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In vitro antifungal susceptibility of the dermatophytes: a single center study from Eastern Black Sea Region of Turkey

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Abstract. – OBJECTIVE: A large number of patients applying to the dermatology clinics are affected by fungal diseases, and a significant portion of which are superficial fungal infections. Dermatophyte infections are a notable public health concern and frequently encountered in clinical practice. Dermatophytosis not only compromises the quality of life but also predisposes individuals to various comorbidities due to its role as a gateway for secondary bacterial agents. This study aims to determine the species distribution of dermatophytes prevalent and assess their susceptibility to antifungal drugs.

PATIENTS AND METHODS: Skin, nail, and hair samples were obtained from patients with a clinical diagnosis of dermatophytosis. Samples were all cultured to isolate and identify the species. *In vitro* liquid microdilution tests were conducted to assess the susceptibility of the isolated strains against terbinafine, fluconazole, griseofulvin, and butenafine.

RESULTS: A total of 353 samples were obtained from the hair, skin, and nail lesions of 326 patients. Dermatophyte was isolated in 71 of the samples (20.1%). The cultured dermatophyte subtypes included *Trichophyton rubrum* (13.8% in 49 samples), *Microsporum audouini* (5.7% in 20 samples), and *Trichophyton mentagrophytes* (0.6% in 2 samples). Antifungal susceptibility testing revealed that terbinafine was the most effective antifungal drug against all dermatophyte species, while fluconazole exhibited the highest resistance.

CONCLUSIONS: The most common dermatophytosis agent in our region is *T. rubrum*. The least antifungal resistance was found against terbinafine. Conducting antifungal susceptibility tests is crucial for selecting effective treatment regimens and early detection of resistance development.

Key Words:
Antifungal resistance, Terbinafine, Griseofulvin, Fluconazole, Butenafine.

Introduction

Dermatophytosis, the most common superficial fungal disease, is a fungal infection caused by dermatophytes in keratinized tissues such as the epidermis, hair, and nails[1,2]. The causative agents of dermatophytes are grouped into three main categories: *Microsporum, Epidermophyton*, and *Trichophyton*[3,4]. Dermatophytosis is an important health concern. Poor hygiene, communal living areas, low socioeconomic status, shared personal items, contact with animals, high humidity, and immunosuppression play crucial role in the transmission and spreading of the disease[4]. Since dermatophytosis is not a notifiable disease, incidence data is limited. It is estimated that 20-25% of the population – approximately 1 billion people – has superficial mycosis all around the world[5,6]. The diversity and prevalence of dermatophytosis vary depending on the region's climate conditions, geographical structure, the lifestyle of people, and their mobility. Each region has its unique dermatophyte flora, and the flora can change over time[5]. Various studies[7-12] conducted in Turkey have found that the most common superficial fungal disease is *tinea pedis*, and the most frequently isolated dermatophyte species is *T. rubrum*.

Topical or systemic antifungal medications are used in the treatment of dermatomycoses. However, treatment can be challenging due to relapses, reinfections, prolonged therapy and anfungal resistance. Dermatophytes can develop resistance to antifungals through various mechanisms[13,14]. Antifungal resistance leads to treatment prolongation and unresponsiveness, which causes an increase in the risk of comorbidities, an increase in the number of hospital admissions, and financial losses. The use of antifungal susceptibility tests

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is important for selecting an effective treatment regimen and early detection of resistance development. However, these tests are not routinely applied in clinical practice[15]. There are several studies[15-17] demonstrating *in vitro* antifungal activity against dermatophytes and the development of resistance in the literature. While there have been a limited number of studies related to the identification of dermatophyte species in the Eastern Black Sea Region of Turkey, no study has been conducted in Giresun province on this topic. Also, there is no study investigating *in vitro* antifungal susceptibility from the Black Sea Region of Turkey[11,12].

The present study's aim was to determine the distribution of dermatophyte species causing dermatophytosis in the Eastern Black Sea Region and assess the *in vitro* susceptibility to antifungal drugs. This research aims to contribute to the planning of the treatment of dermatophytosis infections by selecting appropriate antifungal agents and to contribute to epidemiological studies around the world about this subject.

Patients and Methods

Patient Population

Patients who presented to the Dermatology outpatient clinic with clinical diagnoses of dermatophyte infections such as *tinea capitis*, *tinea pedis*, *tinea unguium*, *tinea manum*, *tinea cruris*, and *tinea corporis*, between January 1, 2021, and January 1, 2023, were included. Patients who declined to participate, those with dermatophyte infections but unable to provide sufficient samples for fungal culture from their lesions, and patients who were using topical or systemic antifungal treatment at the time of examination were not included in the study. Informed consent forms were obtained from the patients who agreed to participate in the study.

Ethics approval was obtained from Giresun University Clinical Research Ethics Committee (Decision Number: 05.12.2019/16). The study was conducted in accordance with the principles of the Helsinki Declaration.

Sample Collection

Prior to sample collection, the lesion and its surrounding area were wiped with 70% ethyl alcohol and left to dry for a few minutes. For nail samples, scraping was performed beneath the nail with a scalpel. In the case of skin infections, a scalpel was used to scrape from the active edge of the lesion towards the center, collecting the squamous material from the lesion. In cases of suspected *tinea capitis*, hair samples were obtained by cutting the hair. The collected samples were placed in sterile Petri dishes.

Culturing

The collected samples were inoculated by touching them to both Dermatophyte Test Medium (DTM) (Biolife Italiana, Milan, Italy), and Sabouraud Dextrose Agar (SDA) plates (Biolife Italiana, Milan, Italy), with a portion of the sample submerged and another portion exposed. Inoculated plates were then incubated at room temperature (22-26°C) and at 37°C. The plates were then examined for growth 2-3 times per week and were left for incubation for duration of 4 weeks. Developed colonies were subcultured onto Potato Dextrose Agar (PDA) plates, as it enhances pigment formation and conidial development. Fungal colonies were stained with lactophenol cotton blue (ChemBio, Istanbul, Turkey). Species identification was conducted based on the characteristics of the hyphal structure, as well as the number and shapes of macro and microconidia. Additional biochemical tests were applied when deemed necessary.

To differentiate between *T. rubrum* and urease-positive *T. mentagrophytes*, a urease hydrolysis test was performed using Christensen's urea agar. The fungal isolates were inoculated onto urea agar plates, and the plates were incubated at 25-30°C for seven days. Changes in color were observed in the agar plates every 2-3 days. If there was no change in the color of the agar, it was considered negative, while a change in color to pink was considered positive.

Additionally, for differentiation, colonies that produced a red pigment on cornmeal dextrose agar supplemented with 1% glucose were identified as *T. rubrum*, whereas colonies that did not produce a red pigment were considered to be *T. mentagrophytes*.

Antifungal Susceptibility Testing

Antifungal susceptibility tests were conducted using the reference liquid microdilution method recommended by the Clinical Laboratory Standards Institute (CLSI) for filamentous fungi. *Candida parapsilosis* ATCC 22019 strains were used as quality control strains.

Drug Dilutions

All drugs [terbinafine (BDLPharm, Shangai, China), griseofulvin (BDLPharm, Shangai, China), fluconazole (BDLPharm, Shangai, China), butenafine (Erregierre SpA, San Paolo D'Argon (BG), Italy)] were dissolved in dimethyl sulfoxide (DMSO). Stock drug dilutions were prepared to be 100 times the final drug concentration in the microdilution test. These stock drug dilutions were stored at -80°C. Before use, they were diluted 1/50 in RPMI-1640 (Sigma-Aldrich, Taufkirchen, Germany).

Preparation of Inoculum

One milliliter of sterile 0.85% saline solution was added to 7-day-old colonies of dermatophytes growing on PDA. The colonies were gently scraped with a loop and transferred to a sterile tube using a Pasteur pipette. The tube was left undisturbed for 3-5 minutes to allow heavy particles to settle. The suspension remaining at the top was transferred to a new sterile tube and vortexed for 15 seconds. The density of the conidial suspension was adjusted to an optical density (OD) of 0.09-0.11.

Liquid Microdilution Test

Sterile 96-well, U-bottom microplates were used. In the first well, 200 µl of drug-free RPMI-1640 was added for growth control. Inoculum and serial dilutions of the prepared drugs were added successively in wells 2 through 11, with each well containing 100 µl of inoculum and 100 µl of the drug dilution. The last well (12^{th} well) contained 100 µl of inoculum and 100 µl of RPMI-1640 as a growth control. The microplates were then incubated for 7 days at 26°C.

Evaluation

Test results were assessed by comparing the growth in the well with growth control well. If necessary, the presence of growth was visually confirmed by observing color changes after the addition of Alamar blue.

For each isolate, the minimal inhibitory concentration (MIC) value was recorded for all four antifungals. Additionally, the MIC_{50} and MIC_{90} values were calculated for each antifungal against all dermatophyte species that exhibited growth. Isolates with MIC values less than 0.5 µg/mL were considered sensitive to the antifungal agent[18].

Statistical Analysis

Data were analyzed using IBM® SPSS Statistics 25 Software (IBM Corp., Armonk, NY, USA). The variables were investigated using visual (histograms, probability plots) and analytical methods (Kolmogorov-Smirnov test) to determine the normality of the data. In descriptive statistics, mean (standard deviation) was used for normally distributed data and median (minimum-maximum) was used for non-normally distributed data. Percentages were used to indicate the frequency of categorical data. As the MIC values were not normally distributed, the Kruskal-Wallis tests were conducted to compare MIC values among the four antifungal agents. The Mann-Whitney U test was performed to test the significance of pairwise differences using Bonferroni correction to adjust for multiple comparisons. A *p*-value of lower than 0.05 was considered to show a statistically significant result.

Results

Of a total of 353 different samples were collected from 326 patients who had a clinical diagnosis of dermatophytosis. Among the patients, 235 had *tinea unguium*, 44 had *tinea corporis*, 38 had *tinea pedis*, 23 had *tinea cruris*, 10 had *tinea manum*, and 3 had *tinea capitis*. The demographic characteristics of the patients are summarized in Table I.

Samples of 299 patients were collected from a single area of the body, while 27 patients' samples were collected from multiple areas. 235 nail specimens (210 toenails and 25 fingernails), 115 scale samples from skin lesions (37 from foot skin, 26 from trunk skin, 12 from gluteal area skin, 11 from arm skin, 11 from inguinal area skin, 10 from hand skin, 6 from leg skin, and 1 from facial skin), and 3 hair specimens were obtained.

In the mycological culture, dermatophyte growth was observed in 71 of the 353 samples (20.1%). The subtypes of dermatophytes grown in culture included *Trichophyton rubrum* (found in 49 samples, 13.8%), *Microsporum audouini* (found in 20 samples, 5.7%), and *Trichophyton mentagrophytes* (found in 2 samples, 0.6%). Apart from dermatophytes, *Aspergillus* spp were cultured in 55 samples, *Penicillium* spp in 6 samples, and *Alternaria* spp in 1 sample. Additionally, 37 samples showed contamination with numerous different molds (Table II).

Table I. Demographic characteristics of patients with suspected dermatophytosis.

Demographics		n = 326
Age/year – median (min-max)		49 (4-87)
Gender	Female	174 (53.4%)
	Male	152 (46.6%)
Comorbidities	Yes	165 (50.6%)
	No	161 (49.4%)
Localization*	Toe nail	210 (59.5%)
	Foot	38 (10.8%)
	Body	26 (7.4%)
	Fingernail	25 (7.1%)
	Gluteal region	12 (3.4%)
	Arm	11 (3.1%)
	Inguinal region	11 (3.1%)
	Hand	10 (2.8%)
	Leg	6 (1.7%)
	Scalp	3 (0.8%)
	Face	1 (0.3%)
Diagnosis*	*Tinea unguium*	235 (66.6%)
	Tinea corporis	44 (12.5%)
	Tinea pedis	38 (10.8%)
	Tinea cruris	23 (6.5%)
	Tinea manum	10 (2.8%)
	Tinea capitis	3 (0.8%)

*Since samples were taken from more than one region of 27 patients, 353 different regions were evaluated.

The highest rate of fungal growth in culture was observed in patients diagnosed with tinea pedis (34.2%), followed by *tinea capitis* (33.3%), *tinea cruris* (26.1%), *tinea corporis* (18.2%), *tinea unguium* (17.9%), and *tinea manum* (10%). *Trichophyton rubrum* was the most frequently cultured dermatophyte in cases of *tinea pedis, tinea unguium, tinea capitis, tinea corporis*, and *tinea cruris*. Only one *tinea manum* sample showed dermatophyte growth in culture, and in this case, *Microsporum audouini* was isolated. The distribution of fungal cultures based on diagnoses is summarized in Table III.

In the second phase of the study, an *in vitro* liquid microdilution test was performed to determine the antifungal susceptibility levels of strains grown in culture against terbinafine, fluconazole, griseofulvin, and butenafine. The minimal inhibitory concentration (MIC) values of the samples are summarized in Figure 1 and Table IV.

When the cut-off MIC value for resistance was taken as ≥0.5 µg/mL, it was found that *T. rubrum* had the highest griseofulvin resistance (91.8%) and the lowest terbinafine resistance (28.6%). It was determined that the antifungals to which

Table II. Mycological culture results.

Mycological culture results		n (%)
Dermatophyte isolated samples		71 (20.1%)
Fungus isolated samples		167 (47.3%)
Fungal species	Contamination	37 (10.5%)
	Aspergillus spp.	52 (14.7%)
	Trichophyton rubrum	46 (13%)
	Microsporum audouini	20 (5.7%)
	Penicillium spp.	5 (1.4%)
	Trichophyton rubrum + Aspergillus spp.	3 (0.8%)
	Trichophyton mentagrophytes	2 (0.6%)
	Penicilium spp. + Aspergillus spp.	1 (0.3%)
	Alternaria spp.	1 (0.3%)

Table III. Mycological culture isolation rates according to diagnoses.

	Dermatophyte isolation rates	*Trichophyton rubrum*	*Microsporum audouini*	*Trichophyton mentagrophytes*	Contamination (mix)	*Aspergillus spp.*	*Alternaria spp.*	*Penicillium spp.*
Tinea pedis (n=41)	13 (34.2%)	7 (18.4%)	6 (15.8%)	0	2 (5.3%)	7 (18.4%)	0	0
Tinea unguium (n=235)	42 (17.9%)	32 (13.7%)	9 (3.8%)	1 (0.4%)	28 (11.9%)	35 (14.9%)	1 (0.4%)	5 (2.1%)
Tinea corporis (n=44)	8 (18.2%)	4 (9.1%)	3 (6.8%)	1 (2.3%)	3 (6.8%)	7 (15.9%)	0	1 (2.3%)
Tinea manum (n=10)	1 (10%)	0	1 (10%)	0	1 (10%)	2 (20%)	0	0
Tinea capitis (n=3)	1 (33.3%)	1 (33.3%)	0	0	2 (66.7%)	1 (33.3%)	0	0
Tinea cruris (n=23)	6 (26.1%)	5 (21.7%)	1 (4.3%)	0	1 (4.3%)	4 (17.4%)	0	0

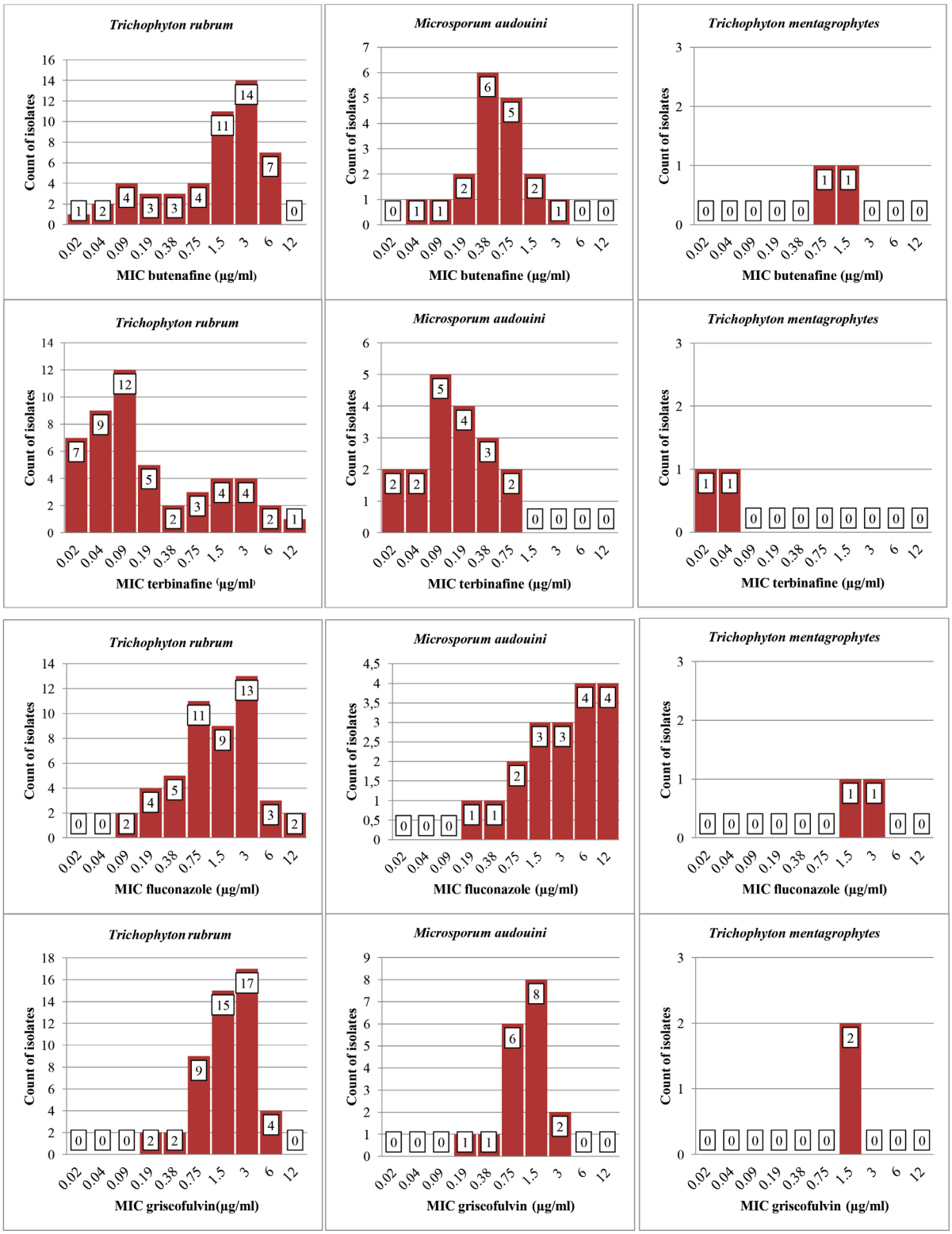

Figure 1. Susceptibility of *Trichophyton rubrum*, *Microsporum audouini* and *Trichophyton mentagrophytes* to butenafine, terbinafine, fluconazole and griseofulvin determined by liquid microdilution tests. Number of the boxes refers to count of isolates with a given minimal inhibitory concentration (MIC).

Table IV. MIC ranges, MIC_{50} and MIC_{90} values of antifungal agents according to the growing dermatophyte strain, and antifungal resistance rates.

	MIC (µg/mL)	*T. rubrum* (n = 49)	*M. audouinii* (n = 18)	*T. mentagrophytes* (n = 2)
Butenafine	≥ 1*	65.3% (32)	16.7% (3)	50% (1)
	≥ 0.5**	73.5% (36)	44.4% (8)	100% (2)
	MIC_{50}	1.5	0.38	0.75
	MIC_{90}	3	0.75	1.5
	Range	0.02-6	0.04-3	0.75-1.5
Griseofulvin	≥ 1**	73.5% (36)	55.6% (10)	100% (2)
	≥ 0.5**	91.8% (45)	88.9% (16)	100% (2)
	MIC_{50}	1.5	1.5	1.5
	MIC_{90}	3	1.5	1.5
	Range	0.19-6	0.91-3	1.5-1.5
Fluconazole	≥ 1*	55.1% (27)	77.8% (14)	100% (2)
	≥ 0.5**	77.6% (38)	88.9% (16)	100% (2)
	MIC_{50}	1.5	3	1.5
	MIC_{90}	3	12	3
	Range	0.09-12	0.19-12	1.5-3
Terbinafine	≥ 1*	22.4% (11)	0	0
	≥ 0.5**	28.6% (14)	10% (2)	0
	MIC_{50}	0.9	0.09	0.02
	MIC_{90}	3	0.38	0.04
	Range	0.02-12	0.02-0.75	0.02-0.04

MIC: minimal inhibitory concentration. *Number of species of which MIC ≥ 1. **Number of species of which MIC ≥ 0.5.

M. audouinii was the most resistant were griseofulvin (88.9%) and fluconazole (88.8%), and the antifungal to which it was most sensitive was terbinafine (10%). Finally, all of the 2 different *T. mentagrophytes* colonies were resistant to butenafine, griseofulvin and fluconazole. It was observed that both colonies were sensitive to terbinafine (Table IV).

When investigating the effectiveness of the four antifungal agents on fungal species, differences were observed among antifungals concerning *T. rubrum* and *M. audouinii* (all $p<0.001$). However, due to the limited occurrence of *T. mentagrophytes* in only two samples, a comparison among antifungals was not feasible. When the median MIC values of four agents against *T. rubrum* were compared, the median MIC value of terbinafine was found to be significantly lower than the other three agents ($p<0.001$). When comparing the median MIC values against *M. audouinii*, terbinafine's median MIC value was significantly lower than the median MIC values of the other four agents. However, butenafine's median MIC value was significantly higher than terbinafine's, and significantly lower than griseofulvin's and fluconazole's MIC values ($p<0.001$) (Table V).

Discussion

Dermatophytosis is the most common fungal disease worldwide[19]. Approximately 40 different dermatophyte species have been identified that can cause superficial fungal infections in humans. Although *T. rubrum*, *T. tonsurans* and

Table V. Comparison of median MIC values of antifungal agents according to the growing dermatophyte strain.

Median MIC values (minimum-maximum)	Butenafine	Griseofulvin	Fluconazole	Terbinafine	*p*
T. rubrum (n=49)	1.5 (0.02-6)[a]	1.5 (0.19-6)[a]	1.5 (0.09-12)[a]	0.09 (0.02-12)[b]	< 0.001
M. audouini (n=18)	0.38 (0.04-3)[a]	1.5 (0.19-3)[b]	3 (0.19-12)[b]	0.14 (0.02-0.75)[c]	< 0.001
T. mentagrophytes (n=2)	1.13 (0.75-1.5)	1.5 (1.5-1.5)	2.25 (1.5-3)	0.03 (0.02-0.04)	NA

MIC: minimal inhibitory concentration, NA: not applicable. [a,b,c]Superscripts show the difference between groups. Different superscript letters indicate statistical significance.

M. canis are the most frequently detected dermatophyte species, dominant species may vary depending on geographical regions[20].

In the present study dermatophyte species were isolated in 20.1% of the samples. Although the largest number of nail samples was collected, the highest rate of dermatophyte isolation was observed in the samples collected from *tinea pedis* (34.2%). In the cultures, *T. rubrum* was the most frequently isolated species.

There are various publications investigating the distribution of species causing dermatophytosis. In a recent study[16] from India it was reported that the most commonly isolated dermatophyte species were, *T. mentagrophytes* (45.6%) and *T. rubrum* (34.4%). In a review investigating the frequency of dermatophytes in the African continent, it was reported that the most common dermatophyte species in countries such as Kenya, Ethiopia, Tanzania, South Africa, Mozambique, Uganda and Zambia was *T. violaceum* (at rates ranging from 56.7% to 95%)[4]. In another review[21] that examined the dermatophyte species observed in Iran, it was reported that the most common dermatophyte species were *T. verrucosum*, followed by *T. violaceum* and *T. mentagrophytes.*

Sahin et al[10] conducted a study to investigate the distribution of dermatophyte species in Düzce province of Turkey. In this study, dermatophytes were isolated in 44% of the samples. The most commonly isolated dermatophyte species was *T. rubrum* (62.2%), followed by *T. mentagrophytes*, which was isolated with a frequency of 16.9%. In the Eastern Black Sea Region, only two publications[11,12] were detected related to this topic. In 1997, Metin et al[11] collected skin and nail samples from patients with dermatophytosis in Samsun, Turkey. They revealed that the most common isolated dermatophyte was *T. rubrum*. Following this, in order, were *T. mentagrophytes, E. floccosum, T. violaceum,* and *T. schoenleinii*. According to their results, dermatophyte growth was observed in 38.5% of the cultured samples.

The other study from the Eastern Black Sea Region of Turkey was conducted in Trabzon in 2000 by Parlat et al[12]. In the study, the percentages of dermatophytes cultured in the mycological culture were as follows: *T. rubrum* (69.5%), *E. floccosum* (18.1%), *T. mentagrophytes* (9.4%), *M. audouinii* (1.3%), *T. violaceum* (1.0%), *T. tonsurans* (0.3%), *M. ferrugineum* (0.3%). To the best of our knowledge there are not any studies examining the distribution of dermatophytes in patients diagnosed with dermatophytosis in Giresun province. This study is the first one investigating the distribution of dermatophyte species in Giresun province. In this study, similar to other two mentioned studies conducted in the Eastern Black Sea Region and in various regions of Turkey, *T. rubrum* was the most common causative agent of dermatophytosis. However, uniquely, it is observed that *Microsporum audouinii* was the second most common pathogen, differing from other literature reports.

In the second phase of our study, *in vitro* antifungal susceptibility tests were performed on the dermatophyte strains using the *in vitro* liquid microdilution method. Butenafine, griseofulvin, fluconazole, and terbinafine were used for *in vitro* antifungal susceptibility tests. It was observed that among these antifungal drugs, terbinafine was the most effective antifungal against all fungal species.

Due to the limited availability of antifungal susceptibility tests in many laboratories, dermatophyte infections are often treated without antifungal susceptibility tests. However, studies[22] have demonstrated an increasing antifungal resistance among dermatophytes over time. In this scenario, the benefits that patients derive from treatment may start to decrease over the years. Therefore, *in vitro* susceptibility tests conducted against antifungals can serve as a valuable guide for making appropriate treatment choices[23,24].

In a study, conducted by Sarıfakioğlu et al[25] from Turkey, nail samples were collected and cultured. *Trichophyton rubrum* (91%) and *Trichophyton mentagrophytes* (9%) were isolated. When antifungal susceptibility testing was performed on these fungal species using terbinafine, fluconazole, and itraconazole, the lowest the MIC value was found for terbinafine, while the highest MIC value was observed for fluconazole. In another study[26] from Turkey, skin and nail samples were collected from tinea pedis and tinea unguium. *Trichophyton rubrum* and *Trichophyton mentagrophytes* were isolated from the samples. Antifungal susceptibility testing was performed with terbinafine, fluconazole, and itraconazole. Terbinafine was found to be the most sensitive antifungal agent against both dermatophyte species.

In a study[27] conducted in Venezuela in 2005, antifungal susceptibility testing was carried out for *M. canis*, *T. rubrum*, *T. mentagrophytes*, and *Epidermophyton floccosum* with griseofulvin, fluconazole, itraconazole, and terbinafine. It was reported that all of the four dermatophyte spe-

cies were resistant to fluconazole, while terbinafine, griseofulvin, and itraconazole were effective against all of the fungal species.

The susceptibility tests for terbinafin, itraconazole, ketoconazole, fluconazole, and griseofulvin against *T. rubrum* and *T. mentagrophytes* were investigated in another study from Brazil[28]. It was determined that terbinafine (MIC_{50} 0.007 mg/ml-1) and itraconazole (MIC_{50} 0.125 mg/ml-1) were the most effective antifungals against both species. Ketoconazole (MIC_{50} 0.25 mg/ml-1) and griseofulvin (MIC_{50} 0.25 mg/ml-1) were slightly lower but still effective antifungals. On the other hand, fluconazole susceptibility, was found to be significantly lower compared to the other antifungals (MIC_{50} 32 mg/ml-1).

There are limited researches about butenafine's resistance of dermatophytes. The first study[29] on this subject was conducted in 1991. MIC of butenafine hydrochloride against dermatophytes was reported to be 0.0015-0.05 µg/ml. In 2003, susceptibility tests were carried out for ciclopirox olamine, econazole, and butenafin against various dermatophytes, bacteria, and fungi. For dermatophytes, the MIC values were reported as follows: ciclopirox olamine, 0.03-0.25 µg/ml; econazole nitrate, <0.001-0.25 µg/ml; and butenafin HCl, 0.03-1.0 µg/ml. It was interpreted that all three agents were effective against dermatophytes[30].

The resistance of nine different antifungal agents against *Trichophyton interdigitale*, *T. rubrum*, *T. tonsurans*, and *Epidermophyton floccosum* was investigated in 2018. The MIC ranges for these antifungal agents were 0.001-0.008 µg/ml for luliconazole, 0.003-32 µg/ml for terbinafine, 0.03-64 µg/ml for griseofulvin, 0.01-16 µg/ml for itraconazole and voriconazole, 0.03-8 µg/ml for ketoconazole, 0.03-32 µg/ml for econazole, 0.03-1 µg/ml for lanoconazole, and 0.01-4 µg/ml for butenafin[31].

In another study[32] conducted in 2020, susceptibility tests were performed with eight different antifungal agents against *Trichophyton tonsurans*. The geometric mean values of the MIC for these antifungals were as follows, from the lowest to the highest: tolnaftate: 0.022 µg/mL, itraconazole: 0.026 µg/mL, terbinafine: 0.033 µg/mL, butenafin: 0.088 µg/mL, griseofulvin: 0.566 µg/mL, sertaconazole: 2.875 µg/mL, clotrimazole: 3.419 µg/mL, fluconazole: 12.540 µg/mL. Based on the results of this study, it was concluded that the most effective antifungals against *Trichophyton tonsurans* were, in order, tolnaftate, itraconazole, and terbinafine, while fluconazole was found to be the least effective antifungal agent. When conducting a literature review, we did not come across any other studies conducted in Turkey that investigated the antifungal sensitivity of butenafine.

When evaluating the results of all the studies examined above, dermatophytes generally appear to be more sensitive to terbinafine. Additionally, it has been observed that the effectiveness of fluconazole against dermatophytes is lower compared to other treatment agents in the literature. Findings of the present study were consistent with these results in the literature.

Limitations

We acknowledge some limitations of this study. The first limitation of the study was our inability to conduct susceptibility testing for antifungals other than the four agents used in the study. Another limitation is that very small amounts of dermatophytes species were isolated in the obtained samples.

Conclusions

As a conclusion, the most common isolated agent for dermatophytosis in Giresun province was *T. rubrum*, followed by *M. audouinii* and *T. mentagrophytes*. Regarding the results of antifungal susceptibility tests, it was determined that terbinafine was the most effective antifungal against all dermatophyte species, while fluconazole was the most resistant. We also believe that the effect of the routine use of antifungal susceptibility tests in clinical practice needs to be investigated in the future.

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Ethics Approval

Ethics approval was obtained from Giresun University Clinical Research Ethics Committee (Decision date / Decision number: 05.12.2019/16).

Informed Consent
Informed consent was obtained from all patients.

Authors' Contributions
Concepts: I.D.O., S.K.; Design: I.D.O., S.K.; Literature search: I.D.O., S.K.; Clinical studies: I.D.O., Ş.D., B.A., S.K., S.A.; Data acquisition: I.D.O., Ş.D., B.A., S.K., S.A; Data analysis: I.D.O., S.K.; Statistical analysis: I.D.O.; Manuscript preparation: I.D.O.; Manuscript editing: I.D.O., S.K.; Manuscript review: I.D.O., S.K.

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Conflict of Interest
The authors declare no conflict of interest.

Data Availability
The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

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