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BJD research letter

Title:
Trends in laboratory-diagnosed onychomycosis between 2006 - 2014 in the South West of England.

Running head: Trends in laboratory-diagnosed onychomycosis

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Dear Editor,

Onychomycosis is a worldwide health problem. Several factors make it a challenging entity: difficulties confirming the diagnosis, potential side effects of antifungals, treatment duration and relapse. Appreciating local pathogens and identifying changing trends are essential for optimal management.

There is no UK data concerning laboratory-diagnosed onychomycosis, therefore we carried out a retrospective analysis in the Southwest of England. Electronic records of all nail specimens received at Public Health England Mycology Reference Laboratory between March 2006 and December 2014 were identified. Data items included age, gender, microscopy/culture results and site of specimen. Statistical analysis was carried out using STATA®.

One-sample t-tests were used to analyse if *Candida* sp. were split between genders in the same ratio as the rest of the samples. A two-sample t-test was used to examine whether age ranges differed between 2006 and 2014. Comparison of proportions were carried out using Chi squared test.

A total of 38,591 nail specimens were received. There was a trend for increased specimen submission over the eight-year period possibly reflecting better awareness of onychomycosis and an ageing population. Data from the Office of National Statistics demonstrates an increase in the population in the Southwest of England by 0.7% between 2004 – 2014, whilst the UK population of those aged 65+ years increased from 16% to 18%.[1] Table 1 lists study population characteristics.

Mean age for microscopy-positive nail specimens was 51.4 years (95% CI 51.2, 51.6). The percentage of microscopy positive samples (60%) was the same for those aged 20-80+ years (p=0.277), and lower at 51% in those aged 0-19 years (p<0.001). Other studies demonstrated an increase in the rate of onychomycosis with age, however this was not a cross-sectional study, possibly explaining our differing findings. During the study period, there was a rise in microscopy-positive samples from adults (patients aged≥16 years, p<0.001). In line with previous data,[2] males were more commonly affected than females (microscopy positive results in males vs females; 54% vs 46%, p<0.001), with no change in this ratio over time.

The presence of fungus was confirmed by direct microscopic examination of fungal hyphae in 22,803 (59.2%) specimens. The causative organism was cultured from 13,684 (35.5%) specimens. In 9,657 cases (25.0%), fungal elements were seen but culture was negative.

Toe nail onychomycosis was much commoner (94.8%) than finger nail infection irrespective of patient age or gender. Thirty-seven fungal species were isolated. As anticipated, dermatophytes were the commonest - reported in 12,577/13,684 (91.9%) cases, non-dermatophyte moulds (NDM) in 771/13,684 (5.6%) and yeasts in 336/13,684 (2.5%) specimens. Of the species isolated we could specify the location of the fungus for 9,845/13,684 (71.9%). Table 2 shows the commonest reported organism was *T.rubrum*; accounting for 48.7% of finger (n=241) and 66.9% of toenail infections (n=6,259). In fingernails the second most common was *Candida* sp. (n=202, 40.6%). In toenails it was *T. interdigitale* (n=2,480, 26.5%) followed by *Fusarium* (n=163, 1.7%). There was no change in the proportion of infections due to *T. rubrum* (p=0.549), *T. interdigitale* (p=0.152) or *Fusarium* (p=0.188) during the study period.

Candida sp. (including C. *albicans*, *C. parapsilosis* and *C. guilliermondii)* were cultured in 202/495 (40.8%) fingernail specimens compared to just 18/9,344 (0.19%) toenail samplings, Table 2. In those with confirmed *Candida* onychomycosis, 76% were female (p<0.001). An Italian study attributed similar findings to occupations such as housework, bar tenders and florists. Diabetes was also a risk factor,[3] something we are unable to comment on. Clinical observation demonstrates that nails are generally longer in females, which predisposes to increased occlusion of the warm damp subungual space. Additionally females are more likely to undertake manicure, which provokes low level tissue damage and hence reduced local immunity.

Onychomycosis caused by NDM depends on diagnostic criteria (Table 2 legend) and geography: in Europe rates range from 1-10%[4] whereas in India, Jamaica, Nigeria, Thailand and Pakistan they range between 10-68%.[5, 6] Over the last two decades there have been reports of increased NDM isolations worldwide including Italy, Greece,[7] Spain[8] the USA[9], however our data did not support this. There is wide geographical variation in causative NDM onychomycosis. In North America, the most frequently isolated genus is *Acremonium* sp.[10], in Europe it is *Scopulariopsis* sp.,[3, 7, 8] and in Asia *Aspergillus* sp.[11] Our study found *Fusarium* sp. to be the most common. This is particularly important to note for clinical practice since such a mould can be life-threatening in the setting of immunosuppression.[12, 13]

We found an increase in the proportion of microscopy-positive cases that were culture-negative during our study period: 34.7% in 2006 vs 41.9% in 2014 ($p<0.001$). This rise in culture failure could be explained in several ways: (a) inadequate nail sample size, highlighting the importance of obtaining generous specimens; (b) more distal nail sample submission, where the organism is likely to be older and hence non-viable, therefore it will be visible on microscopy but will not grow; (c) enhanced sensitivity of fungal microscopy due to calcofluor staining; (d) use of over the counter products, which can kill the fungus in the uppermost layers such that it will not grow on culture yet still be detected on microscopy. Of interest the Medicines & Healthcare products Regulatory Agency granted Galderma (UK) a licence for Loceryl®, amorolfine hydrochloride 5%, nail lacquer for over the counter use in April 2006, since which sales have increased.[14] Considering the growing evidence that dermatophyte onychomycosis is clinically indistinguishable from that caused by other moulds, it is paramount to perform optimal complete mycological studies to identify the causative agent to guide correct treatment and maximise chances of cure.[15]

Although this is the first study of its type from the UK, it has limitations including its retrospective nature and absence of clinical case information. These are potential areas for future study and support the notion of IT-based clinical data recording to access such information.

Performing such studies in individual countries and regions is important for the continued analysis of fungal pathogens to identify changing trends that will influence local guidelines and hence clinical practice.

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Variable	Number	Percentage of total
Gender		
Female	21,342	55.3%
Male	17,199	44.6%
Unspecified	50	0.1%
Mean age (years) (95% CI)	50.9 (50.7, 51.1)	
Age range		
0-16 years	1,434	3.7%
17-64 years	27,144	70.3%
65+ years	9,911	25.7%
Unknown	102	0.3%
Site		
Finger	2,780	7.2%
Toe	25,262	65.5%
Unspecified	10,549	27.3%
Microscopy		
No Fungus	15,727	40.8%
Fungal elements	22,803	59.0%
Yeast	61	0.2%
Culture		
No fungus isolated	24,907	64.5%
Culture positive	13,684	35.5%
Culture results		
Dermatophytes	12,577	91.9%
Non-dermatophyte mould	771	5.6%
Yeasts	336	2.5%
Season specimen received		
Winter	19,432	50.4%
Summer	19,159	49.7%

Table 1. Characteristics of the study population.

Organism	Frequency (% of all cultured organisms) Finger	Toe	Total
Dermatophyte	**269 (54%)**	**8788 (94%)**	**9057 (92%)**
Trichophyton rubrum	241 (48.69%)	6259 (66.98%)	6500 (66.06%)
Trichophyton interdigitale	21 (4.24%)	2480 (26.54%)	2501 (25.42%)
Epidermophyton floccosum	0 (0.00%)	20 (0.21%)	20 (0.02%)
Trichophyton sp.[1]	0 (0.00%)	16 (0.17%)	16 (0.16%)
Trichophyton mentagrophytes	1 (0.20%)	4 (0.04%)	5 (0.05%)
Trichophyton violaceum	2 (0.40%)	2 (0.02%)	4 (0.04%)
Trichophyton erinacei	1 (0.20%)	2 (0.02%)	3 (0.03%)
Trichophyton soudanense	1 (0.20%)	2 (0.02%)	3 (0.03%)
Microsporum audouinii	0 (0.00%)	1 (0.01%)	1 (0.01%)
Microsporum canis	1 (0.20%)	0 (0.00%)	1 (0.01%)
Trichophyton terrestre	0 (0.00%)	1 (0.01%)	1 (0.01%)
Trichophyton tonsurans	1 (0.20%)	0 (0.00%)	1 (0.01%)
Trichophyton verrucosum	0 (0.00%)	1 (0.01%)	1 (0.01%)
Non-Dermatophyte	**229 (46%)**	**559 (6%)**	**788 (8%)**
Candida sp. [#]	202 (40.56%)	18 (0.19%)	220 (2.36%)
Fusarium sp.	18 (3.61%)	163 (1.74%)	181 (1.94%)
Scopulariopsis brevicaulis	0 (0.00%)	93 (0.99%)	93 (0.99%)
Acremonium sp.	1 (0.20%)	77 (0.82%)	78 (0.83%)
Aspergillus versicolor	0 (0.00%)	80 (0.86%)	80 (0.86%)
Aspergillus terreus complex	0 (0.00%)	43 (0.46%)	43 (0.46%)
Neoscytalidium dimidiatum	4 (0.80%)	31 (0.33%)	35 (0.37%)
Aspergillus sp.	0 (0.00%)	24 (0.26%)	24 (0.26%)
Purpureocillium lilacinum	0 (0.00%)	11 (0.12%)	11 (0.12%)
Penicillium sp.	1 (0.20%)	5 (0.05%)	6 (0.06%)
Neoscytalidium hyalinum	0 (0.00%)	5 (0.05%)	5 (0.05%)
Scedosporium apiospermum	0 (0.00%)	3 (0.03%)	3 (0.03%)
Onychocola canadensis	0 (0.00%)	2 (0.02%)	2 (0.02%)
Phoma sp.	0 (0.00%)	1 (0.01%)	1 (0.01%)
Exophilala sp.	1 (0.20%)	0 (0.00%)	1 (0.01%)
Exserohilum sp.	0 (0.00%)	1 (0.01%)	1 (0.01%)
Geotrichum candidum	1 (0.20%)	0 (0.00%)	1 (0.01%)
Lichtheimia corymbifera	1 (0.20%)	0 (0.00%)	1 (0.01%)
Ochroconis sp.	0 (0.00%)	1 (0.01%)	1 (0.01%)
Trichosporon mucoides	0 (0.00%)	1 (0.01%)	1 (0.01%)

Table 2. Species according to site of nail infection. These results exclude the specimens from unspecified sites. [1]this group are likely to be mutant variants of the common species, which were not displaying sufficient morphological characteristics for full phenotypic identification. [#]=yeasts.

Processing of nail samples was conducted in accordance with the standard operating procedures for dermatological samples produced by Public Health

England. Nail fragments were digested in 20% Potassium hydroxide and stained with calcofluor white. They were viewed under a fluorescence microscope and cultured on Sabouraud media (glucose-peptone agar containing 0.05mg/ml of chloramphenicol) with and without acitidione. Fungal growth was examined after 1 and 2 weeks incubation at 30^{o}C. Samples were reported as negative if there was no growth after observation for 2 weeks. Structures visible on microscopy were recorded e.g. arthroconidia (indicative of a dermatophyte infection) or fronding and swelling (indicative of non-dermatophyte mould), or yeast cells only, or yeast cells plus hyphae (indicative of infection with a filamentous yeast). Isolates were identified by means of colonial morphology and direct microscopy to observe the method of spore formation, or in the case of yeast isolates by morphology and the use of commercial carbohydrate assimilation tests or Matrix-Assisted Laser Desorption/Ionisation – Time of Flight (MALDI-ToF) Mass Spectrometry techniques. Local reporting rules were followed which include; always reporting a dermatophyte isolation despite the microscopy result as dermatophytes are not encountered as commensal organisms. In contrast, NDMs would only be reported if grown from at least four specimen portions, in pure culture, from a microscopy positive nail. Non-dermatophyte moulds are frequently cultured from dystrophic or indeed healthy nails, which are contaminated with fungal spores from the environment. However, observation of hyphae within the tissue indicates active growth, sometimes these may be hyphae of an underlying dermatophyte which has damaged the nail allowing secondary colonisation with a NDM. If a dermatophyte and a NDM were isolated the dermatophyte would be considered to be the primary pathogen. Yeasts would only be reported if accompanied by positive microscopy.