REVIEW

Dermatophytes: host-pathogen interaction and antifungal resistance *

Dermatófitos: interação patógeno-hospedeiro e resistência a antifúngicos

Nalu Teixera de Aguiar Peres [1]
Fernanda Cristina Albuquerque Maranhão [2]
Antonio Rossi [3]
Nilce Maria Martinez-Rossi [4]

Abstract: Cutaneous mycoses are among the most common infections in humans and have become an important public health issue because they cause invasive infections in immunocompromised patients. During the infectious process, dermatophyte-host interactions trigger specific metabolic adaptations that allow the pathogen to adhere to and penetrate the host tissue, scavenge nutrients, and overcome the host defense mechanisms. This metabolic shift and the interplay between metabolism, morphogenesis and stress response are important factors that have been extensively studied in several pathogens. Host cells also respond to the pathogen stimuli by activating intracellular signaling pathways that trigger the immune response against the infectious agent. The comprehension of the molecular aspects of these responses may help to establish new therapeutical strategies. In this review, different aspects of the biology of dermatophytes are addressed, with emphasis on the dermatophyte-host interaction and the mechanisms of antifungal resistance.
Keywords: Antifungal agents; Dermatomycoses; Drug resistance, fungal; Host-pathogen interactions

Resumo: As micoses cutâneas estão entre as infecções mais comuns em humanos e se tornaram um importante problema de saúde pública, principalmente por causarem infecções invasivas em pacientes imunodeprimidos. Durante a infecção, a interação dermatófito-hospedeiro desencadeia adaptações metabólicas específicas que permitem aos patógenos aderirem e penetrarem no tecido, remodelando seu metabolismo para captar nutrientes e superar os mecanismos de defesa do hospedeiro. Esse remodelamento metabólico e a inter-relação entre metabolismo, morfogênese e resposta ao estresse são importantes fatores que estão sendo intensamente avaliados em diversos patógenos. As células do hospedeiro também respondem aos estímulos do patógeno, ativando vias de sinalização intracelular que culminam no desencadeamento de uma resposta imune contra o agente infeccioso. O entendimento molecular dessas respostas metabólicas pode ajudar no estabelecimento de novas estratégias terapêuticas. Nesta revisão, são abordados diferentes aspectos da biologia dos dermatófitos, com ênfase na interação dermatófito-hospedeiro e nos mecanismos de resistência a antifúngicos.
Palavras-chave: Antimicóticos; Dermatomicoses; Farmacorresistência fúngica; Interações hospedeiro-patógeno

Approved by the Editorial Board and accepted for publication on 17.06.2010.
* Work conducted at the Department of Genetics, Faculty of Medicine of Ribeirao Preto, University of Sao Paulo - São Paulo (SP), Brazil.
Conflict of interest: None / *Conflito de interesse: Nenhum*
Financial funding: / *Suporte financeiro: FAPESP, CNPq e CAPES*

[1] Ph.D. in Immunology from the Faculty of Medicine of Ribeirao Preto, University of Sao Paulo; Scholarship holder, postdoctorate student, Sao Paulo Research Foundation (FAPESP) - Sao Paulo (SP), Brazil.
[2] Ph.D. in Genetics from the Faculty of Medicine of Ribeirao Preto, University of Sao Paulo; Scholarship holder, postdoctorate student, Federal University of Ceara – Fortaleza (CE), Brazil.
[3] Full Professor at the Department of Biochemistry and Immunology, Faculty of Medicine of Ribeirao Preto, University of Sao Paulo - São Paulo (SP), Brazil.
[4] Full Professor at the Department of Genetics, Faculty of Medicine of Ribeirao Preto, University of Sao Paulo - Sao Paulo (SP), Brazil.

INTRODUCTION

Cutaneous mycoses are among the most common fungal infections and are mostly caused by filamentous keratinophilic fungi that use keratin as a nutrient during skin, hair, and nail infection. These fungi are called dermatophytes and are classified into three genera, *Trichophyton, Microsporum* and *Epidermophyton,* based on the formation and morphology of their conidia (structures of asexual reproduction). In addition, species of dermatophytes are divided into zoophilic, geophilic or anthropophilic, depending on their primary habitat (animals, soil or humans, respectively). Zoophilic species are responsible for about 30% of human dermatophytoses and they often provoke acute inflammation; anthropophilic species represent about 70% of infections on these hosts, causing a chronic infection of slow progression, suggesting that the fungus has adapted to the human host. So far, about 30 species of dermatophytes have been identified among human pathogens. [1]

The transmission of dermatophytoses or tineas occurs by direct contact with infected animals and humans or by indirect contact with contaminated fomites. The clinical forms vary according to the etiologic agent (species) and the anatomical site involved. Symptoms may be mild or severe based on the host's immunologic condition, and invasion of subcutaneous tissues or internal organs normally does not occur. Typical lesions of skin infection are circular, erythematous and pruritic, and are the result of direct action of the fungus or hypersensitivity reactions to the microorganism and/or its metabolic products. In nail infections (onychomycosis) the nail may separate from its bed, may become thick and have white spots or even become distrophic. [2] Although infections by dermatophytes are usually restricted to the superficial epidermis, these fungi may be invasive and cause a severe and disseminated infection in immunocompromised patients, with the development of dermatophytic granulomas. [3]

EPIDEMIOLOGICAL ASPECTS

According to the World Health Organization (WHO), dermatophytes affect about 25% of the world population. It is estimated that from 30 to 70% of adults are asymptomatic hosts of these pathogens and that the incidence of the disease increases with age. Generally, dermatophytes exhibit a cosmopolitan profile, that is, they are found in different regions of the world with variations in the frequency of particular species, as geoclimatic and social conditions interfere with the distribution of dermatophyte species. [4] Climatic factors, social practices, migration and individual characteristics may influence the epidemiology of dermatophytoses. Moreover, some risk factors have been associated with onychomycoses, such as age, abnormalities in nail morphology, genetic factors, poor hygiene conditions, *diabetes melittus*, and immunodeficiency. [4] Reports suggest that hormonal rates affect the frequency of these infections in men and women and that steroid hormones may influence the growth of these pathogens. Some species of *Trichophyton* and *Microsporum* have cytosolic proteins that bind exclusively and with high affinity to progesterone; the latter, in pharmacological and physiological concentration, inhibits the growth of dermatophytes in a dose-dependent manner. Anthropophilic species respond better to the action of steroids than geophilic species. [5]

An epidemiological evaluation involving 16 countries in Europe showed that between 35 and 40% of the analyzed individuals had infection of the foot (*tinea pedis*) caused by dermatophytes. [6] In addition, a study conducted with children in daycares in the United States revealed that between 22 to 50% of them exhibited symptoms of dermatophyte infection of the hair scalp. [7] In Brazil, the Southern and Southeast regions have a high incidence of infections caused by the dermatophyte *Trichophyton rubrum*, followed by *Microsporum canis* and *Trichophyton mentagrophytes*, whereas in the Northeast, *Trichophyton tonsurans, T. rubrum* and *M. canis* predominate.[8, 9] In the State of Sao Paulo, 4,500 children (0-15 years old) were evaluated to determine the incidence of *tinea capitis* from 1996 to 2000. Of the 132 children with suspicion of infection, 112 (85%) received confirmation through direct microscopy and culture. The most common isolates were *M. canis* (70.5%) and *T. tonsurans* (23.2%), followed by *T. mentagrophytes* (3.6%), *Microsporum gypseum* (1.8%) and *T. rubrum* (0.9%). [10]

Among the fungi isolated from skin infections, the anthopophilic dermatophyte *T. rubrum* is the most frequent in clinical cases of *tinea pedis* (feet), *tinea unguium* (nails), *tinea corporis* (body) and *tinea cruris* (groin region). [4] Epidemiological studies conducted with university students from Sao Paulo demonstrated the occurrence of dermatophytes in 18.2% of the studied population; *T. rubrum* was isolated in 80% of the cases and *T. mentagrophytes,* in 20%. [11] Among onychomycoses, *T.rubrum* is also the most prevalent dermatophyte, affecting children and adults in about 33.2% of the cases identified in Sao Paulo, followed by *T. mentagrophytes* (6.3%). [12] In Fortaleza, a three-year study described the isolation of dermatophytes in 12.99% of the cases of onychomycosis; *T. rubrum* was isolated in 9.04% of the patients and *T. tonsurans* and *T. mentagrophytes*, in 2.54% and 1.41%, respectively. [13]

HOST-DERMATOPHYTE INTERACTION AND THE PATHOGENESIS OF DERMATOPHYTOSIS

In the infectious process, dermatophytes must overcome the host's innate defense mechanisms, the initial protection of the organism againts infections, so that tissue colonization occurs. The physical and chemical structure of the skin, constant exposure to ultraviolet light, temperature, lack of humidity, and the presence of normal microbiota make the growth of pathogenic microorganisms difficult. An important defense mechanism of the organism against infectious agents that affect superficial sites is keratinization, the stratum corneum renewal process done by keratinocytes that leads to epithelial shedding and, consequently, to the possible removal of the fungus. [14] Therefore, for the pathogen to reach the epidermis, it should adhere to the surface of the tissue, the arthroconidium should germinate and the hypha quickly enter the stratum corneum, preventing that the fungus be eliminated with epithelial shedding. In the pathogenesis of dermatophytosis, the initial interaction between the arthroconidia and the stratum corneum occurs 3 to 4 hours after contact. Twelve hours after *ex vivo* human skin infection, *T. mentagrophytes* microconidia appear implanted in the outer layer and the germination occurs within 24 hours. [15] In this study the authors report the appearance of a polymeric material between the microconidium and the stratum corneum cell three days after infection; this probably plays an important role in the adhesion of the spores to the skin surface.

It has been recently shown that, during the adhesion of *T. mentagrophytes* arthroconidia to the stratum corneum surface, the formation of elongated fibrillary structures that appear to anchor and connect the arthroconidium to the tissue surface occurs. This may also prevent its removal from the host tissue. However, during the infection of deeper layers of the epidermis, the fibrillary structures become finer and shorter, covering the entire surface of the arthroconidium, which then shows a flat morphology. This would increase the contact surface with the tissue making greater adhesion and acquisition of nutrients possible. The authors report that, in addition to adhesion to the stratum corneum, these fibrillary structures make the connection between adjacent arthroconidia possible, suggesting the formation of a more stable arthroconidia complex that could represent the development of a biofilm or even a mechanism of intercellular communication. [15,16] Studies of *in vitro* interaction with Chinese hamster ovary (CHO) epithelial cells revealed that *T. rubrum* and *T. mentagrophytes* express glycoprotein adhesins, which recognize and bind to mannose and galactose residues in the cell surface. After adhesion, the conidia were endocytosed, suggesting that dermatophytes have the ability to invade cells, since CHO cells are not professional phagocytes. A similar pattern was observed in the interaction with professional phagocytes (macrophages) that mediate immune responses, even when they were pre-treated with cytochalasin D to prevent phagocytosis. [17,18]

After adhesion, dermatophytes have to obtain nutrients to develop and survive, using the macromolecules present in the host tissue as a source of carbon, nitrogen, phosphorus and sulfur. Nonetheless, the selectivity of the cytoplasmic membrane prevents that proteins, starch, cellulose and lipids be transported to the interior of the cell. For these molecules to be used, it is necessary to degrade them into smaller compounds that can penetrate the membranes. Secreted hydrolytic enzymes with different substrate specificities break these molecules. For this reason, the secretion of a great variety of enzymes by dermatophytes, such as proteases, lipases, elastases, collagenases, phosphatases and esterases, is one of the most important factors during the infectious process. [15,19,20] This enzymatic machinery is one of the best-characterized virulence factors of dermatophytes, allowing the hydrolisis of structural components of the epidermal tissue and the invasive character of these pathogens. Among the wide variety of enzymes secreted by dermatophytes, proteolytic enzymes are the most studied, and the importance of keratinolytic proteases to the pathogenicity is well established. It was demonstrated that samples of *M. canis* isolated from cats and dogs showed a higher keratinolytic activity as compared to samples of asymptomatic animals and induced chronic infection in guinea pigs, suggesting a direct relationship between the keratinolytic activity and the pathogenicity of this dermatophyte. [15] Keratin is a fibrous protein molecule of high molecular weight, rich in cysteine, whose disulfide bridges and acetamide bonds guarantee its stability. This protein is produced by humans and other animals and is the main component of skin, nails and shells, having the function to protect and cover. [21]

The keratinases secreted by dermatophytes catalyse the degration of the keratin present in the host tissue into oligopeptides or aminoacids that may be then assimilated by the fungus. However, these enzymes cannot act before the reduction of disulfide bridges inside the compact keratin network that constitutes keratinized tissues. It has been recently suggested that in *T.rubrum* this reduction depends on a sulfite efflux pump, codified by the *TruSsu*1 gene, belonging to the *tellurite-resistance/dicarboxylate* transporter (TDT) family. Sulfite secretion by this transporter allows the cleavage of the cystine present in keratin in cysteine and S-sulfocysteine, making it

accessible to the action of endo and exoproteases and working, at the same time, as a route for detoxification.[22]

It is thought that proteolytic enzymes degrade the protein components of the skin, aiding in the process of penetration in the stratum corneum. Hence, dermatophytes must produce and secrete proteases in response to the presence of the components of the epidermal extracellular matrix during tissue invasion. Induction of these proteases may contribute to the potential of these fungi to degrade components of deeper skin layers, such as dermal elastin, in immunocompromised individuals. Some authors suggest that the proteases secreted by dermatophytes facilitate and are even necessary for an efficient adhesion of these pathogens to the host tissue. [23,24] The pattern of proteases secreted by dermatophytes possibly determines the survival of the fungus in the host tissue and the evolution of the infection, not only providing nutrients despite the keratin barrier, but also triggering and modulating the immune response. It is known that the more severe the lesions (acute infection), the faster the resolution of the infection; therefore, keratinases and the tissue damage they provoke may trigger the inflammatory response and, consequently, activate the immune response. [15]

It has been reported that proteolytic activity is suppressed in *T. rubrum*, among other factors, by the availability of free aminoacids, and that proteases with optimal activity in acidic pH are important virulence factors of dermatophytes. [25] In 2004 we proposed a model of regulation of proteolytic enzymes by neutral pH during the infectious process of dermatophytoses (Figure 1). [26] In the early stages of the infection and in response to the acidic pH of human skin, the pathogen unsuppresses the synthesis of non-specific keratinases and proteases that have optimal activity in acidic pH. They act in substrates, keratinous or not, producing peptides that are hydrolyzed to aminoacids, which are used by the fungus as a source of carbon, nitrogen and sulphur. The metabolization of some aminoacids promotes the alkalinization of the host's microenvironment, making it suitable to the action of keratinases with optimal activity in alkaline pH, which allows the maintenance of the infection. It was shown that *T. rubrum* rapidly responds to changes in neutral pH by modulating its gene expression profile. [27,28] This metabolic machinery allows dermatophytes to use proteins as a source of nutrients in a wide pH spectrum. This makes the complete installation, development and permanence of the dermatophyte in the host tissue possible.

It was also shown that *T.rubrum* codifies a protein homologous to the transcriptional regulator *pacC* (*Aspergillus nidulans)*/Rim101p (*Candida albicans*), which is part of the signaling pathway of neutral pH, whose transcription is autoinduced. The *pacC* lineage of *T.rubrum,* which has this inactive gene, had its infectious capacity reduced when cultured in fragments of human nail, correlating with a sharp reduction of the keratinolytic activity. [29] These works suggest that development in keratin and the consequent degration of this protein are somehow regulated by the *pacC* gene, interfering with the secretion of proteases with optimal activity in alkaline pH. Moreover, pH signaling and monitoring pathways could be considered dermatophyte virulence factors, allowing the development and maintenance of the infection.

Other important components found in the host tissue are lipids, which are also the target of fungal extracellular enzymes in the pathogenesis of dermatophytoses. Studies have demonstrated that the dermatophytes *Epidermophyton floccosum, M. canis, T. mentagrophytes* and *T. rubrum* show lipolytic activity when cultured in different lipid agar sources, confirm-

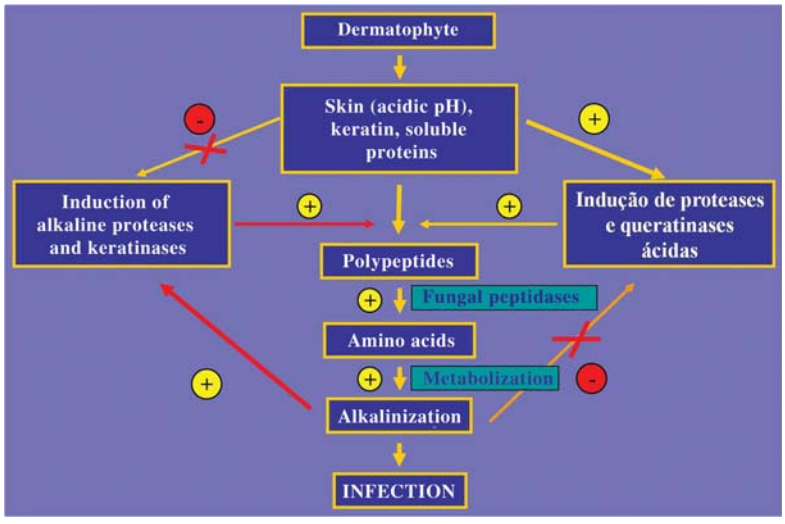

Figure 1: Suggested model for the neutral pH regulation of proteolytic enzymes secreted by dermatophytes. Monitoring of the skin acidic pH in the early stages of the infection activates (+) proteases and keratinases with optimal activity in acidic pH and suppresses (-) keratinases with optimal activity in alkaline pH, releasing polypeptides. Cleavage of these polypeptides in aminoacids, probably by peptidases, will provide the dermatophyte with sources of carbon, nitrogen and sulphur. The use of some aminoacids induces alkalinization of the medium and the consequent suppression (-) of proteases and keratinases with optimal activity in acidic pH and activation (+) of keratinases with optimal activity in alkaline pH. The activation of proteolytic enzymes with optimal activity in alkaline pH allows the maintenance of the dermatophyte in the host tissue.

ing the secretion of lipases and phospholipases by these dermatophytes. [30]

Once in the host tissue, dermatophytes or their metabolites induce an innate immune response by keratinocytes, thus activating immune response mechanisms or mediators. However, the immune response in dermatophytoses is scarcely known, involving unspecific mechanisms, as well as the development of a humoral and cellular response. Studies report that individuals infected with *Trichophyton* may show an immediate or late hypersensitivity reaction in sensitivity tests, proving the existence of an immune response dychotomy in the case of dermatophytoses. It is currently accepted that the cell-mediated immune response is responsible for the control of the infection, as some patients develop a recurrent and chronic infection when this cellular response is suppressed.

Keratinocytes are the most numerous cells in the epidermis, forming a physical barrier against microorganisms and mediating the immune response. These cells secrete various soluble factors capable of regulating the immune response, such as growth factors (bFGF - *basic Fibroblast Growth Factor*, TGF-a - *Transforming Growth Factor*; TGF-β; TNF-α - *Tumor Necrosis Factor*), interleukins (IL-1, IL-3, IL-6, IL-7, IL-8), and colony-stimulating factors – CSFs. [14] Keratinocytes stimulated in vitro for 24 hours with tricophytin, a *Trichophyton* antigen, secret high levels of IL-8, a chemotactic factor for neutrophils, possibly facilitating the acummulation of these cells in the stratum corneum. [31] Therefore, chemotactic factors such as IL-8 and leukotriene B4 can be produced by keratinocytes in response to appropriate stimuli and lead to an inflammatory response in dermatophytic lesions. The addition of a filtrate solution from *T.mentagrophytes* or *T. rubrum* culture to the culture of keratinocytes also increases the secretion of bFGF and IL-1a by epidermal cells. [32] In addition, levels of IL-1a induced by *T. mentagrophytes* were higher than those induced by *T. rubrum.* This corroborates clinical observations of dermatophytosis cases, in which acute infections, such as those provoked by *T. mentagrophytes,* are characterized by the accumulation of neutrophils in the epidermis, whereas infections caused by *T. rubrum* are chronic and characterized by a mononuclear infiltrate.

Recent studies have demonstrated that keratinocytes have a different cytokine expression profile when stimulated by distinct species of dermatophytes. It was shown that *Arthroderma benhamiae,* a zoophilic dermatophyte and teleomorph of *T. mentagrophytes,* induces the expression of various cytokines by keratinocytes, which may be involved in the development of an inflammatory response, typical of these infections. [33] Nevertheless, the authors affirm that these cells, when in contact with *T. tonsurans,* an anthropophilic dermatophyte, showed a limited cytokine expression, which probably explains the low inflammatory response provoked in lesions caused by this species. Other authors also described a difference in the profile of cytokine expression by keratinocytes infected *in vitro* by *T. mentagrophytes*, *T. rubrum* and *T. tonsurans*, which would also explain the difference in the intensity of induced inflammatory responses during infection by these species. 31 Therefore, these studies strengthen the hypothesis that keratinocytes participate in the development of the host's immune response against pathogens that affect the skin, recognizing distinct virulence factors and regulating such responses according to the stimulus they receive.

However, dermatophytes produce substances that reduce the inflammatory response and cellular proliferation in response to the host's defenses. Mannan, a glycoprotein component of the fungus cell wall, appears to be involved in the suppression of the inflammatory response. The incubation of mannan from *T. rubrum*, previously purified, with mononuclear cells, leads to the suppression of the formation of lymphoblasts and inhibition of the inflammatory response to mitogens and a variety of other stimuli. [34] This component also inhibits the proliferation of keratinocytes, allowing the establishment of a persistent chronic infection. Moreover, mannans from different species of dermatophytes have a distinct effect on the inhibition of cell-mediated immune response. In *T. rubrum*, mannan is produced in greater amounts and the inhibition of lymphoproliferation is more intense, as compared with the mannan produced by *M. canis*. This suggests that the higher production and potency to inhibit cell proliferation of the mannan from *T. rubrum* would be another contributing factor to the developmet of chronic and less exacerbated inflammation in infections caused by this species, and would also explain the severe inflammatory reaction caused by *M. canis*.

Studies about host-dermatophyte interaction show that several factors contribute to the intensity and severity of infections, and that the induction of the immune response by keratinocytes also influences the immune response that specifically controls the infection. However, the molecular mechanisms involved in dermatophyte adaptation to the host and the nature of the immune responses that control dermatophyte infections are not clearly understood. Infection models used in molecular studies about host-dermatophyte interactions are limited, and evaluation of the infection *in vivo* is restricted to zoophilic species, since spontaneous cure or even absence of colonization by anthropophilic species are known to occur. [1] An alternative has been the use of

culture media containing molecules of the host's microenvironment, such as keratin and other proteins, delineating the profile of gene and protein expression of *T. rubrum* in the use of these molecules as a source of nutrients. [35] These research studies have contributed to a greater understanding of the molecular strategies of dermatophytes as they use the host's molecules to survive. These studies also reveal interesting genes that should be evaluated as new targets for the development of antifungal drugs. [36] Chart 1 shows determining virulence factors identified in dermatophytes.

The clinical and veterinarian relevance of dermatophytes and advances in genome research have led the *Broad Institute/NIH* to develop a genome sequencing project of five dermatophyte species: *T. tonsurans*, *T. rubrum*, *Trichophyton equinum*, *M. canis* and *M. gypseum* that is currently on the assembly and annotation stage (*Dermatophytes Comparative Genome* - http://www.broad.mit.edu/annotation/genome/dermatophyte_comparative.2). One of the objectives of this project is to use comparative genomics to search for innate characteristics to each species that allow the infection of different hosts and the induction of distinct defense responses, and to reveal genes specific to species and or/ genera and those common to the studied organisms. These data, together with the development of infection models *in vivo* and *in vitro* may unfold the strategies developed by these different species for their development, survival and permanence in the host's tissue, and also promote greater knowledge about the immune response triggered in the control of these infections.

TREATMENT OF DERMATOPHYTOSES AND THE ISSUE OF ANTIFUNGAL RESISTANCE

In general, control of fungal infections initially depends on the host's immune response. The disease develops when a failure in the defenses occurs or when the pathogen evades the immune responses. This requires the use fungicidal or fungistatic drugs that more specifically target the infecting agent to avoid damage to the host. Nonetheless, this specificity is limited due to scarce knowledge about the various areas of pathogen biology, such as factors responsible for the virulence and pathogenicity of fungi and the mechanisms of resistance to the drugs available in the market. In addition, commonly used antifungal drugs have a limited number of cell targets, such as ergosterol and the enzymes involved in its synthesis, nucleic acids and cell wall synthesis, and the formation of microtubules. [37] The treatment of dermatophytoses is often long and expensive, involving the use of drug

Chart 1: Proteins identified in dermatophytes probably involved in virulence

Gene/Protein	Function in the fungi
Isocitrate lyase	Glyoxylate cycle enzyme
Malate synthase	Glyoxylate cycle enzyme
Citrate synthase	Glyoxylate cycle enzyme
Phospholipase B	Gene inactivation attenuates the virulence of *Cryptococcus neoformans* and *C. Albicans*
Subtilisin protease (Sub3)	Sub3 is the main protease secreted by *T. rubrum* during host infection
Subtilisin protease (Sub 5)	Secreted protease is an important virulence factor
Metaloprotease (Mep3)	MEP3 is produced by *M. canis* during infection of Guinea pigs
Metaloprotease (Mep4)	Mep4 is the main metaloprotease secreted by *T. rubrum* during host infection
Carboxipeptidase	It is important to the assimilation of substrates during infection
Dipeptidyl-peptidase V	Potential virulence factors of *M. canis*, helping to degrade substrates
P-type ATPase associated with copper resistance	Gene inactivation attenuates virulence of Listeria monocytogenes and *C. neoformans*
ABC Transporter TruMDR2	Gene inactivation attenuates the virulence of *T. rubrum in vitro*
Mannosyltransferase	Gene inactivation attenuates the virulence of *C. albicans* and *A. fumigatus*
Urease	Gene inactivation reduces the amount of ammonia secreted *in vitro* and attenuates the virulence of *Coccidioides posadasii*
Glucosamine-6-phosphate deaminase	Gene inactivation attenuates the virulence of *C. albicans* in murine model
Glyceraldehyde-3-phosphate dehydrogenase (GAPDH)	GAPDH contributes to the adhesion of *Paracoccidioides brasiliensis* to the host tissue and to the dissemination of the infection
PacC Transcription factor	Gene inactivation attenuates the virulence of *T. rubrum in vitro*
Thioredoxin TrxA	Probable factor of virulence of *T. mentagrophytes*

Source: Peres, et al.[35]

formulations of the allylamine and azole class, especially itraconazole and terbinafine. Combined treatments of topical and oral drugs and anti-inflammatories have been used in an attempt to increase cure rate. Recently, the topical use of amorolfine and ciclopirox to treat onychomycosis has been adopted. [38]

Dermatophytoses are often associated with recidivism after the interruption of antifungal therapy. However, in 2003 a case of clinical resistance to terbinafine, one of the most used drugs to treat dermatophytoses caused by *T. rubrum*, was reported. This resistant lineage was isolated from a patient with onychomycosis whose oral treatment with terbinafine was ineffective, showing cross-resistance to various other inhibitors of squalene epoxidase, including naftifine, butenafine, tolnaftato and tolciclate, suggesting a target-specific resistance mechanism. [39] The main biochemical and molecular mechanisms that contribute to the drug resistance phenotype in eukaryotes are: reduction of drug delivery, metabolic modification or degradation of the drug by the cell, alterations in the interaction between the drug and the target site or other enzymes involved in the same enzymatic pathway, through punctual mutations, overexpression of the target molecule, gene amplification and conversion (recombination); increase of the cell efflux; for instance, through greater expression of efflux pumps such as *ATP binding cassette transporters.* The resistance of dermatophytes to inhibiting agents involves the participation of target-enzyme modifiers, overexpression of *ATP binding cassette transporters* and stress-related proteins. [37] In *T. rubrum*, two *ATP binding cassette transporters, TruMDR1* e *TruMDR2,* were identified showing importance not only in the process of resistance to various antifungal drugs, but also in enzyme secretion and probably in the pathogenicity of this dermatophyte. [40-42] It has also been described that a mutation in the gene that codifies the enzyme squalene epoxidase (ErgA), target of terbinafine, made the fungi *A. nidulans, Aspergillus fumigatus* and *T. rubrum* highly resistant to this drug. [37, 43]

Fungi respond to stimuli from the environment by the activation of several signal transduction pathways responsible for monitoring environmental alterations to allow the functioning of physiological mechanisms that adapt them to stress conditions, developing defense or cell tolerance responses. Antifungal drugs induce cell stress responses necessary to overcome their toxic effects, allowing the survival of the fungus. Knowledge about fungal adaptive responses to adverse conditions promotes a better understanding about the biology of these microorganisms with the possibility of revealing essential metabolic pathways that allow their survival and, consequently, new cellular targets for the development of efficient drugs to control these infections. [37] Transcriptome analyses during exposure of *T. rubrum* to compounds with antifungal activity have led to the identification of genes involved in the adaptation and response to stress, and to the elucidation of the mechanism of action of drugs such as terbinafine, acriflavine, amphotericin B, and fluconazole, among others. [44, 45] Studies about gene expression have also contributed to the evaluation of the effect of new antifungal agents on *T. rubrum*, such as the recently developed PHS11A and PH11B, which inhibit the fatty acid synthase enzyme. 46 In chart 2 the mechanisms of response and resistance of dermatophytes to different antifungal agents - those used in medical practice and those that are still under experimental and clinical research - such as acriflavine and fatty acid synthase inhibiting drugs, are described.

CONCLUSION

Dermatophytoses are the most frequent fungal infections worldwide, affecting individuals in various age groups and leading to these patients' worse quality of life and economic burden due to treatment expenditures. Research about different aspects of dermatophytes, such as physiology, genetics, and biochemistry, as well as the pathogenesis of dermatophytoses and the immune response triggered by these infections, are essential to the development of new therapeutic and prophylactic measures. The development of molecular tools, such as efficient gene transformation methods and *in vivo* and *ex vivo* infection models, have made the identification and characterization of various genes expressed during the infection possible. This research may also help the development of new strategies of diagnosis, therapy and prevention of dermatophytoses.

A combination of different methodologies may lead to the discovery of new antifungal drugs for the treatment of dermatophytoses and other mycoses. The tracking of chemical libraries may help identify candidate inhibitors, whose molecular structure could be modified due to results obtained *in silico*. However, *in vitro* trials and efficacy tests should be performed so that a new inhibitor may be clinically used. The availability of genomic databanks and computational methodologies helps to predict drug properties and their cellular targets. In addition, functional genome analysis about gene function and regulation will bring more understanding about the biology of dermatophytes and their pathogenicity. Also, since resistance of clinical isolates may occur, a process that involves more than one mechanism, the comprehension of the events that promote resistance is essential for the development of structural modifications in the

Chart 2: Mechanism of action, possible mechanisms of drug resistance, and gene expression pattern of *Trichophyton rubrum*

Drugs	Mechanism of action	Likely mechanisms of resistance	Genes associated with resistance
Terbinafine	Inhibition of squalene epoxidase	Modification of the target enzyme by mutation Increase of the drug efflux Adaptation to stress [(b)] Overexpression of salicylate monooxygenase (Degradation of the drug?)	TruMDR2, ABC transporter
Fluconazole	Inhibition of cytochrome P450 14α–lanosterol demethylase	Increase of the drug efflux Adaptation to stress [(b)]	TruMDR1, TruMDR2, ABC transporters
Imazalil	Inhibition of cytochrome P450 14α lanosterol demethylase	Increase of the drug efflux	TruMDR1, TruMDR2
Itraconazole	Inhibition of cytochrome P450 1α-lanosterol demethylase	Increase of the drug efflux	TruMDR1, TruMDR2
Cetoconazole	Inhibition of cytochrome P450 14α-lanosterol demethylase	Increase of the drug efflux Overexpression of the 14α-lanosterol demethylase enzyme	TruMDR1, TruMDR2
Tioconazole	Inhibition of cytochrome P450 14α-lanosterol demethylase	Increase of the drug efflux Adaptation to stress [(b)]	TruMDR2
Anphotericin B	Ergosterol binding and destabilizer of the cell membrane functions	Increase of the drug efflux Adaptation to stress [(b)]	Multi-drug transporters of the RND family/ Multi-drug transporters of the ABC family, Na+ multi-drug efflux pump
Griseofulvin	Inhibition of mitosis	Increase of efflux pumps Adaptation to stress [(b)]	TruMDR1, TruMDR2, ABC transporters
Acriflavine	Inhibition of topoisomerases / DNA and RNA intercalation	Increase of the drug efflux Adaptation to stress [(b)]	TruMDR2
Undecanoic acid	Unspecific cell interactions	Adaptation to stress [(b)]	TruMDR2
Benomyl	Inhibition of mitosis / tubulin binding	Increase of the drug efflux	TruMDR2
Ethidium bromide	DNA and RNA intercalation	Increase of the drug efflux	TruMDR1, TruMDR2
4NQO	DNA modification, mutagenic action	Increase of the drug efflux	TruMDR2
PHS11A	Inhibition of fungal fatty acid synthase	Increase of the drug efflux Adaptation to stress [(b)]	TruMDR1, TruMDR2, Transporters of the ABC family, MFS transporter / Multi-drug transporters of the ABC family

(a) Gene expression was induced or suppressed in response to the drug.
(b) Adaptation to stress unspecific response to drug exposure.
Adapted source: Martinez-Rossi *et al.*[37]

antifungal drugs currently used in medical practice. It is relevant to emphasize that the low diversity of antimicotic drugs may indicate the existence of differences between the pathogen and the host, not yet explored, that may be used in the development of new drugs that interfere with the fungi essential functions.❑

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Mailing address / Endereço para correspondência:
Nalu Teixera de Aguiar Peres
Av. Bandeirantes, 3.900 Monte Alegre
14049 900 Ribeirão Preto – SP, Brazil

How to cite this article/*Como citar este artigo*: Peres NTA, Maranhão FCA, Rossi A, Martinez-Rossi NM. Dermatophytes: host-pathogen interaction and antifungal resistance. An Bras Dermatol. 2010;85(5):657-67.