A survey among dermatologists: diagnostics of superficial fungal infections - what is used and what is needed to initiate therapy and assess efficacy?

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ORIGINAL ARTICLE

A survey among dermatologists: diagnostics of superficial fungal infections – what is used and what is needed to initiate therapy and assess efficacy?

D.M.L. Saunte,[1,2,*] B.M. Piraccini,[3] A.Y. Sergeev,[4] A. Prohić,[5] B. Sigurgeirsson,[6] C. Rodríguez-Cerdeira,[7] J.C. Szepietowski,[8] J. Faergemann,[9] M. Arabatzis,[10] M. Pereiro,[11] M. Skerlev,[12] P. Lecerf,[13] P. Schmid-Grendelmeier,[14] P. Nenoff,[15] R.J. Nowicki,[16] L. Emtestam,[17] R.J. Hay[18]

[1]Department of Dermatology, Zealand University Hospital, Roskilde, Denmark
[2]Health Sciences Faculty, University of Copenhagen, Copenhagen, Denmark
[3]Dermatology, Department of Experimental, Diagnostic and Specialty Medicine, University of Bologna, Bologna, Italy
[4]Department of Clinical Immunology, Sechenov University, Moscow, Russia
[5]Department of Dermatovenerology, University Clinical Center of Sarajevo, Sarajevo, Bosnia and Herzegovina
[6]Department of Dermatology, Faculty of Medicine, University of Iceland, Reykjavik, Iceland
[7]Department of Dermatology, Meixoeiro hospital and University of Vigo, Vigo, Spain
[8]Department of Dermatology, Venereology and Allergology, Wroclaw Medical University, Wroclaw, Poland
[9]Department of Dermatology, Sahlgrenska Academy, University of Gothenburg, Gothenburg, Sweden
[10]Department of Dermatology, Medical School, Aristotle University, Thessaloniki, Greece
[11]Dermatología, Faculty of Medicine, University of Santiago de Compostela, A Coruña, Spain
[12]Department of Dermatology and Venereology, Zagreb University School of Medicine and Zagreb University Hospital, Zagreb, Croatia
[13]Department of Dermatology, University Hospital Brugmann, Université Libre de Bruxelles, Brussels, Belgium
[14]Department of Dermatology, University Hospital of Zurich, Zurich, Switzerland
[15]Laboratory for Medical Microbiology, Mölbis, Germany
[16]Department of Dermatology, Venereology & Allergology, Medical University of Gdansk, Gdansk, Poland
[17]Section of Infectious Diseases & Dermatology, Department of Medicine Huddinge, Karolinska Instituttet, Stockholm, Sweden
[18]Department of Dermatology, Kings College Hospital NHS Trust, London, UK
*Correspondence: D.M.L. Saunte. E-mails: disa@regionsjaelland.dk; ditte@saunte.com

Abstract

Background Superficial fungal infections are common. It is important to confirm the clinical diagnosis by mycological laboratory methods before initiating systemic antifungal treatment, especially as antifungal sensitivity and *in vitro* sus- ceptibility may differ between different genera and species. For many years, the gold standard for diagnosis of superficial fungal infections has been direct fungal detection in the clinical specimen (microscopy) supplemented by culturing. Lately, newer molecular based methods for fungal identification have been developed.

Objective This study was initiated to focus on the current usage of mycological diagnostics for superficial fungal infec- tions by dermatologists. It was designed to investigate whether it was necessary to differentiate between initial diagnos- tic tests and those used at treatment follow-up in specific superficial fungal infections.

Methods An online questionnaire was distributed among members of the EADV mycology Task Force and other der- matologists with a special interest in mycology and nail disease.

Results The survey was distributed to 62 dermatologists of whom 38 (61%) completed the whole survey, 7 (11%) par- tially completed and 17 (27%) did not respond. Nearly, all respondents (82–100%) said that ideally they would use the result of direct microscopy (or histology) combined with a genus/species directed treatment of onychomycosis, der- matophytosis, *Candida*- and *Malassezia*-related infections. The majority of the dermatologists used a combination of clinical assessment and direct microscopy for treatment assessment and the viability of the fungus was considered more important at this visit than when initiating the treatment. Molecular based methods were not available for all responders. Conclusion The available diagnostic methods are heterogeneous and their usage differs between different practices as well as between countries. The survey confirmed that dermatologists find it important to make a mycological diagno- sis, particularly prior to starting oral antifungal treatment in order to confirm the diagnose and target the therapy accord- ing to genus and species.

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Conflict of interests

DMS has received consultant fee for advisory board meeting by AbbVie, Jannsen Pharma and Sanofi and as a speaker for Bayer, Galderma, Astellas, Abbvie and Leo Pharma. RJH has been a consultant for Mayne Pharma, Polychem and Janssen Pharma. PN has received consultant fee for advisory board meeting by Galderma and as a speaker for Almirall Hermal, Galderma, Janssen Pharma, Pfizer, MSD, and Beiersdorf. BMP has received consultant fee for advisory board meeting by Polichem, Legacy Healthcare and Pfizer and as a speaker for ISDIN, Giuliani, Avangarde and Almirall. JF has been consulting for Moberg Pharma, Mylan and Galderma. AYS has received consultant fee for advisory board meetings by Janssen-Cilag, Novartis and Dr Reddy's, and as a speaker for Astellas, Bayer, Galderma, Glenmark, MSD and Pfizer. JCS has been consultant and advisor for AbbVie, Celgene, Dignity Sciences, Leo Pharma, Novartis, Pierre-Fabre, Sienna Pharmaceuticals and Sandoz. He has also been investigator for AbbVie, Actelion, Amgen, GSK, Janssen, Merck, Novartis, Regereron, Takeda, Trevi and speaker for AbbVie, Actavis, Janssen, Leo Pharma, Novartis, SunFarm, Sandoz, Eli Lilly. PSG, RJN, CR-C, MA, MS, AP, PL, BS, LE declares no conflict of interest.

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Introduction

Superficial fungal infections are common mycoses with clinical manifestations that depend on the anatomical site involved and the type of fungus causing the infection.

Although the clinical picture of the different infections is characteristic, it is important to confirm the diagnosis by mycological laboratory methods before initiating systemic antifungal treatment, especially as antifungal sensitivity and *in vitro* susceptibility may differ between different genera and species. Furthermore, a Canadian study has proved that making a mycological diagnosis prior to topical and oral treatment is cost saving.[1]

For many years, the gold standard for diagnosis of superficial fungal infections has been direct microscopy often performed by the clinician, and culture sometimes supplemented by histopathology. Since the first dermatophyte polymerase chain reaction (PCR) was developed in 1996[2] an array of new molecular based methods for fungal identification have been developed with different PCR techniques and lately, matrix assisted laser desorption/ionization – time of flight (MALDI-TOF) mass spec- trometry has also been used.[3–10] Because special equipment is required to perform molecular diagnostics the analysis is per- formed at specialized mycological or microbiological laborato- ries. Unfortunately, the molecular methods used differ between laboratories and the methods are often 'in-house' and have not been standardised for cross comparison. Some of the molecular based methods only detect dermatophytes and relevant fungi such as *Candida*, *Malassezia* and non-dermatophyte moulds such as *Neoscytalidium* is not detected whereas other methods detect all fungi, including fungi which are not clinically relevant, leaving it to the clinician to decide whether the result is relevant for the clinical diagnosis or due to contamination. An overview of the current available diagnostic methods including the pros- and cons is shown in Table 1.

Another important issue is that the clinician is involved in diagnostic mycology in two clinical settings, which require different diagnostic approaches. The first scenario is when the clinician suspects a fungal infection and wants this confirmed before initiation of treatment and the mycological diagnosis may provide a guide to the appropriate choice of antifungal treatment. The other clinical scenario occurs at the follow-up visit where an assessment of treatment efficacy, including clearance of infection, or persistence is evaluated. These two settings require different mycological diagnostic procedures which most of the molecular diagnostic methods are not designed to cover.

This study was initiated to focus on the current usage of mycological diagnostics for superficial fungal infections by dermatologists about both the optimal tests and what they actually use in their daily current practice. It was designed to investigate whether it was necessary to differentiate between initial diagnos- tic tests and those used at treatment follow-up in specific super- ficial fungal infections; onychomycosis, dermatophytosis, *Candida*- and *Malassezia*-related infections.

Methods

An online English questionnaire was distributed among members of the EADV mycology Task Force and other dermatologists with a special interest in mycology and nail disease.

The questionnaire focused on the use of diagnostic methods to confirm a fungal infection as well as the subsequent assessment of treatment. It was divided into questions regarding the

Table 1 The advantages and disadvantages of available diagnostic methods

	Detect viability	Pros	Cons	Other comments
Direct microscopy in KOH	No	Confirms the presence of any fungus Fast Cost-effective	Not genus or species specific	Fluorescence microscopy enhances the detection rate
Culture	Yes	Detects unexpected pathogens Raises the confirmation chance in case of negative microscopy Permits elective antifungal susceptibility testing	Time consuming (days – weeks) Dependent on growth media Requires skilled lab. technicians	
Histopathology	No	Confirms the presence of the fungus in the tissue	Not genus or species specific Expensive	
Molecular based methods	No	Fast (hours – days) Detects both viable and non-viable fungal material and provides identification to genus and species level Not dependent on laboratory technicians skills Requires small amount of material	Pre-defined diagnostic target Contamination risk	No standardization
Woods light	No	Fast Inexpensive Good for screening Tinea capitis: genus differentiation Guide the clinician to the optimal sampling area	Not species specific	

participants views on the optimal diagnostic techniques, their actual use of locally available diagnostic methods and prioritisation of those methods they considered to be the most important in order. They were asked to differentiate between diagnostic requirements in specific clinical settings; onychomycosis, *Malassezia* related diseases (pityriasis versicolor, *Malassezia* folliculitis), dermatophytosis (tinea capitis, tinea of other skin sites) and superficial yeast infections.

Results

An online survey was distributed to 62 dermatologists of whom 38 (61%) completed the whole survey, 7 (11%) partially completed the questionnaire and 17 (27%) did not respond. Some of these non-responders later replied, that they had not provided answers either because they were histopathologists or only spe- cialised in general nail disorders and were therefore not able to answer questions about specialised dermatomycology. The mean age of the participants was 54.5 years (range 31–88 year). The majority of the responders worked in Europe [Belgium (*n* = 2), Bosnia (*n* = 1), Denmark (*n* = 3), Estonia (*n* = 1), France (*n* = 3), Greece (*n* = 3), Germany (*n* = 2), Iceland (*n* = 1), Italy (*n* = 1), Latvia (*n* = 1), Poland (*n* = 1), Portugal (*n* = 2), Rus- sia (*n* = 1), Spain (*n* = 2), Sweden (*n* = 2), Switzerland (*n* = 2), The Netherland (*n* = 3), Turkey (*n* = 2), United Kingdom (*n* = 3)] and fewer from Asia [India (*n* = 1), Japan (*n* = 1), Korea (*n* = 1), Philippines (*n* = 1)] or the Americas [Brazil (*n* = 3), USA (*n* = 1)]. A total of 60% of the dermatologists were employed in public hospitals and 40% worked in private practice.

Nails – when onychomycosis is suspected (*n* = 40 completed the survey, five partially completed)

Before initiation of treatment When onychomycosis is suspected 91% of those asked opted as the ideal diagnostic tests for direct microscopy to confirm the diagnosis and 67% also required the differentiation between yeast, dermatophyte and non-dermatophyte mould to (i) genus (58%) or (ii) species (53%) level (Table 2a, Fig. 1). One third of the dermatologists stated that ideally the clinically suspicion of nail infection should be confirmed by histology too but only 13% were interested in knowing if there was only a single fungus isolated or whether it was viable. The most important pieces of diagnostic information, in order, were considered by the responders to be identification to species level (24%), differentiation between yeasts, dermatophyte and non-dermatophyte moulds (22%), diagnosis at genus level (18%), direct microscopy (18%), histology (11%), viability of the fungus (4%) and the presence of a single culture (2%). In their daily practice, 90% of the responders used culture, 95% performed direct microscopy, 37% histology whereas only 27% used a molecular based method (Table 2b). Six of the dermatologists stated that they performed PCR when the culture results were negative or a mould was cultured [e.g. *Mucor* spp. (where there is a risk of overgrowth of the dermatophyte due to its faster growth rate)]. It was also suggested by some of the responders that an *in vitro* susceptibility test should be per- formed when the isolated fungus was known to have a variable susceptibility profile. Histology was suggested, when the diagno- sis of infection was doubtful. One dermatologist used

Table 2 (a) Diagnostic consideration and treatment decision making among dermatologists. (b) Methods used among dermatologist before and after treatment

Minimum diagnostic information needed before treatment initiation	Onychomycosis % (*n*/total)	Tinea capitis % (*n*/total)	Tinea of the skin* % (*n*/total)	Superficial *Candidasis** % (*n*/total)	Pityriasis versicolor* % (*n*/total)	*Malassezia* folliculitis % (*n*/total)
(a)						
Fungus detected in the specimen (Direct microscopy and/or histology)	91% (41/45)	97% (38/39)	89% (34/38)	82% (31/38)	89% (34/38)	92% (35/38)
Genus/species directed treatment	98% (44/45)	82% (32/39)	ND	100%(38/38)	18% (7/38)	34% (13/38)
Differentiation between Y, D & M	67% (30/45)	NA	NA	NA	NA	NA
ID to genus level	58% (26/45)	67% (26/39)	53% (20/38)	42% (16/38)	13% (5/38)	29% (11/38)
ID to species level	53% (24/45)	64% (25/39)	58% (22/38)	58% (22/38)	13% (5/38)	21% (8/38)
Pattern of resistance	2% (1/45)	ND	ND	24% (9/38)	0% (0/38)	0% (0/38)
Woods light	ND	51% (16/39)	3% (1/38)	ND	21% (8/38)	21% (8/38)
Histology	33% (15/45)	13% (5/39)	16% (6/38)	ND	0% (0/38)	24% (9/38)

Diagnostic tests in every day use	Onychomycosis		Tinea capitis		Tinea of the skin*		Superficial *Candidasis**		Pityriasis versicolor*		*Malassezia* folliculitis	
	Before % (*n*/total)	Follow-up % (*n*/total)	Before % (*n*/total)	Follow-up % (*n*/total)	Before % (*n*/total)	Follow-up % (*n*/total)	Before % (*n*/total)	Follow-up % (*n*/total)	Before % (*n*/total)	Follow-up % (*n*/total)	Before % (*n*/total)	Follow-up % (*n*/total)
(b)												
Direct microscopy	95% (39/41)	75% (30/40)	92% (36/39)	79% (31/39)	95% (36/38)	58% (22/38)	82% (31/38)	42% (16/38)	87% (33/38)	47% (18/38)	87% (33/38)	45% (17/38)
Histology	37% (15/41)	15% (6/40)	13% (5/39)	5% (2/39)	3% (1/38)	3% (1/38)	3% (1/38)	5% (2/38)	0% (0/38)	0% (0/38)	16% (6/38)	8% (3/38)
Culture	90% (37/41)	73% (29/40)	69% (27/39)	72% (28/39)	58% (22/38)	34% (13/38)	68% (26/38)	37% (14/38)	16% (6/38)	3% (1/38)	26% (10/38)	5% (2/38)
Molecular diagnostic	27% (11/41)	8% (3/40)	18% (7/39)	3% (1/39)	16% (6/39)	0% (0/39)	8% (3/38)	0% (0/38)	16% (6/38)	0% (0/38)	5% (2/38)	3% (1/38)
Woods light	ND	ND	41% (16/39)	44% (17/39)	16% (6/39)	5% (2/39)	ND	3% (1/38)	37% (14/38)	3% (1/38)	21% (8/38)	21% (8/38)
Viability important	13% (6/45)	88% (35/40)	21% (8/39)	82% (32/39)	ND	53% (20/38)	ND	39% (15/38)	ND	32% (12/38)	ND	37% (14/38)
Susceptibility test	ND	ND	ND	ND	ND	ND	16% (6/38)	3% (1/38)	0% (0/38)	0% (0/38)	0% (0/38)	0% (0/38)

*The clinical response is the main assessment parameter.

D, dermatophyte; ID, Identification; M, non-dermatophyte mould; Y, yeast.

Before: before treatment initiation. Follow-up: treatment assessment.

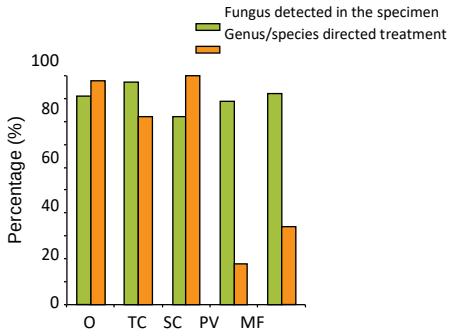

Figure 1 The percentage of dermatologists who consider direct fungal detection in the specimen (microscopy and/or histology) and genus/species directed treatment as sufficient before treat- ment initiation in superficial dermatomycoses. MF, *Malassezia* folliculitis; O, onychomycosis; PV, pityriasis versicolor; TC, tinea capitis; SC, superficial *Candidiasis*.

dermoscopy of the nail (onychoscopy) as a guide to initiating treatment when the clinical suspicion was strong, but direct microscopy with KOH was negative, because culture normally takes 3–4 weeks.

Treatment assessment The dermatologists consulted evalu- ated the treatment efficacy by clinical judgement of the nor- mal regrowth of the proximal nail. As a diagnostic test culture (73%) was still the most popular method as it also confirms if the fungus is viable, which a total of 88% stated to be important. Direct microscopy was performed by 75% and histology and/or a molecular based diagnostic method by 15% and 8%, respectively. (Table 2b).

Hair – when tinea capitis is suspected (n = 39)

Before initiation of treatment The majority of all respondents said that ideally they would use the result of direct microscopy before starting treatment (97%; Table 2a) except those who had access to molecular diagnostic tests (3%). Half of the dermatolo- gists said that they would use Woods light to differentiate between *Trichophyton* and *Microsporum* species and 21% said that it was important to know if the fungus was viable before starting treatment. Only 13% suggested histopathology to con- firm or exclude a diagnosis. In their daily practice, they used direct microscopy (92%), culture (69%), Woods light (41%), molecular diagnostics (18%) and histology (13%; Table 2b). If they had to choose between the diagnostic methods 38% would use direct microscopy, 57% the identification of the organism to genus and/or species level using specific diagnostic methods and 5% wanted to know whether the fungus was viable or make a biopsy. The users of PCR preferred this method because it is very fast, so that treatment can start nearly immediately.

Treatment assessment The majority of the dermatologists used a combination of clinical assessment, Woods light (44%) and direct microscopy (79%) and/or culture (72%). A few reported that they used molecular diagnostic methods (3%) and histology (5%; Table 2b). Half of the participants stated that culture was the most important diagnostic method (51%), fol- lowed by direct microscopy (36%) and only a few dermatologists would choose a molecular based method (5%), histology (3%), and other (unspecified; 5%) if they had to choose between the methods.

Skin – when dermatophytosis is suspected (n = 38)

Before initiation of treatment When a tinea (dermatophyte) infection of the skin was suspected the majority of the dermatol- ogists would like the diagnosis confirmed by direct microscopy (89%) and fungal identification to genus (53%) and/or species level (58%; Table 2a). The most important diagnostic tool was considered to be direct microscopy (45%), followed by culture (29%), a molecular based method (11%) or clinical evaluation (16%). When it came to what was used in practice 95% used direct microscopy, 58% culture, 16% a molecular based method and 16% made the diagnosis based upon Woods light and clini- cal appearance. Only a few used histology (3%; Table 2b).

Treatment assessment Nearly, all (95%) used clinical evalua- tion combined with direct microscopy (58%), culture (34%), Woods light (5%) or histology (3%). Approximately half (53%) said that it was important to know if the fungus was viable (Table 2b).

Malassezia related skin diseases (n = 38) When pityriasis versicolor (PV) is suspected

Before initiation of treatment The clinical picture was rated as the main criterion for diagnosis, but for confirmation direct microscopy was the main (89%) diagnostic tool (Table 2a). A few clinicians wanted a genus and/or species specific (18%) diag- nostic method, 21% used Woods light and 3% used a molecular based method (Table 2a).

When asked to choose between the methods 74% stated that direct microscopy was the most important diagnostic method, 11% the clinical appearance of the patient, 8% culture result, 5% Woods light and 3% molecular diagnostic method. In practice, the majority used direct microscopy (87%) followed by Woods light (37%), culture (16%), molecular diagnostics (16%; Table 2b).

Treatment assessment The majority of dermatologists said that the clinical signs of PV were the most important for confirming treatment efficacy (92%) and 47% used direct microscopy, 3% culture, 3% Woods light for treatment assessment (Table 2b). The majority (71%) of the responders said, that if they had to prioritize between the diagnostic tools, direct microscopy was the most important tool fol- lowed by the clinical appearance (13%), culture (11%), Woods light (3%), and molecular based diagnostics (3%). One third stated that it was important to know if the fungus was viable.

When *Malassezia* folliculitis is suspected (*n* = 38)

Before initiation of treatment For diagnosis of *Malassezia* folliculitis, 92% of dermatologists wanted direct microscopy of the specimen. Genus/and/or species identification was suggested by 34%, histology by (24%), Woods light (21%) and molecular diagnostics (8%; Table 2a). If they could choose between the methods the majority would use direct microscopy (32%), followed by clinical appearance (29%), culture (18%), histology (16%). Some of the dermatologists stated that clinical diagnostic features including distribution and presence of itching was also important and histology was suggested to rule out other diseases.

Treatment assessment The clinical response was used by 87% to evaluate the treatment, 45% also used direct microscopy, 21% Woods light, and fewer histology (8%), culture (5%) or molecular diagnostics (3%; Table 2b). Most (63%) did not consider assessing the viability of the fungus impor- tant.

Skin/mucosa – when a yeast (*Candida*) infection is suspected (*n* = 38)

Before initiation of treatment Most dermatologists ideally required a microscopy result (82%), or identification to genus (42%) and/or species (58%) level before initiating therapy and/or susceptibility testing (24%; Table 2a). The specimen is sometimes sent to a specialist mycology laboratory, as many non-specialist laboratories will not identify *Candida* to species level.

The responders were asked to choose the most important diagnostic information they needed before treatment initiation and the results were in order, species identification (47%), direct microscopy (42%) and genus identification (11%). In practice, direct microscopy was used by 82%, culture (68%), susceptibil- ity test (16%), and a molecular based method (8%; Table 2b). Clinical appearance (8%) and histology (3%) is used more rarely.

Treatment assessment All dermatologists evaluated the patient clinically, supplemented by direct microscopy (42%), culture (37%), histology (5%), susceptibility test (3%) and Woods light (3%; Table 2b). Some stated, that susceptibility testing might be necessary in cases of treatment failure.

Discussion

The development of new molecular diagnostic methods for iden- tification of fungal infections has resulted in many new diagnos- tic opportunities, but it also places a higher demand on the skills of the dermatologist/clinician. If these methods detect all fungal material (Panfungal) the clinician has to be able to distinguish between primary pathogens, colonizing fungi and contaminants for example by microscopy. It is also important that the clinician knows the limitations of the methods for example a dermato- phyte PCR will not detect non-dermatophyte moulds or yeast. The answers by the group showed that, at present, molecular tools are not widely used for diagnosis of superficial mycoses although all recognised that this situation was likely to change with further simplification of the methods and the adoption of this methodology across a wider range of microbiology/mycol- ogy laboratories.

The majority of dermatologists wanted to confirm the fungal diagnosis before treatment initiation by direct microscopy (82–100%) as well as identification to genus or species level (82–100%) except in *Malassezia* related infections, where direct microscopy was thought to be sufficient for fungal identification. In the future this may present a challenge for both clinical diagnosis and training in dermatology because direct microscopy is regarded as a point of care test, in other words a test used by the clinician in clinic, and therefore for the foreseeable future, training in direct microscopy along with other tests which can be deployed in the clinic such as dermoscopy should form part of the training requirements for dermatologists.

This goal of mycological confirmation before treatment initiation was much higher than that reported in previous studies where only 47–60% of dermatologists, 47% of podiatrists and 22% of family practitioners said that they confirmed the clinical diagnosis of onychomycosis by a mycological test.[11–13] A high false negative detection rate when combining direct microscopy and culture in onychomycosis as well as the slow fungal growth may have led to this lower result. The development of molecular based methods, which are fast and have a higher detection rate than conventional methods may be one of the reasons for this as 27% of the dermatologist in this survey used a molecular based method.[14] Other reasons might be that in many societies evidential documentation before treatment initiation is important for legal or re-imbursement reasons. Finally, the responders of this survey are experts in mycology and therefore committed to establishing a

mycological result, and the results of this study may therefore differ from routine diagnosis in general practice.

In onychomycoses, the use of systemic antifungal therapy is common and many of the dermatologists stated, that the planned choice between topical or systemic treatment may affect their preference of diagnostic methods. If the clinician considers topical monotherapy appropriate, the clinical findings are often regarded as sufficient (lower risk of side-effects), but before starting oral treatment, mycological confirmation of diagnosis is required. Another important issue that did not form part of the survey but was highlighted by some in the free comments section is *in vitro* susceptibility testing which is used to detect acquired resistance after antifungal treatment. Unfortunately, susceptibility testing is not available for all clinicians even though terbinafine resistance
has been detected in *T. rubrum*, *T. mentagrophytes* and *T. interdigitale*[15–17] and antifungal resistance is considered to be a growing global problem

In this survey, viability of the infecting organism is considered important in treatment assessment as a positive culture would prolong the duration of the treatment course or lead to a change in anti-mycotic treatment. This is the main reason why molecular based diagnostics were not used as a diagnostic tool in treatment assessment. The incorporation of a viability-test as well as identifying molecular markers of drug resistance in any future battery of molecular diagnostic tests would extend the use of these techniques. In support of this half of the dermatologists surveyed stated that it is important to make a species specific diagnosis in cases of recurrent or treatment resistant infections especially in *Candida* infections as organisms such as *C. glabrata* and *C. krusei* are known to have a lower susceptibility to fluconazole.[18]

Conclusion

The survey reflects the fact that the available diagnostic methods are heterogeneous and their usage differs between different practices as well as between countries. Nevertheless it confirmed that dermatologists find it important to make a mycological investigation, particularly prior to starting oral treatment in order to confirm the diagnosis of fungal infection and target the therapy according to genus and species. When it comes to treatment assessment the clinical response combined with species identification, assessment of viability and susceptibility testing are considered important. Molecular based methods are useful in establishing the diagnosis, but their role in treatment assessment is still limited as they detect both viable and non-viable fungal elements. The future development of methods being able to bridge this current gap would be welcome. These findings have training implications

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