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Tracking down mycoses by gene detection – new and classical pathogens

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Summary

Polymerase chain reaction (PCR) is the most important milestone in mycological diagnostics in recent years. It is not only precise and fast. In contrast to culture, which is falsely negative in about half of all nail samples, PCR has a high sensitivity because it is based on gene detection and is therefore independent of the cultivation of the pathogen and its phenotype. Since it also identifies spore DNA, it is able for the first time to indicate the microbiological end of a therapy. If the result is negative, it is the strongest microbiological argument against the presence of mycosis.

PCR can be carried out in any dermatological practice and is therefore an invaluable asset for maintaining mycological diagnostics in dermatology as a whole.

Keywords

Polymerase chain reaction (PCR), dermatophytes, diagnostics, therapy.

Introduction

The spectrum of cutaneous mycoses and their pathogens is now more diverse and wide-ranging than ever before. Their spread is driven by tourism, migration and animal trade (1). In addition to classical pathogens such as T. rubrum, an increasing number of new fungi from all over the world are coming into the focus of dermatological practice (2). Some dermatophytes are very contagious, while others are highly virulent or can cause suppuration or severe inflammation (3).

Many mycoses are very persistent due to the pathogenic spores and prone to relapse due to lack of immunity (4). At the same time, the possibilities to diagnose and successfully treat mycoses have never been as good as they are today. There have been immense advances in therapy (5), but especially in diagnostics thanks to PCR (6).

The most important classical and new dermatophyte species and their main characteristics are presented here, together with the message that every mycosis is curable if diagnosed correctly.

T. rubrum

The "red fungus" was discovered by *Castellani* in Ceylon in 1911 (7). Today, with a prevalence of one billion people, it is by far the most important global dermatophyte and thus one of the most significant pathogens of our time (8). The fact that it causes two widespread diseases, tinea pedis and onychomycosis, has contributed significantly to this. It also has very resistant spores, does not convey immunity and is thus ideally adapted to human civilisation (4).

Because of these characteristics, it was no coincidence that it displaced the two dominant pathogens until World War II, T. interdigitale and E. floccosum (9).

The "white fungus" T. interdigitale was discovered by *Marie Kaufmann-Wolf* at the Charité in 1914 (10). E. floccosum, the causative agent of epidermophytosis, was about as common as favus in Central Europe until the end of the 19th century. The increasing resistance of T. rubrum to terbinafine is new (11).

Phenotypic characteristics of T. rubrum: anthropophilic dermatophyte, grows slowly (3–4 weeks), forms reddish pigment rings, which may be absent, with mostly sterile aerial mycelium. The germ is generally very difficult to culture. For the most important pathogen in dermatology, PCR is therefore a particularly great asset (Fig. 1).

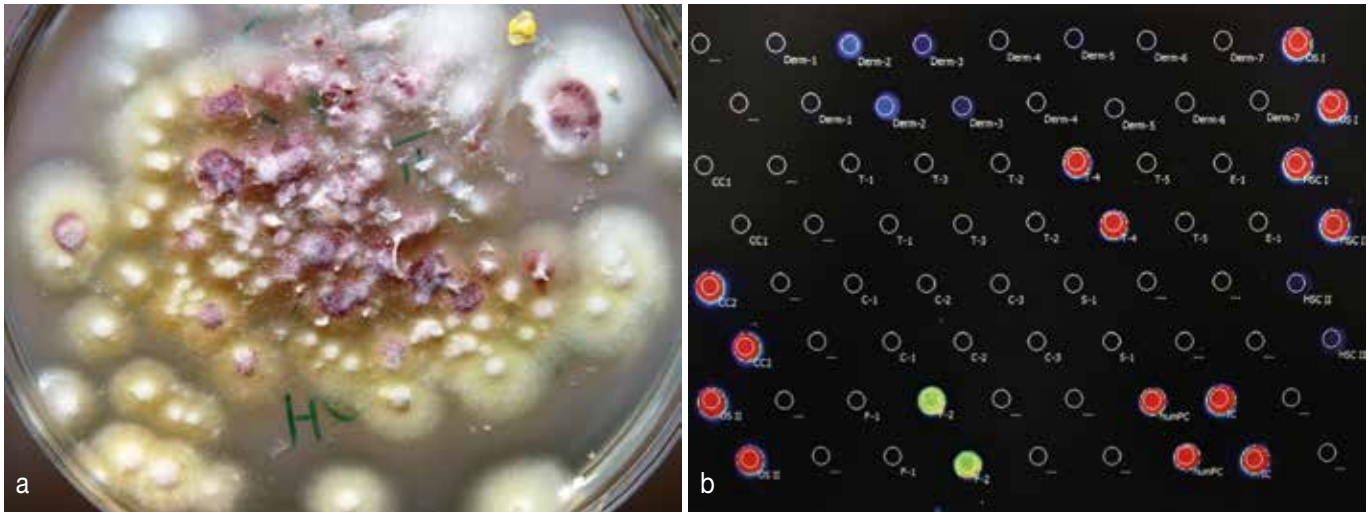
Pathogen changes

But T. rubrum was not the only species that led to a change in mycology and thus also to a change in the spectrum of diseases. Examples include the return of tinea capitis and onychomycosis in children (12, 13), to which the corona measures have also contributed (14), particularly the stay-at-home orders during lockdown and loneliness had an accelerating effect. For instance, the number of pets, including many rabbits and guinea pigs, increased by one million to a total of 35 million in the first year of the pandemic. Children, but also elderly people, sought comfort from their new companions.

Migration remains a significant epidemiological factor, which has caused several pathogens to return to Europe. Exotic animals such as white-bellied hedgehogs or hairless guinea pigs, so called skinny pigs, as well as tourism brought entirely new pathogens to us. All these pathogens arrived before the corona pandemic and are now endemic in Germany.

T. soudanense

This apricot-coloured pathogen is a prime example of how dermatophytes have no longer distinct geographical distributions. The fungus particularly



Figs. 1a and b: T. rubrum, one of the most common pathogens worldwide a) in culture and b) polymerase chain reaction (PCR) (EUROArray Dermatomycosis). The typical pigment of the culture may be missing and about 50% samples do not grow. PCR is independent of this and always positive in the case of infection

affects children of migrants from Africa. However, sporadic infections already occurred in the GDR, which shows that not even fungal pathogens can be confined by walls (16) and that, due to microbial globalisation, the germ is no longer only at home in countries such as Sudan, where its name comes from.

Phenotypic characteristics: anthropophilic, grows very slowly, forms apricot-coloured pigments and crossing-over hyphae (Fig. 2 and 3), hair infection: endotrichous, black light: negative.

T. violaceum

This purple-coloured fungus is considered the twin of T. soudanense. Genetically, both species are almost identical, which is why they were considered one species for a while. However, their phenotype is impressively different (Fig. 2). This germ also mainly infects children's heads and today also comes predominantly from Africa.

It originated in the Mediterranean region, from where it reached Latin America and Eritrea in the course of the migration of many millions of Italians between 1876 and 1915 (17), from where it returned to its place of origin (18).

Phenotypic characteristics: anthropophilic, grows very slowly, produces deep red pigments. The mycelium is mostly sterile, hair infection: endotrichous, black light: negative.

T. tonsurans

There are no longer any limits to the worldwide spread of skin fungi, as this pathogen also proves. From a clinical point of view, it is a fungus with many "talents". From onychomycosis to mycosis of the head, it can cause all forms of tinea. Besides the classic image of a tonsure, which gave the pathogen its name, it can also cause purulent infections. The ways of transmission are just as diverse. Known as "mat fungus" and the causative agent of tinea corporis gladiatorum, it originally migrated from the USA to Germany, where it is still found in all martial arts (19).

The fungus is currently coming to Germany through immigration from Africa and Turkey. It is also increasingly encountered in barber shops, as our own case studies have shown. More worrying, however, are the epidemics in day-care centres and schools (20).

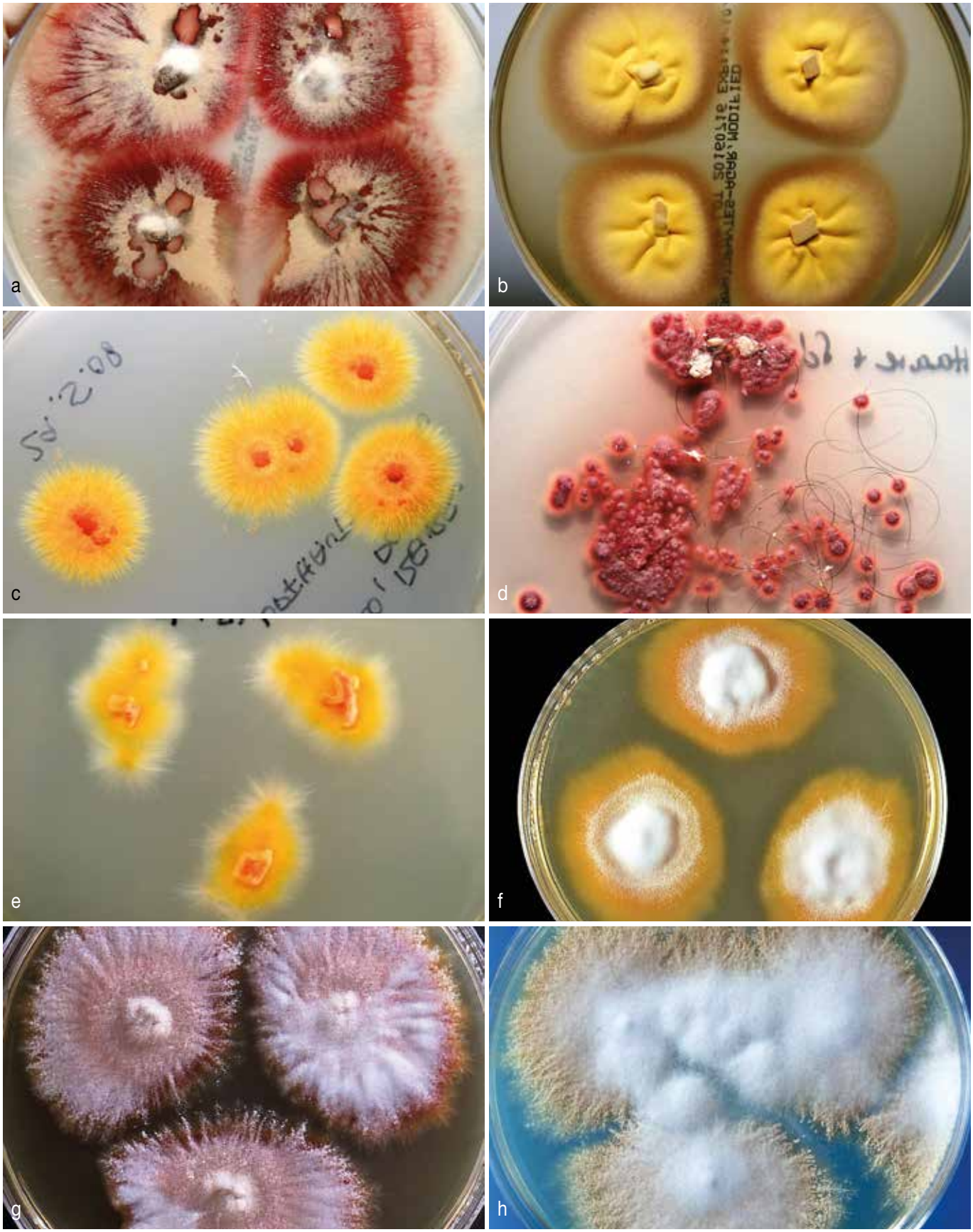
Phenotypic characteristics: anthropophilic dermatophyte, grows slowly (approx. 3 weeks), forms velvety sulphur-yellow colonies (Fig. 2), cylindrical macroconidia, pleomorphic microconidia and large chlamydospores. Hair infection: endotrichous, black light: negative.

M. audouinii

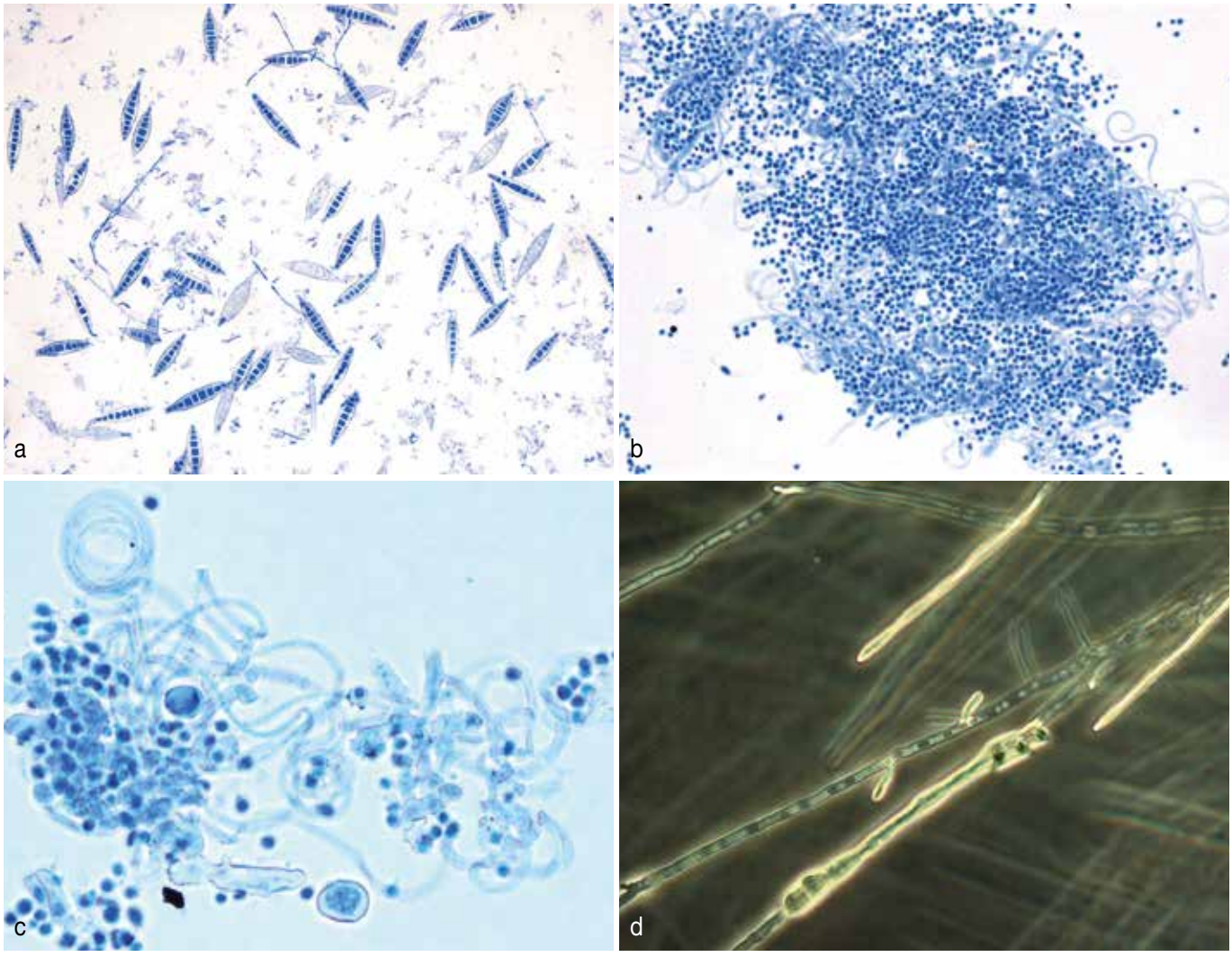
The causative agent of anthropophilic tinea corporis et capitis microsporica is still remembered as the causative agent of "orphanage disease". The pathogen had been largely displaced and is now returning to its origin in Europe, mainly through migration from Africa (2). This pathogen can also be transmitted by scissors.

A significant source is, among other things, hair dressing on the beach of southern holiday paradises. As the fungus is highly contagious, major outbreaks occur time and again, such as in Bonn between 2015 and 2018 (21). Although there is no obligation to report mycoses (22) in Germany, even individual cases of microsporiasis should be reported to the public health department.

Phenotypic characteristics: anthropophilic dermatophyte, grows relatively slowly (up to 14 days), yellow, later greyish-white colonies, hair infection: ectotrichous, black light: green, forms



Figs. 2a–h: Diagnostically relevant pigment formations for conventional determination of dermatophytes. a) T. equinum, b) T. tonsurans, c) T. soudanense, d) T. violaceum, e) M. canis, f) T. erinacei, g) N. persicolor, h) N. gypsea



Figs. 3a–d: Diagnostically relevant microstructures (spindle-shaped macroconidia, spiral hyphae and reflex hyphae) for conventional determination of: a) M. canis, b) T. mentagrophytes type variation granulosum, c) T. mentagrophytes type VII, d) T. soudanense

terminal chlamydospores ("matchsticks") and bizarre, pectinated macroconidia.

M. canis

The causative agent of zoophilic tinea corporis et capitis microsporica is also experiencing a renaissance. It is mainly brought back from southern Europe by holidaymakers as an unwanted souvenir from contact with stray cats. The animals are usually not visibly infected.

However, M. canis is obligate pathogenic for humans, which means that any contact with an infected animal leads to infection, which can then spread rapidly over the entire integument.

The pathogen is transmitted from person to person. There have even been outbreaks in neonatal units due to infected staff (23). Furthermore, the chain of infection does not end with humans. Cats that have stayed at home can easily become infected from their infected owner who has returned from holiday. Stray cats in Germany have now also been identified as carriers (24), which is why Berlin, for example, enacted a cat protection ordinance (25).

Also worth mentioning is a geophilic Microsporum species, now called Nannizzia gypsea (Fig. 2), which occasionally leads to tinea corporis et capitis in children. Table 1 lists the most important dermatophyte species with their sources.

Phenotypic characteristics: zoophilic dermatophyte, grows relatively fast (7 days), golden yellow colonies. Hair infection: ectotrichous, black light: green, forms pointed spindle-shaped macroconidia (Fig. 3).

T. schoenleinii

This germ has achieved cult status in mycology. The first pathogen in medical history, discovered by *Johann Lucas Schönlein,* is also known outside the field of mycology. The discovery that a disease such as favus is caused by a pathogen is considered the birth of medical microbiology (26). *Schönlein's* private assistant *Robert Remak* succeeded in cultivating the pathogen on apple

slices. Out of gratitude, he named it after his teacher (27).

Favus is a special form of tinea capitis and was one of the most common diseases in Europe in the mid-19th century. For a long time, it seemed as if favus had died out in Germany. Only a few foci in the regions of Westphalia and Göttingen were never completely eradicated, which originally came from immigrants from Russia (17).

A re-emergence of T. schoenleinii in Europe must be expected. In North Africa, it is already at the doorstep (28). Phenotypic characteristics: anthropophilic, grows slowly, colonies with crevices, creamy consistency, chlamydospores, hyphae like "antlers", "candlesticks", hair infection: endotrichous, black light: light green.

T. benhamiae and T. mentagrophytes (formerly variatio granulosum)

T. benhamiae was introduced from Japan by "skinny pigs". This breed is very questionable from an ethical point of view and an offence against nature, as these animals are very susceptible to infections.

The fungus quickly conquered domestic guinea pig breeding (29) and within a short time became the most common zoophilic dermatophyte in children (30). It can cause severe infections with suppuration (Fig. 4).

However, it has not displaced the previously dominant indigenous rodent pathogen T. mentagrophytes, so that these animals can now carry two pathogenic skin fungi.

Phenotypic characteristics: both pathogens grow relatively quickly (10–14 days), form white (T. mentagrophytes) to yellowish pigmented colonies (T. benhamiae, Fig. 5), microconidia in clusters, spiral hyphae and cigar-shaped macroconidia. Hair infection: ectotrichous, black light: negative.

Table 1

The most important dermatophyte species and their sources

Pathogen	Source
T. rubrum	Human
T. interdigitale	Human
M. canis	Dog, cat
M. gypseum (new as *N. gypsea)*	Soil
M. audouinii (pathogen of the "orphanage disease")	Human
T. benhamiae	Guinea pig, rabbit
T. mentagrophytes, previously *variatio granulosum*	Guinea pig, rabbit
T. mentagrophytes, genotype VII ("Thailand fugus")	Human
T. indotinea	Human
T. verrucosum	Cattle (especially calves)
T. erinacei	Hedgehog
T. equinum	Horse
T. tonsurans	Human
T. violaceum	Human
T. soudanense	Human
T. schoenleinii (causative agent of "favus")	Human
E. floccosum	Human

T. verrucosum

Calf lichen (Fig. 4 and 5) also belongs to the "pet dermatomycoses". Since calves are no longer vaccinated against bovine trichophytosis and farms have become an increasingly popular holiday destination, the pathogen has experienced a rapid upswing. In the former German Democratic Republic, it was almost eradicated due to the then compulsory vaccination – a proven means of repressing this pathogen. T. verrucosim is the only dermatophyte that grows at 37 °C, penetrates deep into the tissue and thus activates the immune system. For these reasons, vaccination is extremely useful and sustainable. If the infection is recognised too late, the consequences can be severe: fever, swelling of the lymph nodes and scarring (31).

Figs. 4a and b: Misidentified mycoses in children caused by a) T. benhamiae (under antibiotic therapy) and b) T. verrucosum (after triple surgery for suspected pyoderma gangraenosum)

In the boy in Figure 4, despite the positive medical history (reference to the grandparents' farm), mycosis was not considered and several operations were performed. Such surgical interventions are not uncommon. This may be partly because mycology has always been the poor cousin of infectiology and has never had its own academic chair.

Phenotypic characteristics: zoophilic dermatophyte, grows extremely slowly within up to 5 weeks, better at 37 °C, then forms maximally small, warty colonies (Fig. 5), hair infection: ectotrichous, black light: negative, arthrospores from hyphae that disintegrate into chains, chlamydospores.

T. erinacei and T. equinum

These pathogens also enrich the pathogen spectrum due to animal trading. African white-bellied hedgehogs (T. erinacei), for example, are becoming increasingly popular, as are ponies and other mounts, which can be infected with a particularly beautiful fungal pathogen (T. equinum, Fig. 2).

T. mentagrophytes type VII (Thailand fungus)

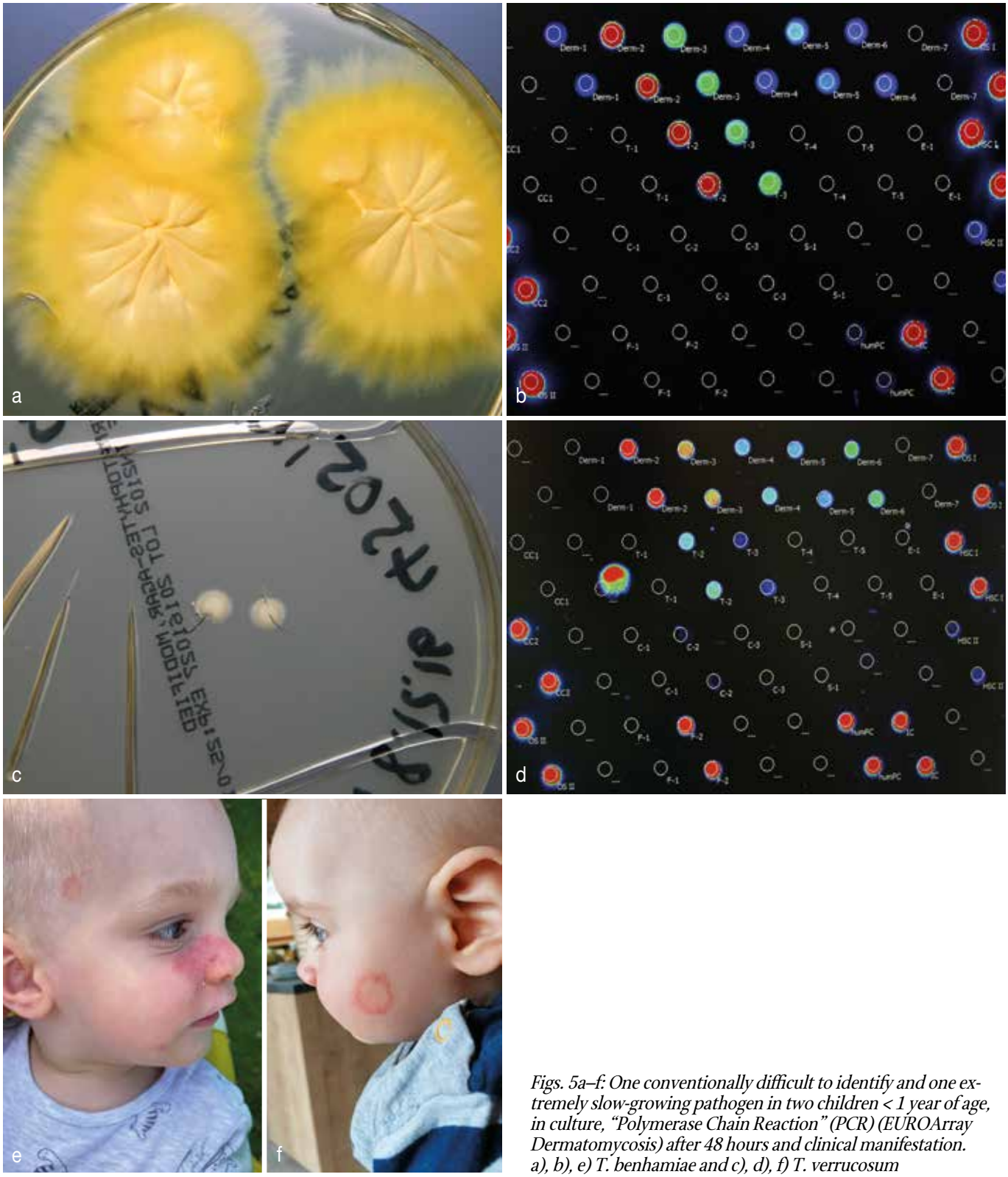
The "Thailand fungus", a novel pathogen with unusually high clinical virulence, originates from the country of the same name (32). Those who have seen it will always remember it because of its impressive clinical symptoms (Fig. 6). Since it is predominantly transmitted through sexual contact, it is the first dermatophyte to belong to the category of sexually transmitted diseases (STD) (33). Due to the increase in illegal prostitution, this pathogen also flourished during the corona pandemic.

Phenotypic characteristics: anthropophilic, grows moderately fast, usually forms spectacular, wine-red colonies, spiral hyphae, chlamydospores, reminds of T. tonsurans.

T. indotineae (previously T. mentagrophytes type VIII)

This novel pathogen also arrived in Germany before the corona pandemic (Fig. 6). It originates from India, where it is by far the most common dermatophyte. It is characterised by extensive and persistent skin manifestations that are difficult to treat (34). A striking feature is its high resistance to terbinafine (35). The pathogen has since become endemic in Germany.

Phenotypic characteristics: anthropophilic, grows moderately fast, mostly unspectacular colonies, occasionally forms spirals.



Figs. 5a–f: One conventionally difficult to identify and one extremely slow-growing pathogen in two children < 1 year of age, in culture, "Polymerase Chain Reaction" (PCR) (EUROArray Dermatomycosis) after 48 hours and clinical manifestation. a), b), e) T. benhamiae and c), d), f) T. verrucosum

Diagnostics and therapy

What all dermatophytes have in common is that, despite their sometimes high virulence and persistence, they are more curable than ever. The greatest progress has been made in diagnostics – the basic prerequisite for successful therapy.

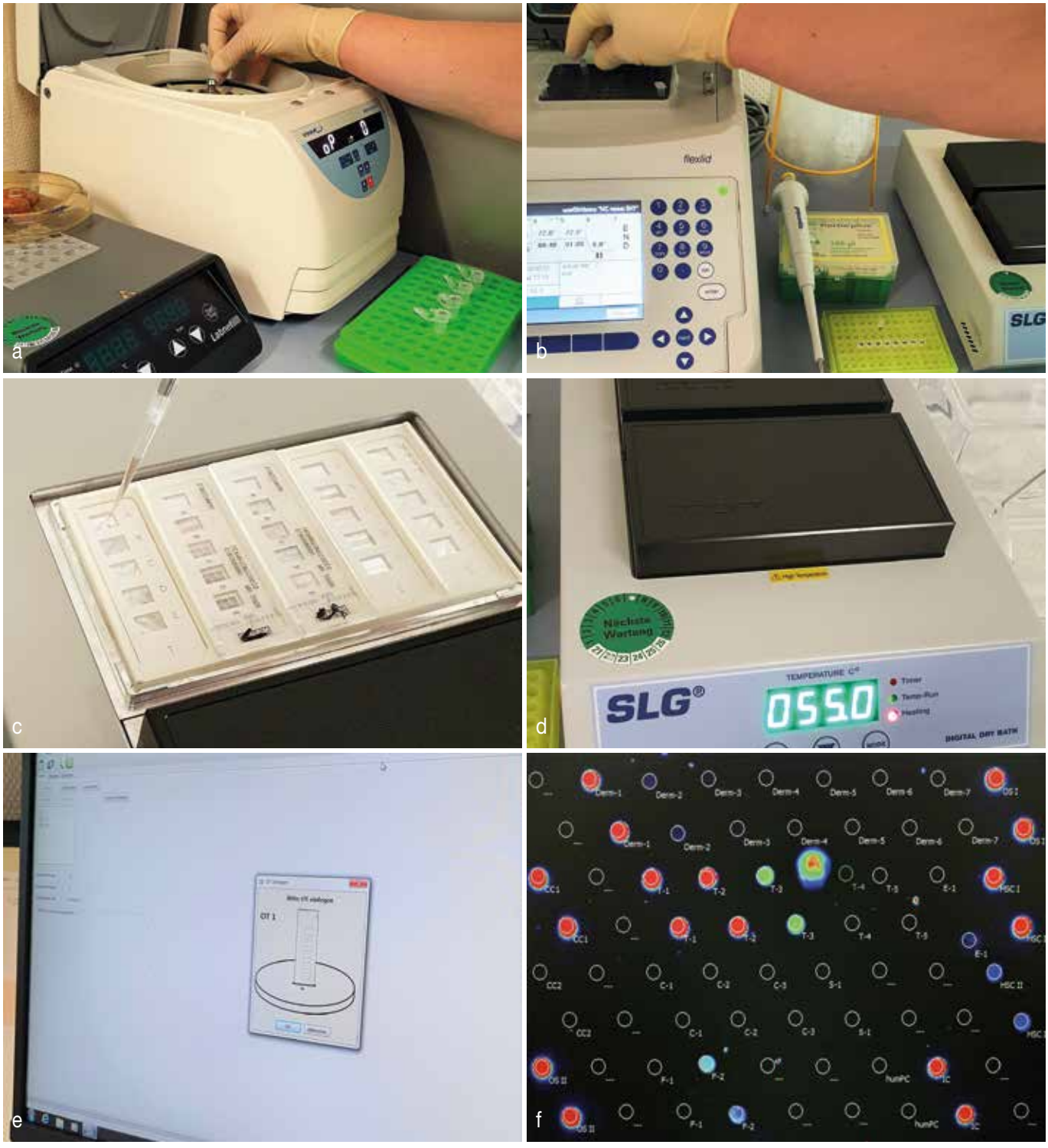
PCR revolutionises fungal diagnostics

The principle is very simple. Like any other "culprit", fungal pathogens also

Figs. 6a–f: Clinical manifestations and cultures of new species. a), c) and e) T. mentagrophytes type VII ("Thailand fungus") in a young woman and b), d), f) T. indotinea in a traveller returning from India

leave a gene trace (their DNA), at the site of action, i.e. the site of infection: in the nail, on hair or in skin scales. The first gene probe for the detection of T. rubrum was developed at the Charité in Berlin in 1999 and published in the "British Journal of Dermatology" (36).

However, decades passed before a practical technique was developed that encompassed the entire spectrum of today's mycology: The EUROArray



Figs. 7a–f: Steps of polymerase chain reaction (PCR): extraction of DNA, multiplication in thermal cycler, hybridisation on slides and computer-based identification of the pathogen. f) Result: T. mentagrophytes type VII

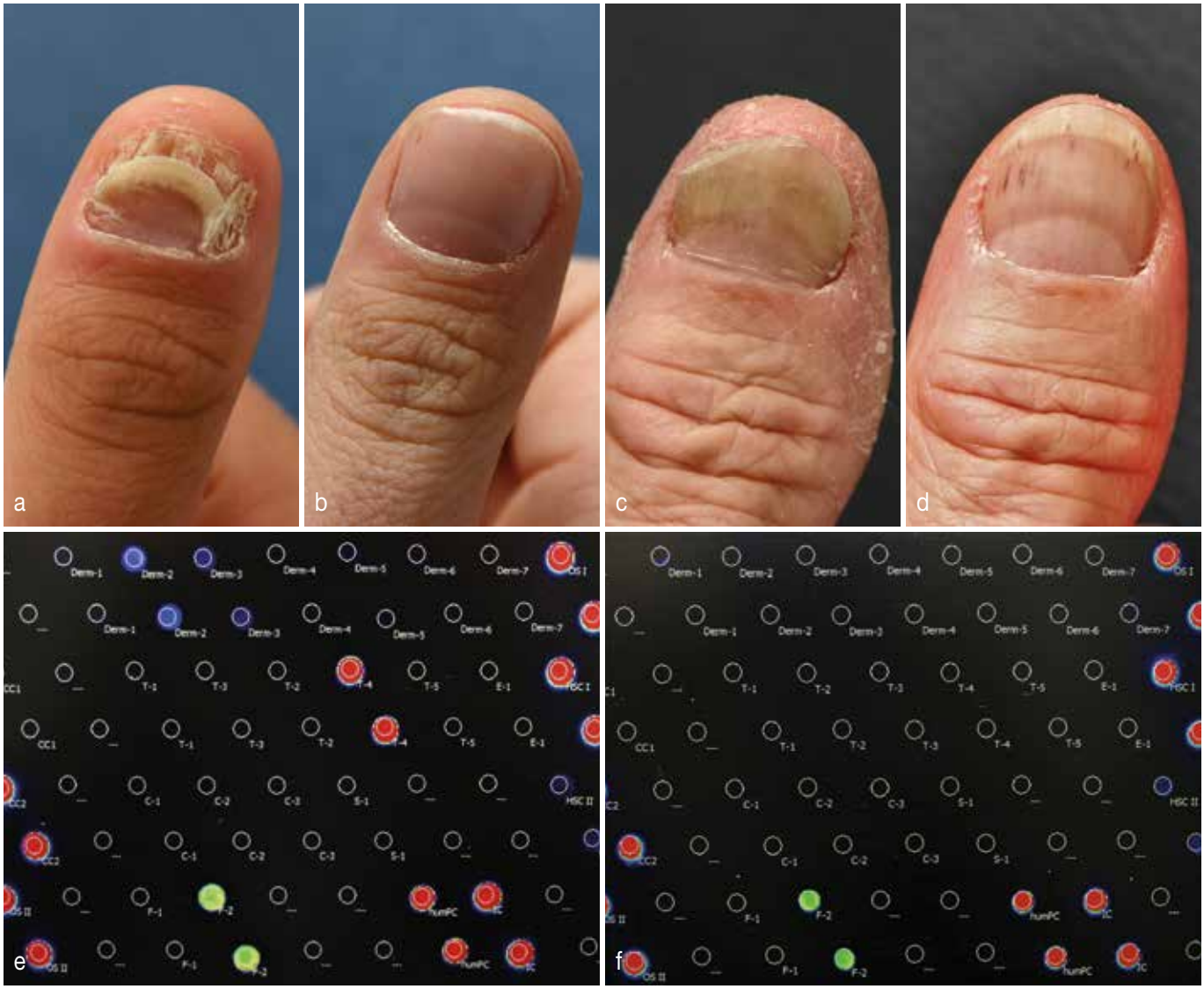
Dermatomycosis, which has a high scientific reputation (37).

The microarray is a slide method based on gene probes with known fungal sequences that are combined with those of the pathogen to be detected. The test procedure consists of 3 steps (Fig. 7):

1. Extraction of the pathogen DNA from the clinical sample.

2. Amplification of the DNA in a thermal cycler, the actual PCR.

3. Hybridisation of the multiplied genes with pathogen-specific probes on a prepared slide followed by



Figs. 8a–f: Therapy monitoring using polymerase chain reaction (PCR) in a) and b) an 8-year-old schoolboy and c) and d) a 70-year-old rock guitarist: a) and c) before and b) and d) after therapy. The microbiological diagnosis was made under therapy with terbinafine. Result in the EUROArray Dermatomycosis: T. rubrum, e) before and f) after the end of therapy with fluconazole (boy) and itraconazole (guitarist)

computer-based identification of the species.

In Germany, the PCR test is fully reimbursed by private health insurance companies. Work is in progress on a remuneration scheme for physicians accredited by statutory health insurance (personal communication *Prof. Nenoff*).

Advantages of PCR

1. The most common pathogen by far, T. rubrum, does not grow in about 50% of samples, despite sophisticated sampling.

2. PCR is also possible during therapy.

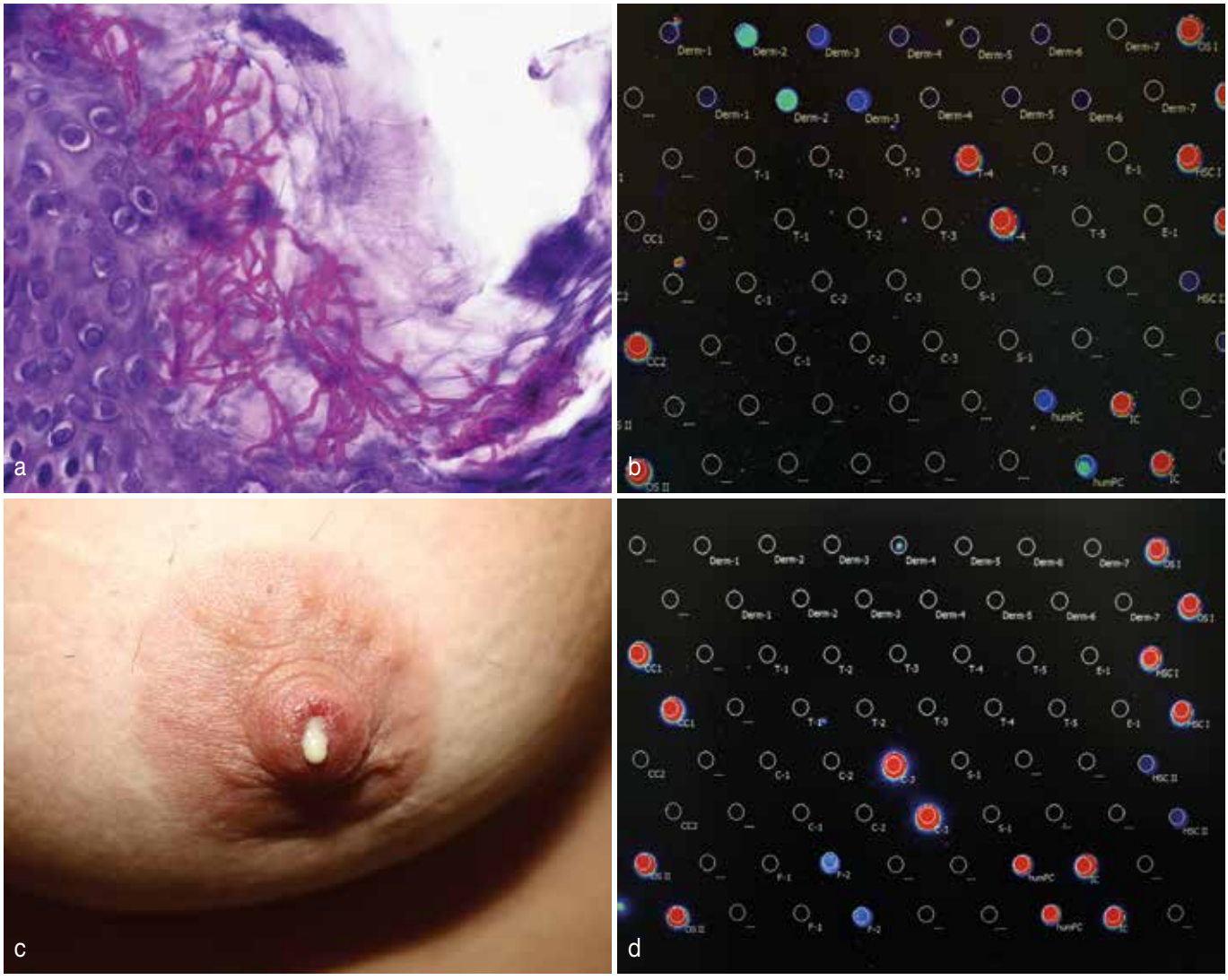
3. Some dermatophytes grow extremely slowly. The PCR delivers the result after only 48 hours.

4. PCR is also useful for pathogens that are difficult to identify by conventional means. There are cases were all structures required for successful conventional diagnostics are missing (Fig. 1–3). PCR is independent of this. It can also be performed from culture.

5. The EUROArray Dermatomycosis has a broad pathogen spectrum and also covers very rare pathogens such as T. simii, T. quinckeanum, T. concentricum, N. persicolor, M. ferrugineum, as well as C. albicans and the only mould that is pathogenic for nails, S. brevicaulis.

6. The acceptance of PCR is also high among patients, to which the Corona pandemic has contributed significantly.

7. PCR is also able to detect spore DNA and it is thus possible for the first time



Figs. 9a–d: EUROArray Dermatomycosis of a) and b) T. rubrum from a paraffin block and c) and d) C. albicans from breast milk. C. albicans does not grow because of the antibodies in breast milk. Histology does not allow identification of the species

to accurately determine the end of a therapy microbiologically (Fig. 8).

8. A negative PCR is also a valuable finding. It is the strongest microbiological argument against mycosis, as about 50 % of nail changes have another cause, which most patients are not aware of (38).

9. PCR replaces pathogen culture, as well as microscopy and histology, which are time-consuming. Requirements imposed by German authorities for the latter are therefore not applicable.

10. PCR is possible from any sample material , e.g. paraffin blocks, brush swabs, dandruffs, hair, nails or body fluids (Fig. 9).

11. PCR can be performed in any dermatological practice.

12. PCR is also financially worthwhile.

In summary, modern pathogen diagnostics belongs to dermatology, starting from the qualified collection of the sample to the clinically plausible interpretation of the laboratory results.

PCR is also recommended as the state-of-the-art diagnostic method in the new guideline on onychomycosis (pers. communication from the chairman *Prof. Nenoff).*

Therapy

The concept of therapy has been greatly simplified and is basically the same for all mycoses (3). It is based on local treatment with highly and broadly effective antimycotics such as ciclopirox, bifonazole or sertaconazole. For every mycosis there is the ideal formulation, from water-soluble nail polish to shampoo.

Systemic therapy has undergone revolutionary changes, both in galenics and in the way it is administered, with only one dose per week, until the patient is clinically and microbiologically cured. With itraconazole, the greatest pharmaceutical advance has been achieved

Table 2

The most important human pathogenic fungal species and their systemic treatment

Preparation	Fluconazole	Terbinafine	Itraconazole*
Pathogen	*T. rubrum* *T. tonsurans* *E. floccosum* *M. canis* *M. audouinii* *C. albicans* *M. furfur*	*T. rubrum* *T. interdigitale* *T. mentagrophytes* *T. benhamiae* *T. verrucosum* *T. equinum* *T. erinacei* *T. tonsurans* *T. violaceum* *T. soudanense* *T. schoenleinii* *E. floccosum*	*T. rubrum* *T. interdigitale* *T. mentagrophytes* *T. mentagrophytes Typ VII* *T. indotinea* *T. benhamiae* *T. verrucosum* *T. equinum* *T. erinacei* *T. tonsurans* *T. violaceum* *T. soudanense* *T. schoenleinii* *M. canis* *M. audouinii* *E. floccosum* *C. albicans* *M. furfur* *S. brevicaulis***
Adults (children > 12 years)	200 mg	250 mg	200 mg
Children (7–12 years)	100 mg	125 mg	100 mg
Children (under 7 years)	50 mg	62,5 mg	50 mg
Wash-in phase	3–7 days daily, then one dose per week		

* Dosage applies to the galenically new itraconazole (Itraisdin®). ** The only human pathogenic mould fungus of the skin and nails

through its incorporation into a stable polymer (39).

The substance also has the broadest spectrum of action, which is an advantage in the case of immediately necessary empirical therapy.

The scheme in Table 2 applies to all mycoses for which systemic therapy may be considered: onychomycosis, pityriasis versicolor (causative agent: M. furfur), intertrigo, chronic nappy rash, recurrent vaginal candidiasis (mostly C. albicans), tinea corporis et faciei.

Systemic therapy is obligatory for tinea capitis (40), even in young children, who are almost exclusively affected, in increasing numbers (Fig. 5). Here, the new itraconazole can be removed from the capsule and mixed with something more palatable (14).

Therapy intervals of one week are not only well tolerated, they also do justice to the biology of the pathogens. This is because they enable the mostly resistant fungal spores to form germ tubes, which are the Achilles' heel of the pathogens from a medicinal point of view.

In this phase, the hyphae generate exponentially many points of attack for the systemic antimycotics. This also explains why these drugs are so well tolerated, because they only work there – only fungi have these structures (ergosterol). Other organisms and cells in the body thus remain unaffected.

Conclusion

With its novel and fascinating pathogens, mycology is not only one of the most beautiful but also one of the most successful fields of infectiology.

Thanks to PCR and simple, universal, well-tolerated treatment options, the prospects for a sustainable cure are better today than ever before.

Conflicts of interest

The author has been working in his own practice with the genetic diagnostics presented here since 2018.

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