

All 50 plates with Sabouraud dextrose agar containing chloramphenicol and gentamicin had multiple fungal colonies (Fig. 3). The macroscopic features indicated that there were many different genera and species, often with multiple colonies of the same organism. It was, however, difficult by macroscopic features to ascertain whether dermatophytes were present because many NDMs grew so readily and rapidly that they filled the petri dishes within a few days. Dermatophytes are generally slower growing[25] and, as such, had little unused agar to germinate in or were mixed in with the NDMs, making it difficult to obtain a usable culture.

The DTM/ESA biplates were more selective and so were used solely as the primary isolation media (Fig. 4). Only four DTM, three ESA, and three DTM/ESA biplates had completely negative indicators. The remaining biplates all had positive indicator changes created by both true- and false-positives (Fig. 4). Forty-three of the 50 dust samples (86%) were positive for dermatophytes, with a total of 151 colonies. The *T mentagrophytes/interdigitale* complexes were the most common species identified, composing 40% of all of the dermatophytes. *Trichophyton rubrum* was isolated (almost) as frequently as *T tonsurans*. Only two isolates each of *T soudanense* and *E floccosum* were found. Forty percent of the dermatophytes were not identified to the species level but based on their macroscopic and microscopic features were assigned to the *Trichophyton* genus category.

Many samples had a variety of species isolated from one sample. For example, sample 23 had six isolates from the *T mentagrophytes/interdigitale* complexes, two *T rubrum*, and four from the *Trichophyton* genus. The same is true of sample 11, which yielded one *T soudanense*, one *E floccosum*, one *T rubrum*, one from the *T mentagrophytes/interdigitale* complexes, two from the *Trichophyton* genus, four *T tonsurans*, and five possible dermatophytes.

However, other samples, such as number 17, yielded exclusively species from the *T mentagro-*



Figure 3. A plate with Sabouraud dextrose agar containing chloramphenicol and gentamicin.

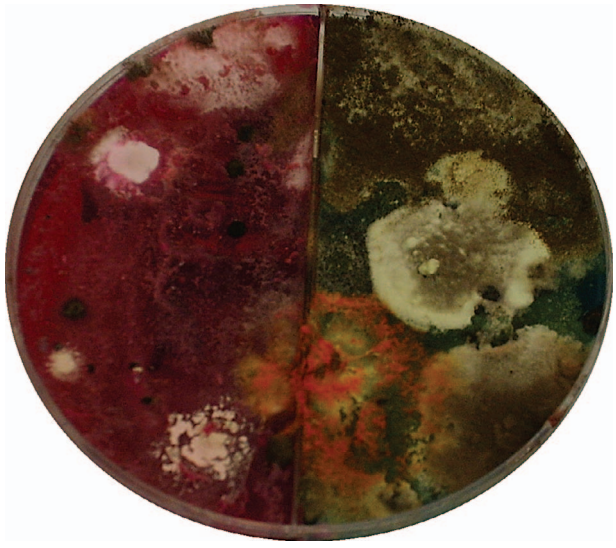


Figure 4. Dermatophyte test medium/enhanced sporulation agar plate.

phytes/interdigitale complexes, with seven separate isolates being cultured. The eighth isolate from this sample had ambiguous features and, therefore, was assigned to the *Trichophyton* genus. Sample 14 grew only one dermatophyte, which was *T soudanense*.

There were 120 isolates identified as possible dermatophytes but that needed further confirmation. Many of these were mixed cultures, with microscopic and macroscopic features associated with dermatophyte characteristics. However, some were nonsporulating, a mixture of different dermatophyte strains or species, or contaminated with one or more NDMs or yeasts. This presented a challenge in the identification macroscopically and microscopically.

Nineteen genera of NDMs were identified. There were also 72 colonies of unidentified NDMs from 74% of the samples (Table 1). The most common identified isolates were *Aspergillus*, *Penicillium*, and *Scopulariopsis* spp, which, although technically breakthroughs, grew readily on the dermatophyte isolation media.[32-34]

Aspergillus spp were isolated from 94% of the samples and were frequently prolific on the plates. The colonies were often varied in their features and had thallus colors ranging from light and dark green to cream, grey, brown, tan, dark brown, bone, white,

Table 1. Nondermatophyte Molds Isolated in This Study in Order of Frequency

Genus	Positive Samples (No. [%]) (N = 50)	No. of Colonies (N = 471)	Onychomycosis Causative Agent	General Medical Significance
Aspergillus	47 (94)	155	Yes	Aspergillosis, hypersensitivity, asthma.[24] Otomycosis.[23]
Unidentified	37 (74)	72	Unknown	Unknown
Penicillium	32 (64)	100	No	*Penicillium marneffei*.[23,24] Other species, rare opportunistic infections.[23]
Scopulariopsis	20 (40)	51	Yes	Onychomycosis and hyalohyphomycosis.[23] Rare subcutaneous infections.[31]
Acremonium	9 (18)	9	Unknown	Opportunistic. Mycetoma, mycotic keratitis, and onychomycosis.[23]
Chrysosporium	8 (16)	11	Unknown	Generally contaminants.[24]
Scedosporium	8 (16)	15	Unknown	Two important species. Mycetomas. Can infect the lungs, eyes, ears, central nervous system, and internal organs.[23,24]
Fusarium	6 (12)	10	Yes	Hyalohyphomycosis, onychomycosis, and mycotic keratitis.[23] Eye infections. Rare mycetoma, septic arthritis, and sinusitis and systemic infection.[24]
Geotrichum	5 (10)	11	Rare	Immunosuppressed patients, rare systemic infections. Endocarditis.[23]
Cladosporium	4 (8)	4	Rare	*Cladophialophora carrionii* causative of chromoblastomycosis in Australia, South Africa, and Venezuela.[23,24]
Fonsecaea	4 (8)	7	Unknown	Chromoblastomycosis, especially lower limbs. Rarely internal infections.[24]
Alternaria	3 (6)	4	Rare	Mycotic keratitis.[23] Asthma and hypersensitivity pneumonitis.[24]
Hortea	3 (6)	3	No	Tinea nigra.[23,24]
Lecythophra	3 (6)	3	Unknown	Rare subcutaneous abscess, endocarditis, sinusitis, peritonitis, endophthalmitis, and corneal infection.[24]
Chaetomium	2 (4)	5	Unknown	Unknown.
Mucor	2 (4)	3	Unknown	Unknown/rare.[23]
Onychocola	2 (4)	4	Rare	Onychomycosis.[23]
Gliocladium	1 (2)	2	No	Unknown.
Malbranchea	1 (2)	1	No	Unknown.
Ochroconis	1 (2)	1	No	*Ochroconis gallopava*; immunocompromised patients; disseminated and subcutaneous infections.[24]

and sulphur yellow (with pigmented agar). Some of the species represented were *Aspergillus fumigatus*, *Aspergillus niger*, *Aspergillus flavus*, *Aspergillus terreus*, and *Aspergillus clavatus* (Fig. 5).[23,24]

Sixty-four percent of the samples yielded *Penicillium* spp, with colonies ranging from greyish to various shades of green. Forty percent of samples were positive for *Scopulariopsis* spp, with colony colors from almost white to tan.[23,24] Seventy-four percent of the samples yielded NDMs that were not identified.

One hundred one colonies of bacteria were recorded, with a large variety of colony characteristics. Thirty-nine colonies of yeasts were isolated. The macroscopic features, such as color, texture, and viscosity, indicate that a variety of genera/species may be present.

Discussion

Although dermatophytes have been extensively studied, they still present many challenges for identification using conventional culture-based methods.[23,35] Genetically, they are a very homogenous group; no one specific test is totally reliable, and identification is often made on balance between the criteria.[23] Many of the isolates do not display key identification features and can be polymorphic, having a variety of crossover features between species/varieties/strains, or become pleomorphic. They can frequently have features that are inconsistent or overlapping.[29,36-40] This has led to many identification systems being developed, such as the *Trichophyton* agars and other supplementary tests, including lactrimel agar, Littman oxgall agar, and urea agar, which are designed to assess a specific biochemical feature to assist in identification. Protocols such as the Kaminski system have been developed that use these media.[23,25] However, it is now becoming increasingly evident that the traditional methods are of limited value and that the development of mass spectrometry, immunologic, and molecular tools is needed to increase the speed and accuracy of identification.[36]

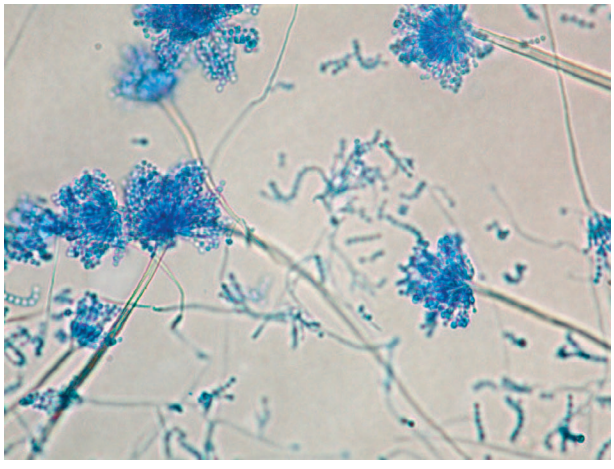


Figure 5. *Aspergillus* conidia.

In the past decade or so, molecular testing of dermatophytes has brought into question the traditional methods of species identification, particularly with the *Trichophytons*.[27,29,36,41] The dermatophytes are very homogenous genetically but have diverse phenotypic expressions. This diversity in morphological expression has led to certain biotypes, based on infection patterns and laboratory morphology, being thought to be separate species. This, however, seems not to be the case, with the number of confirmed species lower than some years ago.[28,29,41] The current position with many of these species/varieties is still in a state of change and development.[23] This growing understanding of the dermatophyte genomes has led to a variety of proposals reviewing the taxonomy. This applies particularly to the species and strains being included in the *T rubrum*, *T mentagrophytes*, *T interdigitale*, and *T tonsurans* complexes.[26,28-30,41]

To our knowledge, this study is the first conducted in Australia using nail and skin dust produced by routine podiatric medical treatments. It analyzes the mycologic content of the nail and skin dust, with particular emphasis on dermatophytes. Although 86% of the samples were positive for dermatophytes, it cannot be concluded that the other 14% of samples did not contain dermatophytes because only 0.76% of the dust from each dust bag was used. All of the samples produced NDMs. Yeasts and bacteria were also isolated from many of the samples.

Attempts to isolate dermatophytes using selective media, especially Sabouraud dextrose agar containing chloramphenicol and gentamicin, were often frustrated by the rapid growth of numerous breakthroughs either overwhelming the dermatophytes or quickly occupying the surface of the agar, leaving little or no room for these slower-growing molds. It is, therefore, possible that because of this, the number of colonies of dermatophytes has been underrepresented from what may have been in the sample, and the frequency of the NDMs isolated was possibly overestimated compared with the dermatophytes. In addition, many dermatophyte colonies were not pure, and microscopically a variety of NDMs and dermatophyte species were frequently

seen mixed together. On many occasions, the obtaining of cultures of dermatophytes that were sufficiently pure for identification was problematic. This presented a challenge in the identification macroscopically and microscopically. Despite this, 86% of the samples in the study, using the DTM/ESA plates only, were found to contain viable dermatophytes, in particular *Trichophyton* spp (Table 2). The changing of the indicators on the DTM/ESA biplates became mostly irrelevant because many of the NDMs, bacteria, and yeasts that grew were capable of producing false-positives.

The NDMs were so prolific and comprised such a broad range of genera and species that although they were technically breakthroughs on the selective media, they were, as much as was practical, identified to the genus level. Those that were not identified were designated as unidentified NDMs. None of the yeasts isolated were identified, and their presence was merely noted. Many bacterial colonies grew on the agars. Again, these were not identified and were recorded only for their presence.

The study did, however, produce enough pure colonies of dermatophytes to be identified using key macroscopic and microscopic features.[23,24,32] Some of the dermatophytes were restricted to genus or possible dermatophyte because of ambiguity, nonsporulation, or crossover features. It is possible that among these were samples of the *Microsporum* genus. It was noted along the way that some of the unidentified dermatophytes had features similar to *Microsporum audouinii*, which frequently does not sporulate.[23,24,35]

The most common isolates were from the *T mentagrophytes/interdigitale* complexes (Table 2 and Figs. 1 and 2), accounting for 40% of the dermatophytes. This is in contrast to many publications naming *T rubrum* as the most common dermatophyte isolated from onychomycosis and tinea pedis.[44-46] Epidemiologically, *T rubrum* is regarded as the most significant anthropomorphic dermatophyte, being globally distributed and increasing in incidence every year.[45,47] This finding may be due to such factors as a sampling or random error; the changing epidemiology of dermatophytes[47-51]; the age group of the general podiatric medical patients reflecting past infection patterns[46]; inhibition of *T rubrum* by NDMs, bacteria, yeasts, or medicaments already present in the nails; or the rate of growth on the agar. Other considerations of relevance may be that *T mentagrophytes* tends to attack the nail plate itself, whereas *T rubrum* tends to infect the nail bed and the inferior surface of the nail plate.[51,52] The grinding process rarely involves the nail bed itself. Scanning electron microscopy has demonstrated that the structures of the hyphal wall of *T mentagrophytes* and *T rubrum* are quite different,[53] and it may be possible that *T rubrum* has a more fragile cell wall and, therefore, would not so readily survive the desiccation and friction heat in the grinding process. In addition, some of the unidentified nonsporulating dermatophyte species, recorded in the classifications of *Trichophyton* genus or possible dermatophytes, may be *T rubrum*. Some molecular studies have indicated that *T rubrum* has more morphological presentations than previously thought and that these atypical strains may be included in the taxonomy of *T mentagrophytes/interdigitale* complexes.

Earlier research on in situ nails and nail dust has demonstrated a broad diversity of microorganisms to be present,[1,5,8,9,16,19,54-58] and this was confirmed by this study. Many of the NDMs isolated in this present research are known to have some degree of medical significance (Table 1).[23,24] The most common NDMs found were *Aspergillus*, *Penicillium*, and *Scopulariopsis* spp. *Aspergillus* was isolated at approximately the same rate as the dermatophytes. This is not surprising as this genus is ubiquitous and some species are keratolytic.[59] *Penicillium* was the second most common genus of NDM isolated. Similar to *Aspergillus*, it is ubiquitous and may be present in the sample from either the nails and skin or in the clinic air. It is thought to be more often a contaminant than a pathogen.[16,60,61] The third most commonly isolated NDM genus was *Scopulariopsis*. Species identification was not performed, but the diverse range of macroscopic and microscopic features indicated that there were a variety of species or strains present in the samples. At least two species have clinical significance. *Scopulariopsis brevicaulis*, a ubiquitous species, has been isolated from mycotic nails.[62] It is known to be keratolytic and has long been regarded as a primary pathogen in onychomycosis.[63-67] *Scopulariopsis brumptii* can have similar clinical patterns, but it is not as frequently isolated in diagnostic laboratories.

In total, there were 19 identified genera of NDMs. Their involvement in onychomycosis can range from contaminant, secondary invader, or saprophyte to primary pathogen. Table 1 records the frequency at which they were isolated in this study and the role they are thought to have in onychomycosis. An ever-increasing number of NDMs have been implicated as either primary or secondary invaders of nails as well

Table 2. Dermatophytes Isolated in the Study of 50 Dust Bag Samples

Dermatophyte	Positive Samples (No. [%])	Total Colonies (No. [%]) (N = 151)	Notes on Identification
Trichophyton mentagrophytes/ interdigitale complexes	22 (44)	60 (40)	These two complexes were combined because they share many morphological features. Sun et al[29] pointed out that the conventional methods of differentiating between the two complexes are frequently confusing and unreliable.
Trichophyton rubrum	8 (16)	13 (9)	Colonies displaying classic macroscopic and microscopic features[23,24] were identified as such. Those that were uncertain or ambiguous were placed in the *Trichophyton* genus. *T rubrum* can have a nonsporulating presentation and so may be underrepresented in the statistics and be recorded in the general category of *Trichophyton* genus. Genetic studies indicate that some strains/varieties can have laboratory presentations similar to the other dermatophytes.
Trichophyton tonsurans	7 (14)	14 (9)	Graser et al,[26] using molecular markers, established this as a distinct species. Standard morphological features were used for the identification.[23,24,26]
Trichophyton soudanense	2 (4)	2 (1)	The descriptions for morphological identification as documented by Frey[42] along with other standard references, were used for the identification. It is a reasonably rare find in Australia, especially in nails.[23,24,42] There is still dispute as to the taxonomy.[28,41] Kong et al[43] recognize this as a distinct species.
Epidermophyton floccosum	2 (4)	2 (1)	The macroscopic and microscopic features outlined in standard reference texts were used.[23,24] Microconidia are always absent.
Trichophyton genus	22 (44)	60 (40)	Organisms that displayed macrocultural and microscopic features consistent with the *Trichophytons*[23,24] but that were not identified to the species level were placed in this category because they were either nonsporulating or had ambiguous presentations.
Possible dermatophytes	120		This category was used when a dermatophyte was considered to be present but, owing to a lack of distinguishing features, could not be reliably assigned to the *Trichophyton* group.

as contaminants.[4,16,20,21,54,68-71] Many authors and researchers have also found many of these NDMs in various types of nail samples.[1,5,8,9,16]

The identified NDMs are generally rated as risk group 1 and risk group 2 organisms, with most known to have some possible medical implications. *Penicillium marneffei* is rated as being risk group 3 and is considered a possible biological hazard to laboratory staff. Seventy-two unidentified NDMs were grown from 37 of the samples. Their role and the reason for their presence are unknown, as is their clinical significance.

The fungal load of the samples seemed to be considerable, but it was not possible in this study to give any quantification. Only approximately 0.76% of each sample material was inoculated, and each plate with Sabouraud dextrose agar containing chloramphenicol and gentamicin with approximately 0.19% of the sample, in most cases filled very rapidly with a large array of fungal growths.

Conclusions

This study confirmed the presence of viable dermatophytes, particularly *Trichophytons*, in human nail

and skin dust produced during routine podiatric medical treatments. It also demonstrated the presence of NDMs, yeasts, and bacteria in these samples. The production of dust by podiatric medical treatments is unavoidable whether by manual or mechanical methods, but the risk to podiatric physicians has been only minimally investigated, and although it is of concern, the risks are largely unknown. Generally, dermatophytes are not airborne and are restricted in their contagion to direct or indirect contact with the outside of the body. A unique circumstance occurs when they are attached to the extremely small, airborne, plate-like keratin particles of the dust with a possibility of reaching sites such as the skin, hair, eyes, and upper and lower respiratory tract.[1,14,15] Rare deep infections have been recorded.[72] The finding of such a large range of NDMs in the dust is also of concern because many of these can cause minor to major infections, with aspergillosis and penicilliosis being potentially fatal.

Onychomycosis is frequently intractable to treatment, with no one protocol being entirely reliable. It is generally assumed that dermatophytes are the main causative agent, but the detection of so many NDMs in nail dust indicates that this needs to be reviewed. Many NDMs are keratolytic and, therefore, capable of causing disease. The role of the interrelationship of so many organisms has not been explored but may give a clue to one of the factors as to why onychomycosis is so resistant to treatment. It raises questions as to whether some of the NDMs are primary invaders or whether they assume a saprophytic or other role once the dermatophyte has infected the nail or nail bed. Future therapeutic research should take into consideration as to whether the right organisms are being targeted in the right way.

Correct identification of dermatophytes using traditional laboratory methods is difficult as well as being cumbersome and time-consuming. Genomic studies have shown that many misidentifications occur because dermatophyte species can have very unreliable morphological and biochemical presentations. The laboratory of the future used by podiatric physicians will need to use molecular tools to overcome this and bring more certainty to diagnosis.[73,74] Ideally this would be done directly from the clinical material, such as nail dust, because the growing of dermatophytes, even on isolation media, can be difficult from nail and skin samples.[74]

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Conflict of Interest: None reported.

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