

Frequency of superficial fungal infections in patients attending the Department of Parasitology-Myiology and Tropical Medicine at the University of Health Sciences, Owendo (Gabon).

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ABSTRACT

Superficial mycoses are widespread skin and integument infections in sub-Saharan Africa, including Gabon. The purpose of this prospective study was to determine the prevalence of fungal agents diagnosed in superficial skin infections among patients attending the Department of Parasitology-Myiology and Tropical Medicine at the University of Health Sciences in Owendo (Gabon). The search for fungal agents was performed on all parts of the body, from the scalp to the sole of the foot. Samples were taken by squama scratching and nail cutting. Fungal agents were diagnosed by direct examination or by culture in appropriate media. Of a total of 141 patients received in consultation, most were female (52%). The mean age of patients was 36.7 years, and the most represented age group was 20-30 years with 28%. Fungal agents were found on the scalp (19.1%), inguinal fold (15.6%) and in the plantar interdigital space (14.9%). The most common clinical signs in patients were pruritus (61.0%) and desquamation (29.8%). Yeasts (59.6%), *Malassezia* sp (22.7%) and *Candida albicans* (14.2%) were the most common fungal group and species identified in patients. Onychomycosis and intertrigo of large folds were the most frequent skin disorders found in patients (21.3% each) and were mainly due to yeasts (Onychomycosis 50.0%, intertrigo 66.7%). Measures to prevent fungal contamination should be implemented to control onychomycosis (aeration of the feet), intertrigo of small folds (reduce the wearing of tight clothing) and yeasts (moderate use of facial oils).



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1. Introduction

Superficial mycoses are infections of the skin and integument due to microscopic fungi [1]. These infections remain at health concern because of the unsightly physical sequelae and pain they may cause [2], [3]. They are cosmopolitan, especially in tropical, subtropical and equatorial regions because of the hot and humid climate which favors the multiplication of micromycetes [4].

Fungal infections are also favored by many other factors including friction between folds, maceration, prolonged wear of socks and closed shoes and tight clothing [3], [5]. In recent years, many fungal agents have emerged with varying prevalence. The global prevalence of superficial mycosis is estimated at 25% [6- 8]. In an earlier study in Senegal, fungal agents accounted for 34.2% of superficial fungal infections [9] while 66.0% of onychomycosis were due to fungal infections [10]. In France, fungal agents were found in 66.6% of plantar mycosis and 10% on the scalp [11]. The fungal agents responsible for mycosis generally belong to the yeasts, dermatophytes and molds [12]. Few studies have been conducted on fungal infections in Gabon these last 10 years [13], [14]. Indeed, mycoses are considered benign in Gabon and some other countries such as Nigeria, India, and Iran and are therefore neglected by clinicians and microbiologists. Although it is important to identify the real spectrum of causative agents and their sensibility to antifungal drugs.

The purpose of this study was to determine the prevalence of fungal agents diagnosed on skin samples of patients attending the Department of Parasitology-Mycology and Tropical Medicine (DPMTM) at the University of Health Sciences in Owendo (Gabon).

2. MATERIAL AND METHODS

2.1 Patients and biological sampling

The study was conducted from September 2018 to September 2019. It involved 141 patients received in consultation at the mycology laboratory DPMTM. Samples were collected in following site: scalp, the face (pustule), the large folds (axillary and inguinal fold), the small folds (intergluteal fold, plantar and palmar interdigital spaces), nails (feet and hands); the soles of feet and the palms of hands. All biological samples were taken using a sterile scalpel blade (hair, squamas and nails). Before removing mothworms and the glabrous skin of the face or face pustules, a paraclinical examination was made with Wood's lamp to determine the type of parasitism through the fluorescence emitted even, in the absence of blunt injury. The lesion was termed Wood positive in case of a green fluorescence appearance and Wood negative in the absence of fluorescence. Only patients with superficial lesions and suggestive symptoms of mycotic origin and who had not taken antifungal therapy during the two weeks prior to sampling were included in the study. The socio-clinical data of the patients were recorded.

2.2 Diagnosis of fungal species

Mycological diagnosis was made by two methods: direct examination and culture. Direct examination was performed with skin squama, hair or nail fragments. It required 30% KOH (potassium hydroxide) (for all samples) and / or black chlorazol (with the exception of hairs). A drop of potash or black chlorazol was deposited on a clean slide then a sample fragment was deposited on the slide, it was triturated, then covered with a coverslip and observed at the objective x10 (tracing) and x40 (identification). For the cultivation of fungal agents, the samples were seeded in Sabouraud-chloramphenicol-actidione (SCA) medium and in Petri dishes containing Sabouraud-chloramphenicol (SC) or tubes containing Sabouraud-chloramphenicol-actidione-olive oil (SCA + OO). Nails and squamas were seeded in SCA and SC media; hair in the SCA medium and scalp squamas and face pustules in SCA + OO medium. Samples for yeast isolation were

incubated at 37°C for 4-7 days; all other samples were incubated at 27-30°C for 21-30 days. The identification was based on macroscopic criteria for all fungi and on microscopic criteria for filamentous fungi (molds, dermatophytes and pseudodermatophytes). For yeast-like fungi, identification was made according to macroscopic criteria, culture in Rice Agar Tween medium (RAT) and Auxanogram (bioMérieux, Marcy-l'Étoile, France).

2.3 Statistical analysis

Data were analyzed with SPSS version 20 (IBM, corporation, USA) and EPI-Info version 6 software (CDC, Atlanta, USA)

3. RESULTS

3.1 Sociodemographic characteristics of patients received

Of the one hundred and forty-one (141) patients received in consultation at the DPMTM; the majority were women (52.0%). The age of the patients ranged from 1 to 77 years with a mean age of 36.7 ± 17.3 years, those aged 20-30 (27.7%) and >50 (22.0%) were the most represented, while the age group <20 years was the least represented (12.1%).

3.2 Pathogenic forms observed on direct examination

Different forms of pathogens were observed on direct examination. Round and budding yeasts were the most frequent fungal forms (45.4%), followed by mycelial filaments (21.3%), Piedra nodules (3.5%), endothrix damage (2.8%) and yeasts clustering (2.1%) while bacterial elements included trichomycosis nodules (5.0%) among others. The cultures were predominantly positive (75.2%).

3.3 Distribution of sampling sites, and clinical signs in patients

Suggestive lesions were present in 12 sites (Table 1) most frequently on the scalp followed by the lower body (Table 1). Lesions on interdigital space were the least frequent (Table 1). According clinical symptoms presented by the patients, pruritus and desquamation predominated as shown in Table 1.

The skin disorders observed in patients were variable, the most frequent being intertrigo of large folds and onychomycosis (21.3% each), followed by intertrigo of small folds (16.3%); Tinea capitis (16.6%); hyperkeratosis (13.5%); and seborrheic dermatitis (4.5%). Tinea nigra was the least observed disorder in patients (1.4%, Table 1).

3.4 Fungal species

Mold, yeasts were the most common fungal group detected. Fold lesions were most frequent, as well as onychomycosis and lesions of the scalp while 25.0% of the samples were free of fungal species according to the diagnosis methods used.

Nine species of yeasts were detected in patients. Of these species, *Malassezia* sp was the most common (22.7%) followed by *Candida albicans* (14.2%), *Candida parapsilosis* (8.5%) and *Candida guilliermondi* (6.4%). The least commonly identified yeast species in patients were *Korhamaea ohmeri*, *Candida rugosa*, *Candida lusitanae*, *Candida famata* (0.7% each, Table 2).

With regard to molds, two species were isolated in patients: *Cylindrocarpon* sp and *Fusarium oxysporum*.

Scytalidium dimidiatum was the only species of pseudodermatophyte identified and was present in 6.4% of

patients (Table 2). Four species of dermatophyte were isolated from patients including *Trichophyton interdigitale* (4.3%) and *Trichophyton soudanense* (2.1%, Table 2).

3.5 Distribution of fungal groups by sampling site

In 141 patients received in consultation, samples were taken from 12 sites ranging from the scalp to the foot plant (Table 3). Dermatophytes were observed on 5/12 sites, mainly on the scalp and in the plantar interdigital space (35.8% each). Yeasts were isolated in all sites with the exception of the palmar interdigital space (11/12 sites). They were found frequently on the scalp (81.5%) and in the inguinal fold (68.2%). Molds were isolated only in the plantar interdigital space (100%). Pseudodermatophytes were isolated from 3/12 sampling sites and mainly on the foot plant (26.8%), followed by the plantar interdigital space (15.8%, Table 2).

3.6 Distribution of skin disorders by age groups

Nine skin disorders were observed in patients. Seborrheic dermatitis was found in patients of all age groups except those >50 years (Table 3). This disorder was observed most frequently in the age group 20-30 years (42.9%). Hyperkeratosis was predominant in patients in the age group 41-50 years (26.1%) but was not found in the age group <20 years. The frequency of intertrigo of large folds was highest in the age groups 20-30 years and 31-40 years (58.3% each) and was absent in patients under 20 years. Intertrigo of small folds was not observed in the age group <20 years but was more common in patients of the age group > 50 years (22.6%). Onychomycosis was found in all age groups with a preponderance in subjects >50 years (32.2%). It was found less frequently in the age groups <20 years and 31-40 years (13.3% each). Pityriasis versicolor was predominantly observed in patients in the age group 20-30 years (5.0%). However, it was absent in patients aged <20 years and 41-50 years. Mothworms with small alopecia patches were found only in patients aged <20 years while *Tinea capitis* was frequently found in patients in the 20-30 years age group (36.4%, Table 3).

3.7 Distribution of skin disorders in patients according to fungal groups

Table 4 represents the distribution of the 9 diseases observed in patients received in clinical consultation according to fungal groups. Seborrheic dermatitis was due solely to yeasts (100.0%). Most fungal groups were associated with hyperkeratosis including yeasts (31.6%) and pseudodermatophytes (26.3%). Only molds were not associated with pseudodermatophytes (Table 4). Intertrigo of large folds was due mainly to yeasts (66.7%) whereas the intertrigo of small folds was associated with all the fungal groups with a predominance of yeasts (43.5%) followed by dermatophytes and pseudodermatophytes (13.0% each). Molds were only observed in the intertrigo of small folds (Table 4). Half of all cases of onychomycosis, all cases of Pityriasis versicolor and *Tinea capitis*, were caused by yeasts, while 80.0% of mothworms with small alopecia patches were associated with dermatophytes (Table 4). Also, 20% of mothworms were not due to any fungal group. Two cases of *Tinea nigra* were observed in patients with one case due to yeasts and another case due to no fungal group identified in culture (Table 4).

4. DISCUSSION

Fungal diseases remain a health problem in sub-Saharan Africa, particularly in Gabon, because of the moist equatorial climate favorable to these pathogens. This study evaluated the frequency of fungal diseases observed in patients received in consultation at DPMTM for one year. We observed that most patients admitted to the consultation were female (53.0%); with an average age of 36.7 years. The 20-30 age group was the most represented among patients. The predominance of females and the young age of patients seen for fungal diseases have been reported in several earlier African studies [13], [14]. This predominance of women among patients is probably due to their greater concern for health and aesthetics, whereas that of the

20-30 age group could be associated with their socio-professional activities [15].

In this study we found 75.1% of pathogenic fungal forms on direct examination with a higher frequency of round and budding yeasts (45.4%) and a low frequency of clustering yeasts (2.1%) while positive and negative cultures were observed in 75.2% and 24.8% of samples respectively. Our results are similar to the fungal frequencies of 74.9% and 76.2% obtained by direct examination and culture in an earlier study on fungal etiologies and factors favoring intertrigos [2]. The negative results obtained on direct examination and in culture could be due not only to patients' unreported self-medication or to the scarcity of fungi in biological samples.

Round and budding yeasts were the most frequently observed pathogenic fungal forms in all types of superficial sampling.

The low prevalence of clustering yeast reflects the rarity of *Malassezia* sp on the body' superficial parts as reported in the literature.

In this study, the search for fungal agents was done on 12 sites located on all the superficial parts of patients' bodies (from the head to the foot plant) with a predominance of the scalp (19.1%). A more significant scalp predominance of 67.0% for fungal agents searching was reported in a previous study involving 9 sampling sites in Senegal [16]. This diversity of sampling sites could be explained by the regions of attack of these pathogens. Micromycetes are microscopic fungi that have an affinity for the skin and integuments. This affinity is expressed in attacks of all parts of the body, with a scalp' predominance.

Pruritus was the most common clinical sign (61.0%). Other secondary signs were desquamation, maceration and erythematous-vesicular signs. A similar prevalence of pruritus of 60.1% was reported in a study in Mali [15]. Frequent scratching of the body increases pruritus by causing pain that in turn stimulates serotonin production. Indeed, a relationship has been shown between serotonin production and increased brain stimuli responsible for pruritus in subjects [17]. This mechanism may partly explain the high rate of pruritus observed in patients in consultation. However, other factors responsible for pruritus such as allergies and filariasis cannot be ruled out in an endemic area such as Gabon [18], [19].

Among the skin disorders found in patients received in consultation, intertrigo of large folds and onychomycosis were more frequent (21.3%, 30/141). In a retrospective study in patients in Dakar between 2011 and 2015, *Tinea capitis* and *Tinea unguium* (Onychomycosis) were the most frequently disorders found with 44.8% and 34.0% [8]. Studies elsewhere than in Africa showed that the most common disorders were *Tinea unguium* (onychomycosis) and *Tinea capitis* in French Guiana with 28.2% and 27.8%, respectively [3], whereas in Brazil *Tinea unguium* (Onychomycosis) and *Tinea pedis* predominated with 62.3% and 25.4%, respectively [20]. The difference found in the distribution of skin disorders in patients could be explained for the intertrigo of large folds by climatic conditions (hot and humid) and for onychomycosis through contamination (obligatory wearing of closed shoes for professional use).

In this study, two main fungal groups were incriminated in superficial mycotic diseases, namely yeasts (59.6%) and dermatophytes (7.8%). These two fungal groups were also the main causes of superficial fungal infections in Senegal and Croatia, with 58.8% and 63.0% for dermatophytes and 36.7% and 36.0% for yeasts, respectively [8], [21]. *Malassazia* sp (22.7%) and *Candida albicans* (14.0%) were the most common fungal species identified in this study. *Candida albicans* was also reported as the most prevalent species of superficial mycosis in Senegal (27.0%) and the United States of America (48.0%) [14], [22]. Its

commensal character may explain the high prevalence of *C. Albicans* and that of *Malassezia* sp by the high prevalence of *Tinea capitis* among patients, of which it is the etiological agent.

Yeasts were isolated on 11/12 sampling sites and the most represented site was the scalp (81.5%). This can be explained by yeast's commensal nature, which is easily found on the mucous membranes and skin. Molds were isolated only in the plantar interdigital space, which agrees with an earlier study that showed that they were only present at sites in contact with the soil [23].

When we assessed the prevalence of skin disorders in different age groups, seborrheic dermatitis was found in all age groups except <50 years old. It was more commonly found in the 20-30 years age group (42.9%). This agrees with the increased secretion of sebum in young adults which could be explained by increased production of hormones during the transition from adolescence to adulthood but also by the use of cosmetics.

Hyperkeratosis was more common in the 41-50 age group (26.1%). Nail hyperkeratosis could be due to a thickening of the nail pad as well as the skin following long-term drug treatments or in certain professions [24].

Intertrigo of the small folds was absent in the patients of the age group <20 years but more frequent among those of the age group >50 years (22.6%). Similar results have been reported in two previous studies and have been explained by the increase in local temperature in tropical zones associated with older people's sedentary lifestyle [25], [26].

The frequency of intertrigo (intergluteal, axillary and inguinal) was highest in the 20-30 years and 31-40 years age groups (58.3% each). A recent study in Ivory Coast showed that the 15-30 years age group was the most affected by intertrigo with 60.5% [27]. These results could be explained by the fact that this age group is the most active professionally and sweats more because they wear tight synthetic fiber clothing resulting in maceration and therefore friction with intertrigo of large folds as consequences [25].

In this study, onychomycosis was found in all age groups with a higher frequency among subjects in the >50 years age group (32,2%). Our results agree with those of [28] but diverge from previous studies that reported a high prevalence of that disorder in young subjects [29], [30]. However, advanced age has been shown to be a risk factor for onset of onychomycosis [31] due to repeated microtrauma, prolonged exposure to fungal agents, and the high activity associated with venous insufficiency in people over 50.0% [31], [32].

Pityriasis versicolor was predominantly observed in patients in the 20-30 years age group (5.0%). Our results do not corroborate those found in a study on this disease conducted in Ivory Coast in which it concerned 54.4% of subjects belonging to the same age group [9]. This could be explained by the relationship between the lipophilic nature of the yeasts involved and post-puberty (20-30 years), which is a period of hormonal stimulation and increased activity of the sebaceous glands resulting in a favorable environment for the proliferation of *Malassezia* spp., the etiological agent of Pityriasis versicolor [20], [33].

In our study, shingling moth with small alopecia patches were found only in patients younger than 20 years of age. Variable prevalence of ringworm of 50-55% has been reported in young patients [34- 36]. On the one hand, this could be explained by a better body hygiene in adult subjects and on the other hand by the spontaneous cure of this pathology at the beginning of the puberty (fungicidal role of the sebum) [34]. *Tinea capitis* has been found frequently in patients in the 20–30-year age group. Our results are similar to

those reported in Algeria where this affection was frequently found in female subjects and in those aged 20-29 years with 56.9% and 41.4%, respectively [37].

We also examined the association between fungal groups and diseases in consulting patients. We found that seborrheic dermatitis was due solely to yeasts. This agrees with the scientific literature that recognizes yeast fungal group as the only cause of facial dermatitis [38].

Hyperkeratosis was due to all fungal groups, mainly yeasts (31.6%) and pseudo-dermatophytes (26.3%). The fungal agents found in hyperkeratosis are the same as previously described by [39].

The intertrigo of large folds was mainly due to yeasts (66.7%). These results are similar to those of a previous study that reported the preponderance of yeasts in intertrigos of large folds. The development of yeasts is favored by heat, humidity, sweat and poor hygiene. A higher prevalence of 83.5% of intertrigos with a yeast frequency of 89.3% was reported in a recent study [27]. Other studies have shown lower involvement of yeasts in intertrigos in Senegal [26] and Burkina Faso [7].

Small-fold intertrigo has been associated with all fungal groups identified in this study. The yeast fungal group was the most observed (43.5%). In a study conducted in Ivory Coast, yeasts were shown to be responsible for 60.8% of intertrigos of small folds [27]. However, other studies have found higher prevalence of dermatophytes compared to yeasts in this skin disorder [2], [40]. The observed differences could be explained by the different climatic conditions between the study areas (Central Africa in our study and West Africa in other studies). Indeed, the hot and humid equatorial climate is more favorable to the growth of yeasts compared to the hot and dry climate of the West African region [2]. With regard to moths with small alopecia patches, 80.0% were due to dermatophytes in our study. These results are in agreement with the results of studies in Morocco [35] and Guinea [34]. We also recorded 24.2% of moths whose cultures were negative. Our results are similar to those of an earlier study that reported 34.6% negative cultures in subjects with ringworm [36]. This absence of fungal agent could be explained by self-medication that could interfere with dermatophyte agent growth. This could also be due to an early infection or a squamous non-mycotic infection such as psoriasis [36].

This study has some limitations. Methods of fungal agent identification, such as MALDI TOF, chromogenic media and agglutination tests were not used. Despite these technical constraints, we have described fungal agents responsible of superficial mycosis in patients attending the Department of Parasitology-Mycology at the University of Health Sciences.

5. CONCLUSION

This study showed that the most common fungal species in patients with superficial mycosis was *Malassezia* sp. Yeasts were the most prevalent fungal group. They were reported on all sampling sites with the exception of the palmar interdigital space. Measures to prevent contamination by fungal agents and mycoses should be implemented including aeration of the feet (onychomycosis), reduced wearing of tight clothing (intertrigo of small fold) and moderate use of facial oils (yeast).

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Table 1. Distribution of sampling sites, clinical signs and skin disorders in patients.

	Number (N)	Percentage (%)
Sampling sites		
Scalp	27	19.1
Palmar interdigito space	1	0.7
Plantar interdigito space	21	14.9
Fingernails	12	8.5
Toenails	18	12.8
Palm of hands	2	1.4
Sole of feet	18	13.5
Axillary fold	3	2.1
Inguinal fold	22	15.6
Intergluteal fold	6	4.3
Face	3	2.1
Pustule	7	5.0
Clinical signs		
Desquamation	42	29.8
Erythematous-vesicular	7	5.0
Maceration	3	2.1
Pruritus	86	61.0
Hyperpigmented spot	3	2.1
Skin disorders		
Seborrheic dermatitis	7	5.0
Hyperkeratosis	19	13.5
Intertrigo of large folds	30	21.3
Intertrigo of small folds	23	16.3
Onychomycosis	30	21.3
Pityriasis versicolor	3	2.1
Mothworm with small alopecia patches	5	3.5
Tinea capitis	22	15.6
Tinea nigra	2	1.4

Table 2. Distribution of Fungal Groups and Species Isolated in Patients

Fungal Groups and species	Number (N)	Percentage (%)
Yeasts	88	59.6
<i>Candida albicans</i>	20	14.2
<i>Malassezia sp</i>	32	22.7
<i>Candida famata</i>	1	0.7
<i>Candida guilliermondi</i>	9	6.4

<i>Candida lusitaniae</i>	1	0.7
<i>Candida parapsilosis</i>	12	8.5
<i>Candida rugosa</i>	1	0.7
<i>Candida tropicalis</i>	1	0.7
<i>Korhamaea ohmeri</i>	1	0.7
<i>Trichosporon sp</i>	6	4.3
Molds	2	1.4
<i>Cylindrocarpon sp</i>	1	0.7
<i>Fusarium oxysporum</i>	1	0.7
Pseudodermatophyte	9	6.4
<i>Scytalidium dimidiatum</i>	9	6.4
Dermatophytes	11	7.8
<i>Trichophyton gourvili</i>	1	0.7
<i>Trichophyton interdigitale</i>	6	4.3
<i>Trichophyton soudanense</i>	3	2.1
<i>Trichophyton tonsurans</i>	1	0.7
None	35	24.8
Total	141	100

Table 3. Distribution of skin disorders by age groups

	Age groups (years)				
	<20	20-30	31-40	41-50	>50
Skin disorders	N (%)	N (%)	N (%)	N (%)	N (%)
Seborrheic dermatitis	1 (5.9)	3 (42.9)	1 (3.3)	2 (8.7)	0 (0.0)
Hyperkeratosis	0 (0.0)	4 (10.0)	4 (13.3)	6 (26.1)	5 (16.1)
Intertrigo of large folds	0 (0.0)	10 (25.0)	10 (33.3)	4 (17.4)	6 (19.3)
Intertrigo of small folds	0 (0.0)	5 (12.5)	6 (26.1)	5 (21.7)	7 (22.6)
Onychomycosis	4 (23.5)	7 (17.5)	4 (13.3)	5 (21.7)	10 (32.2)
Pityriasis versicolor	0 (0.0)	2 (5.0)	0 (0.0)	0 (0.0)	1 (3.2)
Mothworms of small alopecia patches	5 (29.4)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Tinea capitis	7 (41.2)	8 (20.0)	4 (13.3)	1 (4.3)	2 (6.4)
Tinea nigra	0 (0.0)	1 (2.5)	1 (3.3)	0 (0.0)	0 (0.0)

Tableau 4. Distribution of skin disorders by fungal groups

	Fungal groups				
	Dermatophyte	Yeast	Mold	Pseudo-dermatophyte	None
Diseases	N (%)	N (%)	N (%)	N (%)	N (%)
Seborrheic dermatitis	0 (0.0)	7 (100.0)	0 (0.0)	0 (0.0)	0 (0.0)
Hyperkeratosis	1 (5.3)	6 (31.6)	0 (0.0)	5 (26.3)	7 (36.8)
Intertrigo of large folds	1 (3.3)	20 (66.7)	0 (0.0)	0 (0.0)	9 (30.0)

Intertrigo of small folds	3 (13.0)	10 (43.5)	2 (8.7)	3 (13.3)	5 (21.7)
Onychomycosis	2 (6.7)	15 (50.0)	0 (0.0)	1 (3.3)	12 (40.0)
Pityriasis versicolor	0 (0.0)	3 (100.0)	0 (0.0)	0 (0.0)	0 (0.0)
Mothworms with small alopecia patches	4 (80.0)	0 (0.0)	0 (0.0)	0 (0.0)	1 (20.0)
Tinea capitis	0 (0.0)	22 (100.0)	0 (0.0)	0 (0.0)	0 (0.0)
Tinea nigra	0 (0.0)	1 (50.0)	0 (0.0)	0 (0.0)	1 (50.0)