Antimicrobial Susceptibility of Tinea Capitis in Children from Egypt

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Abstract

Background: Dermatophytic fungi of genera Trichophyton and Microsporum are the most important fungal species causing tinea capitis. The aim of this study was to investigate the most prevalent fungal species causing tinea capitis in children from Egypt and the most useful antifungal agent for treatment. Patients and Methods: A total of 100 patients diagnosed clinically with tinea capitis were included in the study. Samples were collected and sent to the microbiology and immunology laboratory for sample processing and fungal identification by routine laboratory techniques. A study of antifungal susceptibility to chosen antifungal medications (fluconazole, ketoconazole, clotrimazole, miconazole, amphotericin, caspofungin, itraconazole, terbinafine, and griseofulvin) was done by minimum inhibitory concentration technique. Results: Our analysis revealed that Microsporum canis is the most commonly isolated strain. Amphotericin was the most effective antifungal agent followed by terbinafine. The most sensitive strain to fluconazole and griseofulvin is Microsporum gypseum, while Microsporum audouinii was mostly responsive to terbinafine. Conclusion: Identification and evaluation of the antifungal susceptibility of the pathogenic species in a certain geographic region is important to achieve a good clinical response.

KEY WORDS: Antifungal, antifungal susceptibility, tinea capitis

Introduction

Tinea capitis is a disease caused by superficial fungal infection that attacks hair shafts and follicles of scalp, eyebrows, and eyelashes.[1] Dermatophytic fungi causing tinea capitis can be divided into anthropophilic and zoophilic; species of genera Trichophyton and Microsporum.[2]

Choice of treatment for tinea capitis is determined by the species of fungus, the degree of inflammation, and in some cases, by the immunologic and nutritional status of the patient.[3]

Despite the introduction of new antifungal medications, antifungal resistance continues to grow and evolve and makes patient management harder.[4] Antimicrobial susceptibility tests are used to determine which specific antifungal agent a particular fungus is sensitive to. These tests can guide the physician to choose the drug and dosage for difficult-to-treat infections. The interpretation of the sensitivity tests usually categorizes each result as susceptible (S), intermediate (I), resistant (R), sensitive dose dependent, or no interpretation.[5]

There had been few previous studies that investigated the causative organisms of tinea capitis in Egypt. The aim of this study was to map the causative organism of tinea capitis in Beni-Suef governorate and to determine the antifungal susceptibility.

Patients and Methods

Patients

A total of 100 children (85 male and 15 female) aging from 1 to 16 years and diagnosed clinically with tinea capitis were included in the study. Samples were collected from the scalp, eyebrows, and eyelashes. The samples were sent to the microbiology and immunology laboratory for sample processing and fungal identification by routine laboratory techniques. A study of antifungal susceptibility to chosen antifungal medications (fluconazole, ketoconazole, clotrimazole, miconazole, amphotericin, caspofungin, itraconazole, terbinafine, and griseofulvin) was done by minimum inhibitory concentration technique.
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Capitis were included in the study. All the children were recruited from the outpatient dermatology clinic, Beni Suef University Hospitals, during a 3 month period, from February 2016. All children were subjected to a full medical history and examination by two dermatologists to confirm the diagnosis of tinea capitis.

Patients receiving any antifungal treatment (topical or systemic) during 1 month prior to enrollment were excluded from the study.

The study was approved by the local research ethics committee of the Faculty of Medicine, Beni Suef University. The purpose of the study was explained and written informed consent was taken from the patients or their parents. The patient data were kept confidential by code number given to each patient.

Methods
Sample collection

The lesional skin was cleaned with alcohol 70%, and with a contact time of 20 s, scrapings were taken from the edge of lesion with the blunt end of the scalpel, the hair were epilated with the help of forceps. The scales were collected in autoclaved paper, which permitted drying of material so that it could be stored for long period without appreciable loss of viability of the fungal agents.

A potassium hydroxide (KOH) (10%) smear of a part of the collected sample was done and the prepared slide was examined under a microscope with reduced light for the presence of hyphae and arthrospores. A high-power objective was used for more minute study.

The rest of the material was sent to the department of microbiology where the material was inoculated into Sabouraud's dextrose agar slope with chloramphenicol (0.5 mg/ml) and cycloheximide (0.5 mg/ml). The inoculation was done with a sterile chromium wire spud at the center of the slope. The tubes were labeled and then incubated at room temperature. The tubes were examined every day for evidence of any growth. They were discarded as negative if there was no growth after 4 weeks. In positive cases, the growths were subcultured on to Sabouraud's dextrose agar slopes without antibiotics.

- The growths in tubes were seen for gross morphological characteristics as follows:
  - Rate of growth
  - General topography, i.e., whether flat, heaped up regularly or irregularly folded
  - Characteristics of colony, i.e., yeast like, powdery, granular, velvety or cottony, pigmentation on the surface, and on reverse.
  - Then, lipid assimilation test was done for strain identification by inoculation of the yeast colony in Wickerham broth (a chemical broth is made in a tube). The yeast is allowed to grow for 48 h and the growth is measured based on turbidity of the solution.

Agar was added to cultured broth, and slant was allowed to form. These could then be stored for months before being used. These slants were usually used with bromocresol blue indicator. When the slants' pH changed (from the neutral 7), it changed the color, indicating a change in the pH and thus a change in the yeast.

Antifungal susceptibility test

In order to obtain conidia for susceptibility testing, we used the method of Ogasawara et al.,[7] incubating the tinea colonies for 6 weeks or longer until conidium-bearing white fluffy colonies appeared on the surface of the original growth. Conidia were harvested to sterile saline by swabbing the colony surface with a sterile swab and were allowed to settle for 10–15 min. Conidium counts were standardized with a hemocytometer, and the suspension was adjusted to $1 \times 10^{-3} - 3 \times 10^3$ conidia/ml in RPMI 1640 medium buffered with MOPS (3-(N-morpholino)propanesulfonic acid; Hardy Diagnostics, Santa Maria, CA). Antifungal powders were reconstituted and serial dilutions were prepared in accordance with CLSI M38A methodology. [7] Serial dilutions of drug (0.125–64 µg/ml for fluconazole, ketoconazole, clotrimazole, miconazole, amphotericin, caspofungin, posafungin, itraconazole, terbinafine, and griseofulvin) and inoculum were combined in 96-well round-bottomed microtiter plates and incubated at 35°C for 4 days. The minimum inhibitory concentration end point was defined as the lowest concentration to inhibit 80% of fungal growth compared to the growth control. Candida parapsilosis ATCC 22019 were included as control.

Statistical analysis

Data were analyzed using the Statistical package for social science (SPSS Inc., Chicago, Illinois, USA) version 19 for Microsoft windows. Frequency distribution with its percentage and descriptive statistics with mean and standard deviation were calculated. Chi-square test, $t$-test, and correlations were done whenever needed. $P < 0.05$ was considered statistically significant.

Results

The disease duration varied from 1 to 36 months, with a mean duration of 2.9 months. Only ten patients (10%) of the studied group were from urban area, while the remaining ninety patients (90%) lived in rural area.

Regarding the type of lesion, the majority of the studied group (97%) showed gray patch lesion, while only three patients (3%) had kerion; 38% of the studied group had single lesion, while 62 patients (62%) had multiple lesions.

Regarding microscopic and culture results, 85% were positive by direct microscopy while 92% had positive culture.
Nearly 79% of samples were positive for both direct microscopy and culture, while only 4% were negative for both.

*Microsporum canis* is the most commonly isolated strain. The isolated strains are presented in Table 1.

**Sensitivity of the isolated organisms to antifungals**

Amphotericin was the most effective antifungal agent tested followed by terbinafine; the susceptibility of evolved strains to various antifungal agents is presented in Table 2.

The most sensitive strain to griseofulvin was *Microsporum gypseum* (83.3%) followed by *Microsporum audouinii* (80%), *T. mentagrophytes* (66.7%), *M. canis* (62.7%), and finally, the least sensitive strain was *Trichophyton rubrum* (57.1%) [Table 3].

The most sensitive strain to terbinafine was *M. audouinii* (100%) followed by *T. rubrum* (85.7%), *M. canis* (76.3%), and finally, the least sensitive strains were *T. mentagrophytes* (66.7%) and *M. gypseum* [Figure 1].

The most sensitive strain to fluconazole was *M. gypseum* (66.7%) followed by *M. audouinii* (60%), *M. canis* (40.7%), *T. rubrum* (28.7%), and finally, the least responding strain was *T. mentagrophytes* (26.7%).

The most sensitive strain to itraconazole was *T. rubrum* (85.7%) followed by *M. audouinii* (60%), *M. canis* (54.2%), *T. mentagrophytes* (46.7%), and finally, the least sensitive strain was *M. gypseum* (33.3%).

The most responsive strain to ketoconazole was *M. audouinii* (60%) followed by *T. rubrum* (57.1%), *M. canis* (50.8%), *M. gypseum* (50%), and finally, the least responding strain was *T. mentagrophytes* (33.3%). On the contrary, the most sensitive strain to clotrimazole was *M. gypseum* (83.3%), while the least responsive strains were *M. audouinii* (60%) and *T. mentagrophytes* (60%).

The most sensitive strain to miconazole was *T. mentagrophytes* (86.7%) followed by *M. audouinii* (60%), *M. gypseum* (50%), *T. rubrum* (42.9%), and finally, the least responding strain was *M. canis*.

There was no significant correlation between strains and clinical and demographic characteristics of the patients.

Table 4 shows susceptibility of *M. canis* to different antifungal agents, where terbinafine was the most effective agent (76.3%).

**Table 1: Number and percentage of evolved strains**

| Strain                     | n (%) |
|---------------------------|-------|
| *Microsporum canis*       | 59 (64.10) |
| *Trichophyton mentagrophytes* | 15 (16.30) |
| *Trichophyton rubrum*     | 7 (7.60) |
| *Microsporum gypseum*     | 6 (6.50) |
| *Microsporum audouinii*   | 5 (5.40) |
| Total                     | 92 (100) |

**Table 2: In vitro susceptibility of evolved strains to different antifungal agents**

| Antifungal agent | Sensitive, n (%) | Resistant, n (%) | Intermediate, n (%) | Total, n (%) |
|------------------|------------------|------------------|--------------------|--------------|
| Fluconazole      | 37 (40.2)        | 39 (42.40)       | 16 (17.40)         | 92 (100)     |
| Ketoconazole     | 45 (48.90)       | 37 (40.20)       | 10 (10.90)         | 92 (100)     |
| Clotrimazole     | 63 (68.50)       | 15 (16.30)       | 14 (15.20)         | 92 (100)     |
| Miconazole       | 46 (50.00)       | 40 (43.50)       | 6 (6.50)           | 92 (100)     |
| Amphotericin     | 84 (91.30)       | 0 (0.00)         | 8 (8.70)           | 92 (100)     |
| Caspofungin      | 32 (34.80)       | 50 (54.30)       | 10 (10.90)         | 92 (100)     |
| Posafungin       | 19 (20.70)       | 57 (62.00)       | 16 (17.40)         | 92 (100)     |
| Griseofulvin     | 60 (65.20)       | 22 (23.90)       | 10 (10.90)         | 92 (100)     |
| Itraconazole     | 50 (54.30)       | 31 (33.70)       | 11 (12.00)         | 92 (100)     |
| Terbinafine      | 70 (76.10)       | 14 (15.20)       | 8 (8.70)           | 92 (100)     |

**Figure 1: In vitro susceptibility of evolved strains to terbinafine. S: Susceptible, R: Resistant; I: intermediate**

**Table 3: In vitro susceptibility of evolved strains to griseofulvin**

| Strain                     | *Microsporum canis, n (%) | *Trichophyton interdigitale, n (%) | *Microsporum gypseum, n (%) | *Trichophyton rubrum, n (%) | *Microsporum audouinii, n (%) | P     |
|---------------------------|---------------------------|-----------------------------------|-----------------------------|----------------------------|------------------------------|-------|
| Sensitive                  | 37 (62.70)                | 10 (66.70)                        | 5 (83.30)                   | 4 (57.10)                  | 4 (80.00)                    | 0.825 |
| Resistant                  | 14 (23.70)                | 3 (20.00)                         | 1 (16.70)                   | 3 (42.90)                  | 1 (20.00)                    |       |
| Intermediate               | 8 (13.60)                 | 2 (13.30)                         | 0                           | 0                          | 0                            |       |
| Total                      | 59 (100.00)               | 15 (100.00)                       | 6 (100.00)                  | 7 (100.00)                 | 5 (100.00)                   |       |
Regarding *T. mentagrophytes*, miconazole was the most effective agent (86.70%) followed by terbinafine (66.70%) and griseofulvin (66.70%) and then clotrimazole (60%) and itraconazole (46.70%).

*M. gypseum* was mostly sensitive to clotrimazole and griseofulvin (83.30% both), while *T. rubrum* was mostly responsive to terbinafine and itraconazole (85.7% both). *M. audouinii* was mostly responsive to terbinafine (100%) followed by griseofulvin (80%).

### Discussion

The KOH microscopy of the studied samples was positive in 85%, while microbiological culture was positive in 92%. Negative cultures could be due to abuse of antifungal agents by the patients and delay in the delivery of the collected samples to the microbiology laboratory and the start of culture procedure.

*M. canis* was the most commonly isolated organism in almost two-thirds of the patients (64%) followed by *T. mentagrophytes* (16.3%), while *M. audouinii* was the least frequently isolated organism.

On reviewing the literature, we found that Bassyouni et al. in 2017 conducted a study on 128 patients in Fayoum governorate (a city close to Beni Suef) and found that *M. canis* was the most prevalent organism (52%), which is consistent with our study.

On contrary to our study, *T. violeceum* was found to be the most commonly isolated organism in studies by Hassan et al. who collected samples from Assuit city in 2012 and the prevalence was (37.5%), El-Khalawany et al. who conducted a multicenter study in Cairo, Alexandria, and Tanta found the prevalence of *T. violeceum* to be 56.9%, and Azab et al. in 2011 in Ismailia, where the organism was isolated from 40.3% of the samples; also in Alexandria, Omar et al. found that *T. violeceum* was the only dermatophyte isolated (100%).

Back in 1965, Abdel Fattah and El-Goithamy found that *T. violeceum* was the most commonly isolated organism (53.3%) in Cairo.

In Europe, Ginter-Hanselmayer et al. found sharp surge of the zoophilic dermatophyte *M. canis* with percentages of 90% in Austria and 54% in Germany. These results are in agreement with our study.

In Africa, on contrary to our study, Dogo et al. found that the most prevalent dermatophyte isolated was the anthropophilic dermatophyte *T. rubrum* from Nigerian patients and Moto et al. found that the anthropophilic dermatophyte *T. tonsurans* was the most frequent in Kenya.

These results suggest that the predominantly isolated organism varies in different geographic regions and also within a given region during different periods – a fact which is influenced by many factors including weather, socioeconomic circumstances, the level of the pathogenicity of the organism, access to treatment, and immune status of the hosts.

As regard *in vitro* antifungal susceptibility testing, antifungals such as fluconazole, ketoconazole, clotrimazole, miconazole, amphotericin, caspofungin, posafungin, griseofulvin, itraconazole, and terbinafine were used against the isolated dermatophyte strains.

We found that the most effective drug tested was amphotericin followed by terbinafine, clotrimazole, griseofulvin, itraconazole, miconazole, ketoconazole, fluconazole, and finally caspofungin and posafungin were the least effective.

As amphotericin, caspofungin, and posafungin are not commonly used for systemic treatment of tinea capitis in children due to its side effects, we can say that terbinafine is the most effective systemic drug that could be used in children followed by griseofulvin, while fluconazole is the least effective drug.

The study revealed that terbinafine is the most effective systemic drug against *M. canis, T. rubrum, M. audouinii, and T. mentagrophytes*, while griseofulvin is the most efficient drug against *M. gypseum*.

These results match with those of Magagnin et al. who found that terbinafine was the most effective agent against all species.

These results were also consistent with the results of González et al. who stated that terbinafine and griseofulvin were the most active antifungal drugs in the treatment of *T. capitis*. On the contrary to our study, Araújo et al. found that fluconazole was the most effective agent against *M. canis, T. rubrum, and T. mentagrophytes* and Zaki et al. found that voriconazole and itraconazole had the greatest
antifungal effect on \textit{M. canis}. Similarly, Afshari et al.\cite{22} found that the highest sensitivity was to ketoconazole and itraconazole in the study of \textit{in vitro} susceptibility of important dermatophytes including those causing \textit{T. capitis}. Significant variations in various reports could be due to the different methodologies, isolation media, and identification procedures employed in each study. Resistance to antifungals may occur when environmental factors lead to colonization or replacement of a susceptible species with a resistant one; for example, the replacement of \textit{T. violaceum} as a causative agent in previous studies in Egypt with \textit{M. canis} in more recent studies.

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Nil.

**Conflicts of interest**

There are no conflicts of interest.

**What is new?**

- \textit{Microsporum canis} is the most commonly isolated strain.
- Terbinafine is the most effective drug against \textit{M. canis}, \textit{T. rubrum}, \textit{M. audouini}, and \textit{T. mentagrophytes}.
- Griseofulvin is the most efficient drug against \textit{M. gypseum}.
- Identification of the antifungal susceptibility is important to achieve a good clinical response.

**References**

1. Fu M, Ge Y, Chen W, Feng S, She X, Li X, \textit{et al.} Tinea faciei in a newborn due to \textit{Trichophyton tonsurans}. J Biomed Res 2013;27:71-4.
2. Hogewoning A, Amaoah A, Bavinck JN, Boakye D, Yazdanbakhsh M, Adegidika A, \textit{et al.} Skin diseases among schoolchildren in Ghana, Gabon, and Rwanda. Int J Dermatol 2013;52:589-600.
3. Gupta AK, Summerbell RC. Tinea capitis. Med Mycol 2000;38:255-87.
4. Pfaller MA, Boyken L, Hollis RJ, Messer SA, Tendolkar S, Diekema DJ, \textit{et al.} \textit{In vitro} susceptibilities of clinical isolates of \textit{Candida} species, \textit{Cryptococcus neoformans}, and \textit{Aspergillus} species to itraconazole: Global Survey of 9,359 isolates tested by Clinical and Laboratory Standards Institute broth microdilution methods. J Clin Microbiol 2005;43:3807-10.
5. Arikian S. Current status of antifungal susceptibility testing methods. Med Mycol 2007;45:569-87.
6. Lin CC, Fung DY. Conventional and rapid methods for yeast identification. Crit Rev Microbiol 1987;14:273-89.
7. Ogasawara Y, Hara J, Hiruma M, Muto M. A case of black dot ringworm attributable to \textit{Trichophyton violaceum}: A simple method for identifying macroconidia and microconidia formation by Fungi-Tape and MycoPerm-blue. J Dermatol 2004;31:424-7.
8. Bassyonyi RH, El-Sherbiny NA, Abd El Raheem TA, Mohammed BH. Changing in the epidemiology of Tinea capitis among school children in Egypt. Ann Dermatol 2017;29:13-9.
9. Hassan A, Mohamed EM, Tawfik KM, Ezzat AA. Tinea capitis in Assuit Egypt. AAMJ 2012;10:45-54.
10. EL-Khalawany M, Shaaban D, Hassan H, Abdalsalam F, Eassa B, Abdel Kader A, \textit{et al.} A multicenter clinicomycological study evaluating the spectrum of adult Tinea capitis in Egypt. Acta Dermato-venereol Alp Pannonica Adriat 2013;22:77-82.
11. Azab MM, Mahmoud NF, Abd Allah S, Hosny AM, Shehata AS, Mohamed RW. Dermatophytes isolated from clinical samples of children suffering from tinea capitis in Ismailia, Egypt. Aust J Basic Appl Sci 2012;6:38-42.
12. Omar AA. Ringworm of the scalp in primary-school children in Alexandria: Infection and carriage. East Mediterr Health J 2000;6:961-7.
13. Abdel Fattah A, El-Gothamy Z. Tinea capitis in Egypt. Mykosen 1967;10:189-94.
14. Ginter-Hanselmayer G, Weger W, Ilkitt M, Smolle J. Epidemiology of tinea capitis in Europe: Current state and changing patterns. Mycoses 2007;50 Suppl 2:6-13.
15. Dogo J, Afegbua SL, Dung EC. Prevalence of Tinea capitis among school children in Nok community of Kaduna state, Nigeria. J Pathol 2016;2016:9601717.
16. Moto JN, Maingi JM, Nyamache AK. Prevalence of Tinea capitis in school going children from Mathare, informal settlement in Nairobi, Kenya. BMC Res Notes 2015;8:274.
17. Ghannoum MA, Hajjeh RA, Scher R, Konnikov N, Gupta AK, Summerbell R, \textit{et al.} A large-scale North American study of fungal isolates from nails: The frequency of onychomycosis, fungal distribution, and antifungal susceptibility patterns. J Am Acad Dermatol 2000;43:641-8.
18. Magagnin CM, Stopiglia CD, Vieira FJ, Heidrich D, Machado M, Vetoratto G, \textit{et al.} Antifungal susceptibility of dermatophytes isolated from patients with chronic renal failure. An Bras Dermatol 2011;86:694-701.
19. González U, Seaton T, Bergus G, Jacobson J, Martinez-Monzón C. Systemic antifungal therapy for tinea capitis in children. Cochrane Database Syst Rev 2007;17:CD004685.
20. Araújo CR, Miranda KC, Fernandes Ode F, Soares AJ, Silva Mdo R. \textit{In vitro} susceptibility testing of dermatophytes isolated in Goiania, Brazil, against five antifungal agents by broth microdilution method. Rev Inst Med Trop Sao Paulo 2009;51:9-12.
21. Zaki SM, Ibrahim N, Aoyama K, Shetaia YM, Abdel-Ghany K, Mikami Y, \textit{et al.} Dermatophyte infections in Cairo, Egypt. Mycopathologia 2009;167:133-7.
22. Afshari MA, Shams-Ghahfarokhi M, Razzaghi-Abyaneh M. Antifungal susceptibility and virulence factors of clinically isolated dermatophytes in Tehran, Iran. Iran J Microbiol 2016;8:36-46.