AIX-MARSEILLE UNIVERSITE

ECOLE DOCTORALE DES SCIENCES DE LA VIE ET DE LA SANTE

FACULTE DE MEDECINE

PARASITOLOGIE-MYCOLOGIE/ IP-TPT UMR MD3

Thèse présentée pour obtenir le grade universitaire de docteur

Mention: Pathologie humaine

Spécialité : Maladies infectieuses

Oumar COULIBALY

DERMATOPHYTOSES EN MILIEU SCOLAIRE AU MALI

Soutenue le 19/12/2014 devant le Jury :

Dominique Chabasse	**Université d'Angers**	**Rapporteur**
Françoise Botterel	**Université Paris-Est Créteil**	**Rapporteur**
Ogobara Doumbo	**Université de Bamako**	**Examinateur**
Renaud Piarroux	**Aix-Marseille Université**	**Examinateur**
Stéphane Ranque	**Aix-Marseille Université**	**Directeur de thèse**

Résumé

Les dermatophytoses sont parmi les maladies les plus fréquentes dans le monde, et en particulier dans les pays en développement. La répartition de ces mycoses varie considérablement en fonction de facteurs épidémiologiques, socio-économiques et géographiques. Pour déterminer la prévalence, les caractéristiques cliniques et les facteurs de risque des dermatophytoses chez les élèves, nous avons effectué trois enquêtes transversales entre décembre 2009 et février 2012 dans des écoles primaires publiques situées dans trois zones éco-climatiques différentes du Mali : zone soudanienne, zone sahélienne et zone soudano-guinéenne. Sur un échantillon aléatoire de 590 élèves (âge moyen de 9,7 ans ; 286 garçons), la prévalence clinique des dermatophytoses était de 59,2%. La teigne du cuir chevelu (39.3%) représentait la forme clinique la plus fréquente ; la prévalence des autres dermatophytoses était de 13,6% avec une prédominance de l'atteinte de la peau glabre (81,3%). Une forte prévalence (59,5%) des cas confirmés de teigne a été enregistrée dans la zone climatique soudano-guinéenne. Nous avons mis en évidence le genre masculin et la résidence dans la zone bioclimatique Soudano-guinéenne comme facteurs de risque indépendants associés à la teigne du cuir chevelu. Les espèces de dermatophytes identifiées étaient *T. soudanense* (41,3%), *M. audouinii* (36,5%), *T. violaceum* (3,7%), *T. mentagrophytes* (2,1%) et l'association de *T. soudanense* avec *M. audouinii* (14,8%) ou *T. mentagrophytes* (1,6%). Par ailleurs, nous avons recherché une contamination des matériels de coiffure par des dermatophytes dans un échantillon aléatoire de cinq salons de coiffure situés en zone soudanienne. Sur 41 instruments de coiffure prélevés, 73,2%, étaient contaminés par deux espèces anthropophiles : *T. soudanense* (53,3%) et *M. audouinii* (46,7%).

Au plan thérapeutique, nous avons évalué l'activité d'un aminostérol, la squalamine, contre des dermatophytes in vitro. Cette molécule a présenté des concentrations minimales inhibitrices variant de 4 à 16 mg/l. Nous avons ensuite montré une bonne tolérance et une efficacité partielle de la squalamine en topique dans le traitement de la teigne du cuir chevelu dans un essai clinique de phase II, randomisé, en double aveugle, contrôlé par placebo.

Mots clés : dermatophytes ; dermatophytoses ; épidémiologie ; teigne du cuir chevelu ; écoles primaires; écoliers; facteurs de risque ; zones climatiques ; aminostérols ; squalamine ; essai clinique; Mali.

Abstract

Dermatophytoses are among the most common diseases worldwide, and particularly in developing countries. The distribution of dermatophytes varies greatly depending on epidemiological, socio-economic and geographical factors. To determine the prevalence, clinical characteristics, and risk factors of dermatophytoses in Malian schoolchildren, we conducted three cross-sectional surveys between December 2009 and February 2012 in three public primary schools located in the Sudan, Sahel and Sudano-Guinean climatic zones. A randomly selected sample of 590 schoolchildren (mean age: 9.7 years, 286 males) participated in this study. Overall, three hundred and twelve participants were diagnosed to have dermatophytosis lesions, giving a 52.9 % prevalence of clinical dermatophytoses. Tinea capitis was the most common clinical presentation, with a 39.3% prevalence, whereas the prevalence other dermatophytoses was 13.6%. A high (59.5%) prevalence rate of confirmed cases of tinea capitis was observed in the Sudano-Guinean climatic zone. Male gender and living in the humid Sudano-Guinean climatic zone were independent risks factors associated with tinea capitis. Mycological culture found *T. soudanense* (41.3%), *M. audouinii* (36.5%), *T. violaceum* (3.7%), *T. mentagrophytes* (2.1%), and the combination of *T. soudanense* with *M. audouinii* (14.8%) or *T. mentagrophytes* (1.6%). In addition, we found a high contamination rate (73.2%), with two anthropophilic dermatophytes: *T. soudanense* (53.3%) and *M. audouinii* (46.7%), of hairdressing tools used in hairdressing salons located in the peri-urban area of Bamako.

Regarding anti-dermatophyte therapy, we showed a significant in vitro activity of squalamine against clinical dermatophyte isolates, with minimum inhibitory concentrations ranging from 4 to 16 mg/l. In a phase II, randomized, double-blind, placebo-controlled, clinical trial, a topical treatment with squalamine ointment was well tolerated and exhibited a partial clinical activity in the treatment of tinea capitis.

Keywords: dermatophytes; dermatophytoses; epidemiology; tinea capitis; primary schools; schoolchildren; risk factors; rural; climatic zone; aminosterols; squalamine; clinical trial; Mali.

Remerciements

Ce travail, loin d'être solitaire, résulte de la collaboration entre le Laboratoire de Parasitologie-Mycologie du CHU de La Timone de Marseille (où ont été réalisés les examens mycologiques, les tests de susceptibilité antifongique in vitro avec les analyses statistiques) et le Département d'Epidémiologie des Affections Parasitaires et Malaria Research and Training Center (DEAP/MRTC) de la faculté de Médecine de l'Université de Bamako (où ont été réalisés la validation des protocoles ainsi que le recueil et la saisie des données). Ce travail doctoral n'aurait jamais pu voir le jour sans le soutien de nombreuses personnes dont l'aide, la générosité, la disponibilité, la bonne humeur et l'intérêt manifestés à l'égard de mes travaux de recherche ont permis ma progression dans cette phase délicate de l'apprentissage.

En premier lieu, je tiens à remercier grandement mon directeur de thèse, Docteur RANQUE Stéphane, pour son encadrement scientifique, pour ses multiples conseils, pour toutes les heures qu'il a consacrées pour diriger cette thèse et pour tout l'intérêt qu'il porte sur le développement de la discipline « Mycologie Médicale » au sein du Département d'Epidémiologie des Affections Parasitaires et du Malaria Research and Training Center (DEAP/MRTC). Il met tient à cœur également de lui dire à quel point j'ai apprécié sa grande disponibilité, non seulement pour mener les différentes enquêtes conduites au Mali dans le cadre des travaux de cette thèse (qui se sont déroulées souvent dans des conditions assez difficiles), mais aussi pour les analyses statistiques et la relecture très rapide des documents. Enfin, j'ai été extrêmement touché par ses qualités humaines, d'écoute et de compréhension tout au long de ce travail.

J'exprime ma reconnaissance au Professeur PIARROUX Renaud, chef de service du Laboratoire de Parasitologie-Mycologie du CHU-Timone (APHM, CHU-Timone-Adultes), pour tout son soutien, son immense contribution, ses multiples conseils, sa disponibilité et son respect sans faille des délais de relecture des documents que je lui ai soumis.

J'exprime toute ma gratitude au Professeur DOUMBO Ogobara K, chef du DEAP/MRTC de la faculté de médecine de l'Université de Bamako, pour son enseignement, ses conseils, son écoute et sa disponibilité.

Tous mes remerciements aux Professeurs THERA Mahamadou Aly et DJIMDE Abdoulaye A du DEAP/MRTC de la faculté de médecine de Bamako, pour leur disponibilité, leur contribution et pour leur respect des délais de relecture des documents qui leur ont été soumis.

J'adresse mes remerciements aux Docteurs GAUDART Jean (Aix-Marseille Université, UM 105 ; Inserm, U1068, F-13009), BRUNEL Jean-Michel (Institut Paoli-Calmettes, Centre de

Recherche en Cancérologie de Marseille (CRCM), CNRS, UMR7258), REBAUDET Stanislas (APHM, Parasitologie-Mycologie CHU Timone-Adultes), AHLANOUT Kamel.

Mes remerciements à tout le personnel du Laboratoire de Parasitologie-Mycologie (APHM CHU Timone Adultes), plus particulièrement à L'OLLIVIER Coralie, CASSAGNE Carole et NORMAND Anne-Cécile.

Mes remerciements vont aussi aux Docteurs KONE Abdoulaye K, GOITA Siaka, COULIBALY Drissa (DEAP/MRTC) et au Docteur TRAORE Pierre du Département de Dermatologie (CNAM Ex Institut Marchoux de Bamako) pour leur active contribution aux différentes enquêtes transversales et descriptives et à l'essai clinique.

Ma reconnaissance au Docteur KONE Issa pour tout son soutien.

J'exprime ma gratitude à tous mes collègues du Centre Hospitalier de Martigues.

Mes remerciements aux personnels de l'école fondamentale de Sirakoro-Méguétana (particulièrement à Monsieur DOUGNON Jean André), de l'école fondamentale Mamadou Tollo de Bandiagara et de l'école fondamentale de Bougoula-Hameau de Sikasso ainsi qu'aux différents guides sur le terrain.

Ma reconnaissance va à ma famille et aux amis qui ont particulièrement assuré le soutien affectif de ce travail.

Remerciements aux membres du jury

C'est un réel plaisir pour moi de remercier très chaleureusement tous les membres du jury, notamment :

-Monsieur le Professeur CHABASSE Dominique, PU-PH, Parasitologie-Mycologie, Institut de Biologie en Santé-CHU Angers.

Je vous exprime mon immense plaisir pour avoir accepté de juger ce travail mais aussi pour tout l'intérêt que vous portez à mon pays, le Mali. C'est un honneur que vous nous faites.

-Madame le Professeur Françoise BOTTEREL, PU-PH, Parasitologie-Mycologie, CHU-Henri Mondor, AP-HP.

Veuillez recevoir ma sincère gratitude pour avoir accepté de juger ce travail et de siéger dans ce jury malgré vos occupations multiples.

-Monsieur le Professeur PIARROUX Renaud, PU-PH, Parasitologie-Mycologie

Chef de service du Laboratoire de Parasitologie-Mycologie, CHU-Timone, AP-HM.

C'est un grand honneur que vous me faites en acceptant siéger dans ce jury, au-delà de toutes vos contributions pour l'amélioration de la qualité de ce travail.

-Monsieur le Professeur DOUMBO Ogabara K., responsable de l'enseignement de la Parasitologie-Mycologie à la Faculté de Médecine et d'Odontostomatologie (FMPOS) de l'Université de Bamako et Directeur du DEAP/MRTC et du Cours d'Epidémiologie pour Cadres Supérieurs de Santé en Afrique.

Je vous exprime toute ma reconnaissance pour tous vos enseignements et pour votre gentillesse d'avoir accepté l'invitation à siéger dans ce jury, malgré vos occupations multiples.

-Monsieur le Docteur RANQUE Stéphane, MCU-PH, Laboratoire de Parasitologie-Mycologie, Timone, AP-HM

Directeur de cette thèse, les mots me manquent ici pour vous exprimer toute ma reconnaissance. Je vous dis tout simplement MERCI pour tout.

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ADN : Acide Désoxyribonucléique

ADNm : Acide Désoxyribonucléique mitochondrial

ADNr : Acide Désoxyribonucléique ribosomique

ARN : Acide Ribonucléique

ARNr : Acide Ribonucléique ribosomique

BCP: Bromocrésol pourpre

BHI : Brain-Heart Infusion

CHU: Centre Hospitalo-Universitaire

CMI : Concentration Minimale Inhibitrice

DTM : Dermatophyte Test Medium

DEAP/MRTC : Département d'Epidémiologie et des Affections Parasitaires / Malaria Research and Training Center

E. : *Epidermophyton*

ELISA : Enzyme Linked Immunosorbent Assay

IFN-γ : Interféron gamma

IL: Interleukine

ITS: Internal Transcribed Spacer

M.: *Microsporum*

MALDI-TOF: Matrix Assisted Laser Desorption Ionization-Time-Of-Flight

PCR : Polymerase Chain Reaction (réaction de polymérisation en chaîne)

PDA: Potato-Dextrose-Agar

RAPD: Random Amplified Polymorphic DNA (ADN polymorphe amplifié au hasard)

RFLP: Restriction Fragment Length Polymorphism

Lymphocytes Th: lymphocytes T helper

T. : *Trichophyton*

TC : Tinea capitis

Introduction

Les dermatophytes, les levures et les moisissures sont des micro-organismes responsables des mycoses cutanées qui sont des infections le plus souvent superficielles, parfois profondes de la peau. En mycologie, l'identification précise de l'agent pathogène responsable et notamment celle des dermatophytes est nécessaire pour des raisons diagnostiques et épidémiologiques. Les dermatophytes sont des champignons filamenteux « imparfaits » (ou Deutéromycètes), kératinophiles et kératinolytiques, appartenant, d'après la classification la plus largement utilisée [1], aux trois genres anamorphiques : *Epidermophyton*, *Microsporum* et *Trichophyton*. Ces agents pathogènes présentent une affinité particulière pour la kératine, expliquant l'atteinte préférentielle de l'épiderme ou des phanères [2]. Ils produisent des kératinases qui sont des enzymes protéolytiques, capables d'hydrolyser la kératine, qui est le constituant majeur de la protéine des cheveux, des ongles et de la peau [3,4]. Selon leur mode de transmission, on distingue les espèces anthropophiles (à transmission interhumaine), les espèces zoophiles (contamination humaine à partir d'un animal et sans risque de transmission interhumaine), et enfin les dermatophytes géophiles (réservoir tellurique avec soit une contamination directe, soit par l'intermédiaire d'un animal porteur). Ce sont des champignons cosmopolites, et leur répartition varie considérablement en fonction des facteurs épidémiologiques, socio-économiques et géographiques [5]. Certaines espèces sont retrouvées dans le monde entier, tels que *T. rubrum*, *T. mentagrophytes var. interdigitale*, *M. canis, M. gypseum* et *E. floccosum* et d'autres ont une restriction géographique partielle, comme *T. schoenleinii* (l'Eurasie, Afrique), *T. soudanense* (Afrique), *T. violaceum* (Afrique, en Asie et en Europe), et *T. concentricum* (îles du Pacifique, en Extrême-Orient et l'Inde) [6,7]. Les infections provoquées par des dermatophytes sont appelées dermatophyties ou dermatophytoses ou encore tinea chez les Anglo-saxons. Les manifestations cliniques varient en fonction des topographies, simulant de nombreuses affections dermatologiques (eczéma, lichen, psoriasis). Ces affections, d'évolution bénigne chez la majorité des sujets, sont très répandues dans le monde, et une augmentation de leur incidence est particulièrement est notée dans les pays en développement [8]. La prévalence mondiale de ces affections oscillerait entre 20 et 25% [9]. A l'échelon mondial, le spectre clinique des dermatophytes peut être résumé comme suit : au sud et dans la ceinture de pauvreté du monde les teignes anthropophiles, au nord, parmi les populations économiquement aisées, le pied d'athlète lié à la pratique sportive répandue. Dans les grandes villes des zones tropicales et subtropicales d'Afrique, les teignes du cuir chevelu constituent un problème endémique important, surtout chez les enfants scolarisés. Ces infections concernent principalement mais

non exclusivement les enfants pré-pubertaires avec des spécificités thérapeutiques. Leur prévalence varie à l'intérieur d'un même pays (d'une zone à une autre) et d'un pays à un autre. Au Mali, une prévalence de 12,4% des teignes du cuir chevelu a été rapportée chez les élèves de la zone rurale de Moribabougou [10]. Cependant, des taux inférieurs de 4,4% et de 2,1% ont été notés respectivement chez les garçons et les filles d'une école de Bamako [11]. Récemment, une prévalence supérieure à 20% a été observée chez les enfants d'âge scolaire au Nigeria [12].

Les raisons d'engager ce travail sont multiples mais trois d'entre elles prévalent : 1/ parce que les données épidémiologiques sur les dermatophytoses sont relativement rares au Mali ; 2 / évaluer la prévalence, les caractéristiques cliniques et les facteurs étiologiques des dermatophytoses chez les écoliers de trois zones bioclimatiques différentes; 3/ évaluer l'activité antifongique des aminostérols contre les isolats cliniques de dermatophytes. Ce travail a abouti à la publication de deux articles, et à trois articles soumis dans les revues de Mycologie et de Dermatologie. Ce travail de Doctorat d'Université s'articule en deux parties. Dans la première partie, nous présenterons les généralités concernant les dermatophytoses. Dans la seconde partie, nous détaillerons nos travaux. Ces travaux seront présentés sous forme d'articles (acceptés, soumis ou presque) dans des journaux scientifiques à comité de lecture, précédés d'un bref commentaire détaillant le plus exactement possible notre participation à chacune des parties de ces travaux.

1. Généralités sur les dermatophytes

1.1. Définition

Les champignons sont des organismes eucaryotes, uni ou pluricellulaires, dépourvus de chlorophylle. Ils sont qualifiés d'hétérotrophes, se nourrissant par absorption et nécessitant pour leur croissance une source de carbone qui provient de matières organiques en décomposition (saprophytes) ou d'être vivants (parasites) [13,14]. La nomenclature des champignons obéit aux mêmes règles et subdivisions que les classifications botaniques. Le règne se répartit en divisions (caractérisées par le suffixe - mycota), séparées en classes (suffixes -mycètes), puis divisées en ordres (suffixe -ales), eux même répartis en familles (suffixe-acées). Une famille est composée de genres qui comprennent des espèces. On distingue donc dans le règne des Fungi, cinq classes (ou phyla) selon leur mode de reproduction : les Chytridiomycotina (Chytridiomycètes) qui ne sont pas impliqués en pathologie humaine ; les Zygomycotina (Zygomycètes) ; les Ascomycotina (Ascomycètes) ; les Basidiomycotina (Basidiomycètes) ; les Deuteromycotina (Deutéromycètes) qui sont des champignons imparfaits.

Les dermatophytes sont des champignons microscopiques appartenant à la classe des Ascomycètes, à l'ordre des Onygénales, à la famille des Arthrodermataceae et au genre des *Arthroderma* [15,16].

Ce sont des champignons filamenteux à thalle septé se multipliant sur le mode sexué, et produisant des ascospores (spores endogènes produites dans les asques disposées sans ordre précis dans des gymnothèces). Leur forme sexuée (ou parfaite ou téléomorphe) étant difficile à obtenir, la classification des dermatophytes repose sur la reproduction asexuée ou conidiogenèse [17,18]

Dans la routine de laboratoire, ne sont étudiées que les formes asexuées (formes dites «imparfaites» ou anamorphes) [13].

Lors de la reproduction asexuée basée sur le mode thallique solitaire, deux types de spores ou conidies sont produits par les dermatophytes: les spores unicellulaires (microconidies ou microaleuries), et les spores pluricellulaires, à base tronquée et cloisonnées transversalement (macroconidies ou macroaleuries) [14,16,17]. Ces deux types de spores, par leur morphologie et leur abondance, sont à la base de la distinction des dermatophytes en trois genres : *Microsporum*, *Trichophyton* et *Epidermophyton*.

1.2. Historique des classifications

En 1910, une classification des dermatophytes en fonction de l'aspect clinique des lésions et du type de parasitisme des cheveux et des poils, a été établie par Raymond Sabouraud, médecin dermatologue français. Reposant sur des critères cliniques, cette classification reste d'un intérêt primordial pour les praticiens [13]. En 1930, une classification botanique (basée essentiellement sur l'observation microscopique des cultures) a été proposée par Langeron, médecin et mycologue français, avec Milochevitch. Cette classification modifiée ultérieurement par Vanbreuseghem, retenait six genres : *Epidermophyton*, *Keratinomyces*, *Langeronia*, *Sabouraudites* ou *Microsporum*, *Trichophyton*, et *Ctenomyces* [13]. En 1934, Emmons publie la troisième grande classification couramment utilisée dans tout le monde Anglo-saxon [13]. Cette classification plus simple reconnaît trois genres : *Microsporum*, *Trichophyton*, et *Epidermophyton* [17,19]. Dans cette classification plus moderne, reposant sur les caractères parasitaires (type d'invasion pilaire) et sur les caractères culturaux des dermatophytes, les genres *Ctenomyces* et *Keratinomyces* précédemment cités ont été rattachés au genre *Trichophyton* [18]. Toutes ces classifications « historiques » ont été établies à partir des formes asexuées c'est-à-dire imparfaites ou anamorphes des dermatophytes.

1.3. Reproduction sexuée chez les dermatophytes

Les champignons se développent par un système de filaments ou hyphes plus ou moins développés et ramifiés, appelé thalle. Chez les dermatophytes, la reproduction sexuée se fait à partir de la confrontation de thalles issus de spores génétiquement différenciées dont les noyaux sont de type « mâle » (+) ou « femelle » (-) : c'est pourquoi ils sont qualifiés d'espèces hétérothalliques [14,17]. La rencontre de deux thalles entraîne la formation des gamétocystes mâles (anthéridies) ou femelles (ascogones) sur les filaments mycéliens. Les noyaux + et – s'apparient et donnent un filament dicaryotique. Ensuite, c'est l'étape de la caryogamie, caractérisée par la fusion des noyaux.

La probabilité d'isoler d'un produit pathologique deux souches de polarité différente est si rare qu'il est impossible en pratique de retrouver les formes parfaites des dermatophytes dans les primocultures au laboratoire [14,17]

1.4. Epidémiologie des dermatophytes

1.4.1. Origine des dermatophytes

L'épidémiologie des dermatophytes est traditionnellement présentée en fonction de leur habitat [14,18] (Tableau 1) :

- les dermatophytes zoophiles, parasites obligatoires des animaux : qui sont pour la plupart les agents de zoonoses (*M. canis, T. mentagrophytes var. mentagrophytes, M. persicolor, Trichophyton gallinae*).

- les dermatophytes anthropophiles, parasites obligatoires de l'homme : ils sont difficilement transmissibles aux animaux (zoonoses inversées rares) (*M. audouinii, T. rubrum, T. mentagrophytes var. interdigitale*).

- les dermatophytes géophiles, qui ont une vie saprobiotique dans le sol et peuvent parfois contaminer l'homme ou les animaux (*M. gypseum, T. mentagrophytes var. mentagrophytes*).

Tableau 1.1- Classification des principaux dermatophytes et leurs modalités de transmission

	Dermatophytes anthropophiles
Genres	**Espèces**
Epidermophyton	*E. floccosum*
Microsporum	*M. audouinii*
	M. ferrugineum
Trichophyton	*T. soudanense*
	T. rubrum
	T. violaceum
	T. tonsurans
	T. schoenleinii
	T. gourvilii
	Dermatophytes zoophiles
Microsporum	*M. canis* (chien, chat)
	M. persicolor (souris)
	M. equinum (cheval)
	M. nanum (porc)
	M. preacox (cheval) (également tellurique)
Trichophyton	*T. mentagrophytes* (chat, lapin, cheval) (également tellurique)
	T. gallinae (volaille)
	T. equinum (cheval)
	T. verrucosum (bovin)
	T. erinacei (hérisson)
	Dermatophytes telluriques
Microsporum	*M. gypseum*
	M. cookei
	M. fulvum
	M. preacox (également zoophile)
Trichophyton	*T. mentagrophytes* (également zoophile)
	T. terrestre (habituellement saprophyte)
	T. ajelloi (habituellement saprophyte)

1.4.2. Adaptation au parasitisme

Les dermatophytes évoluent classiquement vers le parasitisme selon le schéma suivant : transmission du sol à l'animal puis de l'animal vers l'homme. Ces champignons, d'abord saprophytes du sol, se développent aux dépens de la kératine présente dans ce milieu (fragments de peau ou de poils, de cornes, de sabots, de carapaces d'insectes...) puis familiarisés à ce

substrat, par la suite ils parasitent le poil de l'animal (kératine vivante) ou directement l'homme [15].

Une nouvelle approche épidémiologique récente plus évolutive, concernant l'adaptation au parasitisme a été proposée avec distinction de deux principales catégories de dermatophytes [18]: les dermatophytes géophiles et zoophiles (encore associés au sol ou proches de celui-ci) ; les dermatophytes zoophiles et anthropophiles (associés au parasitisme strict, n'étant plus liés au sol).

L'adaptation parasitaire des dermatophytes a des conséquences épidémiologiques, cliniques et biologiques. Sur les plan épidémiologique et clinique, plus une espèce est adaptée à son hôte, plus elle diffuse aisément au sein de la population concernée et plus la tolérance clinique augmente. Sur le plan biologique, on constate une perte de la reproduction sexuée chez les espèces éloignées du sol et bien adaptées au parasitisme [15].

1.5. Pouvoir pathogène des dermatophytes

Les dermatophytes sont des champignons filamenteux ayant une prédilection pour la kératine de la couche cornée de la peau, des poils, des cheveux et des ongles chez l'être humain, et pour la kératine de la peau, des poils et des griffes chez l'animal. Ils sont responsables d'infections cutanées superficielles de la peau et des phanères, mais respectent classiquement les muqueuses [2,16,18–20]. Résistantes, les spores des dermatophytes sont présentes dans l'environnement (sur le sol, dans la terre, dans le pelage des mammifères, sur des objets divers). La contamination se fait par voie directe (interhumaine, animale ou tellurique) ou indirecte (vêtements et chaussures, instruments de toilette et de coiffure, parterres d'établissements sportifs et tapis de salles de gymnastique, bords de piscines de loisirs ou de rééducation, bacs de douches, matériels agricoles, outils, instruments de soins aux animaux, cages d'animaux domestiques, stalles, enclos, …). Nous pouvons ainsi citer quelques exemples classiques de contamination : contacts entre chevelures dans les collectivités d'enfants et teignes trichophytiques (*T. violaceum*, *T. soudanense,* …) ; promiscuité avec un jeune chat et lésions cutanées circinées et/ou teigne microsporique à *M. canis* ; hamster ou cobaye sur l'épaule et lésion cutanée circinée, voire folliculite suppurée du cou (*T. mentagrophytes*) ; jardinage et lésion cutanée circinée à *M. gypseum* ; échanges de chaussures ou de vêtements dans les clubs sportifs (*T. rubrum*, …) ; spores de *Trichophyton* spp. anthropophiles sur les tondeuses de certains coiffeurs ; spores de *T. verrucosum* sur les stalles d'élevages bovins et folliculites suppurées …

L'installation et le développement des dermatophytes sur leurs hôtes sont conditionnés par des facteurs généraux (âge, sexe …) et locaux de défense. Actuellement, ils sont grandement favorisés par les divers savons et topiques, par la diffusion des antibiotiques antibactériens, par l'excès d'utilisation des corticoïdes, et par la multiplication des cas d'immunodéficience. L'implantation d'un dermatophyte nécessite une « permissivité » locale de l'épiderme, du follicule pilaire ou de la matière unguéale, et en premier lieu, l'existence d'une « porte d'entrée » : égratignure (outil de jardinage, peigne, brosse, …), griffure (chat, hamster, …), macération (notamment au niveau des petits plis), absence pré-pubertaire de sécrétion sébacée (teignes de l'enfant), microtraumatismes unguéaux (marche « sportive », …), … Une fois le dermatophyte implanté, ses possibilités de filamentation, puis de dissémination, sont tributaires du niveau de défenses, notamment cellulaires, que peuvent lui opposer la peau et les phanères. Un déficit, même transitoire, de l'immunité cellulaire locale est donc un facteur favorisant la dermatophytose.

Les dermatophytes déterminent ainsi des pathologies cutanéo-phanériennes nommées dermatophytoses. Celles-ci atteignent surtout la peau, les plis, les ongles, le cuir chevelu, et la région de la barbe.

1.5.1. Physiopathologie

Les dermatophytes sont le plus souvent pathogènes chez l'homme, ils sont rarement saprophytes (certaines espèces telluriques) [21,22]. L'étape initiale dans la survenue d'une dermatophytose implique l'adhérence d'une arthrospore (spore provenant de la fragmentation d'un filament mycélien et constituant l'élément infectant) au cornéocyte (couche kératinisée de l'épithélium malpighien pluristratifié). Le principal mécanisme d'adhérence des champignons repose sur la reconnaissance spécifique entre des adhésines fongiques et des récepteurs de l'hôte. Les adhésines identifiées chez les champignons pathogènes, notamment les dermatophytes, sont principalement des protéines et des mannoprotéines, classées en fonction des ligands auxquels elles se lient [22].

Lors de l'étape d'adhérence, les éléments fongiques doivent résister à différents facteurs (les ultraviolets, les variations de températures et d'humidité, la compétition avec la flore résidente et les propriétés locales physico-chimiques de l'épiderme) et les glandes sébacées élaborant des acides gras insaturés à longues chaînes, en particulier les chaînes 7, 9, 11 et 13 qui sont fongistatiques. Ensuite, il se produit la germination des spores et leur pénétration plus rapide que la desquamation dans le stratum corneum. Cette étape est possible grâce à la sécrétion de

protéases, de lipases et d'enzymes mucolytiques qui apportent également des nutriments utiles aux champignons. Les protéases jouent un rôle dans les premiers stades de l'invasion du stratum corneum, en modifiant la surface des kératinocytes ou la paroi fongique. Ces enzymes sont également impliquées dans l'adhérence du champignon aux kératinocytes [21–23].

1.5.2. Réaction de l'hôte

1.5.2.1. Réaction immunitaire non spécifique

La réaction immunitaire débute lorsque le dermatophyte pénètre la couche cornée, il interagit alors avec les kératinocytes. Ces derniers sécrètent des cytokines telles que l'IL-8 en présence des antigènes du dermatophyte notamment la trichophytine (préparation obtenue à partir de cultures de dermatophytes contenant des antigènes de groupe et d'espèce). Ainsi, les kératinocytes en plus de leur rôle mécanique de barrière jouent un rôle dans l'initiation précoce de la réaction inflammatoire. Ces cytokines entraînent l'arrivée de polynucléaires neutrophiles dont le rôle est de phagocyter les éléments fongiques et d'inhiber la croissance fongique, et des macrophages qui vont présenter les antigènes fongiques aux lymphocytes lors de la réponse immunitaire spécifique [24].

1.5.2.2. Réaction immunitaire spécifique

Immunité humorale

Lors de la réaction immunitaire, certains champignons produisent des facteurs chimiotactiques de bas poids moléculaire (identiques aux bactéries) alors que d'autres activent la voie alterne du complément. Une activation prédominante des lymphocytes T de type 2 (Th2) entraîne une réaction immunitaire tournée vers la production d'anticorps et une installation chronique de la maladie. Plusieurs études ont montré que l'immunité humorale n'induisait pas de protection efficace contre les dermatophytes [24,25].

Immunité cellulaire

C'est la seule immunité efficace dans l'élimination des dermatophytes. Le mécanisme passe par une hypersensibilité retardée (type IV). Une activation prédominante des lymphocytes Th1 stimule la réponse immunitaire à médiation cellulaire, caractérisée par la production d'IFN-γ, Il-12 et Il-2, puis la guérison.

Lors du premier contact avec le champignon, la réaction inflammatoire est minime et le test à la trichophytine est négatif. Les antigènes des dermatophytes sont pris en charge par les cellules de Langerhans et présentés aux lymphocytes T dans les ganglions. Ensuite, il y a prolifération

lymphocytaire clonale, avec inflammation puis destruction des champignons. Le test à la trichophytine devient alors positif. Les dermatophytides surviennent chez 4 à 5% des patients : il s'agit de réactions inflammatoires survenant à distance du foyer infectieux telles que des lésions eczématiformes de localisation variée ou des dyshidroses des mains ou des pieds. Ces réactions sont associées à un test à la trichophytine positif [2,14,25].

1.6. Aspects cliniques des dermatophyties

1.6.1. Dermatophyties de la peau glabre

1.6.1.1. Dermatophytie circinée

Sur le plan épidémiologique, il s'agit d'une affection fréquente, pouvant survenir à tout âge. Cependant, dans les études africaines menées essentiellement chez des patients des centres hospitaliers ou des départements de dermatologie, cette affection est principalement rapportée chez l'adulte avec des taux de prévalence variant de 2,2% à 41% dans certains pays Ouest Africains [26–33], et des taux de prévalence variant de 2.4% à 45.9% dans certains pays de l'Afrique du Nord [34–38]. Sur le plan clinique, cette affection se caractérise par des lésions apparaissant 1 à 3 semaines après un contact infectant. Le début est marqué par une petite macule rosée et finement squameuse qui devient à la phase d'état, un peu saillante, en « disque » (figures 1.1.-a et 1.1-b). Des petites vésicules, très évocatrices mais inconstantes, peuvent être visibles à l'œil nu ou à la loupe sur le pourtour des lésions. La lésion est d'évolution centrifuge, pouvant atteindre 2 à 3 cm de diamètre ou parfois davantage. Parfois plusieurs lésions peuvent confluer pour donner des placards polycycliques. Les localisations préférentielles sont les zones découvertes : face, cou, mains, avant-bras, jambes... [2]. Sur le plan microbiologique, les agents dermatophytiques isolés à partir des lésions de dermatophytie circinée varient en fonction des zones géographiques. Ainsi, en Afrique de l'Ouest plusieurs espèces de dermatophytes incluant *T. soudanense, T. rubrum, T. mentagrophytes, T. tonsurans, T. verrucosum, M. audouinii, M. canis,* et *E. floccosum* sont souvent responsables de cette affection [9,26–30,32,39–42], et en Afrique du Nord, *T. violaceum, T. rubrum, M. canis, T. verrucosum, T. mentagrophytes, E. floccosum* [34–38,43] sont principalement incriminés.

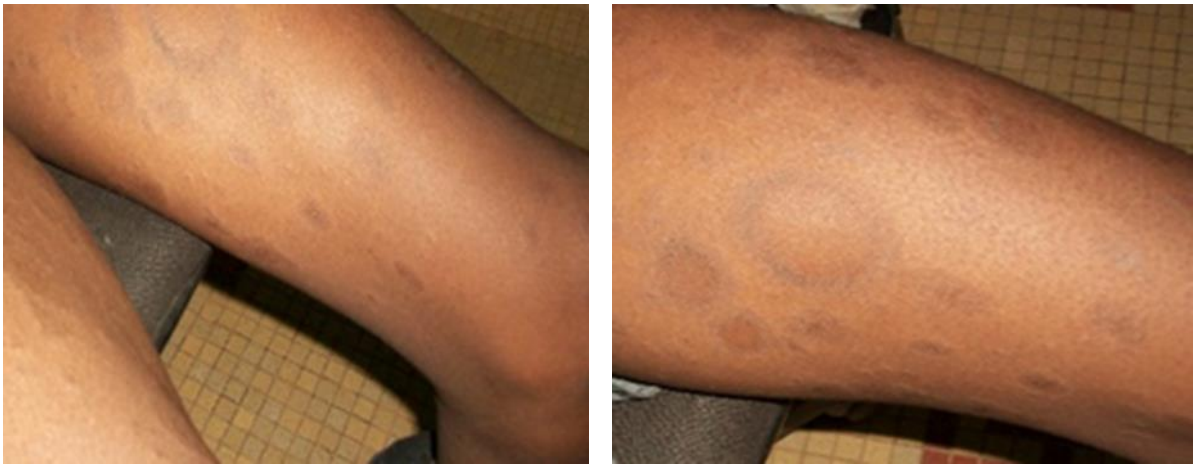


Figure 1.1-a. - Dermatophyties de la peau glabre (topographie : cuisse)

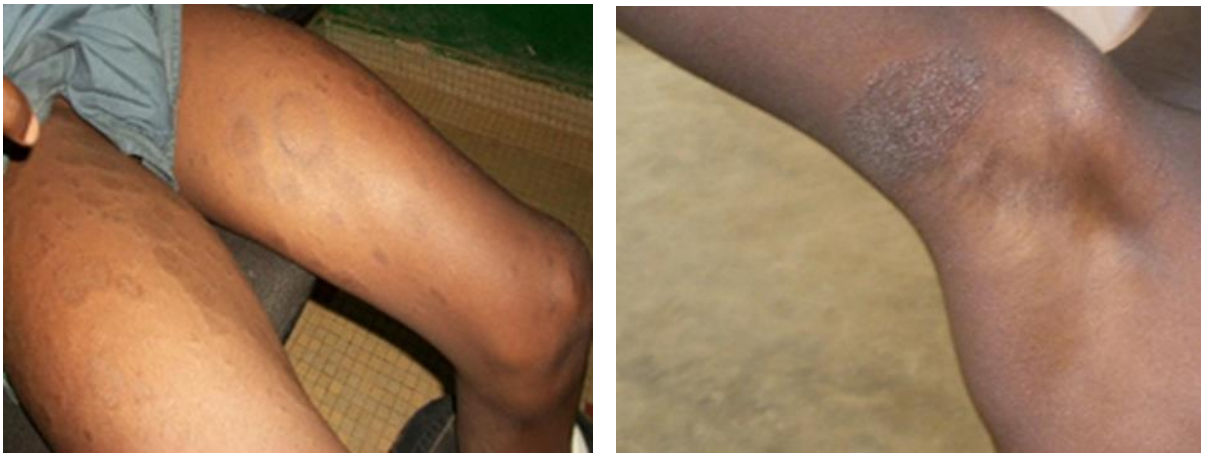


Figure 1.1-b.- Dermatophyties de la peau glabre (topographie : cuisse et bras).

1.6.1.2. Dermatophytie des grands plis

Cette affection, représentée essentiellement par l'atteinte des plis inguinaux (anciennement appelée « eczéma marginé de Hebra ») est plus fréquente chez l'homme que chez la femme ou les enfants. Les dermatophytes anthropophiles sont plus souvent incriminés dans cette topographie. La contamination se faisant par contact interhumain (direct ou indirect par l'intermédiaire des vêtements ou du linge de toilette). La lésion peut être unilatérale ou le plus souvent symétrique, débutant à la face interne des cuisses par une ou plusieurs macules prurigineuses, qui vont confiner pour donner un placard circiné pouvant s'étendre sur la cuisse à partir d'un pli inguinal, et déborder parfois dans le pli interfessier. L'évolution spontanée est chronique, avec alternance des périodes d'amélioration et d'exacerbation [2].

1.6.2. Dermatophyties des pieds et des mains

Ces affections sont plus fréquentes chez l'homme que chez la femme et les lésions concernent plus souvent les pieds que les mains. La contamination se fait essentiellement par contact interhumain indirect. Des cas de contaminations « familiales » sont décrits (surtout à partir des objets de toilette). Certains facteurs comme la chaleur, la transpiration ou la macération, favorisent le développement des lésions. Les symptômes sont représentés essentiellement par un prurit souvent intense, majoré par l'eau et la chaleur. Cependant, l'infection peut rester parfois asymptomatique. Sur les pieds, l'atteinte la plus fréquente concerne les plis interdigitaux (plus spécialement le quatrième espace) et sous-digitaux. Aux mains (*tinea manuum*), la lésion la plus fréquente est l'atteinte hyperkératosique de la paume. Des complications à type de surinfection microbienne, pustules ou une extension mycosique aux ongles peuvent survenir au cours de l'évolution. Les intertrigos dermatophytiques constituent une porte d'entrée classique pour les érysipèles [2].

1.6.3. Teignes du cuir chevelu

Sur le plan épidémiologique, ces affections concernent principalement mais non exclusivement les enfants pré pubertaires surtout des pays en développement. Leur incidence varie en fonction de plusieurs facteurs. Ceci a été confirmé par plusieurs études épidémiologiques qui ont montré, notamment dans certains pays de l'Afrique de l'Ouest la prédominance de ces affections chez les garçons âgés de moins de 10 ans [39,44–47]. Par contre, ces affections sont rarement reportées chez l'adulte, avec des âges moyens variant de 33.1 à 43 ans au Sénégal et en Afrique du Nord [48–50]. En général, la rareté des teignes du cuir chevelu chez l'adulte s'expliquerait par la plus grande résistance des cheveux de celui-ci à la colonisation des dermatophytes, en raison des propriétés fongistatiques de sébum, qui est principalement formé d'acides gras à longue chaîne [48].

Plusieurs études épidémiologiques ont également montré l'impact des facteurs socio-économiques et géographiques (communautés rurales ou urbaines ou péri urbaines, saisons…) [48–50] sur l'incidence des teignes du cuir chevelu. Ainsi, au Nigeria, dans l'État d'Anambra (sud-est du Nigeria), des taux de prévalence de 4,3% et de 8,1% ont été observés respectivement dans les localités urbaine et rurale [44]. Au Togo, une prévalence de teignes de 11% a été notée dans le Nord (zone sèche et urbaine) contre une prévalence de 20% dans le Sud (zone humide et rurale) [48]. Au Ghana, Hogewoning et al [51] ont rapporté une prévalence plus élevée de ces affections dans le milieu scolaire rural que dans le milieu scolaire urbain (10,5% *vs* 6,6%). Une prévalence plus élevée a été également notée chez les élèves provenant d'un milieu socio-

économique faible que chez ceux issus d'un milieu socio-économique moyen ou élevé (10,6% *vs* 4,9%) [51]. Au Gabon, une plus faible prévalence des teignes a été notée dans les écoles urbaines (avec un niveau socio-économique élevé ou moyen) par rapport aux écoles rurales (avec un plus faible niveau socio-économique) (20,4% *vs.* 26,3%) [52].

Le spectre des dermatophytes responsables des teignes du cuir chevelu varie d'un pays à un autre, d'une région à une autre et d'un continent à l'autre. Ainsi, dans certains pays de l'Afrique de l'Ouest, comme le Mali, la Côte d'Ivoire, le Sénégal, certains états du Nigeria, et le Togo, deux dermatophytes anthropophiles (*T. soudanense* et *M. audouinii*) sont le plus fréquemment isolés à partir des lésions des teignes du cuir chevelu [10,11,45,46,53–58]. En Afrique du Nord, les principaux agents étiologiques de ces affections sont essentiellement *T. violaceum* et *M. canis* [39,41,54,65–69]. En Afrique de l'Est, l'espèce anthropophile *T. violaceum* est l'agent étiologique majeur des teignes du cuir chevelu [64–66]. En Afrique Centrale, *T. soudanense*, *M. audouinii, T. tonsurans, T. rubrum* sont des agents étiologiques majeurs des teignes du cuir chevelu [52,67–69]. Dans la plupart des pays de l'Afrique du Sud, *T. violaceum* et *M. audouinii* sont le plus souvent impliqués dans la survenue ces affections [70,71]. En Europe, *M. canis* est l'agent causal fréquent des teignes du cuir chevelu en Autriche, Hongrie, Allemagne et Pologne [4,72]. Cependant l'augmentation globale de l'incidence des dermatophytes anthropophiles est notée dans certains pays Européens: *T. tonsurans* au Royaume-Uni, et *T. soudanense* avec *M. audouinii* en France [72]. Aux Etats-Unis, *T. tonsurans* est signalé comme l'espèce pathogène la plus fréquente chez les enfants et les adultes jeunes [73]. En Asie, au Pakistan, la prédominance de *T. violaceum* et de *T. tonsurans* est rapportée [74], et en Chine, les espèces zoophiles *T. mentagrophytes* et *M. canis* prédominent dans les teignes du cuir chevelu [75].

Sur le plan clinique, selon l'aspect des lésions, les teignes du cuir chevelu sont reparties en teignes tondantes, inflammatoires et faviques.

1.6.3.1. Teignes tondantes

1.6.3.1.1. Teignes tondantes microsporiques

On distingue des teignes microsporiques d'origine humaine et des teignes microsporiques d'origine animale. Dans les atteintes d'origine humaine, les lésions cliniques réalisent des plaques érythématosquameuses uniques ou en petit nombre (4 à 6), de quelques centimètres de diamètre, parfois confluentes (figure 1.2.). Les cheveux atteints, sont cassés à 2 ou 3 mm de leur émergence. En dehors des plaques, les cheveux sont sains. L'examen en lumière de Wood est positif (montrant une fluorescence verte). L'évolution spontanée se fait vers une guérison à

l'âge de 15 ans environ, sans alopécie résiduelle [2]. Dans les atteintes d'origine animale (contamination à partir des animaux de compagnie : chiens, chats, lapins, cobayes…), les plaques sont plus nombreuses, plus petites que dans les teignes d'origine humaine, et les lésions peuvent devenir inflammatoires [3].

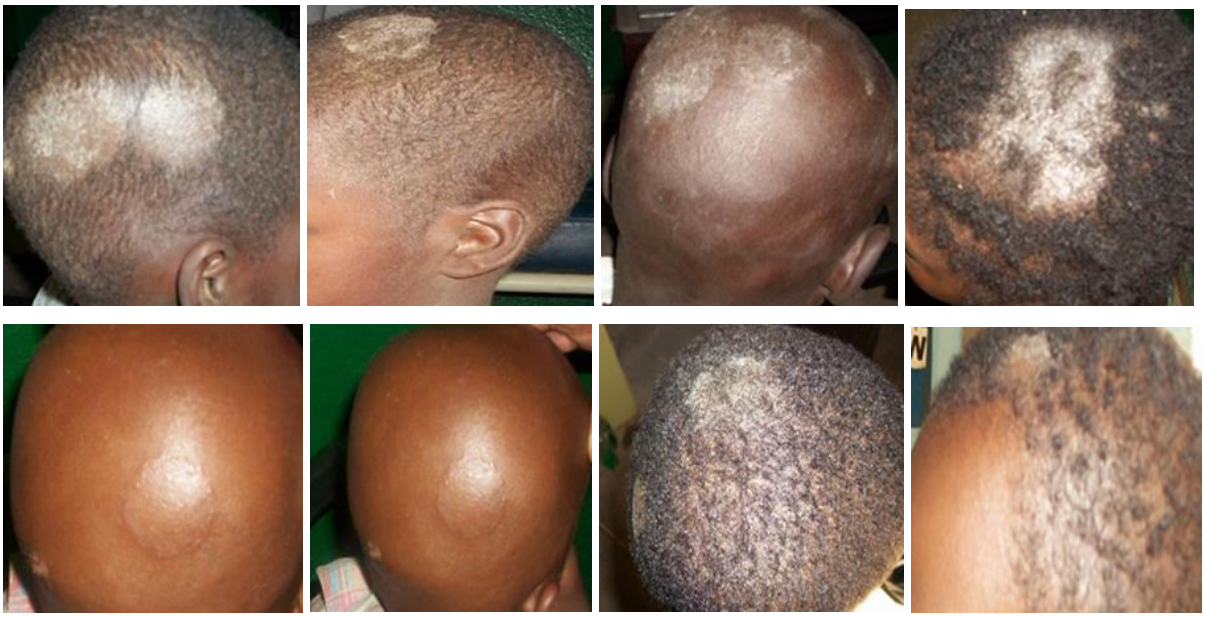


Figure 1.2.-Photographies des teignes microsporiques (différentes topographies)

1.6.3.1.2. Teignes tondantes trichophytiques

Les atteintes trichophytiques réalisent des petites plaques de 1 à 2 cm de diamètre, de forme irrégulière, renfermant des cheveux fragiles se cassant à leur émergence, mélangés à des cheveux normaux (figure 1.3). Les plaques peuvent fusionner en constituant de grandes plaques incomplètement alopéciques. Parfois, il peut s'agir seulement des zones prurigineuses et squameuses bien visibles chez les petites filles africaines au niveau des raies laissées par les coiffures traditionnelles chez les petites filles africaines [76]. Dans ces atteintes, l'examen en lumière de Wood est négatif. Parfois, deux dermatophytes peuvent coexister (*T. soudanense* et *M. audouinii*), rendant alors difficile la classification de la teigne.

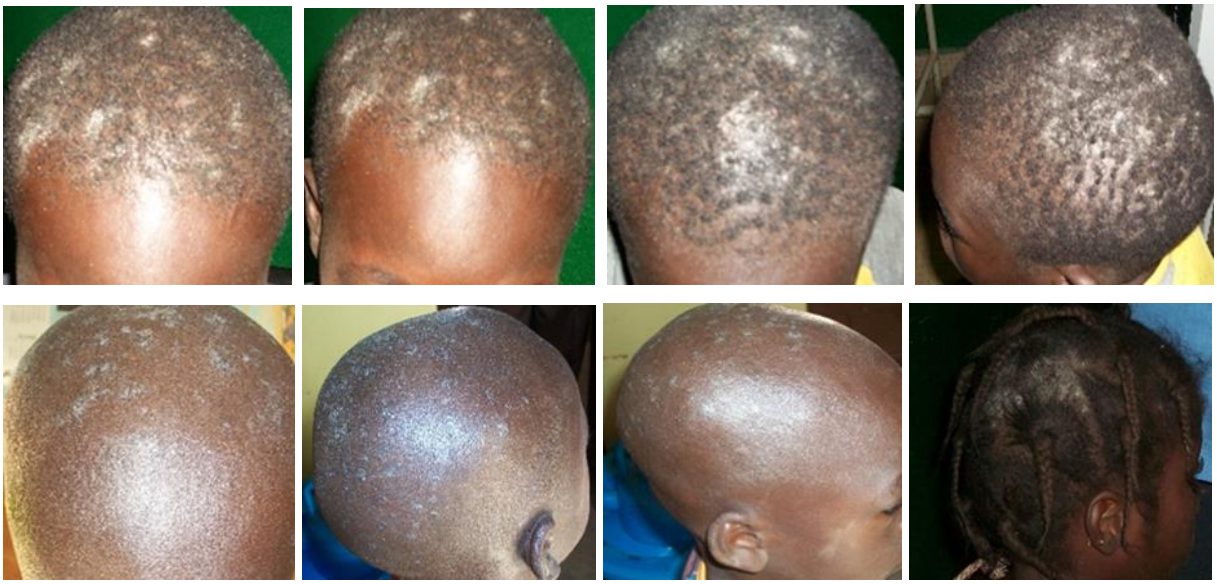


Figure 1.3- Photographies des teignes trichophytiques (différentes topographies)

1.6.3.2. Autres teignes :

Il s'agit des teignes suppurées (ou kérion de Celse) et des teignes faviques (ou favus). Les lésions suppurées sont provoquées principalement par des dermatophytes zoophiles comme *T. mentagrophytes*, ou *verrucosum* (*ochraceum*). Les atteintes faviques, ont pratiquement disparu du fait de l'amélioration de l'hygiène mais de rares cas sont encore rapportés, et l'agent responsable est *T. schoenleinii* [2,77].

1.6.4. Dermatophytoses unguéales (onychomycoses)

Les pieds sont principalement atteints, le tabac et l'artérite sont rapportés comme facteurs de risque [78]. La contamination se fait à partir des sols publics infectés par des débris de kératine contaminée, ou par auto contamination à partir d'un foyer cutané. On distingue trois formes : (i) l'onychomycose sous-unguéale distolatérale (où l'agent pathogène mycosique pénètre sous l'ongle) ; (ii) la leuchonychie superficielle mycosique (se manifestant par des petits îlots blancs et opaques, atteignant progressivement par coalescence toute la surface de l'ongle et celui-ci s'effrite par simple grattage à la curette) ; (iii) et les leuconychies sous-unguéales proximales (envahissement à partir de la face profonde des replis sous-unguéaux) [2].

1.7. Méthodes d'identification classique des dermatophytes

1.7.1. Prélèvement

Etape importante dans l'établissement du diagnostic mycologique, le prélèvement doit être suffisant et de bonne qualité pour permettre l'isolement de l'agent pathogène.

1.7.1.1. Matériel nécessaire

Le recueil de l'échantillon nécessite des instruments divers: la lampe de Wood (pour apprécier l'étendue des lésions, parfois sous-évaluée à l'œil nu) ; les pinces à épiler ou à ongle (sans griffe, de différentes tailles) ; vaccinostyles ou curettes tranchantes ; le grattoir de Vidal ; les ciseaux droits fins ou courbes ; les écouvillons stériles à usage unique ; boîtes de Pétri en plastique ou mieux, en verre ; les carrés de moquette de laine ou les compresses stériles [10,79].

1.7.1.2. Principes généraux des prélèvements

Les prélèvements doivent se faire en dehors de tout traitement par voies générale ou locale (arrêt de 8 jours pour la peau et 1 mois pour les ongles). Une toilette locale préalable à l'aide d'un savon neutre pour éliminer les moisissures de l'environnement susceptibles de contaminer les cultures, mais qui n'est toujours pas réalisée en pratique courante [80]. Pour des lésions multiples (topographies différentes, aspects cliniques différents), les prélèvements ainsi que les identifications doivent être faits séparément. Une bonne connaissance sémiologique des dermatophytoses est indispensable pour réaliser un prélèvement de bonne qualité (prélèvement du matériel vivant, le dermatophyte ayant une croissance centrifuge, les mycéliums vivant se situant donc à la périphérie des lésions cutanées ou à la jonction ongle sain/ ongle mort) [14,18,81].

1.7.1.3. Techniques des prélèvements en fonction de la topographie des lésions

1.7.1.3.1. Prélèvements des lésions cutanées

Les lésions de la peau glabre sont raclées au niveau de leur périphérie, là où le dermatophyte est actif. Les squames sont alors recueillis dans une boîte de Pétri stérile. Deux écouvillons stériles (pour examen direct et la mise en culture) doivent être utilisés pour prélever des lésions et inflammatoires ou suintantes. Dans le cas des lésions sont vésiculeuses, celles-ci doivent être décapitées à l'aide d'une lame de bistouri, et seul le toit sera prélevé (car contenant des filaments). Les compresses stériles peuvent être utilisées avec succès dans les prélèvements des lésions cutanées (recueil des échantillons sur des compresses pour la mise en culture) [79].

1.7.1.3.2. Prélèvements des cheveux

Les cheveux atteints à leur base sont prélevés avec une pince plate (au moins une dizaine cheveux à l'aide pince à épiler), ou mieux après grattage des squames et des croûtes. La lumière de Wood est nécessaire dans les cas des teignes tondantes microsporiques. Le recueil des cheveux peut se faire par traction au niveau des lésions et de leur pourtour (figure 1.4). L'emploi d'un carré de moquette permet de faire des prélèvements par brossage circulaire, notamment

dans le cas d'enquête mycologique (dépistage des porteurs de dermatophytes) chez les enfants contacts ou les animaux domestiques incriminés [2]. Cette technique peut être remplacée avec succès par l'utilisation de compresse stérile [79]. En cas de lésions suppurées, le prélèvement du pus se fait avec un écouvillon.

Figure 1.4- Examen à la lampe de Wood et prélèvements des lésions de teignes sur milieu de culture.

1.7.1.3.3. Prélèvements des onyxis

Les morceaux d'ongle atteint sont coupés à la pince à ongle, en prélevant à la lisière partie malade, partie saine (où le dermatophyte est plus actif). Dans les cas de leuconychies, l'ongle doit être gratté en surface.

1.7.2. Identification morphologique

L'identification morphologique des dermatophytes repose classiquement dans la routine de laboratoire sur l'examen microscopique direct du prélèvement pathologique, la mise en culture de l'échantillon biologique suivie par les identifications phénotypiques (macroscopique et microscopique des colonies).

1.7.2.1. Examen microscopique direct (microscopie optique classique)

C'est un examen indispensable et simple à réaliser qui permet d'observer des filaments mycéliens (confirmation rapide du diagnostic clinique d'une dermatophytose). Pour la réalisation de cet examen, des éclaircissants (contenant le plus souvent de la potasse pour ramollir la kératine : KOH à 10% pour les squames ou à 30% pour les ongles) souvent associés à un colorant sont utilisés. Dans le cadre de nos travaux, le bleu de lactophénol (permettant également de conserver indéfiniment les préparations) a été utilisé comme éclaircissant [79] (figure 1. 5). Cependant, un examen microscopique négatif n'exclut pas une dermatophytose, et la mise en culture du prélèvement est la règle. Un examen direct positif permet d'indiquer seulement la présence d'un champignon, sans préjuger de son espèce.

Figure 1.5.- Prélèvements pour examen direct (par microscopie optique classique)

Dans les squames, l'examen au microscope permet de mettre en évidence des filaments plus ou moins arthrosporés (hyphes) (Figure 1.6). Au niveau des cheveux, cet examen permet d'observer cinq types de parasitisme pilaire (classification de Sabouraud) (Figure 1.7) [13]. Mais les deux types les plus fréquemment observés sont les parasitismes trichophytique et microsporique.

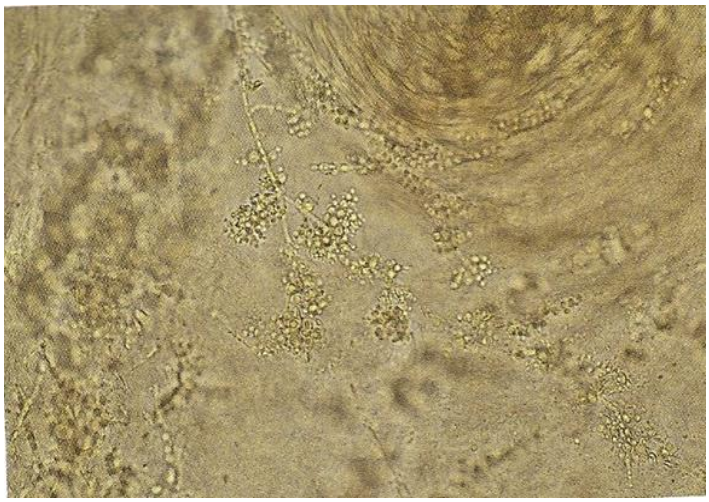


Figure 1. 6- Filaments mycéliens (Source : Encyclopédie Médico-Chirurgicale 8-614-A-10)

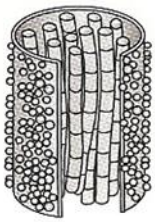
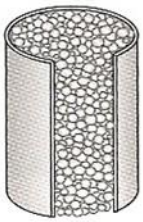
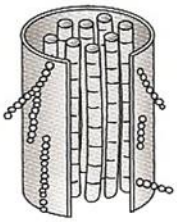
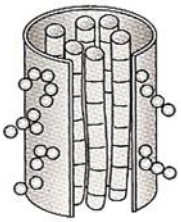
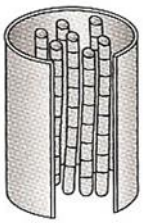
Aspect clinique des lésions	1,2,3 plaques alopéciques de quelques mm de diamètre	Très nombreuses plaques alopéciques de quelques mm de diamètre	Teigne inflammatoire (kérion aigu)	Teigne inflammatoire (kérion subaigu)	Teigne favique
Examen clinique des cheveux	Cheveux cassés à quelques mm de l'émergence	Cheveux cassés très courts englués dans les squames ou aspect de comédon	Cheveux expulsés rapidement	Cheveux cassés court avant d'être expulsés	Cheveux non cassés
Aspect en Wood	Wood +	Wood -	Wood -	Wood -	Wood +
Aspect du parasitisme pilaire à l'examen direct	Microsporique 	Endothrix 	Microïde 	Mégaspore 	Favique 
Étiologies	**Dermatophytes anthropophiles** *M.audouini* *M.langeroni (Afrique noire)* *M.ferrugineum (Extrême-Orient)* **Dermatophytes zoophiles** *M.canis*	**Dermatophytes anthropophiles** *T.tonsurans* *T.violaceum (Méditerranée)* *T.soudanense (Afrique noire)* *T.megninii (Portugal)*	**Dermatophytes zoophiles** *T.mentagrophytes* *T.erinacei*	**Dermatophytes zoophiles** *T.ochraceum*	**Dermatophytes anthropophiles** *T.schöenleinii*

Figure 1.7- Diagnostic clinique et biologique des champignons des teignes (Source : Encyclopédie Médico-Chirurgicale 8-614-A-10).

1.7.2.1. Culture

La mise en culture du prélèvement pathologique est un complément indispensable de l'examen microscopique direct. Sont utilisés des milieux de cultures permettant l'isolement des dermatophytes, et d'autres milieux permettant leur identification.

1.7.2.2.1. Isolement des dermatophytes

L'isolement des dermatophytes se fait habituellement sur des milieux simples contenant un sucre, source de carbone, et un peptone, source d'azote [14]. Le milieu de référence est le milieu de Sabouraud, additionné d'antibiotiques (chloramphénicol et/ ou gentamicine) limitant la pousse des bactéries saprophytes de la peau. Ce milieu peut être rendu sélectif pour l'isolement des dermatophytes en rajoutant de la cycloheximide (Actidione). En règle générale, les dermatophytes poussent à la température du laboratoire (ou mieux à 26-28°C), limitant la pousse des bactéries et celle des champignons non pathogènes. L'aération de cultures est

nécessaire à cause du caractère aérobie des dermatophytes. Les cultures doivent être examinées deux ou trois fois par semaine, pendant au moins 6 à 8 semaines. Lorsqu'une colonie de champignon a poussé sur la boite il existe plusieurs méthodes permettant de l'identifier. Le milieu de Taplin (ou DTM, Dermatophyte Test Medium) peut être utilisé pour l'isolement et l'identification présomptive des dermatophytes. Devant l'alcalisation de ce milieu, contenant un indicateur coloré (rouge de phénol), la présence de dermatophytes est suspectée. Cependant, des faux-positifs et de faux-négatifs ont été rapportés, et malgré les améliorations apportées à cette gélose, son intérêt ne semble pas faire l'unanimité [80,82].

1.7.2.2.2. Identification des dermatophytes et milieux d'identification

Les cultures seront examinées une à deux fois par semaine. Chaque espèce de dermatophyte présente par ailleurs un délai de pousse optimal où l'aspect morphologique est le plus caractéristique. Le milieu d'isolement de Sabouraud permet dans la plupart des cas l'identification des dermatophytes reposant sur un certain nombre de paramètres : vitesse de croissance, aspects macroscopiques et microscopiques [2,14].

L'identification morphologique macroscopique

Elle se base sur plusieurs critères dont la vitesse de pousse d'une colonie adulte, l'aspect macroscopique des cultures (figures 1.8, 1.9,1.10 et 1.11), la couleur de la surface des colonies (brune rouge, noire, verte, grise, blanche…); aspect (duveteux; plâtré, laineux, broussailleux…), le relief (plat), la consistance (friable, élastique, dure, molle…), la forme des colonies (arrondies, étoilées), la taille des colonies (petites, extensives), la présence d'un pigment (couleur, diffusion) au verso de la boîte de culture [83].

Figure 1.8- Cultures sur gélose de Sabouraud : colonies de *T. mentagrophytes* âgées d'une semaine sur SCG (deux premières images) et sur milieu Lactrimel de Borelli (deux dernières images).

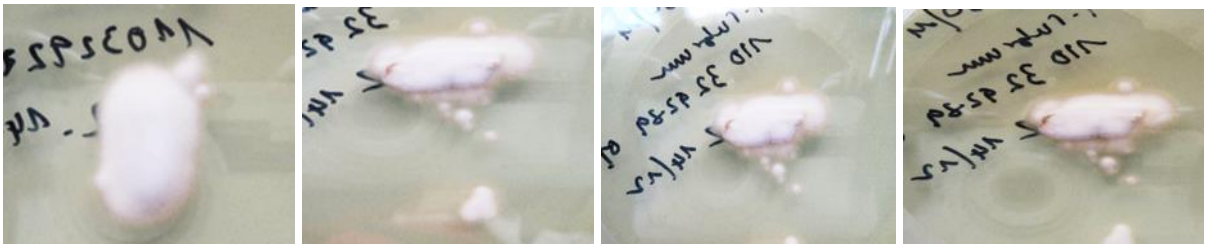


Figure 1.9- Culture sur gélose de SCG : colonies de *T. rubrum* âgées d'une semaine.

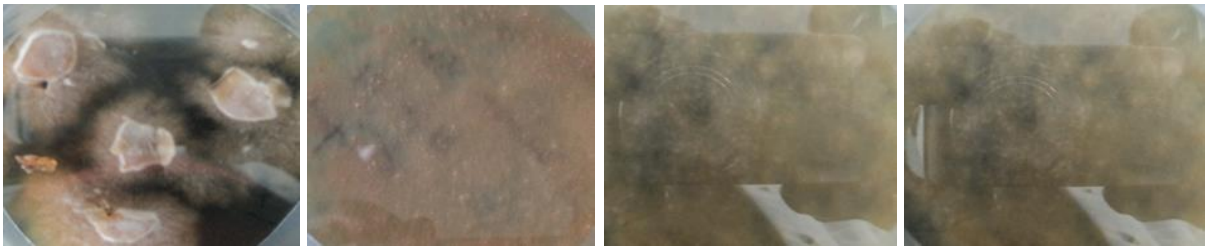


Figure 1.10- Culture sur gélose SCG+Actidione : colonies de *M. audouinii* âgées de 4 semaines.

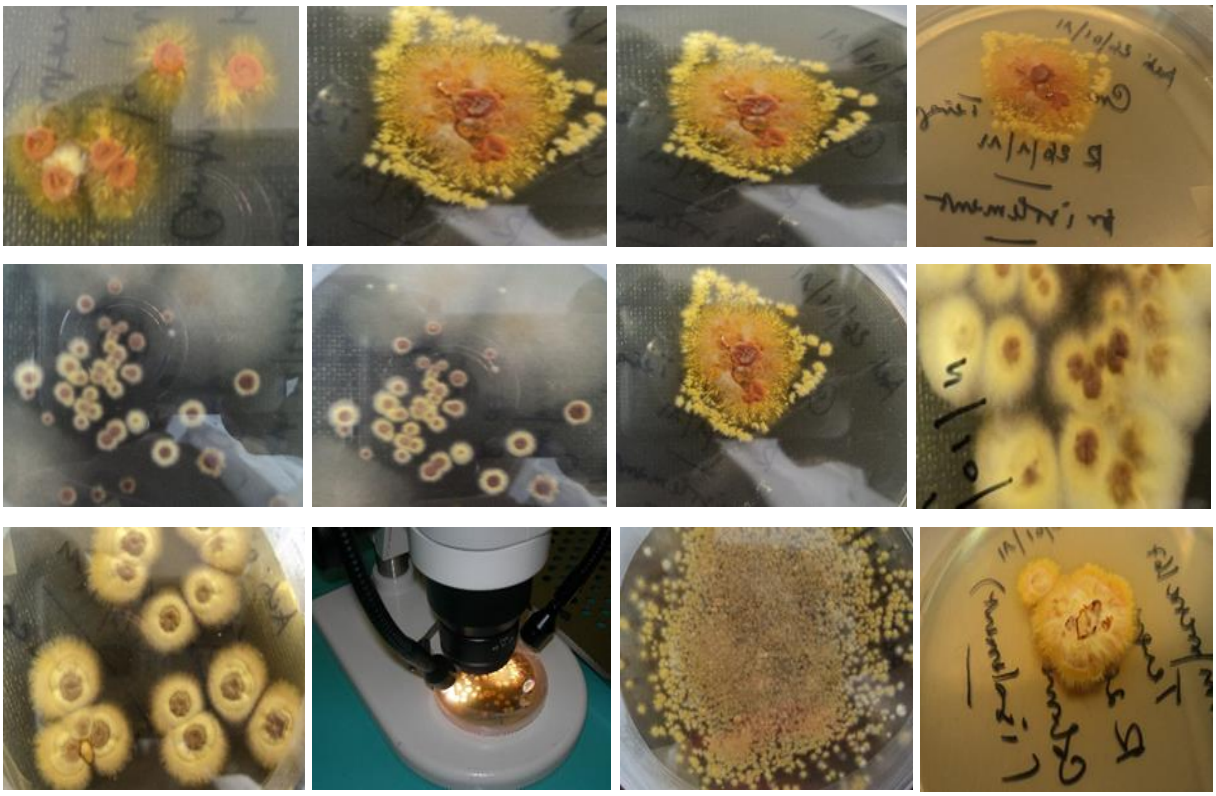


Figure 1.11- Cultures sur gélose SCG : colonies de *T. soudanense* âgées de 15 à 20 jours. Diversité des aspects culturaux avec des colonies jaune paille à rouille (teinte « abricot sec ») avec de nombreuses variantes (teintes violettes et jaune d'or).

L'identification morphologique microscopique

Elle se fait à partir d'un fragment de culture dissocié au bleu lactique ou lactophénol et examiné entre lame et lamelle. Ce procédé peut se faire à partir d'un ruban adhésif appliqué à la surface de la colonie coloré avec du bleu de lactophénol et examiné entre lame et lamelle. Trois éléments servent de base à l'identification du champignon (figures 1.12 et 1.13): les filaments mycéliens qui peuvent être plus ou moins septés dont on étudie le diamètre et la morphologie régulière (aspect en raquette ou aspect moniliforme). L'observation des ramifications permet de décrire des aspects en croix de Lorraine (pour *T. mentagrophytes*), des angles aigus; la présence d'organes de fructification : microconidies à base tronquée, rondes, piriformes ou en suppositoires, disposées en acladium; macroconidies plus grandes, en forme de fuseaux, divisées en logettes par des cloisons transversales, de forme et de taille variables selon les espèces ; les formations environnementales à type de vrille [2].

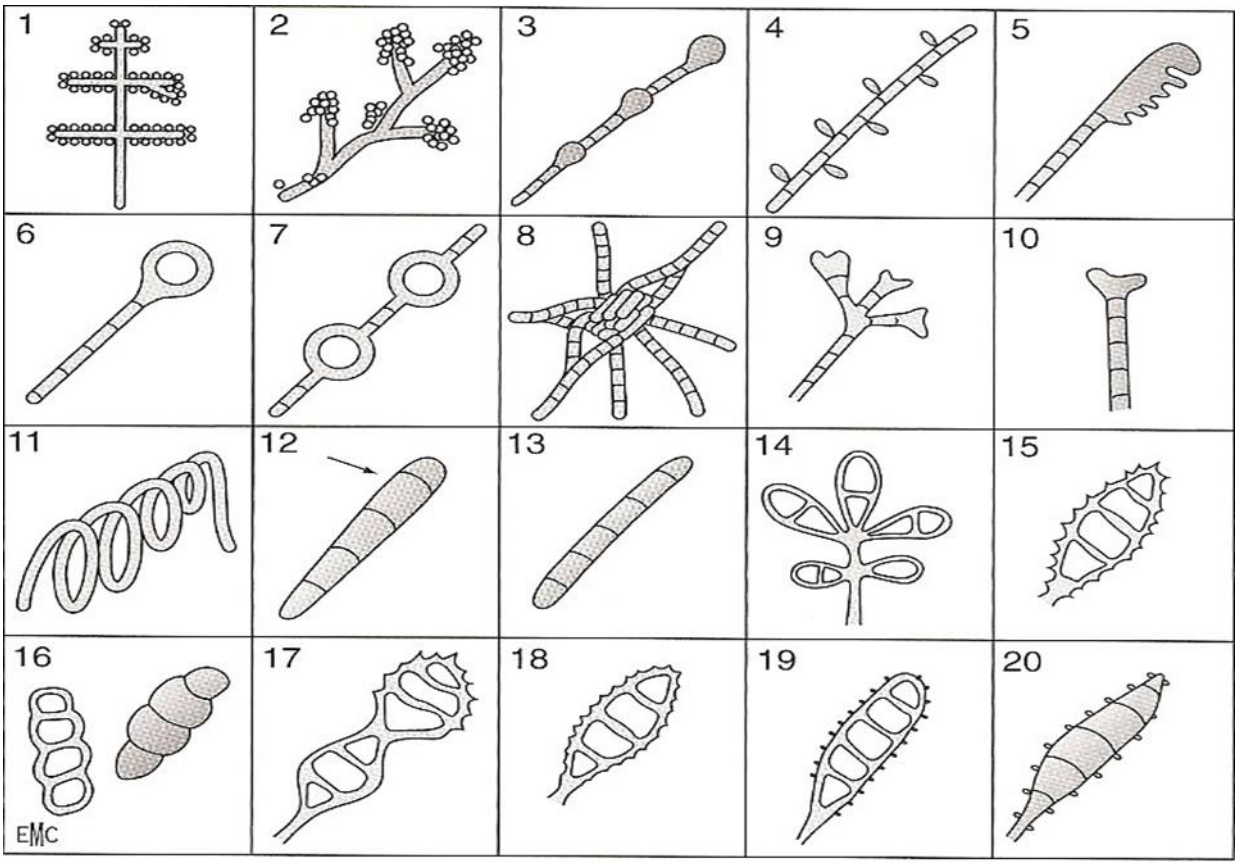


Figure 1.12- Aspect microscopique des cultures : fructifications et formations environnementales. 1. Aspect du mycélium (hyphe) en « croix de Lorraine » (avec microconidies rondes, *T.mentagrophytes*) ; 2. microconidies sphériques en amas ; 3. mycélium

en raquette ; 4. microconidies allongées disposées selon le type Acladium ; 5. mycélium pectiné ; 6. Chlamydospore terminale, à l'extrémité d'un filament mycélien ; 7. chlamydospore intercalaire sur le trajet d'un filament mycélien ; 8. organe nodulaire (*T.mentagrophytes*) ; 9. chandelier favique (*T.schoenleinii*) ; 10. clou favique ; 11. Vrille (*T.mentagrophytes*, *M.persicolor*) ; 12. macroconidie en quenouille de *T. mentagrophytes* ; 13. macroconidie de *T. rubrum* ; 14. macroconidie en bouquet d'*E.floccosum* ; 15. macroconidie de *M. canis* ; 16. macroconidie de *T.tonsurans* ; 17. macroconidie de *M.audouinii* ; 18. Macroconidie de *M.gypseum* ; 19. macroconidie de *M.fulvum* ; 20. macroconidie de *M.persicolor* (Source : Encyclopédie Médico-Chirurgicale 8-614-A-10).

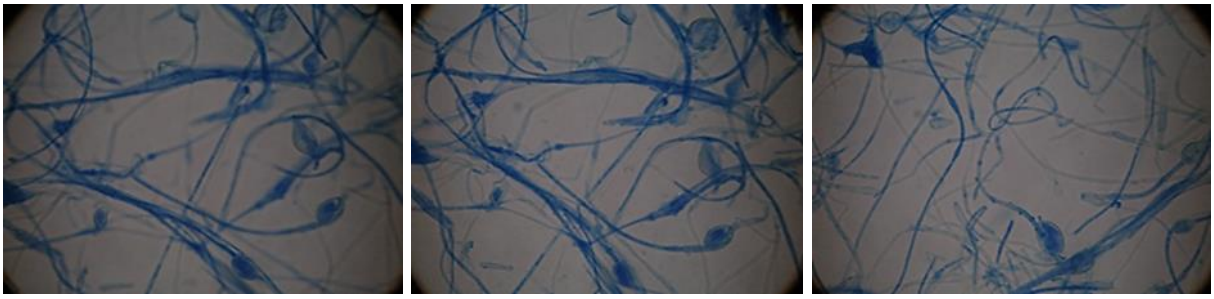


Figure 1.13- Aspect microscopique de *M. audouinii* avec des chlamydospores intercalaires et terminales *(grandissement X 400).*

Figure 1.14. Photographie de quelques écoliers dans la cour de l'école primaire de Sirakoro-Méguétana (localité en périphérie de Bamako, la capitale du Mali).

Les milieux d'identification

Dans des cas de difficultés d'identification du dermatophyte, qui peuvent être liées soit à une souche stérile (dite « pléomorphisée »), soit à une souche présentant des caractéristiques macroscopiques ou microscopiques atypiques [13,14,81], des techniques complémentaires telles que des repiquages sur des milieux spécifiques, dits « d'identification » sont nécessaires parfois pour favoriser la fructification et/ou la production d'un pigment caractéristique [2,17]. Il existe plusieurs types de milieux d'identification, parmi lesquels, on peut distinguer :

-les milieux favorisant la conidiogenèse (stimulant la fructification de la majorité des dermatophytes), comprenant le milieu de Borelli (ou le milieu lactrimel), le milieu de Malt et eau gélosée, la gélose PDA (Potato-Dextrose-Agar), milieu de Baxter ;

-le milieu peptoné à 3% (Sabouraud conservation), permettant de différencier des colonies de *M. persicolor* qui deviennent roses en 8 jours, de celles de *T. mentagrophytes* qui restent blanches ;

- le milieu à l'urée (urée-indole ou Christensen), permettant de différencier *T. mentagrophytes var. interdigitale* et *T. rubrum* ;

- le milieu au Bromocrésol pourpre (BCP caséine), gris au départ, contenant du bromocrésol pourpre et vire au bleu-violacé avec *T. mentagrophytes var. interdigitale*. Il n'y a pas de changement de couleur pour *T. rubrum* et *M. persicolor*. Ce milieu contient également de la caséine que *T. verrucosum* hydrolyse en quelques jours, de même que *T. violaceum var. glabrum* ;

- le milieu BHI (Brain-Heart Infusion) gélosé, qui peut être utilisé pour mettre en évidence *T. verrucosum*. Ce milieu riche, comme les géloses au sang, favorise la croissance de cette espèce zoophile.

1.8. Application des outils de biologie moléculaire aux dermatophytes

Pour pallier au manque de sensibilité et de spécificité des techniques d'identification classique des dermatophytes, plusieurs méthodes moléculaires ont été développées.

1.8.1. Identification moléculaire des dermatophytes

Découverte en 1985 par Kary Müllis, la réaction de polymérisation en chaîne (PCR) est une technique d'amplification des acides nucléiques qui a révolutionné la biologie moléculaire. Elle est basée sur la capacité de l'ADN polymérase à synthétiser le brin complémentaire d'un ADN

matrice, permettant d'amplifier rapidement une quantité importante d'ADN à l'origine peu abondante. C'est un processus de répétition initié par l'hybridation à la séquence cible d'un oligonucléotide synthétique de 15 à 30 bases nommé « amorce ». L'enzyme ajoute successivement les nucléotides complémentaires de la séquence à amplifier et un ADN double brin est ainsi synthétisé. Cette avancée technologique s'est imposée dans les laboratoires et a permis l'essor de nouvelles méthodes ayant un large champ d'applications scientifiques notamment en microbiologie.

1.8.1.1. PCR-RFLP

Cette technique associe l'amplification génomique d'un gène cible par PCR à une digestion enzymatique aboutissant à un profil de fragments d'acides nucléiques qui, après migration électrophorétique en gel d'agarose ou de polyacrylamide peuvent être analysés directement ou hybridés avec des sondes spécifiques, permettant un diagnostic de genres et/ou d'espèces. Plusieurs études ont été menées pour tenter un diagnostic de genre, d'espèce ou même de souche au sein d'une même espèce, par l'analyse du polymorphisme de longueur de fragments de restriction enzymatique en ciblant l'ADN mitochondrial, dans les années 1990 [84–87].

L'utilisation de la cible mitochondriale ne s'étant pas révélée très sensible, des études ont été réorientées vers l'analyse de l'ADN ribosomique, en ciblant les différentes régions de son unité de transcription, dans le but de détecter les dermatophytes et de les identifier, parfois même directement au sein des prélèvements mycologiques [88–93]. Cette cible a montré une meilleure sensibilité que l'ADN mitochondrial, et les méthodes conventionnelles telles que la culture, pour l'identification phénotypique des dermatophytes.

A partir de cette cible ribosomique, d'autres techniques toujours basées sur la PCR ont été développées en quête de spécificité et de sensibilité plus élevées, pour l'identification des dermatophytes.

1.8.1.2. PCR nichée

Les techniques de PCR nichée utilisant deux couples d'amorces lors de deux étapes d'amplifications successives ont également été employées comme outils à visée diagnostique des dermatophytes. En 2007, une étude a montré une bonne sensibilité d'une technique de PCR nichée pan-dermatophytes, chez les patients cliniquement suspects d'onychomycose, en utilisant deux couples d'amorces ciblant la chitine synthase [94]. Pour détecter la présence dermatophytes ou d'autres moisissures impliquées dans les onychomycoses, certains auteurs ont ciblé la séquence du gène de la sous- unité ribosomique 28S de l'ARN [95]. Ces techniques

d'identification utilisées directement à partir de prélèvements se sont révélées être plus sensibles que la culture, mais occasionnant des contaminations importantes, les rendant donc très critiquables en diagnostic. Ainsi, elles ont été peu à peu abandonnées au profit de techniques plus modernes comme la PCR-ELISA mais surtout la PCR en temps réel.

1.8.1.3. PCR-ELISA

Pour le diagnostic d'infections à dermatophytes, des techniques de PCR couplées aux méthodes immunoenzymatiques telles que l'ELISA (Enzyme Linked Immunosorbent Assay) ont été récemment développées pour permettre le diagnostic d'infections à dermatophytes. Dans ce cadre, le kit Onychodiag® (commercialisé par la société BioAdvance) qui a fait l'objet d'une évaluation multicentrique, s'est montré performant pour le diagnostic des onychomycoses à dermatophytes. Cependant, si ce test permet la détection de dermatophyte au sein même du prélèvement, il a comme principale limite : l'incapacité de déterminer le genre ou l'espèce en cause [96,97].

Une technique PCR-ELISA a été mise au point pour permettre l'identification de cinq espèces de dermatophytes à partir de prélèvements cutanés et unguéaux : *Trichophyton rubrum, T interdigitale, T. violaceum, M. canis, E. floccosum* [98]. Cependant, malgré la sensibilité et la spécificité de ce test vis- à-vis de ces espèces données, il ne permet pas d'identifier d'autres espèces de dermatophytes dans les épidermophyties ou onychomycoses. La PCR-ELISA reste cependant, une méthode technologiquement très lourde à mettre en place pour le diagnostic des dermatophytoses, et les kits proposés sur le marché ont un intérêt économique limité dans ce type de pathologie par rapport aux techniques d'identification classique telles que la culture.

1.8.1.3. PCR en temps réel

En raison de sa rapidité, de sa précision excellente et de sa capacité d'analyse à grande échelle, la PCR en temps réel est utilisée dans des domaines variés, notamment en mycologie. Cette technique a été mise en application pour la détection et l'identification de dermatophytes au sein de prélèvements [99–102]. En 2007, une PCR temps réel multiplex recherchant les espèces *T. mentagrophytes* complexe, *T. rubrum, T. tonsurans, T. violaceum, M. canis* et *M audouinii* a été développée[99]. Cette méthode d'identification sensible et spécifique permet un diagnostic des dermatophytoses en moins de 24 heures optimisant ainsi la prise en charge thérapeutique des patients. Dans une étude publiée en 2010, une technique de PCR en temps réel a été mise au point pour la détection et l'identification de 11 espèces de dermatophytes [100,101]. Récemment, Paugam et al [103] ont rapporté une sensibilité de 79% et une spécificité de 73%

d'un test de PCR en temps réel pour la détection de dermatophytes dans des échantillons de peau et des ongles. Cependant, ces méthodes restent coûteuses par rapport aux méthodes classiques, même si elles permettent une détection rapide et une meilleure identification des dermatophytes au sein des prélèvements par rapport à la culture.

1.8.1.4. PCR suivie de séquençage

Le séquençage d'ADN cible consiste à mettre à jour le code génétique d'un gène ou d'une portion de gène ou même d'un génome entier en déterminant l'enchainement de nucléotides de cet ADN, après amplification d'un fragment d'ADN défini par PCR. Cette technique utilise les connaissances acquises depuis plusieurs années sur les mécanismes de la réplication de l'ADN. De par sa précision, le séquençage est un excellent outil tant pour le diagnostic que pour des études épidémiologiques et phylogénétiques. Les inconvénients majeurs sont le coût élevé et le problème du choix percutant des gènes à séquencer en fonction du pouvoir discriminant désiré.

Différents gènes cibles ont été séquencés, notamment le gène codant pour la chitine synthase [104]. Le séquençage de ce gène a permis l'identification en Tunisie d'une souche de *M. ferrugineum* [105]. En 2002, un kit commercial permettant le séquençage d'une portion du gène codant la sous-unité 28S de l'ARNr a été utilisé pour l'identification rapide de certaines espèces de dermatophytes notamment *T. mentagrophytes var. interdigitale* et *T. mentagrophytes var. mentagrophytes* [106]. En parallèle, plusieurs équipes se sont intéressées au séquençage des gènes codant les régions ITS de l'ARN ribososomique pour l'identification des dermatophytes. En 2008, 17 espèces de dermatophytes ont pu être identifiées par des équipes japonaise de Li et française de Bouchara, en utilisant cette technique [107]. La difficulté des méthodes d'identification par séquençage réside dans le fait d'avoir une base de données suffisamment importante pour pouvoir comparer les séquences et permettre une identification précise. Aujourd'hui le « gold standard » pour l'identification d'une souche atypique de dermatophyte en biologie moléculaire est le séquençage de la région ITS [97,108–112].

1.9. Application de la technique de spectrométrie de masse MALDI-TOF à l'identification des dermatophytes

La spectrométrie de masse Matrix Assisted Laser Desorption Ionization-Time-Of –Flight (MALDI-TOF) est une méthode d'ionisation douce permettant la désorption des peptides et protéines de différents micro-organismes. Les ions sont séparés et détectés en fonction de leur masse moléculaire et charge. Les micro-organismes sont identifiés sur la base du rapport masse/charge (m/z). Cette approche donne des spectres qui se composent d'une série de pics.

Chaque pic correspond à un fragment moléculaire libéré à partir des cellules des micro-organismes au cours de la désorption. C'est une technique rapide et sensible qui est récemment devenue un outil populaire pour caractériser les micro-organismes [113]. Cette technique a été plus largement appliquée aux bactéries et a été présentée comme une véritable révolution en microbiologie clinique [114]. L'identification des champignons filamenteux par la spectrométrie de masse type MALDI-TOF est un champ d'innovation en pleine expansion [115,116]. Elle a été récemment appliquée avec succès dans l'identification des isolats cliniques des champignons du complexe *Pseudallescheria/Scedosporium* spp. [117]. Des études plus récentes ont montré une identification rapide et efficace des isolats cliniques de dermatophytes dans la routine de laboratoire, par cette méthode [118–121]. La spectrométrie de masse MALDI-TOF représente ainsi un outil permettant une identification rapide, précise et standardisée des champignons filamenteux. Cependant, les bases des données actuellement disponibles doivent être évaluées dans différents laboratoires de mycologie cliniques et complétées afin d'élargir le spectre des espèces identifiées [115]. L'identification fongique par cet outil s'impose de plus en plus en mycologie médicale.

1.10. Traitement des dermatophyties

1.10.1. Médicaments antifongiques

1.10.1.1. Antifongiques systémiques

1.10.1.1.1. Griséofulvine

Antifongique fongistatique, découvert en 1939, issu du métabolisme de *Penicillium griseofulvum*, la griséofulvine a été utilisée initialement comme antifongique agricole, et c'est à partir de 1958, qu'elle a été développée en médecine humaine, où elle a révolutionné la prise en charge des teignes. Son mode d'action n'est pas parfaitement connu, et plusieurs mécanismes ont été évoqués et aboutissant au niveau cellulaire à une altération de la constitution de la paroi de filament fongique, inhibition de la mitose de la cellule fongique [122]. Son spectre est étroit, limité aux trois genres de dermatophytes : *Epidermophyton, Microsporum* spp. et *Trichophyton* spp. Sur le plan pharmacocinétique, son métabolisme est hépatique avec une élimination rénale sous forme inactive. La griséofulvine est un inducteur enzymatique hépatique, et peut accélérer la transformation de nombreux médicaments en diminuant généralement leur activité. Les effets secondaires de la griséofulvine, plus fréquents chez l'adulte que chez l'enfant peuvent être variables (troubles digestifs, troubles neurologiques, manifestations cutanées, neuropathie périphérique, éruption lupus-like ou aggravation d'un lupus). Ce médicament est contre-indiqué

en cas de grossesse et d'allaitement, chez les patients atteints de porphyrie et en cas de prise de boissons alcoolisées.

1.10.1.1.2. Dérivés azolés

Il s'agit d'une famille de dérivés obtenus par synthèse chimique, et possédant un noyau imidazole. De nombreuses molécules existent, pouvant être utilisées par voie générale ou topique. Leur spectre d'action est très large, incluant les dermatophytes. Leur mode d'action est double : mécanisme physicochimique entrainant une altération des fonctions respiratoires du champignon lors de sa croissance; mécanisme métabolique de type fongistatique entrainant une inhibition de la synthèse de l'ergostérol membranaire.

Le kétoconazole est le premier dérivé imidazolé actif par voie orale. Son métabolisme est hépatique, avec élimination principalement biliaire, sous forme inactive (87%). La toxicité du médicament est représentée essentiellement par le risque de l'hépatite idiosyncrasique de fréquence rare (1/17 000 patients) dont certains cas mortels ont été rapportés. Les hépatites se manifestent le plus souvent après deux semaines de traitement. Le risque de cette toxicité hépatique augmente chez la femme, l'alcoolique, les patients aux antécédents d'atteinte hépatique et aux antécédents de prise de griséofulvine. Aussi une surveillance biologique est indispensable lors de l'utilisation de ce traitement, notamment en cas d'utilisation prolongée. D'autres possibles effets secondaires ont été décrits (troubles digestifs, troubles neurologiques, troubles cutanés, troubles de la libido, oligospermie, alopécie, gynécomastie chez l'homme). Ce médicament est contre-indiqué pendant la grossesse et nécessite l'association d'une contraception chez la femme en période d'activité génitale. Par son mécanisme d'action, le kétoconazole expose aux risques d'interactions médicamenteuses (antivitamine K : potentialisation des anticoagulants ; rifampicine : diminution des taux sériques des deux médicaments ; isoniazide : diminution des taux plasmatiques de kétoconazole ; griséofulvine : aggravation possible du risque hépatique du kétoconazole).

1.10.1.1.3. Allylamines

Cette classe d'antifongiques possède un mode d'action spécifique par blocage de la synthèse de l'ergostérol de la membrane fongique au stade de l'époxydation du squalène. Ces médicaments agissent comme fongicides et la terbinafine est le représentant de cette classe [123]. La pharmacocinétique de cette molécule lipophile montre une absorption de 70% après prise orale, augmentée si la prise a lieu lors d'un repas [124]. Le pic plasmatique est atteint en 2 heures. La liaison aux protéines est forte. Le métabolisme de cette molécule est hépatique

avec élimination majeure par voie urinaire sous forme de métabolites inactifs. Chez l'enfant, la pharmacocinétique est similaire à l'adulte, sauf pour la clairance qui est augmentée [2]. Les contre-indications et précautions d'emploi sont représentées par l'insuffisance hépatique et/ou rénale sévère, la prise du médicament devant évitée ou déconseillée lors de grossesse et d'allaitement. Les indications de la terbinafine sont très centrées sur la prise en charge des dermatophyties cutanées et phanériennes de l'adulte. Les effets indésirables décrits dans la littérature sont des troubles digestifs (nausées, douleurs abdominales, diarrhées, anorexie), des troubles du goût (agueusie ou dysgueusie), réversibles en 1 à 2 mois après l'arrêt du traitement, des éruptions cutanées transitoires (urticaire, rash non spécifiques, pustulose exanthématique), des troubles neurologiques (céphalées, vertiges) et des troubles hépatiques (hépatite mixte à prédominance cholestatique 2,5/ 100000) [125].

1.10.1.2. Antifongiques topiques

1.10.1.2.1. Imidazolés topiques

Disponibles en forme topique, ils possèdent une très faible capacité de passage transcutané, ce qui limite leurs effets secondaires systémiques. Selon les molécules, ces médicaments s'utilisent en une ou deux applications quotidiennes pour des durées de traitement dépendantes de l'indication, voisines de 3 semaines le plus souvent [2].

1.10.1.2.2. Ciclopiroxolamine

Cette molécule appartenant à la famille des hydroxypiridones, inhibe le captage et l'incorporation des substances nécessaires à la croissance et au métabolisme du champignon : altération du transport transmembranaire des ions, des acides aminés, chélation du fer des systèmes enzymatiques cellulaires. De plus, la molécule possède une activité anti-inflammatoire par blocage de la voie des peroxydases et de la lipoxygénase. Le spectre d'action de cette molécule est large, incluant les dermatophytes, les levures, les bacilles à Gram positif et certains bacilles à Gram négatif. [2].

1.10.1.2.3. Amorolfine

L'amorolfine est un dérivé de la morpholine, produit fongistatique et fongicide. Son activité fongicide est liée à une inhibition de deux enzymes impliquées dans la synthèse de l'ergostérol. Du fait de sa toxicité par voie systémique, cette molécule n'existe qu'en forme locale destinée au traitement des onychomycoses. Son spectre d'action est large, comprenant les dermatophytes, les levures, les dématiés et certaines moisissures [126]. Le produit est contre-

indiquée son chez la femme enceinte ou allaitante. La rémanence du produit est telle qu'une application hebdomadaire est suffisante [2].

1.10.1.2.4. Autres molécules

La terbinafine existe en forme topique avec des caractéristiques pharmacocinétiques qui permettent des durées de traitement plus courtes, car des concentrations efficaces supérieures aux concentrations minimales inhibitrices (CMI) des dermatophytes persistent 7 jours après l'arrêt du traitement.

Le tolnaftate qui appartient à la famille des thiocarbamates, comme pour les allylamines, exerce son action fongicide par inhibition de la synthèse de l'ergostérol [2].

1.10.3. Indications des antifongiques

1.10.3.1. Dermatophytie circinée

Sur la peau glabre, un traitement local, basé sur l'emploi d'une crème ou d'une pommade est le plus souvent suffisant. Au niveau des zones cutanées pileuses, l'emploi de gels ou lotions, est nécessaire. Les imidazolés, la ciclopiroxolamine, le tolnaftate s'utilisent pendant 3 semaines, alors que la terbinafine ne nécessite que 2 semaines en application quotidienne, ou 8 jours de traitement en application biquotidienne. En cas d'atteinte très kératosique ou croûteuse, un décapage avec une préparation kératolytique est nécessaire avant de débuter le traitement antifongique. C'est l'extension des lésions qui est le critère principal à considérer pour décider d'un traitement systémique [2].

1.10.3.2. Dermatophytie des plis

Dans l'atteinte des grands plis (où un dermatophyte anthropophile est plus souvent en cause), le traitement local est de règle, sauf en cas de récidives itératives ou de lésions associées qu'il faut rechercher. Le choix de la forme galénique est dans ce cas important, car il faut éviter la macération, source de récidive : gel, lotion ou poudre sont privilégiés. De même, dans l'atteinte des petits plis, le traitement local est la règle avec gel, poudre, lotion, voire crème peu couvrante.

1.10.3.4. Dermatophyties des paumes et des plantes

Dans ces atteintes un traitement systémique est prescrit à causes de l'hyperkératose physiologique est parfois majorée par la maladie dermatophytique. Le traitement de 1ère intention est la terbinafine pour une durée de 6 semaines. Lorsque l'on utilise des traitements systémiques fongistatiques, la durée de traitement est plus longue, et il est préférable d'associer

un traitement local. Le kétoconazole nécessite 1 à 2 mois de traitement et la griséofulvine 1 à 3 mois [2].

1.10.3.5. Teignes du cuir chevelu

Dans les teignes tondantes, les traitements local et général doivent être associés. Les lotions ou shampooings contenant un imidazolé sont à privilégier, car adaptées à une bonne biodisponibilité locale du principe actif. Le traitement général de référence est la griséofulvine pendant 6 à 8 semaines, dont il faut augmenter la posologie lors des atteintes à *M. canis* (20 à 25 mg/kg/j). Le kétoconazole pendant 6 à 8 semaines est utilisable en deuxième intention, et l'intérêt de la terbinafine a été précisé dans cette indication : 4 semaines de traitement pour un *Trichophyton*, et 8 semaines de traitement pour un *Microsporum* [127]. Nous rappelons que la guérison des teignes ne peut-être affirmée que sur la négativité du prélèvement mycologique obtenue à l'issue du traitement.

Dans les teignes suppurées, les mêmes mesures s'appliquent mais l'indication d'un traitement anti-inflammatoire cortisoné pour une durée brève est discutée. Par ailleurs les mesures prophylactiques sont nécessaires : désinfection outils de coiffure, des bonnets, cagoules,… coupe de cheveux autour de la zone malade, examen et traitement des porteurs au sein d'une famille dans le cas d'une teigne d'origine anthropophile [2].

1.10.3.6. Dermatophytes unguéales

Dans l'atteinte distale, le plus souvent, un traitement local est suffisant, basé sur l'emploi d'une forme galénique adaptée : solution filmogène d'amorolfine appliquée une fois par semaine, solution à 8% de ciclopiroxolamine en application quotidienne. Les atteintes leuconychiques superficielles bénéficient également des traitements locaux. L'action mécanique visant à détruire la partie d'ongle malade est indispensable dans tous les cas. La croissance de l'ongle (toujours plus rapide à la main qu'au pied) détermine la durée du traitement. Dans l'atteinte proximale ou totale (atteintes matricielles et atteintes distolatérales étendues), le traitement général est indispensable, et le médicament de référence est actuellement la terbinafine [128]. La durée du traitement dépend de la vitesse de croissance de l'ongle qui détermine la disparition de l'infection au niveau de la matrice unguéale : 3 mois pour les mains, 6 mois pour les pieds, le plus souvent. Aussi l'âge élevé qui retentit sur la vitesse de croissance de l'ongle, doit être pris en compte avant de décider de traiter une onychomycose [129].

Nos travaux

Nous allons maintenant présenter les travaux originaux qui ont fait l'objet de notre travail de thèse. Dans le premier chapitre, nous présentons une revue de la littérature sur l'épidémiologie des dermatophyties en Afrique (Article 1). Dans le chapitre 2, sont présentées les enquêtes transversales descriptives menées dans trois écoles fondamentales situées dans trois zones éco-climatiques différentes du Mali (Article 2). Nous présentons dans le chapitre 3, l'étude menée sur l'évaluation in vitro des aminostérols contre des isolats cliniques de dermatophytes (Article 3) ainsi qu'un essai clinique randomisé, de phase II mené sur la squalamine dans le traitement topique de la teigne, comparée au placebo (Article 4). Enfin, sont présentées en annexes deux publications de Mycologie Médicale dont la première porte sur l'application de la spectrométrie de masse de type MALDI-TOF dans l'identification des champignons du complexe *Pseudallescheria/Scedosporium* spp (sujet de notre Master-2) et la deuxième sur l'identification des isolats cliniques de dermatophytes par cet outil (à laquelle, nous avons participé).

Chapitre 1 : Revue épidémiologique

Les dermatophytes sont des champignons filamenteux cosmopolites. Leur répartition varie considérablement en fonction de différents facteurs : épidémiologiques (sexe, âge…), socio-économiques (pauvreté, mauvaises conditions d'hygiène) et géographiques (zones rurale ou urbaine, saisons, chaleur, humidité…). Certaines espèces sont retrouvées dans le monde entier (*T. rubrum*, *T. mentagrophytes* var. *interdigitale*, *M. canis*, *M. gypseum* et *E. floccosum*), par contre, d'autres ont une restriction géographique partielle, comme *T. soudanense* (Afrique), *T. violaceum* (Afrique, en Asie et en Europe). Les affections cutanées dues aux dermatophytes sont des affections fréquentes, superficielles. Leur épidémiologie se modifie régulièrement et cela se vérifie tout particulièrement pour les teignes, dont les agents pathogènes ont beaucoup changé pendant le XX^{e} siècle en Europe. Cependant, en Afrique, les premières revues critiques sur la distribution géographique des dermatophytes remontent à 1964. Et depuis cette date, de nombreuses études focalisées sur l'incidence, les caractéristiques cliniques et les facteurs étiologiques des infections à dermatophytes ont été menées dans différentes régions du continent Africain. Nous avons procédé à une revue critique (**Article 1**) afin de déterminer les tendances épidémiologiques actuelles de la distribution géographique des différentes espèces de dermatophytes impliquées en pathologie humaine sur le continent Africain. A partir de la base de données PubMed, et en utilisant les termes de recherche limités aux humains, 382 articles ont été identifiés et parmi lesquels, 110 articles répondant aux critères de sélection ont

été évalués. Il ressort que plusieurs études ont été conduites dans différentes régions de l'Afrique et sur des populations différentes. Ainsi, les études conduites chez les enfants, le plus souvent en milieu scolaire notent une prédominance des teignes du cuir chevelu avec mise en évidence de plusieurs facteurs de risque (âge, sexe, niveau socio-économique, zones géographiques). Dans les études menées chez les adultes (essentiellement dans les centres ou départements de dermatologie), la prédominance des autres dermatophytoses est surtout rapportée, parmi lesquelles la dermatophytie de la peau glabre apparait généralement comme la forme la plus fréquente. Globalement, le spectre des dermatophytes varie en fonction des zones géographiques. En Afrique de l'Ouest et du Centre, deux espèces anthropophiles (*T. soudanense* et *M. audouinii*) prédominent dans les teignes du cuir chevelu, tandis que l'espèce anthropophile *T. violaceum* prédomine dans ces affections en Afrique du Nord, du Sud, et de l'Est. Cependant, l'émergence de l'espèce zoophile *M. canis* est de plus en plus signalée en Afrique du Nord. Dans les autres infections dermatophytiques, notamment les atteintes cutanées, plusieurs agents étiologiques variant en fonction des aires géographiques, sont incriminés, notamment *T. rubrum, T. mentagrophytes, T. soudanense, T. violaceum, M. canis, E. floccosum*.

Article 1: Updates on the Epidemiology of Dermatophyte Infections in Africa

Oumar Coulibaly, Coralie L'Ollivier, Renaud Piarroux, Stéphane Ranque*

Article en cours de rédaction

Epidemiology of Dermatophytosis in Africa

Oumar Coulibaly, Coralie L'Ollivier, Renaud Piarroux, Stéphane Ranque#

1- Aix-Marseille Université, IP-TPT UMR MD3, 13885 Marseille, France.

2- APHM, CHU Timone-Adultes, Parasitology & Mycology, 13005 Marseille, France.

 # Corresponding author

Dr Stéphane Ranque

Laboratoire de Parasitologie Mycologie

CHU Timone

264 rue Saint Pierre

13385 Marseille CEDEX 5

Tél. +33491386090

Fax. +33491384957

Email: stephane.ranque@ap-hm.fr

Keywords: Epidemiology; tinea capitis; dermatophytes; dermatophytoses; Africa.

Introduction

Dermatophyte infections, i.e. dermatophytosis, in humans are chiefly superficial fungal infections, as dermatophytes invade and propagate in keratinized tissues such as hair, skin, and nails. According to Emmons (1934) [1] and despite recent significant changes in the taxonomy, around 30 dermatophytes species were classified in three anamorphic genera: *Trichophyton*, *Microsporum* and *Epidermophyton* [2]. Dermatophytosis is common worldwide [3] and has veterinary and public health relevance. In fact, it has been estimated that 20 to 25% of the world populations are infected by dermatophytes and the incidence continues to increase on a steady basis [4]. Dermatophytes can affect different parts of the body. They tend to grow outwards on skin producing a ring-like pattern - hence the term 'ringworm'. Clinical classification of lesion is according to the site: *tinea capitis* for scalp, *tinea manuum* for hands, onychomycosis or *tinea unguium* for nail, *tinea barbae* for beard area, *tinea corporis* for body including arms and trunk. *Tinea capitis* affects predominantly, but not exclusively, prepubertal children. The clinical hallmark of tinea capitis is hair loss, which may be accompanied by signs of inflammation. So, clinical signs may be subtle, with mild flaking of the scalp alone; however, broken-off hairs, patches of frank alopecia, pustules, large inflammatory swellings (kerion), and tender occipital lymphadenopathy may also occur [5]. A particular form of tinea capitis is the favus caused by *T. schoenleinii*. It's a chronic disease characterized by the presence of yellowish, cup-shaped crust called "scutula" on the scalp with severe alopecia. Based on their ecology, dermatophytes have been divided into three groups, as anthropophilic, zoophilic, and geophilic. Anthropophilic dermatophytes are restricted to human hosts and produce mild chronic inflammation. Zoophilic dermatophytes have an animal reservoir, such as house pets, farm animals, horses; they occasionally infect humans who usually develop inflammatory lesions. Geophilic dermatophytes are presents in soil containing keratinous materials (i.e., hair, feathers and horns); they can be transmitted to humans and animals through contact with soil [3,5].

The distribution of these fungi varies considerably, depending on epidemiological factors (i.e., age, sex, seasons, and socioeconomic factors) and on geographical area [6]. Some species are distributed worldwide, such as *T. rubrum*, *T. interdigitale*, *M. canis*, *M. gypseum* and *E. floccosum*. Others have partial geographic restriction, such as *T. schoenleinii* (Eurasia, Africa), *T. soudanense* (Africa), *T. violaceum* (Africa, Asia, and Europe), and *T. concentricum* (Pacific Islands, Far East, and India) [3,7]. Broadly, the current situation can be summarize as follow: *T. rubrum* is the commonest dermatophyte in most developed countries; tinea capitis caused by *Trichophyton schoenleinii*, or favus, declined in most countries [8]; *T. tonsurans* spread in

United States and *M. canis* and *M. audouinii* were the predominant agents of tinea capitis in Europe [9]. But this situation is not static due to immigration-movements, travel activities, changes in the level of surveillance and diagnosis and antifungal treatment.

The first critical reviews of the geographical distribution of dermatophytes in continent of Africa were reported in 1964 and 1974 [10,11]. Since that time, no other review but many epidemiological reports have focused on studying the incidence, clinical characteristics, and etiological factors dermatophytosis in different regions of Africa. So epidemiological data were accumulated, some which were already been collected, analyzed and discussed in various studies. This study attempts to summarize current epidemiological trends on dermatophytes distribution on African continent.

Methods

Definitions and identification

We defined dermatophyte infections (dermatophytosis or dermatophytoses or tinea) such as human infections of hair, skin, or nails caused by dermatophytes, with a mycological confirmation based on direct examination, culture, microscopic examination and/ or sequencing of the ITS2 region of the ribosomal DNA [12].

Search strategy and selection criteria

We searched in the PubMed 'NCBI) database, using the search terms ("arthrodermataceae" [Mesh] OR "dermatophytes" [Mesh] OR "tinea" [Mesh]) AND "Africa" AND Limits: Humans. We also reviewed reference lists to seek data missed by our search. No language restrictions and nor publication date were used. Studies carried on the treatment of dermatophytosis in Africa were excluded. Also, studies carried on histoplasmosis, pityriasis versicolor, or fusariosis, non-dermatophytes, and other skin disorders in Africa were not selected.

Data extraction

Two reviewers examined titles, abstracts, and articles, and selection criteria, and collected data on potential explanatory variables.

Findings

Epidemiological data on the dermatophytoses in the main African regions

The epidemiology of dermatophytoses has been assessed in various contexts such as urban or rural areas, primary or junior schools, various medical institutes, such as primary or tertiary health care health institutions, department of dermatology or dermatology centres, army hospitals, microbiology department,[13,14] in children and adults, schoolchildren, in- or outpatients, and prison inmates. We retrieved in the literature epidemiological data from studies located over the entire African continent, in particular from: ***West Africa***, including Mali, Ivory Coast, Nigeria, Togo, Senegal, Ghana, and Guinea [15–28]; ***North Africa***, including Tunisia, Morocco, Algeria, Egypt, and Libya; ***East Africa***, including Ethiopia, Kenya, Tanzania, and Rwanda; ***Central Africa***, including Gabon, Cameroon, Central African Republic; and ***Southern African*** countries, namely, Zambia, Botswana, Zimbabwe, Malawi, Republic of South Africa, Mozambique, and Madagascar. Because, as it will be detailed below, dermatophytoses features are distinct in children and adults, we will analyze the data observed in children and adults, separately.

Age related particularities.

Over the entire African continent, the prevalence of dermatophytoses, all presentation combined, peak among children aged between 4 to 11 years. In particular this has been evidenced in many studies in West Africa [43,44,48,50,51,56,62–64,146]. Tinea capitis is uncommon in infants [150], as shown in Tunisia, where a retrospective study over a 12 years period showed that only 3.9% infants were diagnosed with tinea capitis [150].

The clinical presentation dermatophytoses varies according to age in North African children: tinea capitis was more frequent before 10 years of age, and after this age, tinea corporis and onychomycoses became more frequent [40,66,67,147,148,150–153]. For example, in Tunisia, a dermatophytoses prevalence of 80.6% was reported among children less than 16-year old; and tinea capitis was the most common type of infection, followed by tinea corporis [67]. Similarly, many studies found that dermatophytoses prevalence peaked among 6 to 11 year-old children, in East African [72,154,155,184]; Central African [73]; and Southern African countries [76,158].

In contrast to tinea capitis, the risk of non–tinea capitis dermatophytosis increases with age. For instance, older age and family history of mycosis were associated with an increased fungal foot diseases risk in a retrospective study on 148 patients in Sfax, Tunisia [173]. Moreover, in 590 patients, aged 16-83 years, in a dermatological unit in Yaoundé or a volunteer service in Douala,

Cameroon, the majority of the 8.8% patients with onychomycosis were older that 50 [177].

Dermatophytoses in African children

In Africa, there are more epidemiological data available on dermatophytoses in children than in adults. Dermatophytoses are frequent childhood diseases and fungal species involved varies according to age and clinical presentation [29]. Tinea capitis is the main dermatophytosis clinical presentation in African children; this has been shown in many studies throughout the whole African continent: in ***West Africa*** [16,18–21,25,30–35]; ***North Africa*** [29,36–43]; ***East Africa*** [44–49]; ***Central Africa*** [8,50,51]; and ***Southern Africa*** [76, 77,158–164].

Many reports evidenced the high prevalence of dermatophytoses in ***West*** and ***North African*** children. Strikingly, Oladele and Dennings [61] estimated that over 15.5 Million children present with tinea capitis in Nigeria only; since tinea capitis occur in at least 20% of school-age children. A 80.6% dermatophytoses prevalence was reported in Tunisia among children less than 16-year old [29]; *tinea capitis* being the most common (69.4%) presentation, followed by *tinea corporis* (20%) [29]. In Libya, 1,180 (52.2%) of 2,224 participants in the region of Tripoli presented with superficial mycoses (*tinea capitis* excluded) that were mainly (52.2%) due to dermatophytes; *tinea corporis* accounted for 45.9% of the superficial mycoses, and 85% of the *tinea corporis* cases occurred in children below 15 years of age. In ***East Africa***, A cross-sectional study on the prevalence of dermatophytoses in primary school children in Kibera (Kenya), showed that the prevalence of dermatophytoses was 11.2% with *tinea capitis* as the dominant clinical manifestation [44]. In ***East African*** cities, dermatophytoses prevalence among schoolchildren ranged from 10.1% to 79.5% [44–46,49,62–64]. For example, in 1993 a large survey of 5,780 children from 13 schools in the rural Kisumu District (Western Kenya), there was a 10.1% dermatophytoses prevalence, and 7.8% tinea capitis prevalence [62]. In contrast, 79.5% the children presented with a clinical dermatophytoses, among whom tinea capitis was the most common (76.5%) presentation in a geographically restricted area in Ethiopia, [63]. Among ***Southern Africa*** countries, a study conducted in Botswana found tinea capitis in 81% the children aged 1-15 years [57]. In primary school children of Dar es Salaam city, Tanzania, 11.4% of 420 children presented with dermatophytoses [45]. In Zimbabwe, 29% of 704 in primary schoolchildren in Harare, had clinical evidence of tinea capitis [53].

Dermatophytes species in African children

Studies including both adults and children in West Africa showed tinea corporis to be the most common dermatophytosis presentation; the most frequent dermatophyte species involved being

T. mentagrophytes complex [33,43,44,145]. In west African children, the two anthropophilic dermatophytes species, *T. soudanense* and *M. audouinii*, were the main etiological agents isolated from tinea capitis [10,11,43,49,50,52,56,59,59,62–64] (Figure 1). One study in Ghana [58] highlighted *T. violaceum* (26%) and another one in Nigeria [146], highlighted *T. schoenleinii* (28.1%) to be the most common fungal pathogen in schoolchildren. In the last study, *T. verrucosum* (20.2%) and *M. gallinae* (18.4%) where the two next more frequent dermatophyte species involved [146]. In Ivory Coast, *T. soudanense* and *M. audouinii* were the most common etiologic agents of tinea capitis in children [50].

The most frequent dermatophyte species isolated were *T. violaceum* and *M. canis* in studies considering both children and adults in North Africa [39,41,54,65–69,191]. In North Africa [53,54,66,170], *T. violaceum* was reported to be the most common fungal pathogen involved in tinea capitis in Morocco [172], Tunisia [54,149], and also in any clinical dermatophytosis presentation in Egypt [41]. Many other species, including *T. verrucosum*, *M. audouinii*, *T. mentagrophytes* complex, *T. schoenleinii*, *T. ochraceum*, *T. rubrum*, *M. gypseum*, *M. ferrugineum*, and *T. tonsurans* have been less frequently isolated from tinea capitis in North African children [54,147,149,170,172]. Interestingly, several reports highlighted the emergence of the zoophilic specie *M. canis* in North Africa [171,172]. Overall, the most common fungal species isolated from tinea capitis were *T. violaceum* followed by *M. canis* [41,170] (Figure 1). The major dermatophyte isolated from tinea capitis was T. violaceum (prevalence ranging from 60 to 77%) [41,170,191]. One retrospective showed that M. canis (13.4%) was second next to *T. mentagrophytes* complex (76.4%) associated with dermatophytoses in Morocco [148].

Converging observations in children indicate that the anthropophilic dermatophyte specie *T. violaceum* was the most common tinea capitis agent in East Africa [70–72,154,165,166,184]. One study in Tanzania showed that M. canis was the first dermatophyte species isolated in a primary schoolchildren population in Dar es Salaam [154]. Another study in Kenya showed the predominance of *T. tonsurans* [155]. Other dermatophyte species, including *M. audouinii*, *T. verrucosum*, *T. mentagrophytes*, *T. terrestre*, and *T. schoenleinii* were rarely reported in East Africa [70–72,154,165,166,184].

Regarding the studies conducted in children from Central Africa, the most frequently isolated species from tinea capitis lesions was *T. soudanense* [61,73,74] (Figure 1). In one study conducted in the Central African Republic, M. audouinii was the first species involved in tinea capitis [75]. *T. tonsurans*, T. rubrum, and *M. audouinii* have been less frequently isolated from children with tinea capitis from Central Africa [61,73,74]. Overall, the two most frequent dermatophyte species involved in tinea capitis among children in Southern Africa were *T.*

violaceum and *M. audouinii* [76,77,158,159,162,192]; with a few exceptions, such as in Mozambique, where a study showed that the most common dermatophytes isolated from schoolchildren were *M. audouinii* (88%) and *T. mentagrophytes* (17%) [160]. The dermatophyte species spectrum was particularly heterogeneous in Madagascar where a study conducted at a primary school in Antananarivo [161] found only *M. audouinii* (100%), and another study conducted at a primary school in Antsirabe highlighted the presence of an anthropophilic dermatophyte specie (T. tonsurans 88%), and two geophilic dermatophyte specie that are rarely involved in human disease, namely *M. boullardii* (=*M. fulvum*, 24%), and *T. terrestre* (6%) [163]. Moreover, other dermatophytes species, including *T. tonsurans*, *T. mentagrophytes*, *T. yaoundei*, *M. gypseum* and *M. canis* were occasionally isolated from tinea capitis in Southern Africa [76,77,159,192].

Dermatophytoses in African adults

In contrast to study among children, that were mainly conducted in schoolchildren populations, the epidemiology of dermatophytosis in ***adults*** was primarily investigated in patients of any age attending various health care facilities (such as university hospitals, dermatology department, microbiology department, dermatology clinic at medical centre, or Army hospitals) and in inmates; in ***West African*** [27,30,34,65–71]; ***North African*** [29,38,39,43,72–86]; ***Central African*** [87,88]; ***East African*** [47,89] and ***Southern African*** countries [90,91].

Strikingly, many reports regarding patients cared for in various health care facilities in ***West Africa*** pointed out a higher prevalence of dermatophytosis in adults than in children [30,66]. For example, in tertiary health care institution patients in the Lagos State (Nigeria), dermatophytes were six times more frequently isolated from adults than from children below 12 years of age. There is a striking predominance of dermatophytoses cases in **North African** adults compared to children in patient attending various health care facilities reports, particularly dermatology departments [73,82–85]. In Egypt, two studies produced conflicting results. The first one found a relatively low prevalence (2.4%) of dermatophytes isolated from skin lesions in Egyptian adults aged at least 55 years [43], and the other found a 79.6% dermatophytosis prevalence in 506 patients attending the dermatology department at a tertiary health care hospital in Cairo [79]. Yet these figures are probably impacted by a selection bias of patients that are referred to tertiary health care facilities, and might thus not be representative of the prevalence in the general population.

Clinical presentation in adults

While *tinea capitis* is the most frequent clinical dermatophytoses presentation in children, *tinea corporis* is most frequent in ***West African*** adults. In ***West African*** cities, *tinea corporis* prevalence in adults range from 2.2% to 41% [23,27,30,65,66,68,69,92].

In ***North African*** cities, *tinea corporis* was the most frequently reported dermatophytosis presentation among adults, with prevalence ranging from 2.4% to 45.9% [73,75,78,79,93]. Tinea pedis and onychomycosis are also among the commonest fungal diseases in the ***North African*** adults [73,82,94]. Another retrospective study, conducted between 1998 and 2007, showed that dermatophytoses was diagnosed in 9960 (39.2%) among 25 432 subjects suspected to have superficial mycoses. The most common clinical presentation was onychomycosis (30.3%), followed by tinea pedis (24.8%), tinea cruris (21.7%), tinea corporis (11.4%) and tinea capitis (9.6%) [73]. Also, in Sfax, the most frequent localizations were tinea pedis, with 60% onychomycosis prevalence [94]. In Tunis, among one hundred outpatients with suspected fungal foot diseases attending the Department of Dermatology of a tertiary hospital, a fungal foot infection was confirmed by positive microscopy or culture in 45.3%, and dermatophytes were involved in 57.1% of confirmed cases [82]. In Cairo, a study carried on 506 patients in the department of dermatology, EL-Houd El-Marsoud Hospital, found 1.2% of onychomycoses among all dermatophytoses diagnosed [79]. In Libya, *tinea pedis* was diagnosed in 8.1% of 2,224 patients attending the dermatology clinic at Tripoli Medical Centre, with suspected clinical symptoms of superficial mycosis [78]. A first study conducted among the military staff attending the Dermatology Department at the Army's Central Hospital in Algiers showed that out 650 male, fungal foot infection (including *tinea pedis* and superficial candidiasis) was clinically suspected in 147 patients, and confirmed by positive culture in 119 patients, resulting in a total prevalence of 18.3% [84]. A second study the clinical diagnosis of *tinea pedis* and/or onychomycosis was suspected in 249 and 72, respectively, of 1300 male outpatients; confirmed by positive dermatophyte culture in 197 (79%) and 60 (83%), respectively, resulting in a global prevalence of 15% for *tinea pedis* and 4.6% for toenail onychomycosis in this population [85].

Tinea capitis in adult

It is noteworthy that tinea capitis also occur in adults, as it has been reported mainly in ***North Africa*** [72,76]. However, even when considering dermatology departments' outpatients population, tinea capitis is relatively uncommon in adults [39,72,77]. In Addis Ababa, a study in a reference centre for dermatological diseases for the whole country of Ethiopia showed that

tinea capitis was diagnosed in only 10% of 165 adults with a clinical dermatophytosis presentation. Overall, tinea capitis in young males and onychomycosis of fingernails in females were the most common manifestations of dermatophytosis in Addis Ababa [47].

Obviously, tinea capitis is uncommon in adults [54]. In 1946, the first cases of tinea capitis in adult were reported by Goodman [205], and then Andrews [206]; thereafter many reports have focused on studying the incidence, clinical characteristics, and etiological factors of tinea capitis in adults [207]. The prevalence of tinea capitis in adults varies in different communities. In the United States, an prevalence of 4.9% in the Southwest area was reported [208], and Silverberg et al reported that 78% of the nine adults diagnosed with tinea capitis over a three-years period were African American women [209]. In Europe, in adult represented only 2.7% of all tinea capitis among Italians [210], whereas the prevalence was higher in Greece (5.8%) [211] and in France (11%) [207]. In Asia, Lee and Hsu [212] showed 63% of 27 culture-proven tinea capitis cases occurred in Taiwanese adults; in China the frequency of tinea capitis in adults ranged from 6% to 13.6% [82]. In Tunisia, Mebazaa et al [53] reported a frequency of 5.27% adults among Tunisian patients. A frequency of 4.2% was reported among Egyptian patients [54]. The overall rarity of tinea capitis in adults in general has been explained by the higher resistance of adults' scalp hair to dermatophyte colonization due to fungistatic properties of post-pubertal sebum, which is mainly formed of long-chain fatty acids [55].

The dermatophytoses generally peak in children aged between 4 to 11 years [50,56,158]. This age pattern has been explained by hormonal factors. The high frequency of tinea capitis in children in general was explained by lower resistance of children's scalp hair to dermatophyte colonization due to low fungistatic properties of sebum (decreased sebum levels during prepubertal period), associated to hormonal factors. Furthermore, these age groups behaviours' makes them more likely to be exposed to dermatophytes sources.

Dermatophytes species in African adults

Several dermatophytes species, including *T. soudanense, T. rubrum, T. mentagrophytes, T. tonsurans, T. verrucosum, M. audouinii, M. canis,* and *E. floccosum*, have been involved in ***West African*** adults' tinea corporis [4,23,25,26,35,65–67,95] (Figure 2) Overall, *T. violaceum*, *T. rubrum*, and *M. canis*, were the three main dermatophyte species involved in tinea corporis in ***North African*** adults [96]. Yet several dermatophytes species, including *T. violaceum, T. rubrum, M. canis, T. verrucosum, T. mentagrophytes*, and *E. floccosum* have been isolated from skin lesions in this population [73,75,78,79,93,96]. Considering tinea pedis in ***North African***

adults, a consistent body of evidence highlight *T. rubrum* as the most frequent species involved [43,73,78,79,82–85,93,94,97,98]. In Egypt, *T. violaceum* was the common fungus (56.9%) isolated from tinea capitis lesions in adults [72] (Figure 2).

Considering ***East African*** adults, one study showed that the most common manifestations of dermatophytosis presentations in Addis Ababa were tinea capitis in young males and fingernails onychomycosis in females [47]. The most frequent dermatophyte species involved was *T. violaceum* [47].

In Cameroon, *Trichophyton rubrum* was the major species involved in tinea corporis, tinea pedis and onychomycosis in adults [87,88]. In this country, among 52 patients with onychomycosis; fingernails were affected in 12; toenails in 30; and both fingernails and toenails in 10. A dermatophyte was isolated in 57.7%, the most common species being *T. rubrum*, involved in 16 cases, and *T. violaceum*, involved in eight, and non-dermatophytic moulds, including *Aspergillus* spp., *Fusarium* spp., and *Scytalidium dimidiatum*, were involved in 10 cases [87].

Regarding all dermatophytoses in ***Southern Africa***, *Trichophyton rubrum* (27%) was the most common fungal pathogen reported in 8-year survey of patients from hospital clinics and private practices in Pretoria seeking specialist dermatological advice for dermatophytoses in Transvaal [91]. In Northern Malawi, between 1.5% and 2.5% of the population in Karonga District, were diagnosed as having tinea faciei, corporis, inguinalis or cruris in population survey carried out between 1987 and 1989; the dermatophytes' epidemiology was characterized by the relative rarity of *T. rubrum* (around 1%) and the predominance of *M. audouinii* (57%). Noteworthy, *E. floccosum* was the most common (56%) dermatophyte species isolate from lesions in the genital area in patients from this part of Africa [89]. In about 500 adults dermatology outpatients in Pretoria (South Africa) *T. rubrum* (27%) was the most frequent dermatophyte species, followed by *T. mentagrophytes* (23%), *M. canis* (19%), *T. violaceum* (18%) and *E. floccosum* (12%). *M. gypseum* occurred in only 1% [91].

Environmental Dermatophytosis Risk Factors

Epidemiological trends of dermatophytoses

Dermatophyte infections are among the most common diseases worldwide and cause serious chronic morbidity [5]. Although dermatophytes are found throughout the world, the most prevalent strains and the most common sites of infection varies by region. Hot, humid climates and overcrowding predispose populations to skin diseases, including dermatophytoses.

Dermatophyte transmission

Dermatophytosis transmission has been reported to be mainly associated with contact with animals in rural areas or with other family members in urban areas [54]. In Madagascar, in a primary school in Antananarivo, a study found that 27 children with Microsporum audouinii tinea capitis, reported a contact with infected classmates, and 19 (70%) reported to have infected brothers and sisters at home [161]. In Egypt, a multicenter study showed that 17.2% of 1380 adult outpatients with tinea capitis reported a history of close contact with animals [54]. The importance of asymptomatic dermatophyte carriers that probably act as reservoir in endemic populations has been highlighted in a study of 510 primary schoolchildren in Alexandria (Egypt). Dermatophytes were isolated in 7.4% of the children; 2.9% had a clinical dermatophytoses while 4.5% were asymptomatic carriers [66]. Recently, a study on 256 adopted children, found that asymptomatic anthropophilic dermatophyte carriers contaminated a family member in 29% of the cases [190].

Spatial risk factors

In Nigeria, epidemiological studies have shown a geographical heterogeneity in dermatophytosis prevalence rates that ranged from 2.7% to 17.3% in schoolchildren from geographical areas located in the Centre, East, Northeast and Southeast of the country [25,26,31,32,99,100]. Similarly, in Togo, dermatophytosis prevalence was 11% and 20% in a dry area in the North and a humid area in the South, respectively [21].

Living in urban or rural areas has been pointed out to significantly influence dermatophytoses prevalence. Cross-sectional population-based studies performed in Ghana, Gabon, and Rwanda,

showed that among 4839 schoolchildren, the highest prevalence of tinea capitis was observed in rural areas [101]. Moreover, a significant difference in dermatophytoses prevalence among children in urban and rural areas was observed in ***West Africa*** [8,21,31,32,52,100,101]. A higher prevalence in rural than in urban areas was observed in the Anambra State, South-eastern Nigeria, 8.1% vs. 4.3% [32], in Ghana, 10.5% vs. 6.6% [22], in Togo, 20% vs. 11% [21], in Cameroon [8], in Gabon, a cross-sectional study conducted with 454 children aged 4-17 years, attending a rural school and an urban school, showed that the prevalence of tinea capitis depended on the school studied and ranged from 20.4% in the urban school with a higher socioeconomic status to 26.3% in the rural school with a lower socioeconomic status [50] and in Lusaka (Zambia) [52].

In contrast, studies found a higher prevalence primary schoolchildren from the relatively more urbanized area in Mali, 54.8% vs. 16.5% [102], in the Illubabor district in South-Western Ethiopia [64], and in two distinct states in Central Nigeria , 44.6% vs. 23.2% [100]. In North Africa two studies were conducted in selected populations. A large tertiary health centre mycology laboratory based-study in Sfax (Tunisia) showed that 81.9% of the patients in whom dermatophytosis was diagnosed originated from urban areas [73]; and a retrospective study on children with inflammatory tinea capitis found that 49.59 % came from a rural area, 34.71% from Tunis City, and 15.7% from an urban area surrounding Tunis City [39].

Geographical /climate trends

In Africa, hot and humid climate appears to favour the growth and spread of dermatophytes [4]. The geographic distribution of dermatophytes species varies from country to country and from continent to continent and over time within a region [103]. In the literature, it appears that the predominance of *M. audouinii* in West, Central Africa and Madagascar was related to the moderate and high level annual average of precipitation.

In Mali, (West Africa), all studies reported the predominance of two anthropophilic dermatophytes species, as *T. soudanense*, and *M. audouinii*; in all dermatophytosis combined, *T. soudanense* is dermatophyte species widely disseminated in tropical Africa [11]. In Mali, the species *T. soudanense* remain clearly predominant in tinea capitis, tinea corporis in both children and adult.

Contrary to Northern African studies, tinea pedis and onychomycosis were rarely reported in

tropical African studies. Although tropical and subtropical climates have an impact on an overall prevalence of skin mycoses, tinea pedis and onychomycosis are rare in India and rural Africa [104]. Aly and al studied the occlusive effects of the army boot on the prevalence of tinea pedis in Columbian soldiers in tropical climates. Those soldiers in high-humidity areas developed tinea pedis more frequently that those in high-temperature, low-humidity areas. Therefore tinea pedis remained at a high level in both climates [105]. In Africa, *M. canis* was almost exclusively present in North Africa: it was a zoophilic species adapted to domestic animals such as cats and dogs which can infect persons, particularly children, who have contact with them. We hypothesize that in North Africa there is a better closer proximity between humans and their pets, with permitted entrance in domestic environment, than in the other part of Africa. It seems that there is a geographical dichotomy between *T. violaceum* and *Trichophyton soudanense* in Africa. But according to Gräser et al (2000) [106] *T. soudanense* was a synonym of *T. violaceum.* So, the geographical difference observed may be biased due to the local identification habits of these two similar fungi.

Individual Dermatophytosis Risk Factors

Gender effect

Overall, many reports observed that male children were generally more infected than the girls [18,25,26,34,99,100], whereas one study in Alexandria found a predominance of girls [37]. Male were generally more infected than girls in ***West African*** children [15,16,18,19,23,25,26,34,100]. In particular, a 3 to 5 times higher prevalence of tinea capitis in boys than in girls has been found in Central Nigeria [34,100]. Similar findings came out of studies conducted in North Africa [38,39,80,81], where for example, in Morocco, the male to female ratio was 1.4 in children under 10 years of age with tinea capitis [38]; in ***East Africa***, where a 2:1 male to female ratio was found in private primary schoolchildren with tinea capitis in Kenya [46]; and, male gender was statistically significantly associated with an increased tinea capitis risk in Addis Ababa (Ethiopia) schoolchildren [63]; in Central Africa [8,51,107], where a statistically significantly higher prevalence of tinea capitis in boys than in girls was observed in schoolchildren (21.2% *vs*. 10.3%) in Gabon [51] and (63.7% *vs*. 36.3) in Cameroon [8]; and in ***Southern African*** countries, where studies among schoolchildren in Lusaka, Zambia [52], and in the Maputo Province, Mozambique [55] showed a higher prevalence of tinea capitis in boys than girls.

Among adults, in contrast to children, dermatophytoses prevalence was significantly higher in females than in males [27,30,66]. This was confirmed, in Tunisia, where a study showed that 63.5% of 255 patients with suspected onychomycosis were females [97], and in Egypt, where 84.5% of the adults outpatients consulting at five dermatology centres were females [72].

Underlying chronic condition

Predisposing host factors play an important role dermatophytosis of the skin and nails. Chronic venous insufficiency, diabetes mellitus, cellular immunity disorders, and genetic predisposition are considered to be risk factors for onychomycosis [108]. Epidemiological studies in diabetes mellitus patients found that a clinical suspicion of fungal foot infection occurred in 61% and was confirmed in 30% of 307 hospitalized patients in Tunisia [83]. In this population, a fungal foot infection occurred in 38% of these patients, and was mostly due to dermatophytes (94%); the commonest localizations of dermatophytes were interdigital toe web spaces (60%) followed by onychomycosis (30%). The main risk factors for superficial fungal infections were age and the duration of diabetes. The frequency of dermatophytes isolation in nails was reported to higher in type 2 than in type 1 diabetes patients; but age might act here as a confounding factor [83]. Other chronic exposition to immunosuppressive therapy, long-term topical or systemic corticosteroid, hepatitis C virus infection, have been associated with an increased tinea capitis risk in adults [72]. Regarding HIV infection, a study in Cameroon found that 53% had at least one superficial mycosis; namely, oral candidiasis in 77%, tinea corporis in 21%, tinea versicolor in 15%, tinea pedis in 13%, and tinea inguium in 12% [88].

Hair dressing practices

Barbing mode, extracurricular activity and cultural habits have been highlighted as dermatophytosis risk factors in West Africa [100,101]. In Mali, Coulibaly et al (2014) [109] showed that public hairdressing practices, home hairdressing practices, traditional braiding practices and head shaving practices were associated with tinea capitis. Also in East Africa, the sharing of hair care equipment, ignorance and poor hygienic practices of hair have been proposed as predisposing factors and shaving of hair at a barber's shop featured as a significant dermatophytosis risk factor [44]. In Ghana most schoolchildren have shaven scalps, which reduces contagiousness, whereas girls in Gabon and Rwanda wear longer hairstyles. The Ghana Education Service requires that boys and girls in basic and secondary public schools cut their hair very short. It seems that this recommendation is actually beneficial [101].

Socio-economic level.

An increased tinea capitis risk has been associated with a low socio-economic level, which might be explained by the poor hygiene condition usually associated with this condition. Population-based studies performed on 4839 schoolchildren in Ghana, Gabon, and Rwanda found the highest prevalence of dermatophytoses in schools serving children living at lower socioeconomic levels [101]. Moreover, prevalence of tinea capitis are higher in schools serving populations of low socio economic level [24,101]: for example in Ghana, 10.3% of the pupils of low and 4.9% of those of middle or high socio economic level presented with tinea capitis [101]; in Lusaka (Zambia), tinea capitis was significantly more frequent in children from squatter and low-cost areas compared to those from high-cost areas [52]; and in Algeria, tinea capitis was associated with low socio economic level in children from both urban and rural areas [80]. Similarly, in Cameroonian adults, onychomycosis was associated with a low social-economic class [87].

Cultural (and religious) practices

Traditional and religious habits such as communal living and the performance of ritual ablutions may affect the prevalence of dermatophytes infections [110]. A study conducted adult Muslim male population regularly attending mosques, in Durban, South Africa, found a high (85%) prevalence of tinea pedis and tinea unguium [90]. Yenişehirli and al (2012) [111] investigated the existence of the potential causative agents of dermatophytosis in the mosque area in Tokat, Turkey. It was reported that contamination of carpets and slippers with dermatophytes may act as a reservoir for transmission of these fungi and that shared slippers was an important source of transmission [111].

Discussion

In Africa, most dermatophytoses are caused by anthropophilic dermatophyte species, but there is a considerable inter- and intra-country variability in these species geographical repartition. *T. soudanense* and *M. audouinii* clearly predominate in West Africa and Central Africa; the geographical distribution of these two species may be associated with the humidity (or annual average of precipitation) of the climate. *T. violaceum* is predominant in East and South Africa. In North Africa, it is *T. violaceum* and *T. rubrum* where recently *M. canis* emerged. These two last species might be associated with urbanisation related life-style changes. The observed change in the dermatophyte species spectrum is observed in Africa but also in the rest of the

world. In Northern countries the main dermatophyte epidemiological shifts is rather associated with importation of anthropophilic dermatophyte species favoured by the development of international travel and immigration.

Insight into the epidemiology of dermatophyte infections in Africa has been limited by the heterogeneity in the study design and especially the variable denominator used to estimate dermatophyte prevalences in the studies include in this review. In most of the studies only prevalences are reported, which entirely depending on the numbers of cases reported to laboratories equipped with appropriate diagnostic facilities. Such studies are inevitably prone to selection bias according to the likelihood of patients presenting for treatment, the ability of the referring physicians to take appropriate diagnostic samples, and by the issue of disease definition.

An increase of dermatophytosis has been noted worldwide, especially in developing countries [3,103,112]. Yet there are remarkable changes in the species distribution occurred over the time 1912, *T. soudanense* was described by Joyeux [113] in Mali. However, the zoophilic species, as *T. verrucosum* and *M. canis* have been found for the first time in Mali in 1973 [95]. And since that time, the disappearance of *T. schoenleinii*, *T. violaceum*, *M. canis*, and *M. ferrugineum*, is certainly the most important change in the spectrum of dermatophytes isolated from tinea capitis in this country. Particularly, in Nigeria, the pattern remains highly heterogeneous. Many species of dermatophytes were found in this country, depending of the time, communities, and geographical areas studied. Several etiological agents of dermatophyte infections have also been observed in different parts of Nigeria [11,103]. However, *T. mentagrophytes* was mostly reported in Nigeria [25–28].

The almost disappearance of *T. schoenleinii* is the most important change in the spectrum of dermatophyte species causing tinea capitis in North Africa. In contrast, the emergence of *M. canis* as a tinea capitis agent has been recently noted [80,81]. Globally, *T. violaceum, T. rubrum,* and *M. canis*, were the predominant etiological agents of tinea corporis in North Africa [73,75,78,79,93]. Many epidemiological reports observed a relatively high incidence of tinea pedis and onychomycosis in North Africa. This has been explained by increased urbanization, use of community showers, indoor sports, and the use of occlusive footwear. These factors were associated to the high prevalence of tinea pedis in certain occupational groups, including marathon runners, miners, and soldiers. Several studies also found high rates of onychomycosis in patients presenting with tinea pedis. In Northern African cities, *T. rubrum* was the most

frequent etiological agent isolated from tinea pedis and onychomycosis. It is the most frequent anthropophilic dermatophyte worldwide.

Developing countries have high rates of tinea capitis; in contrast, developed countries have high rates of tinea pedis and onychomycosis [104]. Our review confirmed the highest prevalence of tinea capitis in Africa, especially among children. In fact, tinea capitis is a significant health problem mainly in school children from tropical regions [22,34,114]. In comparison with African studies, a predominance of *T. violaceum* (69.4%), followed by *T. tonsurans* (16.7%) was reported in both Pakistani children and adults [115]. In contrast, the spectrum of fungal species in adults with tinea capitis among Chinese showed the predominance of *T. mentagrophytes* (55.6%), followed by *M. canis* (13.3%) [116]. In the United States, *T. tonsurans* was reported as the most prevalent causative species in both children and young adults [80].

The change of spectrum in the United States from *M. audouinii* to *T. tonsurans* was explained by the high sensitivity of *M. audouinii* to griseofulvin treatment and the importation of *T. tonsurans* by people emigrating from geographic areas where this species was endemic [220]. In Europe, the largest overall increase in anthropophilic dermatophytes prevalence has been noted with *T. tonsurans* mainly in the UK and with *T. soudanense* and *M. audouinii* in France [117] [118] [119] [120]. In line with the hypothesis that these anthropophilic species have been imported to Europe is that tinea capitis agents as illustrated in Figure 1 are predominantly *T. soudanense* and *M. audouinii* in the former French colonies while *T. tonsurans* has been mainly reported in former British colonies in Africa. Dermatophytosis, and in particular tinea capitis, is a good example showing how the geographical distribution of infectious agents follows worldwide population movements. The restricted temporal and time frame of this review study did not allow highlighting similar phenomenon at the African continent scale. Yet, we might assume that socio-economic changes will alter dermatophyte.

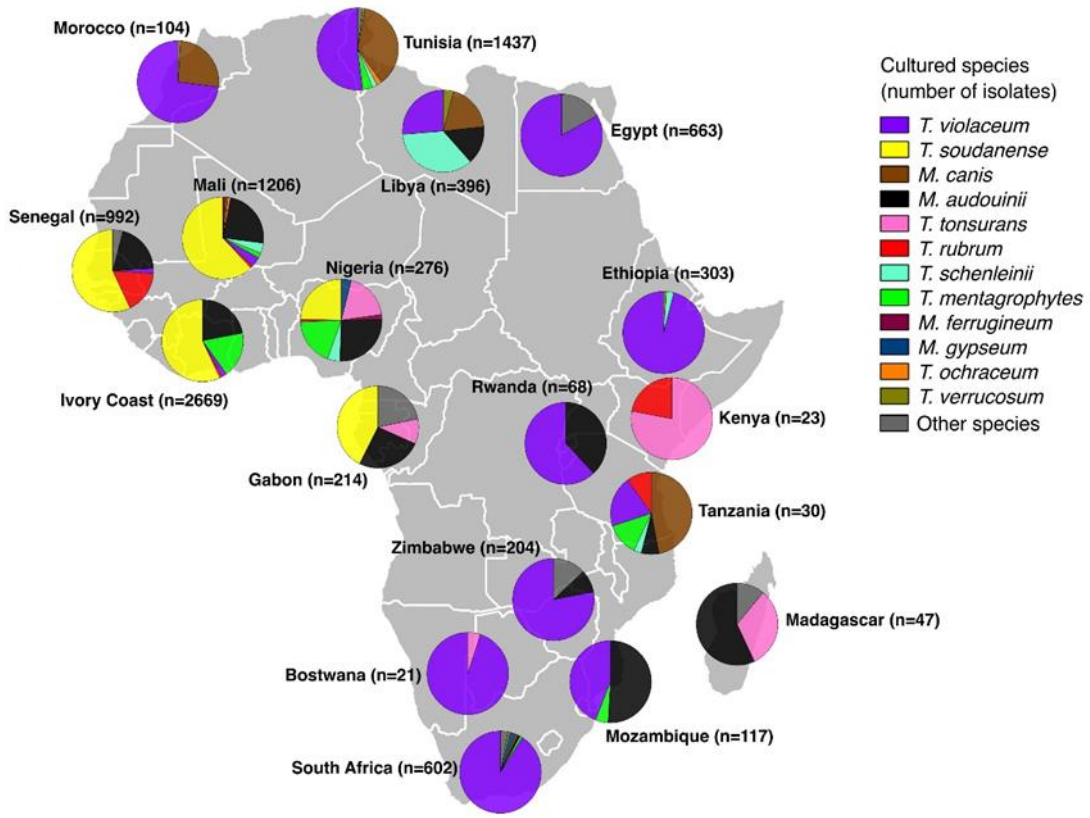


Figure 1-The spectrum of dermatophytes species isolated from tinea capitis in Africa. *Trichophyton soudanense* and *Microsporum audouinii* are frequently isolated in West and Central Africa; predominant in West Africa; *T. violaceum* is predominant in North, East and South Africa.

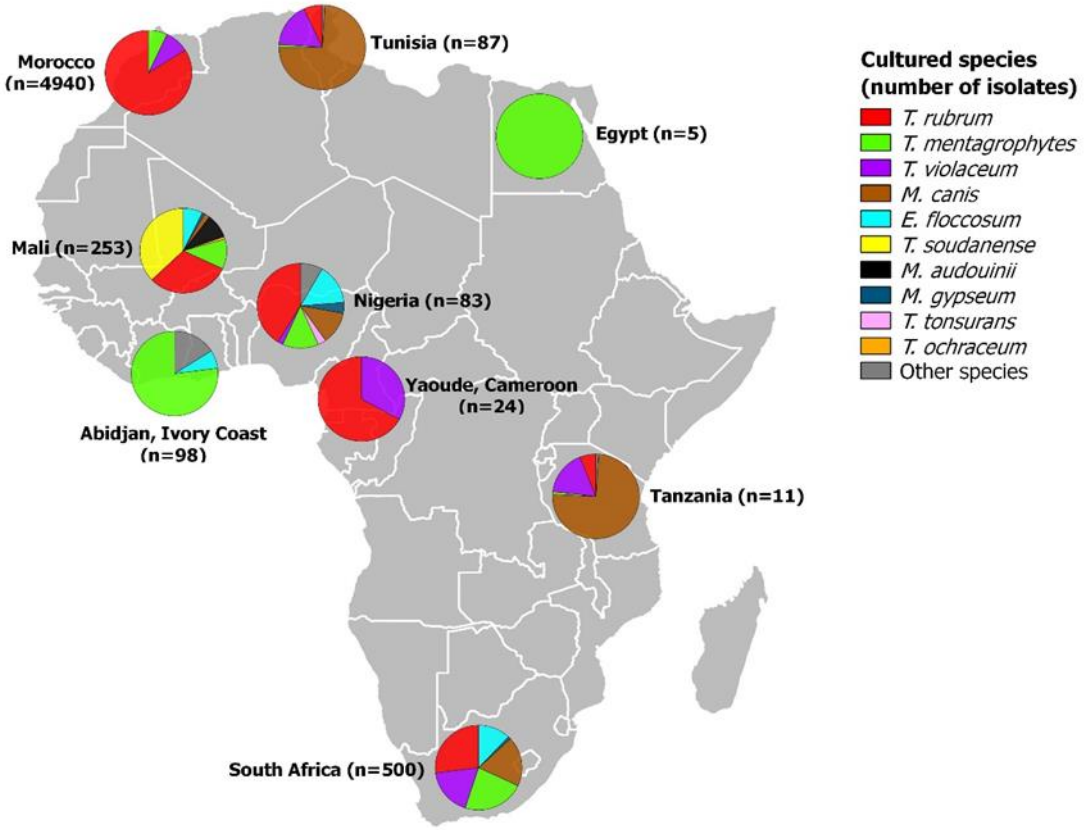


Figure 2- Spectrum of dermatophytes species isolated from tinea corporis in Africa.

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Chapitre 2 : Enquêtes transversales descriptives dans trois écoles primaires de trois zones géographiques distinctes du Mali

Les dermatophytoses sont des affections fongiques superficielles fréquentes. Parmi ces infections généralement superficielles, les teignes du cuir chevelu sont très fréquentes chez les enfants des régions tropicales et subtropicales, où elles sont endémiques. Plusieurs facteurs de risque associés à ces affections ont été suggérés, notamment des facteurs géo-climatiques (saisons, aires géographiques, …). Ainsi nous avons déterminé la prévalence des dermatophytoses chez les élèves de trois zones éco-climatiques distinctes du Mali : zone périurbaine de Bamako, où le type éco-climatique est Soudanien avec une pluviométrie annuelle variant entre 550 et 1400 mm ; ville de Bandiagara, où le type éco-climatique est Sahélien, avec une pluviométrie annuelle de 450 mm ; et la zone périurbaine de Sikasso, où le type éco-climatique est Soudano-guinéen, avec une pluviométrie annuelle comprise entre 1000 et 1400 mm. Nous avons mené trois enquêtes transversales entre Décembre 2009 et Février 2012, incluant 590 élèves, dont 286 garçons et 304 filles, d'un âge moyen de 9,7±2.38 ans. La prévalence globale des élèves ayant des lésions cliniques de dermatophytoses était de 59,2% et la teigne représentait la forme clinique la plus fréquente (39,2%). Nous avons observé une forte prévalence de 59,5% des cas confirmés par culture mycologique de teigne du cuir chevelu dans la zone périurbaine de Sikasso, où le type bioclimatique est soudano-guinéen, caractérisé un niveau élevé d'humidité. L'association entre une prévalence élevée de teignes et l'humidité du climat est renforcée par notre observation de ce que nous pourrions appeler un « effet dose ». C'est-à-dire que la prévalence des teignes augmentait avec l'augmentation de l'humidité du climat, de la zone Sahélienne à la zone Soudano-guinéenne, en passant par la zone Soudanienne dont l'humidité du climat est intermédiaire entre les deux zones précédentes. Cependant, dans la première enquête menée à Sirakoro, nous avons observé un taux relativement élevé de faux-négatifs, qui s'explique par la non-utilisation des compresses stériles dans la procédure de recueil des échantillons pathologiques. Une fois adoptée, la technique de la compresse stérile à permis l'isolement d'un dermatophyte en culture dans 100% des lésions prélevées (**Article 2**). Nous avons mené une autre enquête dans la zone semi-urbaine de Sirakoro-Méguétana, pour évaluer une contamination éventuelle des outils de coiffure. Sur un échantillon aléatoire incluant cinq coiffeurs, et à partir d'un total de 41 outils prélevés, nous avons observé un taux de contamination de 72,3% par deux espèces anthropophiles : *T. soudanense* (53.3%) et *M. audouinii* (46,7%) (**Article 3**). Ma participation a été effective à toutes les étapes des différentes enquêtes : élaboration des protocoles d'enquêtes ; préparation des milieux de culture ; processus d'échantillonnage ; examen clinique des élèves avec les médecins du DEAP ; prélèvements

mycologiques des lésions cliniques évocatrices de dermatophytoses ; examen direct des prélèvements; mise en culture des produits pathologiques ; identification des agents pathogènes; nettoyage et vérification de la base des données saisies au DEAP ; analyses statistiques; et rédaction du premier jet des manuscrits.

Article 2: Dermatophytosis among schoolchildren in three eco-climatic zones in Mali

Soumis

Dermatophytosis among schoolchildren in three eco-climatic zones of Mali

Oumar Coulibaly[1,2], Abdoulaye K. Kone [2], Safiatou Niaré-Doumbo [2], Siaka Goïta[2], Jean Gaudart[3], Abdoulaye A. Djimdé[2], Renaud Piarroux[1,4], Ogobara K. Doumbo[2], Mahamadou A. Thera[2], and Stéphane Ranque[1,4,#]

1- Aix-Marseille Université, IP-TPT UMR MD3, Marseille, France
2- Département d'Épidémiologie des Affections Parasitaires/Malaria Research and Training Center, Faculté de Médecine, Université des Sciences, des Techniques et Technologies de Bamako, Mali
3- Aix-Marseille Université, (SESSTIM), INSERM / AMU / IRD, Marseille, France
4- APHM, CHU Timone-Adultes, Parasitology & Mycology, Marseille, France

[#] **Corresponding author**

E-mail: stephane.ranque@ap-hm.fr (SR)

Keywords: epidemiology, risk factors, climate, Mali, tinea capitis, dermatophytes, dermatophytosis, *Microsporum audouinii*, *Trichophyton soudanense*.

Abstract

Dermatophytosis, and particularly the subtype tinea capitis, is common among African children; however, the risk factors associated with this condition are poorly understood. To describe the epidemiology of dermatophytosis in distinct eco-climatic zones, three cross-sectional surveys were conducted in public primary schools located in the Sahelian, Sudanian and Sudano-Guinean eco-climatic zones in Mali.

Among 590 children (average age 9.7 years) the overall clinical prevalence of tinea capitis was 39.3%. Tinea capitis prevalence was 59.5% in the Sudano-Guinean zone, 41.6% in the Sudanian zone and 17% in the Sahelian eco-climatic zone. *Microsporum audouinii* was isolated primarily from large and/or microsporic lesions. *Trichophyton soudanense* was primarily isolated from trichophytic lesions. Based on the multivariate analysis, tinea capitis was independently associated with male gender (OR=2.51; 95%CI [1.74-3.61], $P<10^{-4}$) and residing in the Sudano-Guinean eco-climatic zone (OR=7.45; 95%CI [4.63-11.99], $P<10^{-4}$).

Two anthropophilic dermatophytes species, *Trichophyton soudanense* and *Microsporum audouinii*, were the most frequent species associated with tinea capitis among primary schoolchildren in Mali. Tinea capitis risk increased with increasing climate humidity in this relatively homogenous schoolchild population, which suggests that climatic factors play a significant role in the epidemiology of dermatophytosis.

Author Summary

We ask that all authors of research articles include a 150- to 200-word non-technical summary of the work, immediately following the Abstract. Subject to editorial review and author revision, this short text is published with all research articles as a highlighted text box.

Distinct from the scientific abstract, the author summary should highlight where the work fits in a broader context of life science knowledge and why these findings are important to an audience that includes both scientists and non-scientists. Ideally aimed to a level of understanding of an undergraduate student, the significance of the work should be presented simply, objectively, and without exaggeration.

Authors should avoid the use of acronyms and complex scientific terms and write the author summary using the first-person voice. Authors may benefit from consulting with a science writer or press officer to ensure that they effectively communicate their findings to a general audience.

Introduction

Dermatophytosis represents one of the most common infectious diseases worldwide and causes serious chronic morbidity [1]. The condition is caused by dermatophytes, which are fungi that require keratin for growth. An increase in the incidence of such infections has been noted worldwide, especially in developing countries [2–4]. In particular, tinea capitis represents a major public health issue among children in developing countries. This dermatophytosis of the scalp and hair shafts is almost exclusively a childhood disease, and evidence suggests that it occurs more often in children of African or Caribbean origin [1]. Many factors including gender, age, urban/rural environment, socio-economic level and cultural habits have been shown to significantly impact the development of dermatophytosis worldwide, especially throughout the African continent [5–9]. It has been hypothesized that climate also plays an important role in the heterogeneity of dermatophytosis epidemiology in Africa [3]. Many studies have described dermatophytosis epidemiology in various geographical settings throughout the African continent. However, the striking differences observed cannot be attributed solely to variations in climate, as heterogeneous study design limits the assessment of numerous potential confounding factors. In fact, no study has been established to specifically address the impact of climate on dermatophytosis presentation. In Mali, the climate ranges from subtropical in the south to arid in the north. Therefore, the current study aimed to assess the prevalence, risk factors and etiological agents of tinea capitis, the most frequent dermatophytosis subtype, among primary schoolchildren in three eco-climatic zones in Mali.

Methods

Study areas and population

Three cross-sectional surveys were carried out between December 2009, December 2010 and February 2012 in three public primary schools in (i) Sirakoro-Meguetana, a semi-urban community located in the suburbs of Bamako, the capital of Mali, in the Sudanian eco-climatic zone; (ii) Bandiagara, a urban community in the Sahelian eco-climatic zone and (iii) Bougoula-Hameau, a semi-urban community located in the suburbs of Sikasso in the Sudano-Guinean eco-climatic zone. The study sites and eco-climatic zones previously defined [10] are shown on the map in Figure 1.

Study participants

Pupils, aged 6 to 15 years, were randomly selected in each primary school using a block randomization design adjusted on the number of pupils in each classroom. Oral informed consent was obtained from the children and their parents or guardians. The exclusion criterion was a history of antifungal treatment (oral or topical, conventional or traditional) within two weeks. Medical history and information concerning exposure to potential dermatophytosis risk factors were recorded, including contact with animals and specific hair grooming habits, and a complete physical examination of the skin and appendages, including fingernails and hair, was performed on all children by one of the investigators. The data were recorded on a standardized clinical report form.

Ethical issue and biological samples collection.

The study protocol was reviewed and approved by the Faculty of Medicine's Institutional Review Board at the University of Bamako, Mali. The study protocol was also approved by the Local Education Authorities at each study site.

Samples were collected from each lesion that was compatible with dermatophytosis. Skin samples were isolated from the peripheral erythematous border of the lesion. Scalp lesions were collected by scraping the area with a sterile curette, and broken and lusterless hairs were selected and plucked using sterile tweezers. One portion of the each sample was used for direct examination via microscopy, while the second portion was inoculated directly onto Sabouraud Dextrose Agar (SDA) (bioMérieux, Marcy l'Etoile, France) with antibiotics and cycloheximide for mycological examination. In the Sahelian and Sudano-Guinean zones, an additional sample was collected directly from the same lesion via sterile gauze. The samples inoculated on SDA were incubated at room temperature before being transferred to the Parasitology-Mycology Laboratory at the University Hospital of Marseilles, where they were incubated at 27ºC for a of 4-6-week period. The sterile gauze samples collected in the Sahelian and Sudano-Guinean zones were stored at ambient temperature in individually sealed plastic bags before being inoculated on SDA agar (BioMérieux) and subsequently incubated for 4-6 weeks at 27°C at the Parasitology-Mycology Laboratory in Marseilles. Dermatophyte colonies were identified based on examination of the macro- and micro-morphological features of the fungus, and those with atypical morphological features were further identified via rDNA internal transcribed spacer 2 (ITS2) sequence analysis as previously described [11].

Statistical analysis

A sample size of 200 children was calculated to estimate a 12% dermatophytosis prevalence rate with a 4.5% precision at α=5%. The data were analyzed using SAS 9.2 for Windows (SAS Institute Inc., Cary, NC, USA). Continuous variables were expressed as the mean (SD), while categorical variables were expressed as proportions and percentages. Continuous variables were compared using ANOVA. Categorical variables were compared using the Chi square or Fisher's exact tests as required. All statistical tests were two-sided with a $P<0.05$ significance level. Univariate and multivariate unconditional logistic regression analyses were performed to estimate odds ratios (ORs) with a 95% confidence interval (CI). All covariates with a $P<0.20$ significance level in the univariate analysis were included in the multivariate logistic regression model. A stepwise selection was performed to retain the most parsimonious model including the covariates that displayed an independent statistically significant ($P<0.05$) effect on tinea capitis risk.

Results

Study population characteristics.

Of the 590 randomly selected schoolchildren, 286 males and 304 females participated in this study, including 190 from Sirakoro-Meguetana, 200 from Bandiagara and 200 from Sikasso (Figure 1). Although the age distribution of the participating children in each eco-climatic zone differed significantly ($P=0.0013$), the mean age of each zone, which ranged from 9.3 to 10.2 years, was quite similar (Table 1). The sex ratio of the participating schoolchildren in each eco-climatic zone did not significantly differ ($P=0.151$). As expected, the ethnic group distribution of each eco-climatic zone differed significantly ($P<10^{-4}$) (Table 1).

Dermatophytosis prevalence.

As detailed in Table 2, 312 children presented with clinical dermatophytosis lesions, thereby yielding an overall dermatophytosis prevalence of 52.9% 95% CI [48.7-57.0] among the entire study population. The most frequent (232/590) clinical presentation was tinea capitis with 39.3% 95% CI [35.4-43.4] prevalence. Therefore, tinea capitis was separately detailed in the study, and all other clinical presentations of dermatophytosis were categorized as non-tinea capitis, which was found in 80 (13.6% 95% CI [10.9-16.6] of the participating children. Among the 80 non-tinea capitis dermatophytosis cases, the most common clinical presentation was

tinea corporis (81.3%), followed by tinea cruris (8.7%), tinea pedis (7.5%) and tinea unguis (2.5%), irrespective of the geographic area.

Tinea capitis prevalence and clinical presentation.

Overall, 39.3% (95% CI [35.4-43.4]) of the schoolchildren presented with clinical tinea capitis lesions. The prevalence of tinea capitis significantly ($P<10^{-4}$) differed depending on the geographic area, with prevalence rates of 17.0% 95% CI [12.1-22.9], 41.6% 95% CI [34.5-48.9] and 59.5% 95% CI [52.4-66.4] recorded in the Sahelian, Sudanian and Sudano-Guinean eco-climatic zones, respectively. The characteristics of the 232 schoolchildren presenting with clinical tinea capitis lesions are detailed in Table 2. The tinea capitis lesions were described as diffuse primarily in the Sudano-Guinean (55.5%) and Sudanian (51.9%) zones, while diffuse lesions were observed only in 26.5% of cases in the Sahelian zone (P=0.0108). Interestingly, this individual marker of infection intensity correlated with prevalence. Inflammatory and suppurative forms of tinea capitis were rarely observed. Tinea capitis presentation in the Sudano-Guinean zone was characterized by the predominance of large lesions (>2 cm) involving *Microsporum audouinii*, in contrast to the Sahelian and Sudanian zones, which were characterized by the predominance of multiple (n>2) lesions involving *Trichophyton soudanense* (Table 2). As expected, the majority of microsporosis lesions (64.5%) and a particularly high proportion (80.3%) of large (>2 cm) tinea capitis lesions, displayed a typical green fluorescence upon Wood's lamp examination, while most trichophytosis lesions (95.5%) did not display this feature. Of note, 30.3% of the diffuse scalp lesions tested positive upon Wood's lamp examination.

Non-tinea capitis dermatophytosis.

Eighty children (13.6%) presented with non-tinea capitis clinical dermatophytosis (Table 3), of which tinea corporis was the most frequent (81.3%) clinical presentation. The prevalence of tinea corporis varied depending on the eco-climatic zone, ranging from 88.4% in the Sudanian to 71.4% in the Sudano-Guinean zone. Overall, six children (7.5%) presented with athlete's foot, of which four (14.3%) originated from the Sudano-Guinean zone and two (4.7%) were from the Sudanian zone.

Mycological findings.

The majority of tinea capitis lesions (81.5%) tested positive upon direct microscopic examination. The distribution of endothrix or endo-ectothrix parasitism type did not

significantly differ between the geographic areas. In contrast, only 3.8% of the non-tinea capitis dermatophytosis samples tested positive upon direct examination.

Dermatophyte culture.

Of 312 samples collected from all clinical dermatophytosis lesions, 247 mycological cultures tested positive for dermatophyte fungus, thereby yielding an overall dermatophyte culture sensitivity of 79.2% (95%CI [74.2-83.5]. Dermatophyte cultures sensitivity for both tinea capitis and non-tinea capitis diagnosis were lowest in the first study site located in the Sudan eco-climatic zone (Tables 2 & 3).

Dermatophytes isolated from tinea capitis.

Mycological culture analysis yielded dermatophytes in 189 of the 232 tinea capitis samples collected, thereby yielding a global prevalence of 32% (189/590) mycologically-confirmed cases. A Positive mycological cultures rate was 100% in the Sahelian and Sudano-Guinean zones but 45.6% (36/79) in the Sudanian zone, where (24% of mycological cultures were negative and 30.4% were contaminated with non-dermatophytes filamentous fungi). Overall, we recovered 24 non-dermatophyte filamentous fungi and 189 dermatophyte isolates of four dermatophyte species, namely *Trichophyton soudanense*, *T. mentagrophytes*, *T. violaceum* and *Microsporum audouinii* (Table 4). In 14.5% of the positive cultures, two dermatophyte species were co-isolated, namely *T. soudanense* together with either *T. mentagrophytes* or *M. audouinii*. The tinea capitis clinical features associated with each dermatophyte species, or combination of species, are detailed in Table 4. *M. audouinii* was isolated primarily from large (>2 cm) lesions (75.4%) and/or microsporic lesions (68.1%). *T. soudanense* was primarily isolated from trichophytic lesions (96.2%). The combination of both *T. soudanense* and *M. audouinii* was found only in trichophytic lesions. The Wood's lamp examination tested positive in 85.5% (P<.0001) of tinea capitis cases involving *M. audouinii* (Table 4).

A predominance of anthropophilic species was observed in the three eco-climatic zones (Table 4). In the Sahelian zone, a clear predominance of *T. soudanense* was observed, which displayed a prevalence of 64.7%, 31.7% and 31.1% in the Sahelian, Sudanian and Sudano-Guinean eco-climatic zones, respectively ($P<10^{-4}$). *M. audouinii* was the most common etiological agent (39.5%) in Bougoula-Hameau. Of note, *T. violaceum* was only isolated in the Sudano-Guinean eco-climatic zone, while *T. mentagrophytes* was found only in the Sudanian zone.

***Tinea capitis risk factors*.**

The global and gender-specific distributions of the assessed tinea capitis risk factors are tabulated in Table 2. The distribution of the potential risk factors and habits associated with tinea capitis among schoolchildren according to their tinea capitis status is shown in Table 5. In the univariate analysis, several hairdressing habits were found significantly associated with tinea capitis; however, the associations were non-significant when gender was considered, which acted as a notable confounding factor. Proximity to cattle was associated with a significant decrease in tinea capitis risk among females but not among males. In contrast, the presence of a dog in the household was associated with increased tinea capitis risk among males but not among females. None of these risk factors were found statistically significant in the multivariate analysis.

The effects of the most significant tinea capitis risk factors are detailed in Table 6. In the univariate analysis, tinea capitis was significantly associated with male gender (OR=7.85; 95%CI [5.22-11.81], $P<10^{-4}$) and residing in Bougoula-Hameau (OR=7.17; 95%CI [4.51-11.4], $P<10^{-4}$). Notably, age had no significant effect on this study population. In the multivariate analysis, both male gender (OR=2.51, 95%CI [1.74-3.61], $P<10^{-4}$) and residing in the Sudano-Guinean eco-climatic zone (OR=7.45, 95%CI [4.63-11.99], $P<10^{-4}$) were statistically significant independent tinea capitis risk factors (Table 6).

Dermatophytes isolated from other dermatophytosis samples.

A dermatophyte was isolated in 58 of the 80 samples collected from children with *tinea corporis*, *tinea cruris*, athlete foot or onychomycosis, thereby yielding a global prevalence of 9.8% (95%CI [7.6-12.5]) of mycologically confirmed non-tinea capitis dermatophytosis cases (Table 3). Two dermatophyte species were predominant, namely *T. soudanense* (63.8%) and *T. mentagrophytes* (5%). The combination of both *T. soudanense* and *T. mentagrophytes* was found in 3.8% of positive cultures. *M. audouinii* was not isolated from non-tinea capitis lesions in this study. Twenty-two (27.4%) non-dermatophyte filamentous fungi were recovered, primarily in the Sudanian eco-climatic zone (Table3).

Discussion

Overall, our study highlights three major findings: a dramatic disparity in tinea capitis epidemiology between distinct eco-climatic zones within Mali, evidence of an increased tinea capitis risk among male children and the dermatophyte species distribution. The

dermatophytosis prevalence variations observed in the current study might be associated with varied exposure of the surveyed schoolchildren in each area to several risk factors, including eco-climatic, socio-economic factors and genetic or ethno-cultural elements. Notably, with the exception of ethno-cultural characteristics, the schoolchildren surveyed in each area were homogenous, especially in regards to age, and each survey was performed at the same period during the dry season. Therefore, the observed geographical differences in tinea capitis epidemiology may be attributed to variations in the local environment, and more specifically eco-climatic differences. Indeed, the highest prevalence of dermatophytosis was recorded in Bougoula-Hameau (59.5%), where the Sudano-Guinean climate is characterized by relatively higher levels of humidity; followed by Sirakoro-Meguetana (41.6%), where the Sudanian climate is characterized by intermediate humidity levels; and Bandiagara (17%), where the Sahelian climate is characterized by relatively lower humidity levels. This association trend of tinea capitis risk that increases with the humidity level of the climate correlates with data from Togo, where tinea capitis prevalence rates were 11% in a dry region in the North of the country and 20% in a humid area in the South of the country [12]. These geographical discrepancies might be associated with hot and humid climates, which favor the growth and spread of fungi and may predispose populations to various skin diseases including tinea capitis [13].

Tinea capitis primarily affects children in developing countries, while tinea pedis and tinea unguis pose the greatest burden to adults and the elderly in developed countries [14]. The high prevalence dermatophytosis rate (52.9%) found in this study is similar with those reported in many studies concerning African schoolchildren [15–18]. In correlation with our findings, tinea capitis was also the most frequent dermatophytosis presentation among children [5–7,19]. Its 39.3% prevalence rate was similar to those observed among outpatients attending the dermatology consultation at the Marchoux Dermatology Institute in Bamako (38.5%) [20] and higher than those reported in previous surveys of schoolchildren in Bamako (7% and 12.5%) [19,21].

In agreement with the majority of African studies, our findings highlight male gender as a significant tinea capitis risk factor among schoolchildren [5–7,15,18,19,21,22]. For example, in Mali, the prevalence of tinea capitis was 4.4 % and 2.1 % ($P< 10^{-6}$) in schoolboys and schoolgirls, respectively [19]. Meanwhile, the prevalence was 3 and 5 times higher in boys than in girls in Abidjan (Ivory Coast) and Central Nigeria, respectively [6,7].

Many potential risk factors for tinea capitis have been proposed [23]. Although our study did not address genetic susceptibility to dermatophytosis, we considered ethno-cultural risk factors,

which are likely to play a significant role in dermatophytosis epidemiology. It has been reported that dermatophytosis prevalence is influenced by the hairdressing mode, extracurricular activity and cultural habits, rather than population density [5,7]. In the present study population, the following factors were associated with tinea capitis: contact with dogs (P=0.0024), public hairdressing practices (P=0.0220), home hairdressing practices ($P<10^{-5}$), traditional braiding practices ($P<10^{-5}$) and head shaving practices ($P<10^{-5}$). In Egypt, El-Khalawany et al (2013) have shown that contact with animals was a common predisposing factor for tinea capitis in rural areas, whereas transmission from other family members was more common among individuals residing in urban areas. However, increased dermatophyte risk due to contact with dogs was unexpected in our study, as we did not isolate *M. canis*, the dermatophyte species usually associated with canines. Further studies are required to assess whether dogs might be involved in anthropophilic dermatophyte species transmission.

One limitation of the study is the relatively high false-negative dermatophyte culture rate in the first study compared with the two other study sites, which is associated with the subsequent introduction of sterile gauze in the sampling procedure. This limitation was taken into account by applying a clinical definition of tinea capitis in the risk factor study. We can reasonably presume that the false-negative culture results occurred at random in Sirakoro-Meguetana and thus did not alter the dermatophyte species distribution evaluation. As observed in other studies [5,7] the most common *Trichophyton* species was *T. soudanense* (36.6%), which was the most common species associated with tinea capitis in the Sahelian climate zone (64.7%). *T. soudanense* is one of the most common clinical dermatophyte species in West and Central Africa, where infections of this anthropophilic species are spread via direct contact between people. Two other species of the *Trichophyton* genus were isolated in this study: *T. mentagrophytes* (exclusively in the Sudanian zone in 6.6% of cases) and *T. violaceum* (only in the Sudano-Guinean zone in 5.9% of cases). The anthropophilic species *T. violaceum* has been shown associated with tinea capitis Conakry-Guinea, where a 56.7% prevalence of this species was reported [24]. However, in Ivory Coast, a low prevalence (2.3%) of this species has also been associated with tinea capitis [6]. It should be noted that *T. soudanense* and *T. violaceum* are phylogenetically very similar and that some experts consider these two taxa to be synonyms [25]. *M. audouinii* was the only species of the *Microsporum* genus isolated in this study. It was the second most common species following *T. soudanense*, which correlates with previous studies of schoolchildren in Bamako [19,21].

Conclusion

Tinea capitis was diagnosed in 39.3% of a representative population of Malian schoolchildren. Two anthropophilic dermatophyte species, *T. soudanense* and *M. audouinii* were isolated in the majority of cases. Male gender and residing in the tropical North Guinea climatic zone of Mali were identified as independent tinea capitis risk factors. Tinea capitis risk increased with increasing humidity among the relatively homogenous populations located in distinct climatic geographic areas, thereby indicating that climatic factors may play a significant role in dermatophytosis epidemiology. Further epidemiological studies are required to elucidate the respective role that climatic and ethno-cultural factors play in dermatophytosis distribution.

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Table 1- Demographic characteristics of the 590 children that participated in the study grouped by geographic area.

Demographic characteristics			**Eco-climatic zones**						
	Total		**Sudanian (n=190)**		**Sahelian (n=200)**		**Sudano-Guinean (n=200)**		
	n	**%**	**n**	**%**	**n**	**%**	**n**	**%**	**P values**
Age Mean (SD)	570	9.7 (2.38)	185	9.3 (2.4)	192	9.6 (2.3)	193	10.2 (2.3)	0.0013
Gender	590	100	190	100	200	100	200	100	0.1506
Female	304	51.5	107	56.3	104	52	93	46.5	
Male	286	48.5	83	43.7	96	48	107	53.5	
Ethnic group	547	92.7	189	99.5	195	97.5	163	81.5	$<10^{-4}$
Dogon	219	40	85	45	134	68.7	0	0	
Bambara	60	11	31	16.4	14	7.2	15	9.2	
Fulani	52	9.5	24	12.7	24	12.3	4	2.4	
Senufo	134	24.5	0	0	0	0	134	82.2	
Other	82	15	49	25.9	23	11.8	10	6.2	

Table 2- Demographic, clinical and mycological features of the 232 children with tinea capitis grouped by eco-climatic zones in Mali.

	Eco-climatic zones								
	Total		**Sudanian**		**Sahelian**		**Sudano-Guinean**		
Demographic data	**n=232**	**%**	**n=79**	**%**	**n=34**	**%**	**n=119**	**%**	**P value**
Age Mean (SD)	232	9.8 (2.4)	79	9.4 (2.5)	34	9.6 (2.4)	119	10.1 (2.3)	0.1319
Sex group	232	100	79	100	34	100	119	100	0.0204
Female	90	38.8	40	50.6	9	26.5	41	34.4	
Male	142	61.2	39	49.4	25	73.5	78	65.6	
Clinical data									
Inflammatory	4	1.7	2	2.5	0	0	2	1.7	0.6370
Suppurative	3	1.3	0	0	1	2.9	2	1.7	0.3870
Diffuse	116	50.0	41	51.9	9	26.5	66	55.5	0.0108
Size >2 cm	100	43.1	17	21.5	12	35.3	71	59.7	<0.0001
Number >2	169	72.8	79	100	27	79.4	63	52.9	<0.0001
Trichophytosis	176	75.9	62	78.5	22	64.7	92	77.3	0.2537
Microsporosis	54	23.3	15	19	12	35.3	27	22.7	0.1664
Hair shaft invasion									
Endothrix	50	20.6	14	17.7	11	32.3	25	21	0.2054
Endo-ectothrix	55	23.7	12	15.2	10	29.4	33	27.7	0.0885
Dermatophytes									
Culture	232	100	79	100	34	100	119	100	
Positive	189	81.5	36	45.6	34	100	119	100	
Negative	19	8.2	19	24.0	0	0.0	0	0.0	
Contamination	24	10.3	24	30.4	0	0.0	0	0.0	
Species	213	100	60	100	34	100	119	100	
M. audouinii	69	32.4	10	16.7	12	35.3	47	39.5	
T. soudanense	78	36.6	19	31.7	22	64.7	37	31.1	
T. violaceum	7	3.3	0	0.0	0	0.0	7	5.9	
T. mentagrophytes	4	1.9	4	6.6	0	0.0	0	0.0	
T. s. + T. m.	3	1.4	3	5.0	0	0.0	0	0.0	
T. s. + M. a.	28	13.2	0	0.0	0	0.0	28	23.5	
NDF[1]	24	11.3	24	40.0	0	0.0	0	0.0	

NDF: non-dermatophyte filamentous fungi

Table 3- Clinical presentation and mycological features of non-*tinea capitis* dermatophytosis according to eco-climatic zones in Mali.

			Eco-climatic zones					
	Total		**Sudanian**		**Sahelian**		**Sudano-Guinean**	
	n=80	**%**	**n=43**	**%**	**n=9**	**%**	**n=28**	**%**
Clinical features								
Tinea corporis	65	81.3	38	88.4	7	77.8	20	71.4
Tinea cruris	7	8.7	1	2.3	2	22.2	4	14.3
Tinea pedis	6	7.5	2	4.7	0	0.0	4	14.3
Tinea unguium	2	2.5	2	4.6	0	0.0	0	0.0
Direct examination features								
Positive	3	3.8	3	7.0	0	0.0	0	0.0
Negative	77	96.2	41	93.0	9	100	28	100
Dermatophytes culture								
Positive	58	72.5	27	62.8	9	100	22	78.6
Negative	10	12.5	4	9.3	0	0.0	6	21.4
NDF*	12	15.0	12	27.9	0	0.0	0	0.0
Fungal species isolated								
T. soudanense	51	63.8	20	40.8	9	100	22	100
T. mentagrophytes	4	5.0	4	8.2	0	0.0	0	0.0
T. s + *T. m*[1]	3	3.8	3	6.1	0	0.0	0	0.0
NDF[2]	22	27.4	22	44.9	0	0.0	0	0.0

1. *T. s* + *T. m*: *T. soudanense* and *T. mentagrophytes*; 2. NDF: non-dermatophyte filamentous fungi

Table 4- Clinical, direct microscopic and Wood's lamp examination features of tinea capitis lesions according to the identified dermatophyte species and non-dermatophyte filamentous fungus.

Species[1]	***M. aud.***		***T. soud.***		***T. menta.***		***T. viol.***		***T. s. + T. m.***		***T. s. + M. a.***		**NDF**		
(n=213)	**n=69**	**%**	**n=78**	**%**	**n=4**	**%**	**n=7**	**%**	**n=3**	**%**	**n=28**	**%**	**n=24**	**%**	**P values**
Clinical features															
Inflammatory (n=4)	0	0.0	0	0.0	2	50.0	2	28.6	0	0.0	0	0.0	0	0.0	<0.0001
Suppurative (n=3)	0	0.0	1	1.3	0	0.0	2	28.6	0	0.0	0	0.0	0	0.0	<0.0001
Diffuse (n=105)	17	24.6	44	56.4	4	100	5	71.4	3	100	18	34.3	14	58.3	<0.0001
Size >2 cm (n=97)	52	75.4	16	20.5	1	25	5	71.4	1	33.3	21	75	1	4.2	<0.0001
Number >2	38	57.1	55	70.5	4	100	4	57.0	3	100	22	78.6	24	100	0.0011
Trichophytosis (n=160)	22	31.9	75	96.2	3	75	7	100	3	100	28	100	22	91.7	<0.0001
Microsporosis (n=51)	47	68.1	1	1.3	1	25	0	0.0	0	0.0	1	3.6	1	4.2	<0.0001
Direct examination features															
Positive (n=99)	48	69.6	27	34.6	0	0.0	3	42.9	2	66.7	14	50.0	2	20.8	0.0001
Endothrix (n=47)	2	2.9	27	34.6	0	0.0	3	42.9	2	66.7	9	32.1	4	16.6	<0.0001
Endoectothrix (n=53)	46	66.7	0	0.0	0	0.0	0	0.0	0	0.0	6	21.4	1	4.2	<0.0001
Wood's lamp examination features															
Wood + (n=72)	59	85.5	3	3.8	0	0.0	0	0.0	0	0.0	9	32	1	4.2	<0.0001
Wood - (n=141)	10	14.5	75	96.2	4	100	7	100	3	100	19	68.0	23	95.8	

1. *M. aud*.: *Microsporum audouinii*; *T. soud*.: *Trichophyton soudanense*; *T. menta*.: *Trichophyton mentagrophytes*; *T. viol*.: *Trichophyton violaceum*; *T. s. + T. m*.: *T. soudanense* and *T. mentagrophytes*; *T. s. + M. a*.: *T. soudanense* and *M. audouinii*; NDF: non-dermatophyte filamentous fungi.

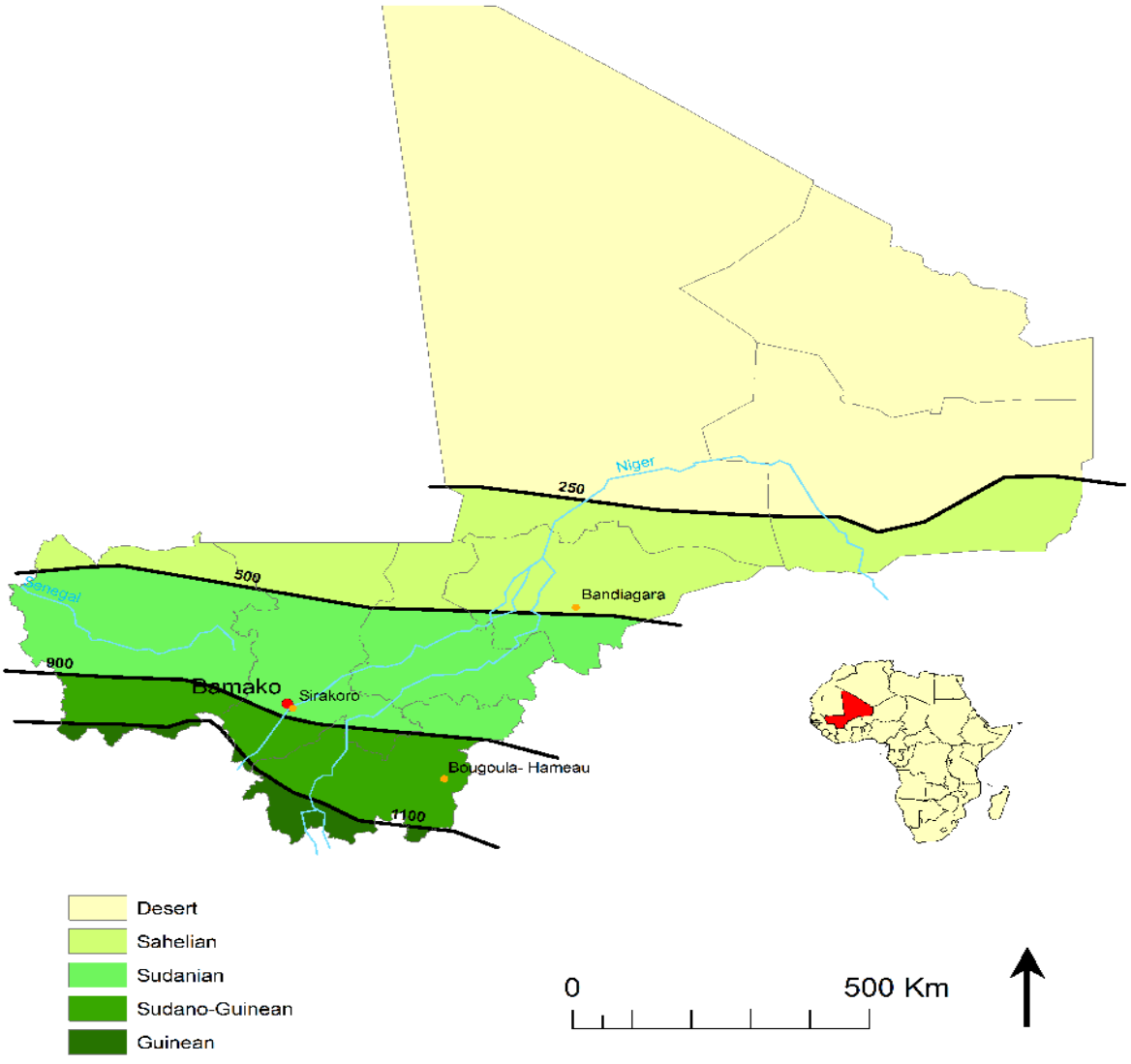
Table 5. Common hairdressing habits and domestic animal contact among the 590 subjects according to gender and tinea capitis status.

Risk factors	Study population					Females (n=304)			Males (n=286)		
	Overall (n=590)		Tinea capitis (n=232)		P value	Tinea capitis (n=90)		P value	Tinea capitis (n=142)		P value
	n	%	n	%		n	%		n	%	
Public hairdressing	253	42.9	174	75	0.0220	4	4.4	0.4895	125	88.0	0.1858
Home hairdressing	342	57.9	129	55.6	$<10^{-5}$	89	98.9	0.6782	18	12.7	0.1941
Traditional braiding	303	51.4	107	46.1	$<10^{-5}$	90	100	0.5576	1	0.7	1.0000
Head shaving	286	48.4	1	0.4	$<10^{-5}$	0	0	1.0000	141	99.3	1.0000
Hairdressing frequency, Mean (SD)	1.7	(0.9)	1.7	(0.9)	0.5311	1.8	(0.9)	0.4298	1.7	(0.9)	0.4079
Nb children sleeping /room, Mean (SD)	3.3	(1.7)	3.3	(1.7)	0.8473	3.4	(1.5)	0.2259	3.3	(1.9)	0.2484
Domestic animals	540	91.5	212	91.4	0.9183	76	84.4	0.0639	136	95.8	0.2229
Contact with animals	409	69.4	174	75.0	0.0220	58	64.4	0.6075	116	81.7	0.0899
Dog	227	38.5	107	46.1	0.0024	36	40.0	0.2929	71	50.0	0.0083
Cat	180	30.5	77	33.2	0.2726	27	30.0	1	50	35.2	0.0949
Horse	41	7.0	11	4.7	0.0991	5	5.6	0.6289	6	4.2	0.1529
Sheep	344	58.3	127	54.7	0.1717	43	47.8	0.0583	84	59.2	0.7169
Goat	158	26.8	64	27.6	0.7753	26	28.9	0.8897	38	26.8	0.5868
Cow	178	30.17	78	33.6	0.1433	27	30.0	0.6775	51	35.9	0.2059
Donkey	264	44.8	113	48.7	0.1276	39	43.3	0.6123	74	52.1	0.2870
Poultry	481	81.5	192	82.8	0.5876	70	77.8	0.7593	122	85.9	0.5169
Proximity to cattle	349	59.2	124	53.5	0.0259	44	48.9	0.0425	80	56.3	0.1834

Table 6- Univariate and multivariate unconditional logistic regression analyses of the *tinea capitis* risk factors identified in this population.

	Univariate analysis			**Multivariate analysis**		
	OR	**95%CI**	P **value**	**OR**	**95%CI**	**P value**
Age (years)						
< 8	0.73	0.43 - 1.24	0.4484			
[8-10]	0.73	0.41 – 1.29	0.4892			
[10-12[	0.81	0.46 – 1.42	0.9838			
≥ 12	-	-	-			
Male gender	2.34	1.67 - 3.29	$<10^{-4}$	2.51	1.74 – 3.61	$<10^{-4}$
Eco-climatic zones						
Sudano-Guinean	7.17	4.51 - 11.4	$<10^{-4}$	7.45	4.63 – 11.99	$<10^{-4}$
Sudanian	3.48	2.18 - 5.55	0.1683	3.81	2.36 – 6.17	0.0849
Sahelian	-	-	-			

Figure 1. Location of three study sites and the isohyets (mm/year) separating the Sahelian, Sudanian and Sudano-Guinean eco-climatic zones in Mali.



Article 3: High dermatophyte contamination levels in hairdressing salons of a West African suburban community

Coulibaly O[1], Thera MA, Piarroux R, Doumbo OK, Ranque S.

Mycoses. 2014 Nov 11. doi: 10.1111/myc.12272. [Epub ahead of print]

mycoses
Diagnosis, Therapy and Prophylaxis of Fungal Diseases

Original article

High dermatophyte contamination levels in hairdressing salons of a West African suburban community

O. Coulibaly,[1,2,3,4] M. A. Thera,[4] R. Piarroux,[1,2,3] O. K. Doumbo[4] and S. Ranque[1,2,3]

[1]*Mycology & Parasitology, CHU Timone-Adultes, Assistance Publique-Hôpitaux de Marseille, Marseille, France,* [2]*Aix-Marseille Université, IP-TPT UMR MD3, Marseille, France,* [3]*Laboratoire de Parasitologie-Mycologie, Marseille Cedex, France and* [4]*Département d'Epidémiologie des Affections Parasitaires, Faculté de Médecine, Université de Bamako, Bamako, Mali*

Summary

Tinea capitis is a dermatophyte infection of scalp is commonly spread by currently infected patients, asymptomatic carriers or by fomites, such as hairdressing tools. However, studies on the risk factors of Tinea capitis remain scarce. The aim of this study was to evaluate the dermatophytes contamination level of the hairdressing tools to which hairdressing salon customers are exposed in Sirakoro-Méguétana, a suburb of Bamako, the capital city of Mali. A total of 41 hairdressing tools were sampled in five hairdressing salons. Two anthropophilic dermatophytes species, *Microsporum audouinii* (53.3%) and *Trichophyton soudanense* (46.7%), were cultured from 30 (73.2%) samples. This first study, addressing hairdressing salons dermatophyte contamination, revealed a strikingly high contamination of hairdressing tools with dermatophyte propagules, which exposes hairdressing salons customers to an important dermatophytosis risk. The sterilisation of hairdressing tools is central to preventing dermatophytoses spreading. Appropriate community information and hairdressers training should be implemented in this view.

Key words: dermatophytosis, *Microsporum audouinii*, *Trichophyton soudanense*, hairdressing salons, hairdressing tools, Mali.

Introduction

Tinea capitis is a common fungal infection of the scalp, hair follicles and hair shafts that is commonly spread by asymptomatic carriers, currently infected patients and, possibly, by fomites such as hairdressing tools.[1] It is almost exclusively a disease of childhood, and tinea capitis is particularly common in school-aged children in Africa.[2] Several investigations in school-aged children suggest infection rates ranging between 10.2% and 57.7% in West African cities.[3–10] It has been hypothesised that this high prevalence may be due to poor socioeconomic status, particular hair style practices and genetic susceptibility.[11] In Mali, tinea capitis is the most common dermatophyte infection of childhood. In 90% of the cases, it is caused by the two anthropophilic species *Trichophyton soudanense* and *Microsporum audouinii*.[12] The risk factors for tinea capitis remain poorly understood. In 2004, a questionnaire-based study provided circumstantial evidences showing that hairdressing salon were probably involved in tinea capitis transmission, with a 4.6 times higher tinea capitis risk in children attending hairdressing salons compared to those who do not.[13] Here, we hypothesised that hairdressing tools may expose the customers to dermatophytosis transmission. The aim of this study was thus to evaluate the dermatophyte contamination of hairdressing tools and assess whether or not this may explain the higher risk of tinea capitis among hairdressing salon customers.

Correspondence: Dr S. Ranque, Laboratoire de Parasitologie-Mycologie, AP-HM Timone, F-13385 Marseille Cedex 5, France.
Tel.: +33 491 38 60 90. Fax: +33 491 38 49 58.
E-mail: stephane.ranque@ap-hm.fr

Submitted for publication 24 June 2014
Revised 24 September 2014
Accepted for publication 22 October 2014

doi:10.1111/myc.12272

Materials and methods

A cross-sectional survey was conducted in December 2009, in Sirakoro-Néguétana, suburban area of Bamako, the capital city of Mali. It included five randomly selected hairdressing salons. After obtaining the hairdressers informed consent, the samples were collected from the various hairdressing tools and other fomites available in the salon: needles used for African braiding; razors; combs; scissors; hair brushes; shea butter; wipes; hair clippers; tables; and synthetic wicks. Samples were collected in the afternoon, before the closing time of the hairdressing salons, via adhesive tape or swabs. They were then plated on Sabouraud agar with antibiotics and cycloheximide (BioRad, France) and incubated up to 28 days at 27 °C. The isolates were identified using morphological: macroscopic and microscopic criteria. Data were entered using MS Office Excel 2007; the Chi-square test computed by SAS, version 9.2 (SAS Institute, Cary, NC, USA) was used for the statistical comparisons. All statistical tests were two-sided with a $P < 0.05$ significance level.

Results

Overall, 41 samples were taken from various fomites present in the hairdressing salons, including: two hair cream pot, five needles used for African braiding; five hair brushes; three cloths; five scissors; one synthetic hair extension; nine combs; two razors; three tables surfaces; five hair clippers; and one shea butter sample. As shown in Table 1, dermatophytes were isolated from 30 fomites, yielding a global contamination rate of 73.2% (30/41). The association between a positive dermatophyte culture and hairdressing salon was not statistically significant ($P = 0.07$). Furthermore, there was no statistically significant association ($P = 0.46$) between the type of hairdressing tool and dermatophyte contamination.

Thirty isolates from two anthropophilic dermatophytes species, 16 *M. audouinii* and 14 *T. soudanense*, were isolated from various hairdressing tools (Table 1). Both species were isolated from table surface samples collected in two hairdressing salons. There was no statistically significant association between the type of hairdressing tool and the dermatophyte species ($P = 0.65$).

Discussion

To the best of our knowledge, this is first study aiming at evaluating the dermatophyte contamination level of hairdressing tools used in hairdressing salons in a tinea capitis endemic area. Our findings of a 73.2% contamination rate by the cultivable anthropophilic dermatophytes of various hairdressing tools sampled in the five hairdressing salons further fuels the hypothesis that attending hairdressing salons is a risk factor for tinea capitis and offers new insights into its transmission mechanisms and the spreading of dermatophytoses among hairdressing salon customers.

However, we would like to point out some limitations of our study. First, only a relatively small number of samples were examined in this exploratory study. Second, the adhesive tape or swab methods we used are possibly be less efficient than the traditional 'carpet technique'.[14] Yet increasing the sensitivity of dermatophyte detection would only tend to increase the observed 73.2% contamination rate and strengthen our finding of a very high contamination with dermatophyte propagules of the fomites in the investigated hairdressing salons.

Although this is an ongoing issue in developing countries, we were struck by both the age and scarcity of studies investigating hairdressing tools dermatophyte contamination and the limited amount of factual evidence regarding its association with tinea captis. Maybe the most convincing evidence come from a study in 1963 where 'hairbrush diagnosis' was successfully used to detect, follow-up and eradicate *T. tonsurans* associated tinea capitis at a girls' residential school.[15] Moreover, a literature review found only three questionnaire-based surveys addressed the issue of association between tinea capitis and hair care practices but none evaluated hairdressing fomites' contamination.[13,16,17] Specifically, no statistically significant association between hair-grooming practices and tinea capitis was found in hospital-based case-control study in the USA.[17] And we have to go back to 1965, to find a hospital-based case-control study in Philadelphia (USA) showing that the tinea capitis risk increased with the sibship size, especially in 5–16 year-old boys, and was associated with boys sharing their combs and caps and having less frequent shampoos during winter.[16] More recently, another hospital-based case-control study conducted in Abidjan (Ivory Coast) reported that the hairdresser's customer is exposed to a 2.5 times higher risk of tinea capitis when a razor is used compared to when a hair clipper is used; and for the children who get their hair cut at home, the use of hair clipper increases by 45.5 times the tinea capitis risk compared to scissor.[13] Consistent with the present study's findings, these authors hypothesised that this increased risk might be related

Table 1 Hairdressing tools contamination with dermatophyte propagules in five hairdressing salons of Sirakoro-Néguétana, a suburb of Bamako, the capital city of Mali.

Hairdressing salon	Collected samples	*N*	Positive	*Microsporum audouinii*	*Trichophyton soudanense*
1	Needle	2	2	2	0
	Hair cream pot	2	2	1	1
	Scissor	2	2	1	1
	Subtotal	6	6 (100%)	4 (66.4%)	2 (3.3%)
2	Needle	3	3	1	2
	Scissor	2	2	1	1
	Comb	3	3	1	2
	Synthetic hair extension	1	1	1	0
	Shea butter	1	1	0	1
	Subtotal	10	10 (100%)	4 (40%)	6 (60%)
3	Comb	2	0	0	0
	Hairbrush	2	2	2	0
	Scissor	1	1	1	0
	Razor	1	1	1	0
	Hair clipper	1	1	1	0
	Wipe	1	1	0	1
	Table surface	1	1	0	1
	Subtotal	9	7 (78%)	5 (71.4%)	2 (28.6%)
4	Comb	2	1	0	1
	Hairbrush	2	1	0	1
	Razor	1	0	0	0
	Hair clipper	2	1	1	0
	Wipe	1	0	0	0
	Table surface	1	1	0	1
	Subtotal	9	4 (44%)	1 (25%)	3 (75%)
5	Comb	2	1	1	0
	Hairbrush	1	0	0	0
	Hair clipper	2	0	0	0
	Wipe	1	1	0	1
	Table surface	1	1	1	0
	Subtotal	7	3 (43%)	2 (67%)	1 (33%)
	Total	41	30 (73.2%)	16 (53.3%)	14 (46.7%)

to the transmission of dermatophyte from one customer to another via insufficiently disinfected tools and the rapid proliferation of the dermatophyte when it is inoculated within micro-lesions in the scalp caused by a hair clipper or a razor blade. Importantly, infectivity of contaminated tools can last for months and even years, because of the high resistance of fungal spores in the environment.[13] Moreover, the same authors reported that using a comb at home, even if shared by other family members, was not a tinea capitis risk factor.[13] Yet a confounding factor might be that very common comb-sharing practices in sibships are probably not altered when one of the siblings develops Tinea capitis.

Another important finding of this study was that only anthropophilic dermatophytes species, which transmission is strictly inter-human, were identified in this study. Furthermore, these two anthropophilic species, namely *T. soudanense* and *M. audouinii*, perfectly matched the local epidemiology of dermatophyte species involved in tinea capitis. Indeed, a concomitant study conducted in pupils of Sirakoro-Néguétana primary school found that 57% and 20% of the dermatophytoses cases were caused by *T. soudanense* and *M. audouinii*, respectively.[12] The present data along with consistent findings in the literature strongly support the critical role that hairdressing tools play in the spreading of tinea capitis, especially among hair dressing salons customers. Therefore, it is mandatory to disseminate to the community, and chiefly to hairdressers, the information that hair dressing tools should be disinfected after each use. In practice, instruments should be washed in water and detergent and the simple use of 1 : 10–1 : 100 dilution of household bleach could be proposed for efficient and cost-effective disinfection.

Conclusion

A strikingly high proportion of hairdressing tools collected in all sampled hairdressing salons in Sirakoro-Méguétana were contaminated by anthropophilic dermatophytes propagules; thus exposing customers to an important dermatophytosis transmission risk. This point to implementing awareness-raising measures about hairdressing tools disinfection aiming to curb the spread of dermatophytoses in the community.

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Chapitre 3: Evaluation de l'activité antifongique des dérivés aminostéroïdiens contre les dermatophytes

Les dermatophytoses sont parmi les maladies les plus fréquentes dans le monde avec une prévalence globale variant de 20 à 25%. En plus, ces infections sont plus fréquentes dans les pays en développement, où la teigne du cuir chevelu constitue une affection endémique chez les enfants, et posant souvent des spécificités thérapeutiques. En effet, le traitement de cette pathologie reste intrinsèquement difficile et prolongée. Les options thérapeutiques actuelles incluent les médicaments oraux et topiques avec des thérapies orales fournissant de meilleurs résultats. La griséofulvine est le traitement de référence pendant 6 à 8 semaines, dont il faut augmenter la posologie lors des atteintes à *M. canis* (20 à 25 mg/kg/j). La durée prolongée de ce traitement et les effets indésirables variables peuvent être associés à des échecs thérapeutiques. Cependant, l'avènement de plusieurs nouveaux agents antifongiques comme l'itraconazole, fluconazole et la terbinafine a élargi l'arsenal thérapeutique au cours des dernières années. Ces agents offrent des intervalles de traitement plus courts, et leurs effets indésirables et les profils d'interaction médicamenteuse semblent bien dans des limites acceptables. Ainsi, le développement d'antifongiques efficaces et bien tolérés semble nécessaire. En plus, au cours de ces dernières années, plusieurs études ont rapporté l'activité antifongique des produits naturels et c'est dans ce contexte que nous avons évalué in vitro l'activité anti-dermatophytique de la squalamine qui est un aminostérol naturel, possédant de nombreuses propriétés thérapeutiques, notamment anti-angiogéniques et antimicrobiennes. Nous avons démontré que la squalamine ainsi qu'un dérivé aminostéroïdien possédaient une remarquable activité in vitro contre 36 isolats cliniques de dermatophytes appartenant à 8 espèces, avec des concentrations minimales inhibitrices (CMI) variant de 4 à 16 mg/l et de 2 à 8 mg/l, respectivement pour la squalamine et le dérivé aminostéroïdien (**Article 4**). Par la suite, la squalamine ayant montré in vitro une activité antifongique significative contre des isolats cliniques de dermatophytes, nous avons évalué in vivo la tolérance et l'efficacité de cette molécule dans le traitement local de la teigne du cuir chevelu chez les élèves d'une communauté semi-urbaine de Bamako dans un essai clinique randomisé de phase II contrôlé par placebo. La squalamine a montré une bonne tolérance, ainsi qu'une efficacité clinique partielle, caractérisée par un meilleur score de repousse des cheveux chez les enfants traités (**Article 5**). Ma participation s'est matérialisée par l'identification des différents isolats cliniques, les tests de sensibilité in vitro des souches cliniques aux antifongiques ; vérification et nettoyage de la base des données saisies au DEAP ; analyses statistiques; et rédaction du premier jet des manuscrits.

Article 4: *In vitro* activity of aminosterols against dermatophytes

Oumar Coulibaly, Kamel Alhanout, Coralie L'Ollivier, Jean-Michel Brunel, Mahamadou A Théra, Abdoulaye A. Djimdé, Ogobara K. Doumbo, Renaud Piarroux, Stéphane Ranque*

Med Mycol. 2013 Apr; 51(3):309-12. doi: 10.3109/13693786.2012.724773. Epub 2012 Sep 24.

*Corresponding author

Dr Stéphane Ranque

Laboratoire de Parasitologie Mycologie

CHU Timone

264 rue Saint Pierre

13385 Marseille CEDEX 5

Tél. +33491386090

Fax. +33491384957

Email: stephane.ranque@ap-hm.fr

Medical Mycology April 2013, **51**, 309–312

informa
healthcare

In vitro activity of aminosterols against dermatophytes

OUMAR COULIBALY*†, KAMEL ALHANOUT‡, CORALIE L'OLLIVIER*, JEAN-MICHEL BRUNEL‡, MAHAMADOU A THERA†, ABDOULAYE A DJIMDÉ†, OGOBARA K. DOUMBO†, RENAUD PIARROUX* & STÉPHANE RANQUE*

Laboratoire de Parasitologie-Mycologie, Aix-Marseille Université, AP-HM, CHU Timone, Marseille CEDEX 05, France, †Département d'Epidémiologie des Affections Parasitaires, Malaria Research and Training Center, Faculté de Médecine, Université de Bamako, Mali, Western Africa, and ‡URMITE UMR 6236, CNRS-IRD, Faculté de Médecine et de Pharmacie, Marseille, France

We recently reported that aminosterols are fungicidal due to their disrupting the outer membranes of yeasts and that they have a significant *in vitro* activity against various mould species. Yet, their activity against dermatophytes had never been tested. This study's objective was to evaluate the *in vitro* activity of squalamine and a synthetic aminosterol derivative (ASD) against various dermatophytes. Susceptibility testing of squalamine, ASD, terbinafine, and griseofulvin was performed, in triplicate, in accord with the Clinical Laboratory and Standards Institute's M38-A2 procedure, using an 80% growth inhibition endpoint. The studies included the following dermatophytes: *Trichophyton rubrum, T. mentagrophytes, T. soudanense, Microsporum canis, M. audouinii, M. persicolor; M. cookie* and *M. gypseum*. Squalamine and ASD showed significant *in vitro* activity against these dermatophytes. The minimum inhibitory concentrations (MICs) ranged from 4–16 mg/l and from 2–8 mg/l for squalamine and ASD, respectively. These findings support further clinical studies of aminosterols activity against superficial dermatophyte infections.

Keywords Squalamine, aminosterol synthetic derivate, antifungals, terbinafine, griseofulvin, CMI

Introduction

Dermatophytes are a group of closely related fungi capable of invading keratinized tissues such as hair, skin and nails, to cause infections referred to as dermatophytoses. Epidemiological studies show that this pathology is among the most prevalent in the world and is considered the second most common skin disease in the adult population. Although many antifungal agents have been developed during the last decades and have become available to treat dermatophytosis, they are members of a relatively limited number of chemical groups. In addition, dermatophytosis treatment failures have been associated with drug-related adverse events or resistance of the etiologic agents [1]. Thus, the development of effective antifungals, which should be both highly active and safe, is needed.

In recent years, several reports have described the antifungal activity of natural products [2]. Squalamine is a natural aminosterol, first isolated from the tissues of the dogfish shark *Squalus acanthias*, which possesses numerous therapeutic properties such as antiangiogenics and antimicrobial [3]. A phase II study evaluating the antiangiogenic effect of squalamine demonstrated that this aminosterol compound could be safely administered to cancer patients in a continuous five-day infusion of 300 mg/m^2/day [4]. The synthesis of squalamine is complex and costly, while many squalamine-related compounds, namely aminosterol derivatives, have been synthesized from easily available and inexpensive starting materials, some of which also have demonstrated and interesting antimicrobial activities [5,6]. Aminosterols possess antibacterial properties, as determined with various clinical isolates of multidrug-resistant bacteria [7]. We recently

Received 16 April 2012; Received in final revised form 18 July 2012; Accepted 23 August 2012

Correspondence: Stéphane Ranque, Laboratoire de Parasitologie-Mycologie AP-HM Timone, F-13385 Marseille Cedex 5, France. Tel.: +33 491 386090; Fax: +33 491 384958; E-mail: stephane.ranque@ap-hm.fr

DOI: 10.3109/13693786.2012.724773

reported that aminosterols are fungicidal through their disruption of the outer membrane of yeasts [8], as well as having a significant *in vitro* activity against several moulds isolated from cystic fibrosis patients [9]. However, the question of whether aminosterol derivatives have the potential to be developed as clinically useful anti-dermatophyte agents remains to be addressed. Hence, this study aimed to test the *in vitro* activity of squalamine and a synthetic aminosterol derivative (ASD) against clinical dermatophytes isolates.

Materials and methods

Squalamine was a generous gift from Pr. M. Zasloff (Georgetown University, Washington, USA). Aminosterol derivative (ASD), namely 7β-(1,4-Diaminobutane)-3β-hydroxycholestane, was synthesized as previously reported [8–10]. The antifungal agents tested were terbinafine and griseofulvin (Sigma-Aldrich, France). Stock solutions of squalamine, terbinafine, griseofulvin, and ASD were prepared in methanol.

The dermatophytes isolates were recovered from *tinea capitis* samples referred to the Parasitology-Mycology Laboratory at the University Hospital of Marseille and from samples collected from school children in Bandiagara, a rural town of Mali, West Africa [11]. The dermatophytes were identified using both macroscopic and microscopic morphological characteristics and the sequence analysis of the rRNA genes' ITS regions [12]. *Candida albicans* ATCC 90028 was used for quality control.

Standard antifungal susceptibility testing was performed in accord with the Clinical and Laboratory Standards Institute's (CLSI) document M38-A2 [13], using RPMI 1640 medium (Sigma-Aldrich) with L-glutamine, without sodium bicarbonate, buffered at pH 7.0 with 0.165 mol/l morpholinepropanesulfonic acid (Sigma-Aldrich). Seven to 14 days-old dermatophyte PDA cultures grown at 27°C were used for inocula preparation. After scraping each plate, the suspension of conidia and hyphal fragments in sterile 0.9% saline was adjusted to 1 McFarland and further diluted to 1/10th with RPMI 1640 medium to obtain the final test inocula [13]. The following drugs – squalamine, aminosterol derivative (ASD), terbinafine and griseofulvin – were dissolved in 100% dimethyl sulfoxide, followed by further dilutions in RPMI 1640 medium to yield twice the final concentration required for the assay. Aliquots of 100 µl of two-fold drug dilutions were transferred to the U-shaped wells of a sterile, 96 round-bottomed microplate by means of a multichannel pipette. Each well was then inoculated with 100 µl of the diluted inocula suspension to achieve drug dilutions ranging from 0.25–32 mg/l. Growth and sterility controls were included for each assay. The plates were incubated at 27°C and read visually after 4–7 days, depending on the fungal growth observed in the drug free control wells. The minimum inhibitory concentration (MIC) endpoint was defined as the lowest concentration that inhibited 80% of fungal growth relative to the growth control [14]. All experiments were performed in triplicate.

Results

Thirty-six clinical isolates of eight dermatophytes species (three – *Trichophyton mentagrophytes*, 14 – *T. soudanense*, three – *M. canis*, nine – *M. audouinii*, one –*M. persicolor*, one – *M. cookei*, two – *M. gypseum* and three – *T. rubrum*) were evaluated. The MICs of squalamine and ASD were relatively similar but did differ slightly depending on the dermatophyte species. Table 1 shows that MICs of squalamine and ASD against tested isolates ranged from 4–16 mg/l and from 2–8 mg/l, respectively. MICs of griseofulvin ranged from 1–4 mg/l and those of terbinafine were < 0.25 mg/l.

Discussion

This study demonstrated for the first time that squalamine and ASD have remarkable *in vitro* activity against the main medical important dermatophyte species. Their *in vitro* activity was reproducible within one dilution and similar against all isolates. Unsurprisingly, terbinafine was found to be the most effective in these *in vitro* antifungal studies as has been reported previously [15–17].

Recently, some authors have described that antifungals such as terbinafine and itraconazole hold great promise for the treatment of dermatophytosis. However, these agents have drug-drug interactions and monitoring of liver enzymes is required during the protracted treatment of hair and nails infections. Therefore, new antifungal agents with distinctly different mechanisms of action could be useful alternatives if they are safe to use and are of lower cost. Aminosterols compounds are known to possess antibacterial activity [7]. Recently, we demonstrated their *in vitro* fungicidal effect against yeasts and moulds [6,8,9]. In the present study we found that both squalamine and ASD had potent *in vitro* antifungal activity against clinical dermatophyte isolates. In line with previous yeasts and moulds *in vitro* susceptibility testing findings, the MICs of ASD were repeatedly about one concentration lower than those of squalamine (Table 1). Squalamine and ASD had higher MICs compared with the classical anti-dermatophyte drugs, e.g., terbinafine and griseofulvin. ASD MICs were about one dilution higher than those of griseofulvin and terbinafine MICs were always below 0.25 mg/l. Among all dermatophyte species tested, the single *Microsporum persicolor* isolate

Table 1 Minimum inhibitory concentration values of squalamine; an aminosterol synthetic derivative (ASD); terbinafine; and griseofulvin against 36 clinical isolates of eight distinct dermatophytes species and a *Candida albicans* quality control strain. MIC50 and MIC90 were calculated by grouping the isolates of the two genera: *Trichophyton* and *Microsporum.*

Dermatophyte species (number of strains tested)	MICs values (mg/l)			
	Squalamine	ASD	Terbinafine	Griseofulvin
Trichophyton mentagrophytes ($n = 3$)	8	2–4	<0.25	4
Trichophyton rubrum ($n = 3$)	16	8	<0.25	2
Trichophyton soudanense ($n = 14$)	8	2–8	<0.25	1–2
Trichophyton spp. ($n = 20$)				
MIC_{50}	8	2	<0.25	1.5
MIC_{90}	16	8	<0.25	4
Microsporum canis ($n = 3$)	8–16	4–8	<0.25	1–2
Microsporum audouinii ($n = 9$)	8–16	4	<0.25	2
Microsporum gypseum ($n = 2$)	8–16	4	<0.25	1
Microsporum cookei ($n = 1$)	8	4	<0.25	2
Microsporum persicolor ($n = 1$)	4	4	<0.25	1
Microsporum spp. ($n = 16$)				
MIC_{50}	8	4	<0.25	2
MIC_{90}	16	4	<0.25	2
Candida albicans ATCC 90028	8–16	8	>32	>32

was particularly susceptible to aminosterols compounds. Noticeably, the MICs of squalamine against the 14 *Trichophyton soudanense* isolates were very homogenous.

This study's findings are in line with those of our previous study on the antifungal activity of aminosterols compounds against various clinical yeasts and moulds species with MICs ranging from 8–16 mg/l and 2–4 mg/l for squalamine and ASD, respectively [8,9]. We previously showed that these agents were active against fungi resistant to polyene and/or azole antifungals, which suggests that aminosterols have a different mechanism of action [8], i.e., probably acting by disrupting the membranes of Gram-negative bacteria, yeast, and filamentous fungi in a detergent-like non-specific manner [7,8,18].

Concerning the topical use of aminosterol derivatives we have recently demonstrated the efficiency of a squalamine for *Staphylococcus aureus* skin decolonization in a mouse model [19]. It is noteworthy that no significant differences were noticed in terms of the appearance of treated or untreated skin patches, and no lesions or inflammation were encountered after the application of aminosterol derivative ointments. Moreover, squalamine has reached Phase III trials for the treatment of age-related macular degeneration and prostate cancer without any major side-effects, as it appears to be well tolerated even at doses 250 mg/day in adults, suggesting that an ointment for local use could be totally safe. Furthermore, an ointment will consist in a 1% squalamine-formulated petrolatum-based cream as typically used in topical applications which will exceed 1000 times the *in vitro* MIC values suggesting a good efficiency for a topical use.

In conclusion, our findings indicate that aminosterols compounds have an interesting *in vitro* activity against all clinical dermatophyte isolates tested. Further studies are needed to characterize the antifungal effects of aminosterols in dermatophytes. Their relatively higher MICs than those recorded with available systemic anti-dermatophyte agents advocate using the local route in further clinical studies of these aminosterols against dermatophytosis.

Acknowledgments

We thank the DEAP/MRTC staff for their help in collecting the isolates from Bandiagara, Mali. We thank JP Bouchara and N Contet-Audonneau for graciously providing dermatophyte strains. No specific funding was received for this study.

Declaration of interest: The authors report no conflicts of interest. The authors alone are responsible for the content and the writing of the paper.

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This paper was first published online on Early Online on 24 September 2012.

Article 5: A double-blind randomized placebo-controlled clinical trial of squalamine ointment for tinea capitis treatment

Coulibaly Oumar, Thera Mahamadou A, Koné Abdoulaye K, Goïta Siaka, Traoré Pierre, Djimdé Abdoulaye A, Brunel Jean-Michel, Gaudart Jean, Piarroux Renaud, Doumbo Ogobara K, Ranque Stéphane*

[#]**Corresponding author**
Dr Stéphane Ranque
Laboratoire de Parasitologie Mycologie
CHU Timone
264 rue Saint Pierre
13385 Marseille CEDEX 5
Tél. +33491386090
Fax. +33491384957
Email: stephane.ranque@ap-hm.fr

Submitted to Mycopathologia

A double-blind randomized placebo-controlled clinical trial of squalamine ointment for *tinea capitis* treatment

Running title: Clinical trial of Squalamine against *tinea capitis*

Coulibaly Oumar[1,2], Thera Mahamadou A[2], Koné Abdoulaye K[2], Goïta Siaka[2], Traoré Pierre[3], Djimdé Abdoulaye A[2], Brunel Jean-Michel[4,5], Gaudart Jean[6], Piarroux Renaud[1,7], Doumbo Ogobara K[2], Ranque Stéphane[1,7].

5- Aix-Marseille Université, IP-TPT UMR MD3, 13885 Marseille, France.
6- DEAP/MRTC, Faculté de Médecine, Université de Bamako, Mali.
7- Department of Dermatology, CNAM Ex Institut Marchoux. PO Box 251 Faculty of Medicine, University of Bamako, Mali.
8- Institut Paoli-Calmettes, Centre de Recherche en Cancérologie de Marseille (CRCM), CNRS, UMR7258, Marseille, France.
9- Aix-Marseille Université, UM 105 ; Inserm, U1068, F-13009, Marseille, France.
10- Aix-Marseille Université, UMR912 (SESSTIM), INSERM / AMU / IRD, Marseille, France.
11- APHM, CHU Timone-Adultes, Parasitology & Mycology, 13005 Marseille, France.

[#]**Corresponding author**
Dr Stéphane Ranque
Laboratoire de Parasitologie Mycologie
CHU Timone
264 rue Saint Pierre
13385 Marseille CEDEX 5
Tél. +33491386090
Fax. +33491384957
Email: stephane.ranque@ap-hm.fr

Keywords: tinea capitis; squalamine; aminosterols; placebo-controlled clinical trial; Phase II clinical trial; topical treatment; dermatophytes; dermatophytoses;

Summary

Background. Novel treatments against for tinea capitis are needed and the natural aminosterol Squalamine is a potential topical antidermatophyte drug candidate.

Objectives. This phase II randomized double-blind placebo-controlled clinical trial aimed at testing the efficacy and safety of a three-week squalamine ointment regimen for the treatment of *tinea capitis*.

Patients Male aged 6-15 years presenting with *tinea capitis* were treated with either topical squalamine ointment or placebo for three weeks. The primary endpoint was complete clinical cure. The secondary endpoints were the occurrence of: local and/or systemic adverse events; mycological cure; partial clinical response. Prospective follow-up of clinical adverse events was performed daily.

Results. Five patients were treated with 1% squalamine ointment and 15 with placebo. No complete cure was observed. No clinical or biological adverse event was recorded. A significantly (p=0.03) better hair-growth score, indicating a partial clinical improvement of the *tinea capitis* lesion, was observed in the patients treated with squalamine compared to those treated with placebo.

Conclusions. This three-week squalamine ointment regimen was well tolerated and showed an encouraging partial clinical activity for the treatment of *tinea capitis*. Further studies are needed to evaluate the efficacy of topical squalamine alone against *tinea corporis* or in combination with a systemic antidermatophyte drug against *tinea capitis*.

Trial registration: www.pactr.org PACTR201306000522355

Tinea capitis is a common fungal infection of the scalp, hair follicles, and hair shafts [1]. Although it can affect any age group, it is particularly common in school-aged children. It is almost exclusively a disease of childhood, and current evidence suggests that it occurs more often in children of African or Caribbean extraction [2]. In the United States, tinea capitis is the most common dermatophyte infection of childhood, with 90% of infections caused by the anthropophilic fungi *Trichophyton tonsurans* [3, 4]. A recent survey carried in Mali, a country in western Africa, found a high prevalence rate of 55% of tinea capitis in children attending at primary school in a suburbs of Bamako (the capital city of Mali), with a predominance of anthropophilic dermatophytes [5].

Treatment of tinea capitis is inherently difficult and protracted. Current treatment options included oral and topical drugs with oral therapies providing better outcomes. Griseofulvin remains the only licensed treatment for children in the UK. Nonetheless, many dermatology units in the UK use terbinafine as first line treatment because it is highly effective, well tolerated, and adherence to treatment is high [2]. However, in developing countries, the cost of these treatments remains very high. In this context, developing an effective and affordable topical therapeutic seems necessary. In recent years, several reports have described the antifungal activity of natural products [6]; among them squalamine was a good drug candidate [7, 8]. Squalamine is a natural aminosterol, first isolated from the tissues of the dogfish shark *Squalus acanthias*, which possesses numerous therapeutic values including antiangiogenic properties [9]. The aim of this study was to evaluate the efficacy of topical administration of squalamine compared with placebo in the treatment of tinea capitis in children. Secondary objectives were to assess the safety, and to estimate the relative efficacy of squalamine against placebo.

Patients and Methods

Study design

This was a randomized double-blind placebo-controlled clinical trial conducted in primary school in Sirakoro-Meguetana, a semi-urban community located at about 4 km of Bamako. The trial was registered under the PACTR201306000522355 identification number in the Pan African Clinical Trial Registry (www.pactr.org). The trial included an approximate 2-week screening period and 3-week treatment period. Patients were randomized to receive one of two possible treatment: squalamine (petroleum jelly with 1% squalamine ointment); or placebo (petroleum jelly ointment). Treatment evaluated and placebo were applied once daily on the lesions for three weeks. The investigator, wearing gloves, first roughly cleaned the scalp and then applied the ointment using his finger. Patients were followed-up until four weeks after the first dose. Patients were to receive their medication once a day, preferably in the morning. A thin layer of ointment (either squalamine or placebo) was applied topically by the investigator.

Study patients

The study protocol was approved by the Ethical Committee of Faculty of Medicine and Dentistry of the University of Sciences Techniques and Technologies of Bamako (N° 10-82/FMPOS), and carried out in accordance with the Good Clinical Practice standards. Written informed consent was obtained from each patient and their parents before enrolment. Eligible patients aged 6-15 years had a clinical mycological diagnosis of tinea capitis. Patients underwent complete clinical examination, collection of material from lesions of tinea capitis for mycological examination, and blood samples for biochemical and hematological tests.

Inclusion criteria were: male children, aged from 6 to 15 years, attending the primary school of Sirakoro-Meguetana, with both clinical and mycological diagnosis of tinea capitis, normal liver and renal function tests, and no history of allergy.

Non-inclusion criteria were: children with dermatophyte infection of the skin and/or who received systemic antifungal therapy within three months before inclusion or topical antifungal therapy within one month before inclusion. Patients were required to shave their heads at day-0. Because shaving of head is usual practice for boys but not for girls in this population, girls were not included to avoid stigmatization.

Randomization and blinding

The blinding and randomization process was as follows: JMB added 1% squalamine to 5 out of 20 randomly selected tubes containing 20 g of petrolatum-based ointment. The tubes were anonymized and identified with a unique number from 1 to 20. Only JMB knew the randomization key; he was neither involved in the drug administration nor the clinical monitoring of patients. Twenty patients received daily topical application (either squalamine or placebo) during three weeks. The treatment evaluated and placebo had the same visual appearance. Neither the clinician in charge of the patient's treatment follow-up, and nor the patient knew the exact nature of the administered treatment. The unblinding occurred after the "freezing" of the database before the statistical analysis.

Patient's follow-up

At day-0, the patients were clinically examined by qualified physicians, and dermatophytes lesions were characterized. Blood samples were collected to evaluate kidney and liver functions, and blood cell count. Wood's light examination was performed in a darkened room to detect ectothrix hair invasion caused by *Microsporum* spp. Samples were collected from dermatophytoses lesions. Tinea capitis lesions were collected by scraping with a sterile curette and a sterile compress. In areas of alopecia, scales and broken hair were collected and used for direct examination. A second sample collected directly with the sterile compress was kept at room temperature in sealed plastic bags and then transported to the Parasitology-Mycology

Laboratory at the University Hospital of Marseille where samples were inoculated onto Sabouraud Cycloheximide Agar medium (bioMérieux, France). Cultures were maintained for 6 weeks for positive culture identification. Positive cultures were examined macroscopically and microscopically for species identification on the basis of cultural characteristics, pigment production, and microscopic examination in lactophenol cotton blue preparation. Treatment was started after shaving of the patients' skull and was administered once daily. Clinical efficacy and adverse events were also monitored daily.

At day-7, samples were collected from tinea capitis for mycological examination. Blood sample was collected for laboratory tests. Finally, administration of treatment was continued in the absence of cure or major adverse effects.

At day-21, the samples were collected from lesions of tinea capitis. Blood samples were also collected to monitor kidney and liver functions and blood cell counts. Treatment was ended. The patients with residual tinea capitis were then treated with 20mg/kg/d oral griseofulvin for six to eight weeks.

Efficacy evaluations

At each study visit, signs and symptoms of tinea capitis were assessed and samples of tinea capitis lesions were collected by the investigator for mycological analysis, as detailed above. The primary efficacy end-point was a complete clinical cure at week 3. The first secondary efficacy end-point was mycological cure at week 3. Complete cure was defined as negative mycology (negative culture) and clinical cure (no clinical symptoms of tinea capitis, and normal hair growth). Mycological cure was defined as negative culture. The second secondary efficacy end-point was partial clinical response, which was quantified using a hair-growth score and the diameter of lesions. The diameter of lesions was measured using a sliding caliper gauge. Hair

growth was assessed clinically using a score of 0 (no growth) to 5 (uniform growth) in comparison with healthy areas of the patient's scalp.

Safety evaluations

Adverse events were assessed by the investigators at each visit. Safety variables assessed included vital signs measurements, physical examination and laboratory test results. There was a particular focus on aspartate aminotransferase (AST), alanine amino-transferase (ALT), alkaline phosphatase and total bilirubin, and hematological parameters (white blood cells count, packed cell volume and platelet counts).

Susceptibility testing

In vitro susceptibility testing of squalamine against the patients' isolates was performed as described in [6] using the M38-A2 micro-broth dilution protocol of the Clinical and Laboratory Standards Institute.

Statistical analysis

Patients were randomized in a 1:3 ratio in the group treated with squalamine or in the placebo group. To test the difference between 2% cure rate in placebo group and 80% cure rate in the squalamine group, with a 5% type I error and 90% power, number needed to treat was 20 patients: five in the squalamine group and 15 in the placebo group. All data were collected onto case report forms that were kept confidential and checked daily by a study supervisor. Data were entered into a MS ACCESS^{TM} database and checked by the supervisor. The data were presented as mean (± standard deviation) for quantitative variables and percentages for qualitative variables. The primary endpoint was complete cure. The secondary endpoints were: safety, mycological cure (negative mycology), and partial clinical response (hair-growth score, and/or reduction of the diameters of lesion). The proportions of patients with an endpoint in either squalamine or placebo treatment groups were compared using the Fisher's exact test. The

same analysis was performed considering each lesion of tinea capitis, using the generalized estimating equations option to account for the non-independence of multiple tinea capitis lesions in a given patient. Statistical analyses were performed using SAS, version 9.2 (SAS Institute, Cary, NC). All tests were two-sided with a $p<0.05$ significance level.

Results

The study was conducted in May 2011. Twenty male patients aged 6 to 15 years were included. Five were treated with topical squalamine and 15 with placebo. The data at days-0, -7, and -21 of both squalamine and placebo groups are tabulated in Table 1. There were no lost to follow-up. The patients' baseline characteristics were homogenous among treatment groups (Table 1). At day-0 there were 10 and 27 lesions of tinea capitis, mean number of lesions per child 2 ± 1.2 and 1.8 ± 0.8 ($p> 0.05$), in the squalamine and placebo groups, respectively.

Microsporum audouinii was the predominant pathogen in this study. The MIC of terbinafine were <0.25 mg/l in all 21 isolates. All patients in the squalamine group were infected by *Microsporum audouinii*. The means of MICs of squalamine were 11.2 ±4.4 and 12.4 ±4.2 ($p=0.38$) for *Microsporum audouinii* isolated in patients in the squalamine and the placebo groups, respectively.

At day-7 (Table 1**)**, mean diameters of lesions were 4.9 ±4 and 4 ±2.7 ($p=0.78$) in the squalamine and placebo groups, respectively. The hair growth scores in each group did not differ (p=1.00). Neither complete nor mycological cure occurred in any patient in this study.

At day-21 (Table 1**)**, means diameters of lesions were 3.9 ±3.2 *vs*. 4 ±2.5 ($p=0.92$) in the squalamine and placebo groups, respectively. As illustrated in Figure 1, the mean hair growth score was significantly higher (2.1 ±8.9 *vs*. 1.1 ±0.6, $p=0.039$) in the squalamine group than in the placebo group. A score >1 for hair growth occurred in 70% (95% CI [0.35 - 0.93]) *vs*. 18.5% (95% CI [0.06 - 0.38], p=0.005) in lesions of tinea capitis in the squalamine and placebo groups,

respectively. Complete cure was not recorded. However, a negative mycological culture of four tinea capitis lesions was observed in two (10%) patients in the placebo group (p=1.00). With respect to safety, no clinical or biological adverse event was prospectively recorded during the entire study.

Discussion

In this study, although no complete tinea capitis cure occurred in the patients who were treated with topical squalamine, a statistically significantly higher hair-growth score within the tinea capitis lesions, indicative of a partial clinical response, was observed in the patients treated with squalamine. In fact, the currently available topical treatment are effective against tinea capitis only when combined with a systemic antidermatophyte drug, most probably because the dermatophytes are located within the hair [2]. Although systemic therapy, such as oral griseofulvin, effectively penetrates the infected hair shaft, topical shampoos, including ketoconazole and selenium sulfide, are recommended as adjunctive therapeutic options and to prevent spread of infection [10, 11, 12]. This partial efficacy of topical squalamine against dermatophytes was in keeping with recent experimental data on the use of topical squalamine treatment for *Staphylococcus aureus* skin decolonization in mice [13]. In this study, a negative dermatophyte culture occurred in two patients with stable clinical lesions in the placebo group. The most probable explanation is a false negative culture results due to relatively low sensitivity of culture for the diagnosis of dermatophytosis [14], mainly because of the difficulties in obtaining good quality samples for dermatophyte culture.

However, this study showed a favorable safety profile of squalamine when administered topically as no clinical or biological adverse event was recorded in the treated patients. Yet, this finding should be interpreted taking into account the small number of patients treated. The favorable safety index of topical squalamine was in line with the recent findings of the absence

of genotoxicity and its low cytotoxic activity against CHO cells suggesting a low cytogenetic impact on eukaryotic cells [15]. A phase II study demonstrated that this aminosterol compound could be safely administered to cancer patients in a five-day continuous infusion regimen [16]. Moreover, squalamine well tolerated without any major side-effects at doses up to 250 mg/day in adults included in phase III trials for the treatment of age-related macular degeneration, and prostate cancer [13]. Although no pharmacokinetic data are available, it is likely that squalamine plasma levels when administered topically are much lower than those obtained when administered intravenously; thus suggesting that a squalamine ointment could be safe for topical use. Aminosterols also possess interesting *in vitro* antibacterial and antifungal activities; especially squalamine minimum inhibitory concentrations (MICs) ranged from 4 to 16 mg/l against various clinical dermatophytes species [7, 8]. Thus using a 1% squalamine-formulated petrolatum-based cream ointment, as in the present study, the local squalamine concentration would exceed more than 1000 times the dermatophyte MIC, suggesting a good microbiological efficiency for a topical use [13]. With respect to the *in vitro* activity of aminosterols against dermatophytes, we recently showed that another aminosterol derivative had lower MICs than squalamine [7] and might have an enhanced activity against dermatophytes.

There were three major limitation of this phase II clinical trial. First, the children who were randomized in the squalamine group were infected by the single *Microsporum audouinii* species. Thus, this study's findings can only cautiously be extrapolated to tinea capitis caused by other dermatophytes; although *in vitro Trichophyton soudanense* was at least as susceptible to squalamine as *Microsporum audouinii*. Second, squalamine ointment was administered once in a day, which could have been insufficient, regarding the inefficacy of the available antidermatophyte topical treatments alone against treat tinea capitis to date [2]. Currently, the recommended therapy for tinea capitis in children is a 6- to 8-week course of oral griseofulvin [17]. This study's objective to cure 80% of the children in three weeks can retrospectively be

regarded as too optimistic and even unrealistic. Nonetheless, considering the randomized double-blind design, the partial clinical response evidenced in this study should be considered as a very encouraging result. In 2007, a Cochrane review of systemic antifungal treatment for tinea capitis in children concluded that newer treatments, including terbinafine, have equivalent efficacy and safety profiles to griseofulvin and allow shorter treatment [2]. Lipozencic *et al* [18], in a randomized trial, double-blind, carried in children with tinea capitis due to *Microsporum* spp, concluded that terbinafine therapy for 6 weeks could represent an alternative to griseofulvin for the treatment of *Microsporum* tinea capitis. The third limitation is that the dose of squalamine applied daily on the lesions was not quantified. A number of antifungal drugs with proven efficacy are available for the treatment of tinea capitis. However, varying dosage schedules, changes in epidemiology, and increasing drug resistance are factors that might hamper treatment's efficacy [19]. The partial clinical response found in this phase II study suggests that topical squalamine would be active against tinea corporis, which is usually treated with topical antifungals alone, or against tinea capitis in combination with a systemic antifungal. In our setting, systemic antidermatophyte agent such griseofulvin should be preferred because of its relatively lower cost and the high prevalence of *Microsporum sp*. in this population.

Conclusion

This phase II study evidenced the safety of topical squalamine treatment and a partial clinical efficacy of a three-week regimen for the treatment of tinea capitis lesions. This encouraging result supports further studies, which should aim at evaluating the efficacy of various doses of squalamine ointments as monotherapy against tinea corporis or in combination with a systemic antifungal like griseofulvin against *tinea capitis*. In both situations, squalamine has the potential to shorten dermatophytosis treatment duration with subsequent improvement of compliance to treatment schedules and costs reduction.

Acknowledgements

We would like to thank the school principal Jean-André Douyon for his help.

Conflict of interest

None to declare

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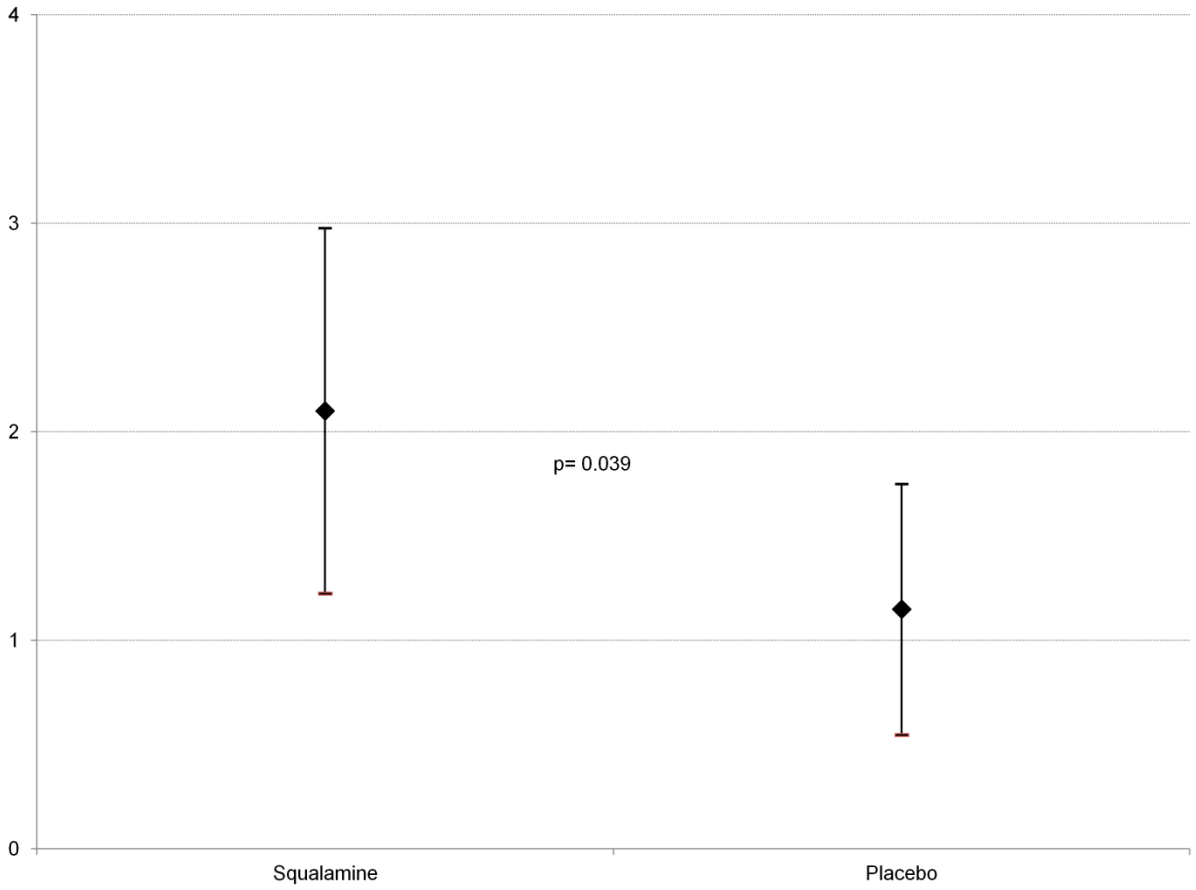

Figure 1: Mean (±SD) hair growth score in the tinea capitis lesions treated for three weeks with squalamine ointment (n=10) or placebo (n=27).

Conclusion

Les dermatophytoses sont des mycoses fréquentes en Afrique. Il semble exister une variabilité inter et intra-régionale considérable de l'incidence des dermatophytoses sur le continent Africain. La distribution des agents étiologiques est hétérogène et varie d'un pays à un autre, et d'une sous-région à une autre. En outre, les formes cliniques prédominantes varient selon la localisation géographique. Deux espèces anthropophiles, incluant *T. soudanense* et *M. audouinii*, prédominent en Afrique de l'Ouest et en Afrique Centrale. La plus forte incidence de *T. violaceum* et *T. rubrum* est observée en Afrique du Nord, ainsi que l'émergence de l'espèce zoophile *M. canis*. En Afrique de l'Est et en Afrique du Sud, *T. violaceum* est prédominant. Au Mali, deux espèces anthropophiles, *T. soudanense* et *M. audouinii*, sont les principaux agents étiologiques isolés à partir des teignes du cuir chevelu chez les élèves de trois zones bioclimatiques différentes. L'étude épidémiologique que nous avons menée a montré une prévalence significativement plus élevée des teignes du cuir chevelu dans le Sud du pays où le type éco-climatique est Soudano-guinéen et humide que dans la région Sahélienne et sèche, alors que la prévalence était intermédiaire dans la zone Soudanienne. Cette corrélation entre la prévalence des teignes du cuir chevelu et l'humidité suggère clairement un effet des facteurs climatiques sur l'épidémiologie des dermatophytes. Sur le plan thérapeutique, nous avons objectivé une activité antifongique significative in vitro de la squalamine et d'un autre dérivé aminostéroïdien contre les isolats cliniques de dermatophytes. Dans un essai clinique de phase II, la squalamine a montré une bonne tolérance et une efficacité clinique partielle dans le traitement topique des teignes du cuir chevelu. Ces résultats nous encouragent certes à évaluer cette molécule en traitement topique seul et/ou en combinaison avec un traitement systémique dans les teignes du cuir chevelu. En parallèle au traitement de ces infections, il est nécessaire de promouvoir l'information de la population et l'implémentation de mesures prophylactiques.

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ANNEXES

Annexe 1: Identification par Spectrométrie de Masse MALDI-TOF des champignons du complexe *Pseudallescheria/Scedosporium* spp.

Nous avons démontré dans cette étude, la faisabilité de l'identification des champignons du complexe *Pseudallescheria/Scedosporium* spp. regroupant des champignons filamenteux, Ascomycètes, appartenant à la famille des Microascaceae, saprophytes, ubiquitaires, cosmopolites, et opportunistes. Dans la perspective d'utiliser la spectrométrie de masse MALDI-TOF dans le diagnostic d'espèce de ces champignons, nous avons testé l'influence de plusieurs conditions de culture. Nous avons montré que les profils protéiques des différentes espèces testées étaient stables dans le temps et que leur identification n'était pas influencée ni par le milieu de culture et ni par la technique d'extraction protéique. Nous avons obtenu par cette procédure 68% d'identification correcte d'isolats cliniques alors que la bibliothèque des spectres était incomplète.

Medical Mycology August 2011, **49**, 621–626

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Pseudallescheria/Scedosporium complex species identification by matrix-assisted laser desorption ionization time-of-flight mass spectrometry

OUMAR COULIBALY*, CARINE MARINACH-PATRICE†, CAROLE CASSAGNE*, RENAUD PIARROUX*, DOMINIQUE MAZIER† & STEPHANE RANQUE*

**Laboratoire de Parasitologie-Mycologie, CHU Timone, Université de la Méditerranée, Marseille, and †Immunité & Infection, INSERM/UPMC UMRS 945, CHU Pitié-Salpêtrière, Paris, France*

Because timely and accurate identification of members of the *Pseudallescheria/Scedosporium* species complex (PSC) is clinically relevant, the objective of this investigation was to study the stability and influence of the main variable factors in the routine clinical laboratory to the potential use the Matrix-Assisted Laser Desorption Ionization-Time-Of-Flight (MALDI-TOF MS) in the identification of these fungi. Twenty-two PSC reference strains, three clinical isolates, an αHCCA matrix, and an Autoflex I spectrometer with BioTyper software (Bruker) were employed in this study. Intra-and inter-specimen composite correlation indices for each MS spectrum as compared to a reference spectrum were computed. MS identification was stable after the fungi were subcultured over a 1-month period. While neither culture medium (Sabouraud vs. Malt extract) nor protein extraction methods (formic acid vs. trifluoroacetic acid) significantly influenced the quality of the MS identifications, they were considerably increased from day 3 to day 6 of incubation. MALDI-TOF MS can be used in the routine clinical laboratory in the identification of members of the complex provided that valid spectra libraries are developed. Although preliminary results are encouraging, further studies are warranted to demonstrate whether MS can distinguish the species that have recently been described using multilocus sequence analysis within *P. boydii sl.* and to validate its use in the routine clinical laboratory for identifying clinically relevant moulds.

Keywords MALDI-TOF Mass spectrometry, moulds, *Scedosporium, Pseudallescheria*, fungi identification

Introduction

Moulds of the *Pseudallescheria/Scedosporium* complex (PSC) are involved in a wide spectrum of diseases called pseudallescheriosis or scedosporiosis [1]. In immunocompetent hosts they cause white-grain mycetoma and lung infections in near-drowning syndromes [2,3]. In addition they can colonize the airways of patients with underlying lung disease, notably in 0.7–9% of cystic fibrosis patients [4]. Furthermore, they can cause life-threatening disseminated infections, chiefly among immunocompromised patients, such as solid organ or bone-marrow transplant recipients, and patients with hematological malignancies or AIDS [5–8].

Members of the PSC used to be divided into two species, i.e., *Pseudallescheria boydii* (anamorph, *Scedosporium apiospermum*) and *Scedosporium prolificans*. However, *P. boydii* was recently divided on the basis of multilocus sequence analysis [6], into six distinct species, i.e., *P. boydii s.l.*, *P. africana*, *P. minutispora*, *S. prolificans*, *S. dehoogii*, and *S. aurantiacum*. Each of these new species has now been associated with distinct clinical manifestations, prognosis, and antifungal drug susceptibility profiles. In Australia, for instance, *S. prolificans* was more

Received 27 September 2010; Received in final revised form 27 December 2010; Accepted 13 January 2011

Correspondence: Stéphane Ranque, Laboratoire de Parasitologie-Mycologie, AP-HM Timone, F-13385 Marseille Cedex 5, France. Tel: +33 491 38 60 90; fax: +33 491 38 49 58. E-mail: stephane.ranque@ap-hm.fr

DOI: 10.3109/13693786.2011.555424

frequently involved in invasive infections than *P. boydii* or *S. aurantiacum* and was the only species recovered in culture from blood, whereas *S. apiospermum* and *S. aurantiacum* were more common in respiratory specimens [2]. *S. prolificans* also has the particularity of being naturally resistant to all available systemic antifungal agents [9]. Therefore, it is important that these species be rapidly and accurately identified to improve patient management. However, such identification remains a challenge in the clinical laboratory setting as it requires morphologic studies of isolates recovered in culture which could lead to erroneous results [10]. Molecular identification by multilocus sequence analysis also allows accurate species identification [6], but the several-day turnaround time required is detrimental to patient care.

Recently, Matrix-Assisted Laser Desorption Ionization-Time-Of-Flight (MALDI-TOF) Mass Spectrometry (MS) has emerged for microorganism identification. MS provides protein spectra from crude extracts or intact cells and as each species has a characteristic spectrum, it can be identified within minutes by comparison of its spectrum to that in a reference spectra library. Simplicity and rapid turnaround time are the main advantages of MALDI-TOF MS over current microorganism identification methods. MS has already been successfully used for bacteria [11,12] and more recently for yeast identification [13–18]. Its practicability in daily clinical laboratory routine has been demonstrated [19]. Yet, data remain scarce on the use of this novel technology for mould identification and in particular it has never been used to identify members of the PSC. Previous attempts at the characterization and identification of some moulds, including *Penicillium*, *Aspergillus*, *Fusarium*, *Trichoderma* and dermatophytes, were encouraging [13, 20–22] but there remain major issues relative to the lack of standardized protocol and validated commercial reference spectra library. This study addresses the feasibility of identifying members of the PSC by MALDI-TOF MS after the development of standardized operating procedures (the choice of culture media, incubation time and protein extraction method) and validated reference spectra of these moulds. As the first step, the influence of growth and sample treatment conditions on spectra stability was tested. In a second step, a reference spectra library was built and used for tentative identification of three PSC clinical isolates.

Materials and methods

Pseudallescheria/Scedosporium strains

The 22 PSC strains (Table 1) used in this study were maintained in the BCCM/IHEM Biomedical Fungi and Yeasts Collection (Brussels, Belgium) collection and kindly provided by the *Pseudallescheria/Scedosporium* working group of the International Society for Human and Animal Mycology (ISHAM). Two type strains (IHEM 4429, *Pseudallescheria boydii*, type strain of *Petriellidium angustum*, and IHEM 21148, type strain of *Pseudallescheria minutispora*) were used to optimize the experimental conditions, following which the 22 reference strains (Table 1) were used to build a reference spectra library.

Experimental flow chart

As the first step in our investigation we studied the effect of conditions that usually vary in the routine clinical laboratory setting to ultimately determine optimal technical conditions for MALDI-TOF MS moulds identification (Fig. 1). In the second step, clinical isolates were tentatively identified using the MALDI-TOF MS assay.

Any microorganism species identification assay based on spectra comparisons with those in a MSP reference library aims to achieve both the highest intra-specific and the lowest inter-specific correlations. The BioTyper 1.1 (Bruker Daltonics, Bremen Germany) software calculates Composite Correlation Indices (CCI) between the spectra

Table 1 CBS and IHEM collection numbers of reference strains, kindly provided by the *Pseudallescheria/Scedosporium* working group of the International Society of Human and Animal Mycoses. (T: type strain).

IHEM no.	CBS no.	Species
4429	254.72	*Pseudallescheria boydii* (T of *Petriellidium angustum*)
14076	116906	*Scedosporium prolificans*
14263	116898	*Pseudallescheria boydii*
14268	116899	*Pseudallescheria boydii*
15933	101.22	*Pseudallescheria boydii* (T of *Allescheria boydii*)
21147	116910	*Scedosporium aurantiacum* (T)
21148	116911	*Pseudallescheria minutispora* (T)
21159	116892	*Pseudallescheria boydii*
21162	695.70	*Pseudallescheria apiospermum/Scedosporium apiospermum* (T of *Acremonium suis*)
21163	116779	*Pseudallescheria apiospermum/Scedosporium apiospermum*
21165	108.54	*Pseudallescheria boydii*
21166	116894	*Pseudallescheria minutispora*
21167	116403	*Pseudallescheria boydii*
21168	116895	*Pseudallescheria boydii*
21170	116897	*Pseudallescheria boydii*
21172	116900	*Scedosporium prolificans*
21174	116902	*Scedosporium prolificans*
22912	116896	*Pseudallescheria apiospermum/Scedosporium apiospermum*
22913	117407	*Pseudallescheria apiospermum/Scedosporium apiospermum* (T)
22915	106.53	*Pseudallescheria boydii* (T of *Petriellidium fusoideum*)
22919	418.73	*Pseudallescheria boydii* (T of *Petriellidium ellipsoideum*)
22923	117406	*Scedosporium dehoogii* (T)

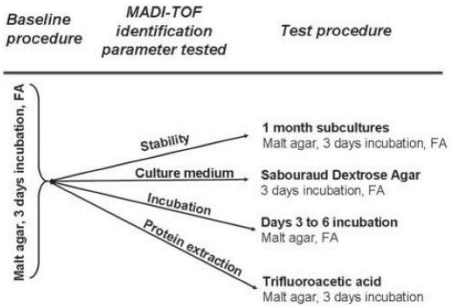

Fig. 1 An Experimental flow chart showing the stability and effects of culture media, incubation time, and protein extraction (FA: Formic acid, or trifluoroacetic acid) on MALDI-TOF Mass Spectrometry's identification quality. Each experiment was performed in quadruplicate on the *Pseudallescheria boydii* (IHEM 4429) and *Pseudallescheria. minutispora* (IHEM 21148) type strains.

obtained from the tested samples and reference spectra obtained under identical conditions. Intra-strain correlation was between one strain spectrum and its own reference spectrum, while the inter-strain correlation was between one strain spectrum and the reference spectra of the other strains. We considered the optimal condition to be one that both maximized intra-strain correlations and minimized inter-strain correlations.

MALDI-TOF MS analysis

MS analysis was performed using a MALDI-TOF Autoflex I (Bruker Daltonics, Bremen Germany) mass spectrometer with an α-cyano-hydroxy-cinnaminic acid matrix.

The effects of culture media, incubation time, and protein extraction on MALDI-TOF MS's identification quality were evaluated with the two type strains (IHEM 4429 *P. boydii* and IHEM 21148 *P. minutispora*). Unless otherwise stated, malt agar cultures were incubated at 27°C for 72 h, with each experiment performed in quadruplicate.

To test the culture media effect, strains were grown on two classical mycological culture media, i.e., malt extract agar (AES, France) and Sabouraud glucose agar with chloramphenicol and gentamicin (AES, France). Proteins were extracted with formic acid (FA) and subjected to MALDI-TOF analysis [14].

To evaluate the duration of incubation, two samples were taken daily from day 3 to day 6 from each plate, extracted with FA, and subjected to MALDI-TOF analysis. The spectra were compared with reference spectra obtained after three days of incubation.

To study the protein extraction protocol, four samples were taken from each plate and extracted with either FA or trifluoroacetic acid (TFA). Each spectrum was compared with reference spectra obtained with an identical protein extraction protocol.

To investigate the stability of the spectra, the fungi on each plate was subcultured every three days for 1 month, at the end of which two samples were taken from each plate, extracted with FA and subjected to MALDI-TOF MS analysis. Each spectrum was compared with reference spectra obtained at day 3 of incubation.

Statistical analysis

The influence of each tested variable on performance of MALDI-TOF MS identification was evaluated by comparing the intra- and inter-strain spectra correlation. The CCIs' distributions were non-Gaussian, hence data were presented as median and inter-quartile range, and non-parametric statistical tests were used. Univariate analysis was based on the Kruskal-Wallis H test. Multivariate analysis was performed by Friedman's Q test to adjust on the effects of strains and intra- or inter-strain comparisons. All statistical tests were two-sided with a $P < 0.05$ significance threshold. Analysis was performed using SAS Stat 9.1.3 (SAS Institute Inc., Cary, NC).

MALDI-TOF MS identification of clinical strains

For the second step tentative identification of the clinical strains using the MALDI-TOF MS assay, we incorporated the spectra of all 21 reference strains in the reference spectra library (Table 1). Duplicate analyses of 10 spectra from five distinct experiments for each strain were used to build the reference main spectra (MSP) library as previously described [22]. The following three fungi which were morphologically identified as PSC members in the Mycology Laboratory of the University Hospital of Marseille (France) were analyzed; 877899-374F08 recovered from respiratory samples in a 19-year-old man with cystic fibrosis, 984729-604F09 isolated from a 73-year-old woman with bronchiectasis, and 979215-369F09 found in specimens from a 65-year-old man with septic arthritis of the elbow. These isolates and the reference strains were subjected to both rRNA gene ITS region sequence analysis [23] and to MALDI-TOF MS analysis. A neighbor-joining distance tree based on the ITS sequences was computed using the MEGA4 software [24]. MALDI-TOF MS analysis was performed as previously described on samples taken after 72 h incubation at 27°C on Sabouraud dextrose agar with antibiotics, and extracted with FA. The clinical isolates MALDI-TOF MS spectra were compared to the reference MSP in the library. Identification quality was measured by a matching score based on identified masses and their intensity correlation that was generated and used for ranking of the results by the BioTyper software (Bruker).

Results

The strains displayed distinct MALDI-TOF mass spectra when cultured on malt extract agar or on Sabouraud Dextrose agar with antibiotics. However, MALDI-TOF MS identification performance was not significantly altered by culture medium. Irrespective of the culture medium, median and inter-quartile ranges of intra-strain correlations were higher than those of inter-strain correlation. The culture medium effect was not significant in univariate ($P = 0.4290$) or in multivariate analyses ($P = 0.4001$).

MALDI-TOF spectra analysis of culture samples collected daily from day 3 to day 6 indicated that the intra-strain correlations increased but the inter-strain correlations decreased with incubation time (Fig. 2), suggesting that identification quality increased with the duration of incubation. Incubation time effect was not significant ($P = 0.2112$) in univariate analysis, but was in multivariate analysis ($P = 0.0040$).

Samples from the same culture displayed distinct MALDI-TOF spectra when proteins were extracted using either TFA or FA. Irrespective of the protein extraction method, median and inter-quartile ranges of intra-strain correlations were higher than those of inter-strain correlations. The protein extraction effect was not significant in univariate ($P = 0.3791$) or multivariate analyses ($P = 0.3945$).

Stability of MALDI-TOF identification was assessed by comparing the spectra obtained before and after ten subcultures over a 1-month period. The time effect was not significant in univariate ($P = 0.737$) or in multivariate analyses ($P = 0.052$), indicating relative stability of the spectra after ten subcultures. However, we did observe a trend toward improved identification after sub-culturing, with both increasing intra-strain and decreasing inter-strain correlations (data not shown).

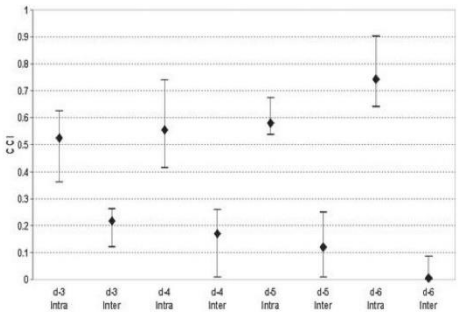

Fig. 2 Median and inter-quartile range of intra-and inter-strain composite correlation indices (CCI) as a function of incubation time from days 3–6. The intra-strain correlation increased while the inter-strain correlation decreased with incubation time, indicating that MALDI-OF MS identification quality improves with incubation time ($P = 0.0040$).

The ITS sequence distance tree (Fig. 3) illustrates that isolates 979215-368F09 and 877899-374F08 clustered within distinct clades of *Pseudallescheria apiospermum* complex reference strains, and isolate 984729-604F09 clustered with the *Scedosporium aurantiacum* type strain (IHEM 21147). The MALDI-TOF MS identification assay results were concordant with ITS sequence analysis. For isolate 979215-369F09, the highest score was 2.295, matching with the reference IHEM 22912 (*P. boydii sl*; for isolate 877899-374F08 the highest score was 2.268, matching with the IHEM 22913 (*P. boydii sl.*); and for isolate 984729-604F the highest score was 1.944, matching with IHEM 21147 (*Scedosporium aurantiacum*).

Discussion

This is the first proof-of-concept validation of a method based on MALDI-TOF MS analysis for the accurate and rapid identification of PSC moulds in the clinical laboratory setting. In agreement with others [22,25] we observed that MALDI-TOF mass spectra differed depending on culture, incubation time, and protein extraction conditions. However, neither culture medium extraction method nor incubation time significantly influenced identification quality, provided that the reference library contains MSP spectra of reference strains processed in identical conditions. In particular, we demonstrated that the performance of MALDI-TOF MS identification was independent from the protein extraction (FA or TFA) method. We thus chose FA, which is less toxic and faster than TFA. Otherwise, our data show that even if the identification quality increases significantly with the culture incubation time, 3-day incubation was enough to obtain MALDI-TOF mass spectra useable for identification purpose. In this study 72 h was the minimal incubation time tested because we needed a sufficient amount of mycelia and conidia to replicate the experiments. Incubation times of less than 72 h could be of interest in clinical practice but were not tested here and whether younger cultures might be used in MS identification assays remains an open question. Our findings also showed that spectra remain stable after 10 subcultures. This is in accordance with Marinach *et al.*, who found that spectra of *Fusarium dimerum* and *Fusarium verticilloides* strains remained similar after 13 subcultures [22]. Consequently, in a given laboratory routine, it would be possible to subculture the samples that are difficult to identify using the classical morphological approach and to send them to a reference laboratory for MALDI-TOF MS identification.

The tentative identification of clinical isolates by MALDI-TOF was concordant with ITS sequence analysis results. The three clinical isolates studied were adequately identified using a spectra library of 21 reference strains. The maximum

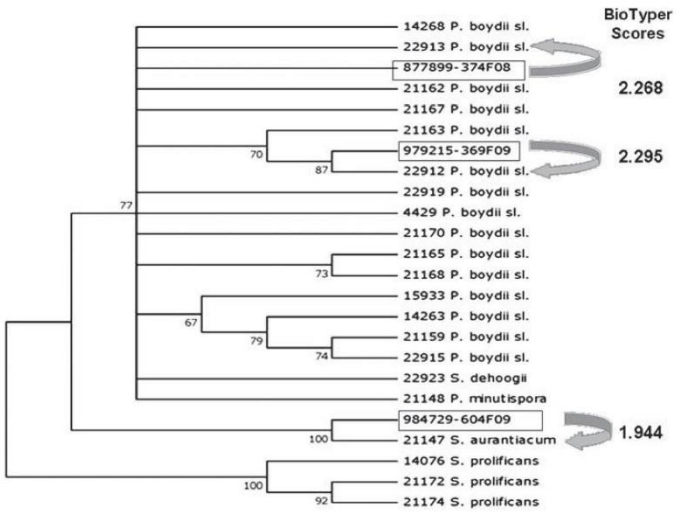

Fig. 3 Neighbor-joining distance tree of the 21 reference strains identified with their IHEM number (see Table 1) and the three clinical isolates described in the results section. Bootstrap values >65% of 500 resampled data sets and the best-match BioTyper score for the identification of the clinical isolates are shown.

matching score, between 1.9 and 2 for the 984729-604F isolate, is usually considered suboptimal for species identification with respect to standards validated for bacteria identification [19]. The matching score threshold for mould identification remains to be defined; and one can expect that MALDI-TOF MS identification of *Scedosporium aurantiacum* specimens will be improved when greater numbers of reference spectra of this species are included in the library. It should be noted that spectra from clinical moulds are poorly represented in commercially available libraries, which is one of the major drawbacks to MALDI-TOF MS identification of moulds in the clinical laboratory setting.

In conclusion, species identification within the PSC appears feasible with MALDI-TOF MS. Identification quality was similar for the different conditions tested. We thus used those that were most workable in our routine procedures, i.e., culture on Sabouraud dextrose agar with antibiotics and FA protein extraction. MS identification quality increased significantly with incubation time, which conflicts with the aim of reducing identification turnaround times to improve patient management. Fortunately, samples from 72 h cultures were adequate for identification purposes. If necessary, subsequent testing after a few additional days of incubation might resolve substandard MS identifications. The adequate identification of a limited number of clinical isolates is encouraging and should be extended to a larger sample. Indeed further studies are warranted to demonstrate whether MS-based identification can distinguish the species that have recently been described using multilocus sequence analysis within *P. boydii sl.* and to validate its use in the routine clinical laboratory.

Acknowledgments

We thank the BCCM/IHEM Biomedical Fungi and Yeasts Collection (Brussels, Belgium) for kindly providing us the set of reference strains of the ECMM/ISHAM Pseudallecheria/Scedosporium Infections working group.

Declaration of interest: The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

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This paper was first published online on Early Online on 04 February 2011.

Annexe 2: A MALDI-TOF MS procedure for clinical dermatophyte species identification in the routine laboratory

Le temps de croissance, parfois long des dermatophytes, et la nécessité d'une identification precise ont conduit à une étude dont le but était d'évaluer l'application de la technique de spectrométrie MALDI-TOF dans l'identification des dermatophytes dans le cadre de la routine de laboratoire. L'étude a montré que la procédure de spectrométrie MALDI-TOF permettait une identification parfaite des isolats cliniques de dermatophytes. Cette technique a été évaluée sur certains isolats cliniques issus des élèves atteints de teigne du cuir chevelu au Mali.

Medical Mycology October 2013, **51**, 713–720

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A MALDI-TOF MS procedure for clinical dermatophyte species identification in the routine laboratory

CORALIE L'OLLIVIER*, CAROLE CASSAGNE*, ANNE-CECILE NORMAND*, JEAN-PHILIPPE BOUCHARA†‡, NELLY CONTET-AUDONNEAU§, MARIJKE HENDRICKX#, PATRICK FOURQUET^, OUMAR COULIBALY*, RENAUD PIARROUX* & STEPHANE RANQUE*

**Laboratoire de Parasitologie-Mycologie, Aix-Marseille Univ., Marseille; AP-HM, CHU Timone, Marseille cedex 05, †Laboratoire de Parasitologie-Mycologie, Institut de Biologie en Santé-PBH, Centre Hospitalier Universitaire, Angers cedex9, ‡Groupe d'Etude des Interactions Hôte-Pathogène, Université d'Angers, L'UNAM Université, Institut de Biologie en Santé-IRIS, Centre Hospitalier Universitaire, Angers cedex9, §Laboratoire de Mycologie, Hôpital de Brabois, CHU de Nancy, rue du Morvan, Vandœuvre-les-Nancy, France, #BCCM/IHEM: Scientific Institute of Public Health, Mycology & Aerobiology Section, Brussels, Belgium, and ^Service Protéomique, Centre d'Immunologie de Marseille Luminy, Marseille, France*

The conventional identification of dermatophytes requires a long turnaround time and highly skilled mycologists. We have recently developed a standardized matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) assay to routinely identify molds of potential clinical significance. This study objective was to determine if this same assay could also be employed to identify clinical dermatophytes in the routine laboratory setting. The effects of the inclusion of cycloheximide in the culture medium and incubation time were tested after building a reference spectra library that included 48 well-characterized isolates of 17 dermatophyte species. Then these same isolates were prospectively identified using this library. MALDI-TOF MS-based identification was effective regardless of the presence of cycloheximide or incubation time as 130/133 (97.8%) of the clinical isolates were appropriately identified. Two *Microsporumcanis* isolates yielded uninformative spectra and one *M. audouinii* isolate was misidentified. Since one only requires a small colony for MALDI-TOF MS analysis, accurate identifications were obtained in 3–6 days and, specifically, before the appearance of their characteristic morphological features. Consequently, identification turnaround time was dramatically reduced as compared to that needed for conventional morphological identification. In conclusion, this standardized MALDI-TOF MS-based identification procedure for filamentous fungi effectively identifies clinical dermatophyte isolates and drastically reduces the response times in the routine clinical laboratory.

Keywords clinical dermatophytes, MALDI-TOF mass spectrometry, standardized procedure, routine, identification

Introduction

Dermatophytoses in humans are chiefly superficial fungal infections restricted to skin, nails and hair. Dermatophytes are distributed worldwide and species vary according to geographic area [1]. According to Emmons [2] and despite recent significant changes in the taxonomy, around 30 clinically important dermatophyte species can be grouped into three anamorphic genera, i.e., *Trichophyton*, *Microsporum* and *Epidermophyton* [3]. However, the

Received 17 July 2012; Received in final form 18 January 2013; Accepted 26 February 2013.
This study was presented in part at the 5th Trends in Medical Mycology (TIMM), 2–5 October 2011 in Valencia, Spain, and at the 18th Congress of the International Society for Human and Animal Mycology (ISHAM), 11–15 June 2012 in Berlin, Germany.
Correspondence: Stéphane Ranque, Laboratoire de Parasitologie-Mycologie AP-HM Timone, F-13385 Marseille Cedex 5, France. Tel: +33 491 386090; Fax: +33 491 384958; E-mail: stephane.ranque@ap-hm.fr

DOI: 10.3109/13693786.2013.781691

taxonomy, identification and nomenclature of the pathogenic species have been amended by the recent phylogenetic species concept, based on DNA sequence analysis [4].

Classically, the major dermatophytes are identified by skilled mycologists on the basis of macroscopic and microscopic of colonies growing in culture. Sometimes, complementary identification techniques, e.g., subculturing on specific culture media (lactrimel, potato-dextrose, malt extract, etc.) or the ability to degrade keratin, are needed to successfully identify the isolate. Since dermatophytes grow slowly, reliable identification may require several weeks. Moreover, conventional identification techniques cannot be employed with some isolates which may not produce characteristic morphological features in culture. Most of the clinical dermatophytes can otherwise be identified to the species level by using either DNA sequencing [5,6] or specific PCR assays [5,7]. However, DNA sequence-based identification, the recommended gold standard, is relatively costly and time-consuming in the routine clinical laboratory setting. Thus matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) for rapid, simple, reproducible and reliable identifications has raised considerable interest in the clinical microbiology community. This assay generates spectra, comparable to protein fingerprint signatures of microorganisms which can then be identified within minutes by comparing their spectra with those in a reference spectra database. For the past few years, MALDI-TOF MS has enhanced routine bacterial identification in the clinical microbiology laboratory [8–14]. More recently, MALDI-TOF MS has been applied to the routine identification of yeasts [15] and molds [1,10]. We have recently developed a standardized MS-based assay suited to identifying a comprehensive array of molds species in the routine clinical laboratory setting [16] but without addressing dermatophyte identification. The present study thus aimed to evaluate this standardized MALDI-TOF MS-based identification assay for the identification of dermatophytes in the routine clinical laboratory setting.

Materials and methods

Dermatophytes reference spectra library

A reference spectra (MSP) library was built that included 48 isolates of 17 dermatophyte species, as detailed in Table 1. All were identified using both conventional and molecular procedures, as described below. This library included *Trichophyton mentagrophytes* complex members with *T. mentagrophytes*, *T. interdigitale*, *T. erinacei* and *T. simii* and *Arthroderma otae*-related species including *Microsporum canis*, *M. audouinii* and *M. langeronii*. Two non-pathogenic dermatophytes, *T. terrestre* and *T. ajelloi*, were also included in the investigation.

Table 1 Characteristics of the dermatophyte strains included in the reference spectra library.

Species	*N*	Strain no.	GenBank ref. with ≥99% identity
Epidermophyton flocossum	2	50600264	
		59700858	
Microsporum audouinii	8	xTA	AJ252334.1
		1043189	AJ252334.1
		013M	AJ252334.1
		001M	AJ252334.1
		027M	FJ479802.1
		171M	AJ252334.1
		50500597	
		50600031	
M. audouinii var. *langeronii*	2	50002007	
		59700523	
Microsporum canis	3	1100085	
		50500921	
		249D	AY213709.1
Microsporum cookei	2	IHEM 14180	
		94.548	
Microsporum gypseum	4	250D	EU151494.1
		50500961	
		93.629	
		94.446	
Microsporum nanum	1	IHEM 21111	
Microsporum persicolor	2	92.136	
		59900598	
Onychocola canadensis	1	161D	AY123784.2
Trichophyton mentagrophytes	5	1036900	AB566295.1
		1039316	HQ014710.1
		1036279	AB566295.1
		1042599	AB566299.1
		IHEM 4270	
T. mentagrophytes var. *ajelloi*	1	IHEM 17776	
T. mentagrophytes var. *erinacei*	2	IHEM 15931	
		IHEM 20118	
T. mentagrophytes var. *interdigitale*	1	IHEM 620	
Trichophyton rubrum	3	1043024	FM178326.1
		235D	AJ270806.1
		1041710	FM178326.1
Trichophyton schoenleinii	1	1100084	
Trichophyton simii	1	IHEM 15735	
Trichophyton soudanense	5	243D	FJ409221.1
		1100082	
		110 31 66 05	
		110 32 11 05	
		115 33 73 57	
Trichophyton terrestre	1	LMA 92 347	
Trichophyton tonsurans	2	90D	AB220045.1
		1100081	
Trichophyton verrucosum	1	IHEM 5480	
Trichophyton violaceum	1	IHEM 13459	

Clinical dermatophyte isolates

From November 2009 to December 2011, a total of 133 isolates were recovered from nail, hair or skin samples of individual patients in the medical mycology laboratories of university hospitals in three French cities (Angers, Marseille and Nancy; Table 2). They were identified to the

Table 2 Identification of 134 clinical dermatophyte isolates. (NB. *Onychocola canadensis* is a non-dermatophyte fungus implicated in nail infections).

Species	Number of clinical isolates tested	Number of strains in the library	Number of corrrect MALDI-TOF MS identifications	
Epidermophyton flocossum	1	2	1	
Microsporum audouinii	32	8	31 (97%)	
M. audouinii var. *langeronii*	2	2	2	
Microsporum canis	6	3	4 (67%)	
Microsporum cookei	0	2	-	
Microsporum gypseum		3	4	3
Microsporum nanum	0	1	-	
Microsporum persicolor	3	2	3	
Onychocola canadensis	1	1	1	
Trichophyton mentagrophytes	21	4	21	
T. mentagrophytes var. *erinacei*		0	2	-
T. mentagrophytes var. *porcellae*		0	1	-
Trichophyton rubrum	52	3	52	
Trichophyton schoenlenii	0	1		
Trichophyton soudanense	12	5	12	
Trichophyton terrestre	0	1	-	
Trichophyton tonsurans	2	2	2	
Trichophyton verrucosum		0	1	-
Total	134	45	129	

species level using both conventional methods and MALDI-TOF MS.

Growth conditions

The fungi were cultivated at 30°C for three or 15 days on both Sabouraud gentamicin-chloramphenicol (SGC; Bio-Rad, France) and Sabouraud gentamicin-chloramphenicol with cycloheximide (SGCc; Bio-Rad) agar plates. This allowed testing the effects of culture conditions, e.g., culture medium, incubation time on MALDI-TOF identification results.

Conventional morphological identification

All fungi were evaluated both macroscopically, in terms of colony growth and pigment production and microscopically to detect the presence and morphology of macro- and/or microconidia and any other key characteristics following the keys of the *Atlas of Clinical Fungi* [17].

DNA sequence-based identification

Fungal DNA was isolated with NucliSENS EasyMAG (bioMérieux, France) according to the manufacturer's recommendations which yielded approximately 1 µg of high molecular weight DNA. Internal transcribed spacer (ITS) 2 region of the rDNA were amplified using ITS3 (gCATC gATgAAgAACgCAgC) and ITS4 (TCCTCCgCTTATT gATATgCTTAAgT) primers [18–20] in PCR reactions of 50 µl containing 10 ng template DNA, 20.0 pmol of each primer, 0.2 mM dNTPs, 2.5 mM MgCl2, 10 mM Tris-HCl, pH 8.3, 50 mM KCl and 1 U Taq polymerase (Applied Biosystems, France). Fragments were amplified in a T1 thermocycler (Biometra, Göttingen, Germany) with the following reaction profile: 10 min 94°C, then 40 cycles of: 30 s, 94°C; 30 s, 55°C; and 60 s, 72°C. The resulting amplicons were purified with UltraClean GelSpin DNA purification kit® (MO BIO laboratories, Carlsbad, CA, USA) and sequenced with Big Dye Terminator v1.1 Cycle Sequencing kit (Applied Biosystems, France) in a 3130 Genetic analyser (Applied Biosystems, France). Homology searching with the ITS region sequences was performed against sequences registered in GenBank/NCBI using BLAST (http://blast.ncbi.nlm.nih.gov/Blast.cgi).

Protein extraction and target plate preparation

Dermatophyte proteins were extracted using the procedure previously described for molds [16]. Briefly, a piece of mycelium was gently scraped from the culture plate with a scalpel and suspended in 900 µl absolute ethanol (ethyl alcohol anhydrous, Carlo Erba SDS, Val de Reuil, France) and 300 µl HPLC water (Water HPLC, Prolabo BDH, Fontenay-sous-Bois, France). The sample was centrifuged at 13,000 *g* for 10 min, with the resulting pellet resuspended in 12.5 µl of 70% formic acid (Sigma-Aldrich, Lyon, France) and the solution then incubated for 5 min at room temperature. Subsequently, 12.5 µl of 100% acetonitrile (Prolabo BDH, Fontenay-sous-Bois, France) was added during 10 min at room temperature and the sample then centrifuged at 13,000 *g* for 10 min. One µl of supernatant

was spotted onto a MTP 384 target plate polished steel TF (Bruker Daltonics GmbH, Bremen, Germany) and allowed to air dry. Then, the spot was covered with 1 µl matrix solution [alpha-cyano-4-hydroxycinnamic acid (Sigma-Aldrich, Lyon, France) saturated in 50 acetonitrile: 25 HPLC water: 25 10% TFA and was allowed to air dry. A bacterial test standard (Bruker Daltonics) was used for instrument calibration.

Mass spectra data acquisition

The MALDI-TOF MS assays were performed on an UltraFlex (Bruker Daltonics) mass spectrometer, according to the manufacturer's instructions, and analyzed using the MaldiBioTyper v2.1 software (Bruker Daltonics). The spectra were acquired after 650 shots in linear mode in the ion-positive mode with a 337 nm nitrogen laser, using the following adjustments: delay time: 170 ns; acceleration voltage: 20 kV; ion source 2 voltage: 18.5 kV; mass spectral range: 2–20 kDa, acquisition time: 30–60 s per laser pulse. The TOF measurements were converted to m/z values and all raw spectra were automatically processed by the Flexcontrol v2.4 (Bruker Daltonics) AutoXecute software. The resulting peak lists were exported to the MaldiBioTyper v2.1 software (Bruker Daltonics) but only peaks with a signal/noise ratio ≥ 10 were considered.

Reference spectra (MSP) library creation

Reference spectra were created from fungal isolate cultured at 30°C for three days using the procedure described above. The library included four MSPs (each of them derived from 10 raw spectra of four culture replicate using the 'MSP creation' function of the MaldiBiotyper software) of each of 48 strains (Table 1) grown under two conditions, i.e., SGC/3-days and SGCc/3-days. To assess the validity of this library, the 48 test isolates were sub-cultured again, with four spots prepared for each isolate and submitted for MS identification using this 48 reference spectra library. Results of the matching process were expressed as LogScore (LS) values, computed by the MaldiBiotyper software, ranging from 0 (no spectrum match) to 3.0 (perfect match). Two LS values, indicative of identification strength, were recorded for each spot which included the best-match LS value for the 1st identified species and the best-match LS value for the 2nd identified species. A cut-off value for reliable species identification was defined taking into account both LS values.

MALDI-TOF MS-based identification of clinical isolates

In parallel to the conventional identification, clinical isolates grown on SGC or SGCc agar were analyzed in quadruplicate (four spots each) by MS. An LS value was obtained by comparing the unknown fungus spectra with the reference spectra in the MS library using the 'start identification' function of the MaldiBiotyper software. MS identification was considered concordant (or informative) when at least three out of the four spots matched with the reference spectra of the same species and at least one of these spots yielded a best-matched LS value above the defined cut-off. MALDI-TOF MS-based identification results were considered adequate when they agreed with those of morphological identification. When the initial LS value was below the cut-off, the isolate was re-extracted 2–4 days later and reanalyzed using an identical procedure.

Statistical analysis

Data were expressed as mean ($\pm$ SD) LS; or proportion of correct identifications. Effect of the culture medium was analyzed by comparing the LS values obtained from dermatophytes grown on both SGC and SGCc culture media to a library of reference spectra obtained on SGC medium. Incubation time effect was analyzed by comparing the LS values obtained for 3–6 or 15-day-old cultures to a library of reference spectra obtained from 3-day-old cultures. Generalized estimating equations were used to account for the non-independence of spectra from the same isolate. All statistical analyses were performed with the SAS 9.2 (Cary, NC, USA) statistical software. All statistical tests were two-sided and $P < 0.05$ was considered significant.

Results

Reference spectra library validation

Spectra of the 48 strains (Table 1) grown on both SGC and SGCc agar plates for three days were included in the library resulting in 96 reference spectra. The validity of this library was controlled using the raw MALDI-TOF spectrum of a subculture of each of these 48 strains on both culture media. The use of 380/384 spots tested (eight spots per strain; four spots per each of the two media) led to exact identification at the species level with high best-match LS (mean LS = 2.33 ± 0.32 and 2.15 ± 0.34 from SGC and SGCc plates, respectively). In contrast, the second best-match LS score corresponding to a misidentification (i.e., the best-match LS score with a reference spectrum from a distinct species) was much lower (mean LS = 1.37 ± 0.26 or 1.40 ± 0.22 from SGC or SGCc plates, respectively). Four of the eight spots for *T. violaceum* IHEM 13459 strain were misidentified with a high best-match LS (2.27 and 2.23) with the *T. violaceum* reference strain being found to be *T. soudanense*. Therefore, as

plotted in Figure 1, the distributions of exact and false best-match LS values were distinct and allowed us to choose a LS cut-off ≥1.9 for species identification in subsequent experiments. It is noteworthy to indicate that the LS scores obtained by comparing the spectra of all the *T. mentagrophytes* complex's strains were very similar.

Culture medium effect

When using a reference library of spectra obtained from isolates grown only on SCG culture it should be noted that the identification of the isolates grown on SGCc was significantly better ($P = 0.0036$). However, the magnitude of this discrepancy was of minimal biological significance as the mean LS were 2.25 (± 0.32) and 2.32 (± 0.33) for SGC and SGCc, respectively. As a LogScore (LS) >2 indicates a very good match between the analyzed spectrum and the reference spectrum in the library, these findings indicate that the presence of cycloheximide in the culture medium only marginally altered MS-based identification results for the evaluated species. Noticeably, we found that the spectra of fungi grown with or without cycloheximide were visually similar.

Incubation-time effect

The effect of incubation-time on MS-based identification results was tested on 15 isolates of seven species: *M. audouinii* (n = 3); *M. canis* (n = 1); *T. mentagrophytes* (n = 2); *T. rubrum* (n= 2); *T. soudanense* (n = 5); *M. gypseum* (n = 1) and *T. tonsurans* (n= 1). The mass spectra obtained from three- or 15-day-old cultures on SGC medium were visually very similar. When using a reference library of spectra obtained after three days of incubation, identification of isolates was significantly better ($P = 0.0119$) than those obtained for 15-day-old cultures. However, the magnitude of this discrepancy was of minimal biological significance as the mean (± SD) LS were 2.30 (± 0.32) and 2.09 (± 0.27) for three- or 15-day-old cultures, respectively, which suggests that MS-based identification was effective, irrespective of incubation time. More importantly, because a small colony was enough for MALDI-TOF MS analysis, dermatophytes isolates could be identified after only three days of culture and, specifically, before the appearance of their characteristic morphological features.

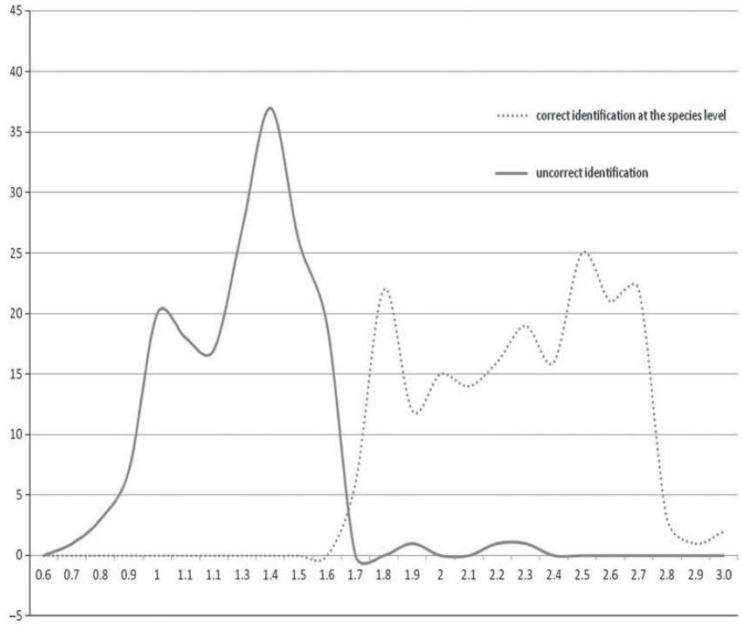

Fig. 1 Distribution of the best-match LogScore (LS) values issued from the MALDI-TOF MS-based identification of the 48 reference library strains. The dotted line plots the best-match LS values of concordant spots whereas the solid gray line plots those of the discordant spots. Concordant and discordant spots best-match LS value distributions were almost distinct and allowed us to choose an LS cut-off ≥1.7 for species identification.

MALDI-TOF MS identification of sequential clinical isolates

From the 133 clinical isolates collected, the spectra generated for 130 (97.76%) allowed for their identification at the species level with a sufficient LS score (Table 2). The failures of MALDI-TOF MS identification corresponding to three isolates are detailed in Table 3. The LS-score was below the 1.7 threshold in two that were identified, by both conventional and DNA sequence-based identifications, as *Microsporum audouinii*. The last isolate was falsely identified as *Microsporum audouinii* (with a mean best-match LS of four concordant spots = 1.757 ± 0.08) whereas phenotypic and DNA sequence-based identification yielded *Microsporum canis*. Although MS-based species identification was accurate in 130 isolates, it could not discriminate varieties among species. Indeed, *Trichophyton mentagrophytes* complex members also matched together especially *T. mentagrophytes* with *T. interdigitale* and *T. simii*.

Discussion

The present study demonstrates that the same standardized MALDI-TOF procedure that has been used for mold identification in the routine clinical laboratory setting [16], is also effective in the identification of clinical dermatophyte isolates. Thus, the two reference libraries validated in Marseille's University Hospital mycology laboratory can be combined into a unique comprehensive library suitable to identify clinical filamentous fungi, including dermatophytes. The major benefits of the use of MS-based identification in the routine clinical laboratory are that it allows an accurate recognition to the species level, without the need of a skilled mycologist and with a dramatic reduction of turnaround times. MALDI-TOF is available in a growing number of microbiology laboratories, and starting from pure cultures, MS-based identification procedure is straightforward, faster and less expensive than DNA-sequencing [8,15].

The first investigations of the identification of clinical dermatophytes using MALDI-TOF MS demonstrated that *T. rubrum*, *T. interdigitale*, *T. tonsurans* and *Arthroderma benhamiae* could be appropriately recognized after one month of culture [21]. Then, Theel *et al*. [22] prospectively identified clinical dermatophyte isolates to the species level with a sensitivity of 59.6% using the commercial standard Bruker library (MBL), supplemented with additional dermatophyte spectra obtained from clinical isolates and 20.5% using the MBL alone. The comprehensive reference spectra library used in the present study included 384 spectra of 48 strains of 17 dermatophytes species. It allowed identifying 97.8% of studied clinical isolates belonging to nine distinct dermatophyte species. Species identification by MALDI-TOF MS is largely limited by the number of spectra in the reference library. In the present library, dermatophytes species commonly isolated in the clinical laboratory, e.g., *T. rubrum*, *M. audouinii* and *T. mentagrophytes* have more reference spectra than those isolates infrequently seen in the clinical setting. Theoretically, a higher number of spectra entries per species would encompass more intraspecies diversity occurring due to variable protein expression, growth conditions, or age of culture [8]. In this way, we chose to build the reference spectra library by including multiple references derived from four subculture replicate under two culture conditions. In the study of Theel *et al*., the reference supplemented library was limited in replicate number of spectra per strains and per species, and some important factor, as the age of the culture used by the manufacturer to build the original MBL are not mentioned. The better percentage identification elicited with our study is likely due to a number of factors. As mentioned above, the added dermatophyte spectra per strains likely enhanced the species-specific diversity covered by the library. Surprisingly, neither culture medium nor incubation time, both factors that are known to alter fungal protein profiles, significantly impacted the dermatophyte MALDI-TOF MS identification.

Table 3 Details of the best-match and second-match identifications and LogScore (LS) values (one for each of the four spot tested) of the three MALDI-TOF MS-based identification failures.

Isolate	Best-match LS	Species	Second match LS	Species
Microsporum audouinii	1.45	*Microsporum canis*	0.976	*Trichophyton rubrum*
	1.07	*Microsporum canis*	1.048	*Trichophyton rubrum*
	1.37	*Microsporum canis*	0.871	*Trichophyton rubrum*
	0.94	*Microsporum audouinii*	0.915	*Trichophyton rubrum*
Microsporum canis	1.727	*Microsporum audouinii*	1.609	*Microsporum canis*
	1.817	*Microsporum audouinii*	1.444	*Microsporum canis*
	1.662	*Microsporum audouinii*	1.607	*Microsporum canis*
	1.823	*Microsporum audouinii*	1.533	*Microsporum canis*
Microsporum canis	1.590	*Microsporum nanum*	1.13	*Microsporum audouinii*
	1.599	*Microsporum audouinii*	1.416	*Trichophyton mentagrophytes*
	1.017	*Microsporum audouinii*	0.905	*Trichophyton mentagrophytes*
	1.343	*Trichophyton mentagrophytes*	1.293	*Microsporum canis*

Reference spectra included in our library were usually processed after three days of culture and for some strains after three and 15 days. In our study, clinical strains were processed when an adequate colony size, corresponding to a young culture, was present to sample for MALDI-TOF. This may explain the high level of identification obtained with our library as the protein spectra of clinical samples may be significantly identical to those found in the library.

To note that among the clinical isolates, 21 were not correctly identified by a first MS-based identification assay, but only after re-extraction and reprocessing the MS-based identification assay. This limitation has previously been described [22] by Nenoff *et al*. [23] who constructed a mass spectra database with 285 isolates of which 164 of the species identification were confirmed by sequence analysis. The study showed that conventional identification and DNA-based identification matched the results of MALDI-TOF MS for 78.2% and 99.3%, respectively, of the 285 isolates tested. *T. violaceum* could not be identified by the MALDI-TOF MS technique as during the validation step of our reference database library, this dermatophyte was misidentified as *T. soudanense* in about half (4/8) of the tests. Yet, *T. violaceum* and *T. soudanense* are very close phylogentically and whether they are distinct species is disputed [4]. The findings from two recent studies, using various MALDI-TOF MS systems, indicated that the procedure is a fast and very specific method for species differentiation of dermatophytes grown in culture [23,24].

In this study, we could not differentiate species within the *T. mentagrophytes* complex. Since the complex includes both anthrophilic and zoophilic specie, it is important to have a reliable method of identifying the human-pathogenic species. The taxonomy of this complex was largely debated [27–29] because the species members were phylogenetically closely related together. The taxonomy was based among other factors on the dichotomy between zoophilic and anthropophilic species indicating a preference for a particular host. However, this feature is not convincing as it does not provide for sufficient differentiation as does habitats. It is known that *T. simii* was closely related to *T. mentagrophytes* [4] and MALDI TOF results yielded similar LS values for both species.

Preliminary data indicate that this limitation of MALDI-TOF-based dermatophyte identification can be addressed by increasing the number of reference strains of these species in the library. It is now well known that the mass spectra are influenced by culture conditions, protein extractions procedures, the type of the matrix and the spectrometer used [25,26]. It was thus very encouraging that MALDI-TOF MS-based dermatophyte identification performed well with colonies grown on both SGC and SGCc agar as they are the most widely employed for recovery of dermatophytes in the clinical setting. Interestingly, dermatophyte identification from selective SGCc agar plates was efficient when molds were concomitantly present in the clinical sample. More importantly, dermatophyte species identification was obtained within 3–6 days turnaround time with MALDI-TOF MS, which was considerably faster than the conventional morphological identification that may take up to two or three weeks, in particular when complementary techniques requiring a subculture are used. The MALDI-TOF MS identification method was easily applicable to a routine laboratory because regents' costs and hands-on time are considerably lower than with the conventional method. At this time, the major limitation of this method was the architecture of the references spectra database. Identification results are improved when the spectra included in the reference database and those obtained from the sample to be identified are processed with the same culture and extraction protocol.

In conclusion, MALDI-TOF MS-based identification proved efficient and dramatically reduced response time for identification of clinical dermatophytes. This standardized procedure is suited to the first-line identification of filamentous fungi, including dermatophytes. Ongoing work aims to enhance MALDI-TOF MS-based identification of filamentous fungi by increasing the number of quality-controlled reference spectra in the library.

Declaration of interest: The authors report no conflicts of interest. The authors alone are responsible for the content and the writing of the paper.

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This paper was first published online on Early Online on 18 April 2013.