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Dermatophytosis, Trends in Epidemiology and Diagnostic Approach

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Abstract Dermatophytes are amongst the most common fungal agents causing superficial skin infections. The epidemiology of dermatophytosis has changed during the last century under the influence of socioeconomic factors, modern life, intensification of travel, migration of populations from the southern to the northern hemisphere. As result, *Trichophyton rubrum* has become the most frequent species worldwide, causing mainly tinea pedis and tinea unguium, while *Microsporum canis* is still the main agent in tinea corporis and capitis in Mediterranean countries. However, the prevalence of anthropophilic dermatophytes causing tinea capitis in young children is increasing overall in the big cities of Europe and America, causing epidemics and becoming a public health concern. This review summarizes the current status of dermatophyte infection in Europe, Africa, Asia and America and gives an overview of the most recent molecular methods currently available for the laboratory diagnosis of dermatophytosis.

Keywords Dermatophytosis · Dermatophytes · Tinea epidemiology · Molecular methods · Diagnosis

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Introduction

Dermatophytes are a group of filamentous fungi referred to as the ringworm fungi. They are keratinolytic and invade keratinized tissues causing mostly superficial infections involving the skin, hair and nails. They are amongst the most common causes of skin disease in the world, and the real prevalence is probably underestimated. Onychomycosis is the most common nail disorder in adults causing about 50 % of all nail diseases. Large-scale studies on onychomycosis conducted in the US and Canada in the late 1990s showed a prevalence rate of 14 % [1] and 8 % [2], respectively. In Europe, the prevalence rate is even more variable: 2.7 % in the UK [3] and Spain [4], 8.4 % in Finland [5], 12.4 % in Germany [6••] and 16.8 % in France in a more recent study [7]. The Achilles project, the largest survey on foot disease undertaken in Europe, conducted in 20 European countries during 1997–1998, showed a particularly high prevalence of fungal foot disease and onychomycosis which accounted for 40.6 % and 28 %, respectively (data extracted from study II, including clinical and mycological examination of patients consulting a dermatologist) [8]. This survey was divided into two studies: in the first, patients visiting a general practitioner or a dermatologist were clinically checked for foot disease, and in the second, only patients visiting a dermatologist were examined for foot disease and a sample taken for culture. However, patients consulting a dermatologist for foot disease were not excluded from the study, creating an inclusion bias, in contrast to north American studies which excluded this population [1, 2].

Dermatophytosis or infections due to dermatophytes are called *tinea* according to the site of infection as for example tinea corporis, involving the arms, trunk and legs, tinea capitis (TC), involving the scalp, and tinea pedis involving the foot. In some cases, a misdiagnosis followed by inappropriate

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topical use of corticosteroids results in an atypical clinical presentation (*tinea incognito*) making the diagnosis more difficult. Complications such as bacterial secondary infection and allergies can also complicate an unrecognized chronic tinea [9, 10]. Depending on the climate and culture, the picture can differ: tinea pedis and onychomycosis are the most prevalent clinical forms in Western countries while TC and tinea corporis are the most frequent forms in tropical areas. For a few years, small epidemics of TC due to anthropophilic dermatophytes have been emerging in different European countries [11, 12].

Dermatophytes are divided into three closely related genera: *Epidermophyton*, *Trichophyton* and *Microsporum*. The main characteristic of these fungi, with the exception of keratinophily, is their membership of a group that depends on their normal habitat: geophilic dermatophytes are naturally present in the soil, zoophilic in animals, and anthropophilic in humans. The fungal pathogens that infect humans belong mostly to the second and third groups, geophilic dermatophytes being more rarely involved in human disease. Zoophilic and anthropophilic dermatophytes evolved from a geophilic origin, with the anthropophilic dermatophytes being the most highly specialized group. They rarely infect other animals and they are also restricted to some body parts. Some species including *Microsporum audouinii*, *Trichophyton tonsurans* and *T. soudanense* mostly cause TC and are rarely isolated from other body sites. Other species are responsible mostly for onychomycosis; these include *T. rubrum* which is the main agent, followed by *T. interdigitale*. Finally, *Epidermophyton floccosum* infects only the skin.

The dermatophytes normally develop in the dead part of keratinized tissue of the stratum corneum, within and around hair and in the nails [13••]. In these tissues, growth is associated with the production of hyphae and arthroconidia, this characteristic being used as a diagnostic feature. The pathogenicity of dermatophytes is associated to different factors including the production of keratinolytic enzymes [14, 15], a genetic predisposition and the presence of host factors [16••]. Numerous host factors have been associated with tinea pedis and onychomycosis such as circulatory disorders, diabetes mellitus, ichthyosis, psoriasis, disorders affecting cellular immunity, to the extent that onychomycosis is now considered to be a predictor of diabetic foot syndrome [17]. The prevalence of onychomycosis in diabetics is higher than in the normal population, with high prevalence rates of, for example, 20 % in Denmark (20 %) and 51 % in Japan [18, 19]. Indeed, due to the increasing number of diabetics in the world [20], it is likely that the prevalence of onychomycosis and tinea pedis will continue to rise in the future.

This review considers the recent changes in epidemiology and the new diagnostic tools applied to the laboratory diagnosis of dermatophytes.

Trends in Epidemiology

Evolution of Dermatophytosis in European Countries

During the last 100 years, the dermatophyte spectrum has markedly changed over the world with differences depending on the geographic area and other factors such as immigration. The most common dermatophytosis and dermatophytes involved according to the country are summarised in Table 1. In central and northern Europe, the example of Germany, which has been widely reviewed by Seebacher et al. [21••] and Nenoff et al. [16••], shows that the predominance of *E. floccosum* and *M. audouinii* as causal agents of tinea corporis and TC, respectively, in the 1920s has been progressively replaced by that of *T. rubrum* which since the 1950s has been the most prevalent dermatophyte in Europe, causing mainly tinea pedis and tinea unguium. This species has evolved since the nineteenth century as a cause of chronic tinea corporis from the endemic areas in South Asia [22]. Since the 1950s it has progressively replaced anthropophilic *T. interdigitale* as the aetiological agent in tinea pedis and unguium throughout Europe [21••]. This trend is particularly marked in northern Europe. A retrospective study performed in Stockholm (Sweden) found that *T. rubrum* was the main causal agent of fungal skin infections being associated with 83 % of infections [23]. In a large survey performed by the Mycology Reference Laboratory, Bristol, UK, from 1980 to 2005, *T. rubrum* was the most frequently isolated dermatophyte (70 % in 2005), followed by *T. interdigitale* (20.8 %) [24]. In Germany this species is responsible for 91 % of onychomycosis [16••]. In Belgium, *T. rubrum* has been isolated from nails in 76 % of onychomycosis and *T. interdigitale* in about 22 % (personal data, Belgian National Reference Center, Liège; 2012 annual report). In Poland, *T. rubrum* and *T. mentagrophytes* represented 90 % of all dermatophytes isolated from superficial fungal infections for the period 2005–2010 with, however, a less marked predominance of *T. rubrum* [25]. In a survey in Slovakia conducted during the period 1994–1999 *T. rubrum* accounted for 81.61 % of all dermatophytes isolated [26].

This predominance of *T. rubrum* suggests a great capacity of this species to spread as attested by the findings of recent molecular studies showing that some strains of *T. rubrum* have a significantly higher capacity to spread than others [27]. The most common source of infection is the private bath that is contaminated by family members, with vertical transmission being more common than horizontal transmission [16••]. However, other factors may explain the rise of this species. First, the evolution of a life-style marked by the increasing use of sports facilities including public pools, fitness studios and martial arts facilities where the main sources of transmission are changing rooms, showers and mats. Another factor is the use of occlusive footwear which causes humidity leading to

Table 1 Most common dermatophytosis and dermatophytes involved according to the country

Most common dermatophytosis	Most common dermatophyte	Region	Country	Reference
Tinea pedis + onychomycosis	*T. rubrum*	Europe	UK	24
			Sweden	23
			Germany	16
			Belgium	Personal data[a]
			Poland	25
			Slovakia	26
			Spain	35
			Greece (Crete)	33
		Middle East	Turkey (Dusce)	54
			Iran (Tehran)	50
		North and Central America	USA	37
			Mexico	47
		Asia	Japan	65
Tinea corporis	*T. mentagrophytes*	Middle East	Lebanon	51
			Saudi Arabia	53
	T. verrucosum		Northern Iran	48
	M. canis	Europe	Italy	36
	T. rubrum	Asia	India	63, 64
Tinea capitis	*T. tonsurans*	Caribbean	Haiti	45
	M. audouinii	Africa	Mali	55
	T. soudanense + *T. tonsurans*		Nigeria	57
	M. audouinii			58
	T. soudanense		Senegal	59
	T. violaceum		Ethiopia	60, 61
			Botswana	62

[a] Personal data: 2012 CNR Mycosis Belgium

maceration which promotes the emergence of tinea pedis and toenail onychomycosis. Indeed, tinea pedis is also called a "pedal fungus reservoir", spreading to other parts of the body and causing tinea manuum, inguinalis and unguium [28].

Secondly, the introduction of griseofulvin in 1958 as a systemic antifungal agent for the treatment of tinea corporis and TC led to the disappearance of both *M. audouinii* and *M. schoenleinii* from central Europe [29]. Indeed neither species has been isolated in Germany since 1967: in a large study performed in East Germany between 1967 and 1971 including 38,738 patients with dermatophyte infection, only 18 patients had TC all of which were caused by *M. canis*. In another study in another area of Germany performed in 1976 and 1985, no *M. audouinii* isolate and only one *T. schoenleinii* were found [30]. A review by Seebacher at al. showed similar results for central European countries [21••]. All these results demonstrate the effectiveness of griseofulvin in paediatric TC and this is still considered the "gold standard" treatment in some countries.

In Mediterranean countries however, the situation is variable depending on the country considered. In Greece, *T. rubrum* is also predominant, as demonstrated in a study conducted in Crete between 1992 and 1996 in which *T. rubrum* was the most frequent dermatophyte (44.4 %) isolated, followed by *M. canis* (24 %), *T. interdigitale* (3.4 %) and *T. verrucosum* (1.8 %) [31]. A few years later (1997–2003), the same authors reported the same dermatophyte distribution: *T. rubrum* (48 %), *M. canis* (17.9 %), *T. interdigitale* (14.2 %), *E. floccosum* (6 %) [32, 33]. In Spain, while few studies are available, a 5-year retrospective survey performed in a dermatology clinic in Zaragoza during the period 1991–1995 showed a predominance of *M. canis* (44 %) associated with tinea corporis, followed by *T. mentagrophytes* and *T. rubrum* (18.6 %) [34]. However, a study conducted in the University Hospital of Cadiz from 1998 to 2008 showed a predominance of *T. rubrum* (38.2 %) with an increasing incidence from 2000, *M. canis* being only the second most frequently isolated dermatophyte (22.8 %) [35]. In Italy, *M. canis* is still the most frequently isolated dermatophyte at 88.9 %, as found by Panasiti et al. [36] in a study conducted in Rome between 2002 and 2004. This was associated with a predominance of tinea corporis, with tinea pedis accounting for only for a small percentage (6.7 %).

The Situation in the US and Central America

The most recent study is that of Foster et al. published in 2004 [37], an epidemiological surveillance study conducted at the Center for Medical Mycology in Cleveland, Ohio, from 1995 to 2002. In that study, *T. rubrum* was the most prevalent fungal pathogen with an increasing incidence observed between 1999 and 2002 from 32 % to 47 %. Conversely, *T. tonsurans* which has been the predominant causal agent of TC in the US for a long time [38–40], decreased from 32 % to 17.9 % during the study period [37]. This contrasts with the findings of a previous survey of dermatophytes in the US for the period 1993–1995 published in 1998 [41] in which *T. tonsurans* was the most frequently isolated dermatophyte (44.9 %), followed by *T. rubrum* (41.3 %). This survey was performed by the Dermatophyte Survey Committee of the Medical Mycological Society of the Americas, and is probably more representative than the study performed in the state of Ohio. Further studies are needed before a conclusion can be drawn. Indeed, *T. tonsurans* is considered to have entered the southwestern US from Central America and the Caribbean in the 1950s, and within a decade, it had established itself in urban regions [42, 43]. Moreover, infection is more common in the American black African population. More, recently (2010) an outbreak of tinea corporis due to *T. tonsurans* was reported among health-care workers in a freestanding paediatric hospital [44]. The index case was a 2-year-old child with recalcitrant infection of the scalp and arm and who was hospitalized many times. This outbreak highlights the risk for nosocomial infection due to dermatophytes in medical institutions, which is rarely reported.

In Haiti, *T. tonsurans* emerged in Port-au-Prince in 2005 after a slow increase from 1988 [45]. Interestingly, this emergence was due to the increase in the number of Haitians travelling from and to North America as well as in the Caribbean. Indeed many Haitian immigrants living in North America began to return to Haiti for vacations or to resettle after the end of the dictatorship in 1986. Because they were living in big cities such as Miami, New York, Boston and Chicago, where *T. tonsurans* was prevalent, it is likely that they were infected in the US and brought the epidemic to Haiti [45]. The predominance of *T. tonsurans* as a major agent in TC has also been reported for neighbouring countries such as the Dominican Republic (61.1 %), with a marked prevalence in rural areas (87 %) in comparison with urban areas (39.7 %) [46]. In Mexico, different studies since 1940 have shown a constant increase in the prevalence of *T. rubrum* in parallel to an increase in tinea pedis and tinea unguium and a decrease in TC. This was confirmed in a recent survey over a 10-year period (1996-2006) that showed a marked dominance of *T. rubrum* (71.2 %), followed by *T. tonsurans* (6.9 %), *T. mentagrophytes* (5.5 %), *M. canis* (4.5 %) and *E. floccosum* (1.9 %) [47]. In two-thirds of TC infections, *T. tonsurans* was the main agent, followed by *M. canis* in one-third.

Any Change in the Middle East, Africa or Asia?

Middle East

The epidemiology of dermatophytosis in the Middle East is highly variable according to geographical area. In Iran, as reported by Naseri et al. [48], different studies have shown that tinea corporis is the main clinical form of dermatophytosis among different species according to regional particularities. For example, in 1999–2001 in Tehran, *E. floccosum* was the main dermatophyte isolated (31.4 %), followed by *T. rubrum* (18.3 %) [49]. In a more recent study conducted in Mashhad, northern Iran, Naseri et al. [48] found that TC accounted for 32.5 % of infections while tinea corporis was still the main clinical form (33.1 %), and tinea pedis accounted for 3.4 %. In that study, *T. verrucosum* was the most prevalent species, followed by *T. violaceum* and *T. mentagrophytes*, with *T. violaceum* accounting for the majority of TC infections (27 %). However, 10 years later in Tehran (2013) tinea pedis (43.4 %) and tinea unguium (21.3 %) were the most prevalent clinical forms [50] as in European countries, but with *T. interdigitale* as main causal agent (43.5 %), *T. rubrum* being less represented (34.5 %).

In Lebanon, in 2004 tinea unguium (44.2 %) and tinea corporis (43.2 %) were found to be the main clinical forms and *T. tonsurans* was the most prevalent species (54.8 %), followed by *T. mentagrophytes* (24.5 %), *M. canis* (7.7 %), *T. rubrum* (5.3 %) and *T. verrucosum* (4 %) [51]. The distribution of dermatophytes differed from that found in a study conducted about 40 years earlier (1962) in which *T. rubrum* and *E. floccosum* were found to be predominant [52]. In Saudi Arabia, a study performed in the Riyadh Military Hospital during the period 2003–2005 showed that onychomycosis was predominant (40.3 %), followed by TC (21.9 %). However, the main causal agents were found to be *T. mentagrophytes* and *M. canis*, with *T. rubrum* not reported [53]. Among predisposing factors associated with onychomycosis, diabetes mellitus which affects 25 % of the population of Saudi Arabia may certainly play a significant role. Furthermore, the traditional and religious habit of ablution without drying the extremities is another risk factor for acquiring tinea pedis [53]. This was recorded in a randomized study conducted in the rural area of Duzce, Turkey, published in 2004 (no study period available) where tinea pedis (49.1 %) and tinea unguium (35.8 %) were predominant. Furthermore in that study, *T. rubrum* (62.2 %) was the main aetiological agent, followed by *T. mentagrophytes* (16.9 %) [54].

Africa

In Africa, dermatophytoses are common, but are often undetected and consequently undertreated. The patient may also not be able to afford the cost of treatment. Very little published material on the status of dermatophyte infection in Africa is available. The most prevalent clinical form is TC and most of the reports concern only this clinical condition. The findings of a recent study performed in hairdressing salons in Bamako, Mali, attest to the involvement of hairdressing tools in propagation of fungal spores or propagules from one customer to the next. *Microsporum audouinii* (53.3 %) and *T. soudanense* (46.7 %) were cultured from 73 % of the hairdressing tools sampled [55].

The fungal species involved vary according to the region considered as reviewed by Nweze in western Africa [56]. In Nigeria for example, the findings of the most recent publications attest to the predominance of TC, mainly in children under the age of 10 years, and depending on the region, different species are recorded as the main causal agent: *T. soudanense* and *T. tonsurans* in Abia state Nigeria [57], and *M. audouinii* in Anamba state [58]. In Senegal, in a study performed at Le Dantec Hospital from 2007 to 2011 TC was the most prevalent clinical form [59]. The main species isolated were *T. soudanense* (52.78 %), followed by *T. rubrum* (30.94 %), *M. canis* (4.89 %), *T. mentagrophytes* var. *interdigitale* (4.50 %), *M. langeronii* (3.54 %), *T. mentagrophytes* var. *mentagrophytes* (1.82 %). In Ethiopia, East Africa, high incidences of TC mainly caused by *T. violaceum* have been recorded in the southeastern and southwestern parts of the country [60, 61]. Ethiopia has a young population (44 % younger than 15 years, data from 2001), and the impact of this on the infection rate is high since children are mainly affected by TC. In Botswana, a recent study conducted during the period 2009–2010 showed that *T. violaceum* was the main agent of dermatophytosis in TC as well in other clinical forms [62].

Asia

In India, a study conducted in a tertiary care centre in a rural area in southern India showed tinea corporis and TC to be the main clinical forms, followed by tinea cruris and TC [63]. *Trichophyton rubrum* (58.9 %) was the main agent found in the study, followed by *T. mentagrophytes* (24.6 %), and *T. tonsurans* was predominant in TC (4/17 infections). Similar epidemiology has been recorded in Tiruchirapalli, Tamil Nadu, India [64]. Tinea corporis (35.4 %) was the predominant clinical condition, followed by tinea cruris (16.8 %) and TC (16.7 %). *Trichophyton rubrum* (32.8 %) was the predominant dermatophyte, followed by *T. mentagrophytes* (29.2 %). However, *M. gypseum*, *T. mentagrophytes* and *M. canis* were the main agents causing TC.

In a large survey conducted in Japan including 63,029 patients from 16 dermatological clinics in Japan, tinea pedis was the main clinical form, followed by tinea unguium [65]. *Trichophyton rubrum* was also the most frequently isolated causal species except in TC. However, an increasing number of *T. tonsurans* infections in members of combat sports clubs (wrestlers, judo athletes and sumo wrestlers) and family members has been reported since 2000 [66]. The presence of numerous asymptomatic carriers and the paucisymptomatic character of *T. tonsurans* infection makes it a very challenging public health problem in Japan.

Increase in Anthropophilic Dermatophytes Causing TC in Europe

TC represents about 1 % of superficial fungal infection in Europe and affects mainly prepubescent children [67]. Worldwide, TC is mostly caused by *M. canis*, *T. mentagrophytes* and *T. verrucosum*, while the European picture includes anthropophilic species in the list as well as *M. canis*, followed by *T. tonsurans*, *T. violaceum*, *T. verrucosum* and *T. soudanense* [67].

In Austria, a retrospective study performed between 1985 and 2008 showed a predominance of zoophilic dermatophytes (76.3 %) with *M. canis* as the main agent causing TC (84.4 %), anthropophilic dermatophytes (*T. soudanense*, *T. violaceum*) accounting only for 4.5 % of the infections [68]. However, a great change in the epidemiology of TC has been reported throughout Europe. For example, in Italy the re-emergence of previously eradicated anthropophilic dermatophytes such as *M. audouinii*, *T. violaceum* and *T. tonsurans* has been recorded over the last two decades [69]. Indeed, in the 1980s, *M. canis* was the main dermatophyte isolated in dermatophytosis in Italy causing tinea corporis and TC.

In a study performed in Florence from 1985 to 1990 [70], *M. canis* accounted for 96 % of the TC infections and no anthropophilic dermatophytes were isolated during this period. In a survey performed over a 10-year period in Rome (1985 to 1995), 50 % of all dermatophytes were *M. canis* isolates, followed by *T. rubrum* (27 %), and again *M. canis* was the main dermatophyte causing 91 % of TC infections [71]. A small percentage of TC infections were due to anthropophilic dermatophytes such as *T. violaceum* (3.1 %) and *T. tonsurans* (0.5 %). In a more recent study performed in Rome during the period 2002–2004, Panasiti et al. found TC in 29 patients with *M. canis* as the causal dermatophyte in the majority (44.6 %), followed by *M. audouinii* (27 %), demonstrating a new trend with an increase in anthropophilic species with the exception of *T. violaceum* [36]. But more recent studies have shown that *M. canis* has lost its predominant position as the causal agent of TC in Italy.

In a study in Milan during the period 2004 to 2011 including adults and children under the age of 16 years suffering from dermatomycosis, TC was found in 86 patients [69]. Among 70 infections with a positive culture, the majority (47.2 %) were due to *T. violaceum*, followed by *M. canis* (37.1 %). The authors found a marked change in epidemiology during the period 2008–2011. While the number of TC infections did not increase from 2004 to 2011, the number of infected non-Italian children increased parallel to the number of infections due to *T. violaceum*. A majority (58.5 %) of TC infections were found in non-Italian children, among whom 91 % were infected with *T. violaceum*. The majority of non-Italian children originated from African countries including Ethiopia (16 children), Egypt (8), Senegal (1), Congo (1) and Eritrea (1). In the majority of TC infections in Italian children the causal agent was *M. canis*, demonstrating that the recent change in epidemiology is directly associated with the increasing immigration from African countries to Europe, Italy often being the nearest destination for those coming by boat.

A similar picture has been reported in other European countries. In Stockholm, Sweden, a retrospective analysis conducted at the Karolinska University Hospital over a 5-year period (2005 to 2009) of dermatophytosis showed onychomycosis to be the most prevalent clinical form with a prevalence of 14.1 % and *T. rubrum* remained the main agent isolated, accounting for 83 % of infections, followed by *T. mentagrophytes* (14 %) [23]. The authors noted, however, an increase in TC from 1.4 % in 2005 to 2.7 % in 2009. Moreover, anthropophilic dermatophytes were the main aetiological agents isolated in TC with *T. violaceum* accounting for 63.8 % of the infections, followed by *T. soudanense* (17.2 %), *M. audouinii* (8.2 %) and *T. tonsurans* (5.8 %), while *M. canis* accounted only for 0.4 %. The increasing incidence of *T. violaceum* was also reported previously at the same hospital, with an increase from 5 cases for the period 1989–1999 to 92 cases for the period 1999–2001, *T. violaceum* representing 68 % of TC isolates [72]. This increasing number of *T. violaceum* infections was linked with immigration from northeastern Africa including Ethiopia where this species predominates in school-age children [73].

In other European countries *M. audouinii* is the emerging anthropophilic pathogen. For example, an outbreak was recently reported in Munich (Germany) in kindergartens and elementary schools that included 20 patients (16 children and 4 adults) [11]. In Switzerland, a retrospective study conducted in Zürich from 2006 to 2013 showed an increase in TC in parallel with an increase in *T. violaceum* isolates [74]. Interestingly, 30 % of the infected population originated from Eritrea, where *T. violaceum* is endemic. Another small outbreak of TC due to *M. audouinii* was reported in Zürich, involving three children attending the same after-school care facility [12]. The screening of all the classmates and family members led to the detection of five and three asymptomatic carriers, respectively. These reports show that asymptomatic carriers play an important role in transmission and should be detected to avoid the spread of infectious agents.

In Belgium, a study performed at the Free University of Brussels over a 1-year period (2001–2002) showed the predominance of anthropophilic dermatophytes that accounted for 89.3 % of TC infections, and the following species distribution: *M. audouinii* (39.3 %), *T. soudanense* (28.6 %), *T. violaceum* (18 %) and *T. tonsurans* (3 %) [75]. The same species were recorded by Detandt et al. in a survey conducted in Brussels schools and nurseries over a 2-year period [76]. In this study, up to 40 % of the screened materials from the environment, including bedclothes, deckchairs, toys, and play mats, were contaminated, demonstrating the place of indirect transmission and the need to establish specific measures of disinfection particularly if an infection has been detected. Activity reports for the years 2012 and 2013 published by the National Reference Center (NRC; data from NRC Liège and Leuven, Belgium) confirm the predominance of *M. audouinii* as the main agent of TC in Belgium. The findings of a national study conducted in 2013 attest to the high number of *M. audouinii* and *T. violaceum* infections recorded in Belgium. Molecular studies have demonstrated that different genotypes coexist according to the geographic area, the ethnic origin of the infected population and the degree of environmental adaptation of the strains [77].

In the UK, *T. tonsurans* has emerged as the leading cause of TC (and tinea corporis) accounting for 50 % to 90 % of the cases [78–81]. It affects mainly African and afro-Caribbean boys in urban areas. The management is crucial to avoid further spread of this highly contagious species. Outbreaks are still occurring in UK as reported very recently by Gray et al. [82] who investigated a large outbreak of TC and tinea corporis that occurred in 2011 in an urban day-care centre population.

In Spain, there is still a predominance of zoophilic dermatophytes such as *M. canis* which is the main agent of TC, followed by *T. mentagrophytes*, as demonstrated in a retrospective study covering 30 years from 1977 to 2006 [83]. However, the authors noted a trend towards anthropophilic species with an increase in *T. violaceum* and *T. tonsurans* species, but the proportion of migrants included was probably too low so that the change in the species distribution was not marked. Other reports attest to the rise in anthropophilic dermatophytes in Spain, particularly in urban areas where immigration (particularly from Africa) is high, as reviewed by del Boz-González [84]. For example, a survey performed in a school in Madrid showed an increase in *T. tonsurans* (12 of 33 infections), but *M. canis* remained the predominant species in TC with 16 of 33 infections [85]. The same species was recorded in studies conducted in different areas of Spain as the main agent of TC [35, 84]. *Trichophyton violaceum* also has been recorded as the

main agent of TC in Spain [86]. However, a cross-sectional study conducted among 1,305 children in Barcelona with the highest immigrant population showed only a small percentage of TC (0.23 %) [87]. The findings of these studies attest to an increase in anthropophilic aetiological agents of TC; however, no predominant position has yet been noted.

In France, *M. canis* was the main agent of TC up to the 1970s. However, since 1980, *M. audouinii* and *T. soudanense* have been predominant in connection with immigration from West Africa. A15-year retrospective study performed at the St Louis Hospital (Paris) from 1996 to 2010 showed that TC was the most prevalent clinical form (65.5 %), followed by tinea corporis (22.3 %) [88]. There was a consistent increase during the study period in *T. tonsurans* isolates that accounted for 19.1 % of TC isolates in 2010, in contrast to 44.4 % for *M. audouinii* and 36.1 % for *T. soudanense*. Patients with *T. tonsurans* infection originated equally from the Caribbean islands and from West Africa. A study performed in Créteil near Paris, from 1998 to 2002, showed a majority of anthropophilic species with *T. soudanense* and *M. audouinii* predominant. Although the majority of studies have been performed in the Paris suburbs, the results from other regions show another picture, with *M. canis* remaining the main agent of TC accounting for 60 % of the infections, but followed by anthropophilic species (32 %) such as *T. violaceum* (19 of 38 infections) and *M. audouinii* (15 of 38 infections) [89].

Laboratory Diagnostic Methods: New Developments

Various studies including a recent review by Nenoff et al. have investigated the methods currently used in the laboratory for the diagnosis of dermatophytosis [6••, 90]. This paper focuses only on molecular methods directly involving the sample or those used for fungal detection and identification. However, sample quality is first addressed.

Quality of the Sample

It is important to ensure that the quality of the sample is adequate for culture-based and molecular methods because of the potential repercussions of a poor quality sample on the final result. The sampling method and the device used for transport to the laboratory may be sources of contamination from environmental fungi. Indeed, and this is particularly relevant to the sampling of nail clippings, it is mandatory to clean the nails before sampling. First, it is recommended that the patient washes his or her hands or feet before coming to a consultation or just before sampling [91]. Secondly, the clinician should always first remove potential contaminants by cleaning the nails with a gauze impregnated with a 70 % alcoholic solution. This simple precaution helps avoid the development of environmental moulds which can be falsely interpreted as pathogens rather than contaminants by less skilled mycologists. After sampling, the use of a sterile device such as those used to collect urine samples, for example, are much better than the envelopes which are often use by dermatologists to send their samples including nails, skin material or hair. Indeed the use of a screw-top device avoids any further contamination after sampling and seems to be the best way to transport such biological material.

Current diagnostic methods for dermatophyte identification rely on macroscopic and microscopic observation of hyphae/spores from lesional material and from in vitro cultures [92]. These methods often give false-negative results, and they are also time consuming. Indeed, some culture-based identification methods can take more than 3 weeks. Conventional methods also require a high degree of specialist skill. Moreover, for some atypical and unusual isolates, in vitro characteristics are not easily interpretable. In some cases macro/microconidia are rare or not produced, making culture identification difficult, even impossible. Several external factors such as temperature and chemotherapy can also highly affect in vitro characteristics.

In recent years, the development of molecular biology techniques for the investigation of superficial mycosis has revolutionized the detection and identification of dermatophytes. PCR methods are intrinsically more specific and more accurate than conventional phenotypic methods as genotypic features are less likely to be affected by external features.

Molecular Tools for the Detection and Identification of Dermatophytes Using DNA Extracted Directly from Infected Tissues

For the successful treatment of onychomycosis as for other dermatophytoses, there is a need for accurate and rapid diagnosis. Culture has a low sensitivity (±75 %) and is time consuming, particularly for slow-growing dermatophytes such as *M. audouinii* and *T. verrucosum* (2–4 weeks). This is why a lot of recent work has focused on the detection of dermatophytes directly on sample material such as nails, hair and skin scrapings. Molecular tools offer the ability to rapidly diagnose dermatophytosis within 48 h. The power of these methods is increased by direct extraction from clinical specimens which allows time-consuming culture to be bypassed. Many "in-house" methods and less frequently commercial PCR tests have been developed, and are summarized in Table 2.

A modification of the PCR approach for biological material is the nested PCR in which conventional PCR is followed by another amplification of a smaller region inside the initial amplified fragment. Pan-dermatophyte nested PCR was evaluated in 2007 by Garg et al. for the diagnosis of onychomycosis. Primers targeting the pan-dermatophyte specific sequence chitin synthase I (CHSI) were used and compared with KOH microscopy. This team concluded that pan-

Table 2 Representative molecular biology methods for the identification of dermatophytes using DNA isolated from nail, skin and hair samples

Method	Key features	Target	Organisms identified	References
Nested PCR	Conventional PCR followed by amplification of smaller region inside the initial amplified fragment	Chitin synthase I	*T. rubrum*, *T. mentagrophytes*, *T. tonsurans*, *T. violaceum*	92, 93
		ITS1	*T. rubrum*, *T. mentagrophytes*	94, 95
PCR	Conventional real time PCR	ITS1 + ITS2	*T. rubrum*, *T. interdigitale*, *M. audouinii*, *T. violaceum*	96
		ITS1	Dermatophyte species	97, 98
		Chitin synthase I + ITS1	*T. rubrum*, *T. mentagrophytes*	99
	Commercially available kits			
	Dermatophyte PCR kit (Statens Serum Institute)	Chitin synthase I + ITS2	*T. rubrum*	104, 105
	FTD dermatophyte kit (Fast-track diagnostics)	No data available	*T. mentagrophytes* complex, *T. tonsurans*, *T. violaceum*, *T. rubrum* complex, *M. canis*, *M. audouinii*	No reference
	Dermatophyte real-time PCR test (IDEXX Laboratories)	No data available	*M. canis*, *M. gypseum*, *M. audouinii*, *M. ferrugineum*, *T. mentagrophytes*, *T. rubrum*, *T. schoenleinii*, *T. tonsurans*, *T. megninii*, *T. violaceum*	No reference
	DermaGenius Nail plus multiplex (PathoNostics)	ITS region	*T. rubrum*, *T. mentagrophytes* complex, *C. albicans*, *C. parapsilosis*	110
PCR-RLB	Membranes with immobilized probes hybridized to denatured PCR products. Detection with streptavidin peroxidase and chemiluminescence	ITS	*T. rubrum*, *T. interdigitale*, *T. mentagrophytes*, *T. violaceum*, *M. audouinii*, *T. tonsurans*, *M. canis*, *E. floccosum*, *T. verrucosum*	100
PCR ELISA	PCR amplification followed by hybridization of digoxigenin-labelled PCR products to an array of biotin-labelled probes	Topoisomerase II	*T. rubrum*, *T. interdigitale*, *T. violaceum*, *M. canis*, *E. floccosum*	101, 102
	Onychodiag (Bio Advance)	Hypervariable V4 domain of 18S rDNA	*T. rubrum*, *T. interdigitale*, *M. gypseum*, other nondermatophyte species	106
PCR-RFLP	Restriction enzymes producing different fragment patterns depending on the species after digestion	ITS + 18S	*T. rubrum*, *T. mentagrophytes*, *E. floccosum*	103

PCR polymerase chain reaction, *RLB* reverse line blot, *ELISA* enzyme-linked immunosorbent assay, *RFLP* restriction fragment length polymorphism

dermatophyte nested PCR could be considered as the gold standard for the diagnosis of dermatomycosis in nails, as the method shows a higher sensitivity than KOH microscopy [93]. In 2009, the same nested PCR (directed against CHTI) was used for direct detection and identification of dermatophytes in skin and hair. The sensitivity of the method was 83.8 % which was better than that of KOH microscopy and culture [94]. The same year, a nested PCR directed against internal transcribed spacer 1 (ITS1) was also reported to efficiently detect *T. rubrum* and *T. mentagrophytes* in nails and skin samples [95]. Nested PCR with *Trichophyton*-specific primers directed against ITS1 allowed investigators to detect the causal organism (*T. rubrum*) without resorting to culture from paraffin-embedded material [96].

In 2007, Arabatzis et al. reported the development of a multiplex real-time PCR for the direct detection of dermatophytes in nail and skin clinical specimens. ITS1 and ITS2 regions were the two targeted regions. Real-time PCR detected and correctly identified the causal agent in specimens with cultures positive for *T. rubrum*, *T. interdigitale*, *M. audouinii* or *T. violaceum*, and also identified a dermatophyte species in an additional seven specimens that were negative on microscopy and culture [97]. Later, Bergmans et al. reported the development of a single-tube dermatophyte-specific qPCR assay based on ITS1 sequences that allows the rapid detection and identification of 11 clinically relevant species within the three dermatophyte genera *Trichophyton, Microsporum* and *Epidermophyton* in nail, skin and hair samples within a few hours [98]. This real-time PCR method directed against ITS1 for use with skin, nails and hair was compared with conventional methods by Wisselink et al. The real-time PCR showed a sensitivity of 97 %, representing a significant increase in the detection rate for dermatophytes in clinical samples compared with culture [99]. In 2014, a multiplex PCR based on chitin synthase I and the ITS region was developed for detection and identification of *T. rubrum* and *T. mentagrophytes* in nail specimens. The sensitivity of the method was 97 % in contrast to 81.1 % for conventional methods. Specificity was also excellent [100•].

A PCR reverse-line blot assay (PCR-RLB) based on ITS sequences has been developed and was reported in 2008. It allows the detection and identification of nine relevant dermatophyte species in nail, skin and hair samples within 1 day (Table 2). Membranes containing immobilized oligonucleotide probes are exposed to denatured PCR products. After hybridization and several washes, detection is performed using streptavidin peroxidase and chemiluminescence. This method showed good sensitivity and specificity [101]. However, the method is labour-intensive and difficult to standardize in a diagnostic setting, with a high risk of amplicon contamination and false-positive results [98, 99].

A 24-h PCR ELISA method was developed a few years later for direct detection of five common dermatophyte species (*T. rubrum*, *T. interdigitale*, *T. violaceum*, *M. canis* and *E. floccosum*) in clinical samples. The method consists of PCR amplification of the topoisomerase II gene region, followed by hybridization of the digoxigenin-labelled PCR products to an array of biotin-labelled probes. The sensitivity of this method compared to fungal cultures is around 90 %. Specificity of the method is good as no cross-hybridization was observed with one of the five dermatophyte species or with human DNA [102]. A few years later, a German team evaluated the same PCR ELISA method in nails in comparison with conventional methods, and found a sensitivity of 79.0 % and a diagnostic specificity of 85.5 % [103].

Recently, PCR-RFLP has been developed to detect dermatophytes directly from nails, skin or hair. Elavarashi et al. associated a pan-fungal primer targeting the ITS region and optimization of PCR-RFLP using a dermatophyte-specific primer targeting the 18S ribosomal DNA unit for direct identification of dermatophytes from clinical specimens. *Trichophyton rubrum*, *T. mentagrophytes* and *E. floccosum* were successfully detected by this method but no strain variations were detected among these species [104•]. This method is, however, quite complex and laborious and not easily applicable for routine use.

In addition to these in house trials, that are sometimes difficult to reproduce, several commercial kits have been developed for molecular identification of dermatophytes. The Statens Serum Institute in Denmark has developed a duplex PCR kit to identify dermatophytes in general (pan-dermatophyte PCR) and specifically *T. rubrum* in nails within 5 h. Primers amplify chitin synthase I for the detection of all dermatophytes and ITS2 for the identification of *T. rubrum* [105]. Kondori et al. compared this method with conventional methods and found that the positive predictive value, negative predictive value, specificity and sensitivity of the duplex PCR were 93 %, 87 %, 94 % and 85 %, respectively, when confirmed by positive culture, microscopy or both. These values raise the interesting possibility of the use this method for routine investigations of onychomycosis [106]. The disadvantage of this method is that only *T. rubrum* can be identified from nails even if it is the main aetiological agent.

Fast-track diagnostics (Sliema, Malta) have developed the FTD dermatophyte kit which provides a two-tube multiplex PCR for the detection of *T. mentagrophytes* complex, *T. tonsurans*, *T. violaceum*, *T. rubrum* complex, *M. canis*, *M. audouinii* and *M. ferrugineum* by real-time PCR. The kit is designed for use with extracted nucleic acids from skin scale specimens, hair, nails, culture and swabs. IDEXX Laboratories (Wetherby, UK) uses the same technology for its real-time PCR kit. Still no studies have evaluated the efficiency of these products for the detection and identification of dermatophytes in dogs and cats.

Bio-Evolution (Bry-sur-Marne, France) has developed a commercially available RT-PCR kit called “Dermatophyte”

for the detection of dermatophytes in skin, hair and nail samples but without more precision regarding species. This kit is under clinical evaluation. A major criticism is that detection to the species level cannot be achieved, which is necessary for successful treatment of dermatophyte infections in skin and hair.

A multiplex real-time PCR kit (DermaGenius; PathoNostics, Maastricht, The Netherlands) targeting yeasts (*C. albicans*, *C. parapsilosis)* and dermatophytes including zoophilic and anthropophilic species has recently become commercially available (Table 2) [107]. The results of clinical evaluation are pending. Another commercially available kit for the diagnosis of fungal infections of the nails is Onychodiag (Bio Advance, Bussy-Saint-Martin, France). It was designed to detect dermatophytes using a PCR ELISA in nail samples. This test shows a sensitivity of 83.6 % and a specificity of 100 % [108].

In conclusion, the place of multiplex dermatophyte PCR in the identification of the causal agents in superficial fungal infections will increase in parallel with the development of simple, rapid and sensitive commercial kits. Because they are also more sensitive than the other conventional diagnostic methods, it is likely that laboratory diagnosis will reveal a greater prevalence of dermatophytes and/or *Candida* infections in superficial infections as reported by Wisselink et al. [99].

Molecular Tools for the Identification of Dermatophytes Using DNA Obtained from Isolated Cultures

Most of the recently described methodologies are based on DNA amplification and sequence analysis as summarized in Table 3. Indeed at the end of the twentieth century, PCR fingertyping emerged as a tool for molecular biology. Arbitrarily primed PCR/random amplified polymorphic DNA (RAPD) has been applied for dermatophyte DNA fingerprint generation. A Japanese group used this method with a random primer (OPAA11) in the arbitrarily primed polymerase chain reaction (AP-PCR). Except for *T. rubrum* and *T. gourvilli*, and three *T. mentagrophytes* varieties, most of the dermatophyte fungi investigated formed distinct DNA band patterns on gel electrophoresis (Table 3) [109]. Other investigations by this group have highlighted the interest in AP-PCR for dermatophyte identification [110–113]. Faggi et al. have also described the use of PCR fingerprinting for the identification of species and varieties of common dermatophytes. A single primer, the simple repetitive oligonucleotide GACA4, was used to generate DNA fingerprints. Species-specific profiles were obtained for *M. canis*, *M. gypseum*, *T. rubrum*, *T. ajelloi* and *E. floccosum* [114, 115].

Several years later, one specific study confirmed interest in this method for *E. floccosum* identification [116]. Using non-specific primers including (AC)10, (GTG)5, M13 core sequence and AP3, characteristic PCR profiles were generated for 17 dermatophyte species by Graser et al. [117]. Intraspecies variability could be distinguished for some *T. mentagrophytes* varieties but not for *T. tonsurans*. The commercial DiversiLab system (bioMérieux) is also able to generate DNA fingerprints for dermatophytes based on the rep-PCR principle: random primers hybridize on repetitive sequences interspersed in the fungal genome and produce fragments of different sizes depending on the species considered. Pounder et al. found that the performance of the DiversiLab system for identification of dermatophytes commonly encountered in a clinical mycology laboratory (*T. mentagrophytes*, *T. rubrum*, *T. tonsurans*, and *M. canis*) was excellent [118]. Although no reports have yet been published, this method has been shown to be able to distinguish intraspecies variabilities in the species *M. audouinii* [77].

Restriction fragment length polymorphism (RFLP) is an alternative method also used for dermatophyte identification. This method is based on the choice of several restriction enzymes that produce different fragment patterns after enzyme digestion according to species or strain. Kamiya et al. targeted the DNA topoisomerase II using RFLP. Six dermatophyte species (*T. rubrum*, *T. mentagrophytes*, *T. tonsurans*, *M. canis*, *M. gypseum*, and *E. floccosum*) were distinguished by this method [119]. Machouart et al. used the hypervariable V4 domain of the small ribosomal subunit 18S gene as target and showed that the method was able to distinguish nine different species of dermatophytes [120]. Jackson et al. used digestion of 18S rDNA and ITS regions. Among 50 random clinical isolates of *T. rubrum*, 14 individual RFLP patterns were recognized. Digestion of the amplified ITS products with the restriction endonuclease MVAI produced unique and easily identifiable fragment patterns for the majority of species. However, some closely related taxon pairs, such as *T. rubrum*, *T. soudanense* and *T. quinkeanum*/*T. schoenleinii* could not be distinguished [121]. The ITS region was again used by another team as the target with the RFLP method. The RFLP patterns obtained were specific for many species including *T. interdigitale*, *T. rubrum*, *T. violaceum*, *M. persicolor*, *M. audouinii*, *M. nanum* and *E. floccosum*, but were similar for some closely related species such as *M. canis*/*M. ferrugineum* [122]. Several other teams have used this method for dermatophyte identification [119, 123, 124].

The twenty-first century has seen the advent of genome sequencing technologies. A lot of work has shown that ITS between rRNA genes show a high degree of polymorphism sufficient to identify dermatophytes at the species level. These ITS sequences have the advantage that they are present in all dermatophytes. The amplification and sequencing of the ITS1 and/or ITS2 region is frequently used for dermatophyte identification. Multiple sequence alignment has demonstrated that some dermatophytes show specific barcode sequences in the ITS1 and/or ITS2 regions. Some studies have used the

Table 3 Representative molecular biology methods for the identification of dermatophytes using DNA isolated from fungal cultures

Method	Key features	Target	Organisms identified	Reference
PCR RAPD	Amplification of random segments of genomic DNA with single primer of arbitrary nucleotide sequence	Random primer OPAA11, OPD18, OPAA17, OPU15	*T. . verrucosum*, *T. schoenleinii*, *T. terrestre*, *T. mentagrophytes* complex, *T. concentricum*, *T. ajelloi*, *M. canis*, *M. gypseum*, *M. fulvum*, *M. audouinii*, *M. nanum*, *M. cookei*, *E. floccosum*, *M. persicolor*, *T. equinum*, *T. soudanense*, *T. tonsurans*, *T. violaceum*, *M. ferrugineum*	107, 108
		Random primer (unnamed)	*T. mentagrophytes* var. *mentagrophytes* and var. *interdigitale*	109
		Random primer (unnamed)	*T. rubrum*, *T. tonsurans*, *T. mentagrophytes*	111
		Random primer OPD18	*T. rubrum*, *T. soudanense*, *T. gourvilli*	110
		Random primer GACA 4	*M. canis*, *M. gypseum*, *T. rubrum*, *T. ajelloi*, *E. floccosum*	112, 113
		Random primer AC10, GTG5, M13, AP3	*T. verrucosum*, *T. rubrum*, *T. schoenleinii*, *T. terrestre*, *T. mentagrophytes*, *T. tonsurans*, *T. concentricum*, *T. ajelloi*, *M. canis*, *M. gallinae*, *M. gypseum*, *M. fulvum*, *M. audouinii*, *M. persicolor*, *M. vanbreuseghemii*, *E. floccosum*, *E. stockdaleae*	115
Rep-PCR (Diversilab® system)	Random primers hybridize on repetitive sequence on the fungal genome. Fragments of different size are produced depending on the species	All genome	*T. rubrum*, *T. mentagrophytes*, *T. tonsurans*, *M. canis*	116
PCR RFLP	Restriction enzymes producing different fragment patterns after digestion	DNA topoisomerase II	*T. rubrum*, *T. mentagrophytes*, *T. tonsurans*, *M. canis*, *M. gypseum*, *E. floccosum*	117
		Hypervariable V4 domain of 18S rDNA	*E. floccosum*, *M. canis*, *M. audouinii*, *T. soudanense*, *T. rubrum*, *T. mentagrophytes*, *T. tonsurans*, *T. violaceum*	118
		18SrDNA + ITS	*M. audouinii*, *M. canis*, *M. gypseum*, *M. persicolor*, *E. floccosum*, *T. mentagrophytes*, *T. terrestre*, *T. verrucosum*, *T. violaceum*	119
		ITS	*T. interdigitale*, *T. rubrum*, *T. violaceum*, *M. persicolor*, *M. audouinii*, *M. nanum*, *E. floccosum*	120
PCR and gene sequencing	PCR amplification of specific genes followed by sequencing and comparison of sequences in a database	ITS	*E. floccosum*, *T. rubrum*, *T. mentagrophytes* complex, *M. canis*, *M. gypseum*, *T. tonsurans*, *T. violaceum*, *M. praecox*, *M. audouinii*, *M. cookei*, *M. ferrugineum*, *M. nanum*, *M. persicolor*, *T. erinacei*, *T. schoenleinii*, *T. soudanense*, *T. terrestre*, *T. verrucosum*	123–127
		28S rRNA	*T. mentagrophytes* var. *mentagrophytes* and var. *interdigitale*	128
		Chitin synthase	*T. mentagrophytes* complex, *T. rubrum*	131
		DNA topoisomerase II	*T. rubrum, T. mentagrophytes, T. tonsurans, M. canis, M. gypseum*, *E. floccosum*	117
Oligonucleotide array	PCR amplification followed by hybridization of the digoxigenin-labelled PCR products to an array of oligonucleotides on a nylon membrane	ITS1 and ITS2	*T. verrucosum*, *T. schoenleinii*, *T. terrestre*, *T. mentagrophytes* complex, *T. concentricum*, *T. ajelloi*, *M. canis*, *M. gypseum*, *M. fulvum*, *M. nanum*, *M. cookei*, *E. floccosum*, *M. persicolor*, *T. equinum*, *T. soudanense*, *T. tonsurans*, *T. violaceum*, *M. ferrugineum*, *M. audouinii*	132

PCR polymerase chain reaction, *RAPD* random amplified polymorphism DNA, *RFLP* restriction fragment length polymorphism

amplification of both regions [125–127]. But it was noted that the ITS1 region is better for the differentiation of *T. rubrum*, *T. soudanense* and *T. violaceum*, given that these species have very similar ITS2 regions. In contrast, ITS2 seems better for the differentiation of *M. canis*, *M audouinii* and *M. ferrugineum* complex, as this sequence shows few single nucleotide polymorphisms [128, 129••]. One recent study compared the efficiency of several ITS primer pairs and concluded that ITS86F/ITS4 amplifying the ITS2 region is the most efficient primer pair leading to good amplification and identification rates [129••].

Amplification and sequencing of other specific gene regions can be used for dermatophyte identification such as 28S rRNA (allowing the distinction between *T. mentagrophytes* var. *mentagrophytes* and *T. mentagrophytes* var. *interdigitale*), chitin synthase I and DNA topoisomerase II [119, 130–133]. Li et al. designed an oligonucleotide array for dermatophyte identification [134]. The method is based on the ITS1 and ITS2 sequences of the rRNA genes, and allows the identification of 17 dermatophyte species (Table 3). The method consists of PCR amplification of the ITS regions using universal primers, followed by hybridization of the digoxigenin-labelled PCR products to an array of oligonucleotides (17-mer to 30-mer) immobilized on a nylon membrane.

Conclusions

Molecular methods applied to the detection and identification of dermatophytes have recently become increasingly available driven by the fact that they ensure fast and accurate identification. However, these methods have not been introduced in many clinical laboratories. Sequencing methods targeting the ITS region are the most popular techniques used for definitive identification of a fungal strain. However, only reference laboratories and laboratories with a large PCR platform can use this tool. In the near future, many smaller laboratories will use PCR assays for the detection of dermatophytes directly on nail, skin and hair samples because more and more commercial kits are being validated. The use of such methods will reduce the turn-around time from that seen with culture-based identification methods, particularly when full automation is achieved, and their use will also increase because they are much more sensitive than culture-based methods and because skilled technicians in mycology are scarce. However, these molecular methods applied directly on the sample cannot replace microscopic and histopathological examination particularly to assess the involvement of contaminants/pathogens such as *Fusarium*, or other nondermatophyte moulds, and also because a microscopic examination provides a faster result than any PCR assay.

New trends in epidemiology of dermatophytosis have been driven by two major factors: modern life in developed countries has promoted the increase in the prevalence of tinea pedis and onychomycosis throughout the world. This may also be enhanced by several risk factors amongst which the increase in the number of diabetics in the population is the most significant. The second important factor is the increase in TC due to anthropophilic dermatophytes in Europe. This has occurred in parallel to the increase in immigration from countries where TC is endemic. Consequently, it is important to be aware and to organize screening of children in schools where numerous migrants are present in order to avoid the spread of very contagious and difficult to treat dermatophytes such as *T. tonsurans* which can promote epidemics.

Compliance with Ethical Guidelines

Conflicts of Interest Marie-Pierre Hayette and Rosalie Sacheli declare that they have no conflicts of interest.

Human and Animal Rights and Informed Consent This article does not contain any studies with human or animal subjects performed by any of the authors.

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