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Superficial mycoses in the Nis region, Southeast-Serbia

Research Article

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Abstract: The aim of the study is to investigate the most frequent cause of superficial mycoses in patients from the territory of city Niš Southeast Serbia in the period from 1998 to 2010. A total of 3223 samples from 2887 patients with suspected dermatomycoses were examined. Superficial mycoses were diagnosed using standard microbiology techniques (conventional microscopy and cultivation). Dermatophytes were determined on the basis of their macroscopic and microscopic morphological and morphometric characteristics. Morphometric characteristics were obtained by Laboratory Universal Computer Image Analysis system (Lucia M, 1996). Species of genus Candida were identified using the test of production of germ tube in sera, by growth on comertial chromatogen medium (Chromotogenic Candida, Liofichem/Bacteriology products, Italy) and by using Auxacolor TMBioRad, France. The results were elaborated with the statistical method of descriptive and quantitative analysis (SPSS 14.0 for Windows 2003). The prevalence of superficial mycoses was 25,1%. Dermatophytes were identified in 67.6% of all positive cultures. Microsporum canis was the most prevalent (50.3%) dermatophyte isolated, followed by Trichophyton metagrophytes var. mentagrophytes (35.4%). Yeast genus Candida has become a more frequent cause of superficial fungal infection since 2001. and C. albicans was the dominant yeast (61.1%).

Keywords: *Superficial mycoses • prevalence • dermatophytes • Candida spp.*

1. Introduction

Medical science is often attracted to the investigation of serious and severe illnesses, especially those that may pose a serious detriment to world populations. Superficial mycosis is such an illness, that is considered to be a major public health problem in many parts of the world. The prevalence of superficial mycotic infections has risen to such an extent that skin mycoses now affect more than 20-25% of the world's population, making it one of most frequent forms of infection [1-3].

The cause for this increase in prevalence of skin mycoses may be found in the socioeconomic statuses of the general public, life-style, type of population, migration of people and climatic conditions [4].

Superficial mycosis involves the keratin-containing structures of the body. The infection generally is considered to cause cosmetic problems, and is not life

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– threatening. The disease processes very rarely spread to other tissues, but can in fact do so in extremely immunocompromised individuals [1-3,5,6].

The major causes of superficial mycosis are *Trichophyton, Microsporum, Epidermophyton* species which collectively are referred to as dermatophytes, then *Malassezia furfur* and *Candida species*. Other fungi that can cause an opportunistic mycosis (*Cryptococcus spp., Aspergillus spp., Geotrichum spp., Trichosporon spp.*) several genera belonging to the class Zygomycetes can, albeit rarely, cause primary or secondary skin and nail infection [1,6].

Dermatophytes can be classified according to their usual habitat into anthropophilic, zoophilic and geophilic organismas. Anthropophilic fungi cause superficial dermatomycoses characterised by relatively low inflammatory activity. In contrast, the zoophilic germs are associated with highly imflammatory and potentially highly contagious skin infections. Geophilic fungi grow in the soil and only sporadically infect humans. When they do, the results vary from high to low grade inflammation [1].

In addition to dermatophytes, yeast of Candida genus can also infect the skin and nails. Candida spp. are a part of transient or commensal flora in specific parts of the body. Candidosis is usually an endogenous infection, however yeast genus Candida can be transmitted by direct and indirect contacts [1,6].

There are approximately 40 different species of dermatophytes, but a majority of superficial fungal infections are caused by five to six species [1,6].

By evaluating numerous studies of a large group of authors, it is discovered that the most comon cause of superficial mycosis in European countries are the species *T. rubrum, M. canis, T. mentagrophytes var granulosum* and *T. verrucosum* [7-10].

Species M. audouinii, T. soudanense and *T. violaceum*, causes of so-called immigrational mycosis, can rarely be detected in this area of our continent, considering the fact that endemic zones for these species are in Africa and some regions of Asia [1]. It is supposed that there will be an incident increase of mycoses caused by this species, due to a greater migration of people and development of tourism.

T. rubrum is proven to be the most common cause of dermatomycosis in developing countries in Europe [1,7-10]. This species is a frequent pathogen in Germany, Finland and Russia urban regions [1-4,7-9]. However in the United Kingdom [7] the most prevalent species is *T. tonsurans*, while in France [2] those are *T. soudanense* and *M. audouinii*.

Nevertheless in these European countries it was found that the zoophilic species such as *M. canis, T. mentagrophytes* and *T. verrucosum* are the most frequentely isolated dermathophytes from the material of patientes who live in rural areas [1-4,7-10].

Epidermophyton flocossum, was detected in a significant percentage as the cause of superficial mycosis in Finland and Germany [8,9]. From 1960 to 1990 in Belgium and Netherlands the prevalence of superficial mycosis caused by *M. canis* increased from 9% to more than 40%, after a long period of *T. verrucosum* predominance [10]. In the East Europen countries, Italy just like in rural area of developed European countries, zoofilic species of dermatophytes are a common cause of superficial mycosis in humans [1,11,12]. In former Yugoslav republics, in a significantly higher percentage, the cause of superficial mycosis is *Microsporum canis (M. canis)* [13,14].

For many years, dermatophytes were the most frequent cause of superficial mycosis in our region. However, the prevalence of fungal infection caused by dermatophytes, such as the distribution of dermatophytes strains isolated from skin and nail lesions has changed significantly within last 50 years [15].

Similar to other countries of Europe, in the period between 1950 and 1980, *Trichophyton spp*. was the predominant pathogen causing dermatophytoses in patients from territory of the city of Nis. In the period to follow, however, a steep increase in the prevalence of *M. canis* infection was discovered and since then this zoophilic species has been one of the most common species isolated from material of our patients [15,16].

Beside the changes of *M. canis* prevalence, from the beginning of 2000 until today, the prevalence of superficial candidosis has permanently increased.

Considering the fact that in available literature there are no data of the prevalence of superficial mycoses in our country, this study attempts to summarize etiological and epidemiological characteristics of superficial fungal infection in this region. The study is based on the mycological data obtained from the Department of Microbiology and Parasitology, Medical faculty-Nis (South-east Serbia).

2. Materials and methods

From 1997, until the end of August 2010, we examined 3,223 samples of 2887 patients with suspected dermatomycoses referred to mycological laboratory.

Clinical and mycological examination was done in the laboratory of the Department for mycology of the Medical faculty in Nis. The isolation and identification of fungi was established using the standard mycological procedure. The diagnosis involved the usage of

the conventional microscopy (CM) of the preparations prepared with a patients material (skin scars, nails, hair fragments) and an addition of chlorine-lactoferol or 20% KOH.

Another part of material were inoculated onto Sabouraud glucosa-agar (with added chloramphenicol and cycloheximide); Dermatophyte (D.T.M.) agar (Liofichem/Bacteriology products, Italy). Both mediae were incubated at 27°C for up to 5 weeks and cultures were checked for growth once a week. Dermatophytes were identified on the basis of characteristic growth on D.T.M. agar (medium changed to a red color when positive for dermatophyte growth) and their morphological and morphometric characteristics [17]. Since 2003 morphometric characteristics were obtained by television image analysis system Lucia M (Nikon, Japan). Subcultures on special media were done when necessary.

Material was inoculated onto Sabouraud glucosa-agar, liquid Sabouraud glucosa medium and a commercial chromatogen medium (Cromogen albicans, Parquetecnologico de Madrid, Spain, and Chromotogenic Candida media Liofichem/Bacteriology products, Italy) for isolation of *Candida spp*. (incubated at 37°C for 10 days). Cultural, macroscopical, microscopical morphological characteristics, made posiblle the determination of *Candida spp*. Species of genus Candida were identified useing the test of germination in sera, the Chromotogenic Candida media Liofichem/Bacteriology products, Italy and Auxacolor™, BioRad, France.

2.1. Statistical analyses

The results were elaborated with the statistical method of descriptive and quantitative analysis [Statistical Package for Social Sciences (SPSS) software 14.0 for Windows 2003]. A chi-square test for trend was used to compare proportions over time using each year as a different exposure level. A $p<0.05$ was considered statistically significant.

3. Results

Using the CM and cultivation of the material positive mycological results are confirmed in 726 patients. In 241 (33.2%) the mycoses was determined only with CM. Positive cultures were obtained in 485 (66.%) cases. The annual rate of positive cultures increased from 63% to 73% in the last 4 years. Dermatophytes were isolated from the material of 328 patients (67.6%), while

Table 1 Dermatophytes and Candida spp. isolates from material of patients from Niš

	Positive findings N (%)
Dermatophytes	328 (100%)
Microsporum canis	165 (50.3%)
Trichophyton mentagrophytes	116 (35.4%)
Trichophyton rubrum	22 (6.7%)
Epidermophyton floccosum	15 (4.6%)
Microsporum audoinii	3 (0.9%)
Microsporum gypseum	4 (1.2%)
Trichophyton violaceum	1 (0.3)
Trichophyton verrucosum	1 (0.3)
Trichophyton tonsurans	1 (0.3)
Candida spp.	157
C. albicans	96 (61.1%)
C. krusei	32 (20.4%)
C. parapsilosis	2 (1.3%)
C. tropicalis	2 (1.3%)
Non – albicans Candida spp.	25 (15.9%)

superficial candidosis was diagnosed in 157 (32.4%) examines. In any case, we did not find mix infection.

Other fungi such as molds *Aspergillus spp*. *Curvularia spp., Fusarium spp., Alternaria spp, Penicillium spp.* were identified in 26 samples. In 92.8% of positive cultures, microscopical examination was also positive. The most frequent dermatophytes isolated were zoophylic species *M. canis* that was identified in 50.3% of all dermatophytes recovered, and *T. mentagrophytes var. mentagrophytes* (35.4%). There followed *T. rubrum* (6.7%), *Epidermophyton floccosum* (4.6%), *M. gypseum* (1.2%). Other dermatophytes such as *M. audoinii, T. verrucosum, T. violaceum* and *T. tonsurans* were rarely isolated (Table 1).

The most common cause of superficial candidosis is *C. albicans* (96/61.1%) followed by *C. krusei* (32/20.4%), *C. parapsilosis* (2/1.3%), *C. tropicalis* (2/1.3%), non-albicans *Candida spp.* (25/15.9%). Table 2 illustrated the most common superficial mycosis: tinea capitis (t.

Table 2 Prevalence of superficial mycoses

Superficial mycoses	Number	%
Tinea capitis	161	33.2%
Tinea corporis-imbricata	36	7.4%
Tinea corporis-arms, legs	46	9.5%
Tinea faciei	34	7%
Onychomycosis (fingernail)	120	24.7%
Onychomycosis (toenail)	42	8.7%
Tinea manus	19	3.9%
Tinea pedis	10	2.1%
Tinea cruris	13	2.7%
Tinea barbae	5	1%

Table 3 Number of positive dermatophytes and Candida findings by location of infection

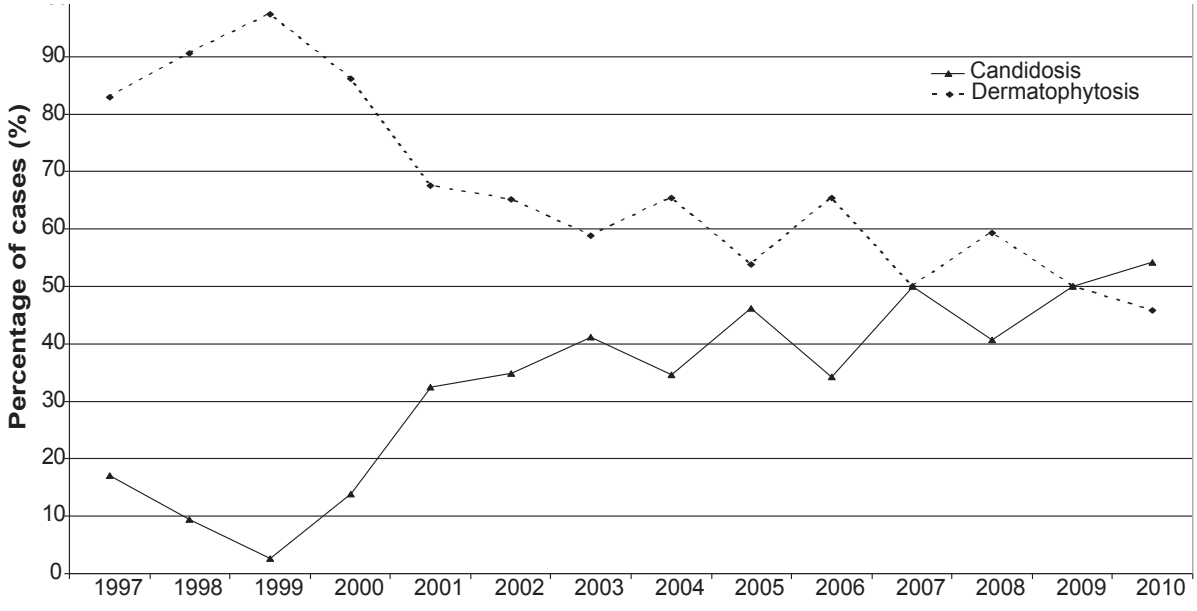
	T. capitis	T. corporis imbricata	T. corporis arms,legs	T. faciei	Onycho-mycosis toenail/ fingernail	T. manus	T. pedis	T. cruris.	T. barbae
Microsporum. canis	124	18	17	3	1/1	1			
Trichophyton mentagrophytes var. mentagrophytes	27	13	20	25	6/12	6	1	2	4
Candida spp.	6	4	3	2	34/103	2	3		
Tr. rubrum			4		1/1	3	6	7	
E. floccosum				3	/3	6		3	
M. audouini	3								
M. gypseum	1	1	2						
T. violaceum				1					
T. verrucosum						1			
T. tonsurans								1	

capitis, 161; 33.2%) and onychomycoses (162; 33.4%; fingerneils 120 and toenails 42). T. cruris, t. faciei, t. pedis, t. manus, and t. barbae were the diagnosis in significant lower percent of examined patients.

The frequency of isolated fungi by infection site is presented in Table 3. *M. canis* was the major pathogen responsible for t. capitis and t. corporis. T*richophyton mentagrophytes var. mentagrophytes* was the most frequent species identified in t. corporis and t. faciei and major pathogen responsible for t. barbae. *Trichophyton rubrum* and *Epidermophyton floccosum* were more frequently in t. manus, t. pedis and t. cruris.

Rare species, such as *T. violaceum, T. verrucosum* and *T. tonsurans*, were determined as the cause of t. faciei, t. manus and t. cruris, respectively. Figure 1 shows the prevalence of dermatophytes and *Candida spp* as a cause of superficial mycotic infection in the last 14 years in our patients. It can be seen that in the last 10 years dermatophytes were in permanent decrease, while yeast genus *Candida* become a more frequent cause of fungal skin and nail infection. Statistical analysis show that there is the significant differences in frequencies for both candidiasis and for dermatophytosis during the studied period ($p<0.001$).

Figure 1 Frequency of dermatophytosis and candidosis during the period 1998/2010



4. Discussion

As in other parts of Europe and in the southeastern Serbia, after the Second World War, superficial mycoses caused by anthopophilic species of dermatophytes had a significant incidence and epidemic character [18]. After the post – Second World War period, in the fifties and sixties, there were no significant epidemics, there has been an ongoing decline in prevalence of dermatomycoses caused by anthropophilic species of dermatophytes, and *T. mentagrophytes* was the most common cause of skin and nail infections in patients of our region. This zoophilic species was the cause of as much as 93.8% cases. In that period, *T. verrucosum, T. violaceum, T. tonsurans, T. quinceaneum, T. rubrum, M. gypseum i M. ferrugineum* were the species that have been isolated from our patient samples [19,20].
In the following years of the last century a significant increase in the number of infected by *T. rubrum* was diagnosed and the most frequent manifestation of this infection was t. pedis [16,21].

In the seventies, an extensive systematic research in the area of South-Eastern Serbia was conducted. The research study included the monitoring of the prevalence of superficial mycoses and the most common cause of these infections in more than 70,000 adolescents. In addition to this, studied was also the determination of most common causes of mycoses in domestic animals (investigation included more than 32,000 animals), and to investigate the presence of fungi in the soil and agricultural products [16,19,21,22].

After this extensive study, it was proven that infections were caused predominantly by by *T. mentagrophytes* and *T. rubrum*. Domestic animals usually had the infection caused by *T. verrucosum, T. mentagrophytes* and *M. gypseum*. In the soil and agricultural products *T. mentagrophytes* and *M. gypseum* were more prevalent species [16,19,21,22].
Despite regional characteristics and predispositions for dermatophyte infection, the spectrum of dermatophytes is not static. Climatic changes, mass tourism, international sports activities and increasing migration mean that less common or forgotten species are being imported and disseminated. Also, it is certainly true for our country, very low socio-economic standard caused by the civil war, years of economic sanctions, a large number of refugees from the territory of former Yugoslav republics, are some of the possible risk factors for a significant change in the prevalence of some of dermatophytes species in the area of our city.

Since the early nineties it was determined that previously less recovered species *M. canis* became the predominant cause of superficial mycoses in our patients [15,16]. In the last 14 years compared with all dermatophytes, isolated *M. canis* was the cause of superficial mycoses in 50.3% cases. This species was the cause of t. capitis in 77% patients and in 42.7% of examinees with t. corporis.
Epidemiologically, *M. canes* infections are usually associated with poor hygienic living conditions, living in rural areas, keeping livestock and low socio-economic status. However almost all of patients with *M. canis* infection were from urban region of our city and they are living in satisfactory social and economic condition.
Out of the total number of dermatophytoses, the prevalence of infection by T*richophyton mentagrophytes var. mentagrophytes* was 35.4% and it can be said that the frequency of this zoophylic species has been high in the last 60 years in our region.
According to patients anamnestic data, stray dogs and cats were implicated as the main source of *M. canis* and *T. mentagrophytes var. mentagrophytes* infections. However 15% of patients or parents denied any contact with animals. Until today, we have not had the measures to prevent dermatophytoses caused by zoophilic strains, because of the economic troubles and because stray animals are very difficult to bring under control.
Contrary to the findings in the last half of the twentieth century, the tendency of increase of *T. rubrum* infected in investigated period did not continue. This species was the cause in only 6.7% of all investigated with dermatophytoses.
Epidermophyton floccosum has not been isolated or identified in our patients since 2005.

Considering the fact that dermatophytes are the most common cause of dermatomycosis, and superficial mycosis therapy is advanced, we rarely need laboratory isolation and identification of the fungi for the diagnosis of a skin and adnexa fungal infection. It is recommended that general practitioners can establish the diagnosis and start with therapy, based on the findings of the fungal elements at the microscopical native preparation from the patient samples. The therapy can start the same day after these findings, without cultivation of the fungi [5,6].

Starting antifungal therapy before mycological analysis, self-treatment with antimycotics and using the cosmetic preparations with antifungal substances available commercially can be the possible reasons for the low prevalence obtained for *T. rubrum* infection, and absence of *E. floccosum* in recent years. However these can be the reasons behind failed cultivation of fungi, in spite of the mass of fungal elements observed on the direct microscopic preparation. The encouraging fact is that in the last 4 years the

percentage of unsuccessful isolation or identification of fungi was prooved to be significantly reduced.

Besides dermatophytes, *Candida spp*. is a common cause of superficial mycoses and the prevalence of this infection in the 14-years period was similar to the percentage in previous long periods of time [15,18-20]. However, in the last 10 years, the prevalence of superficial candidosis of our patients has been permanently increasing.

Superficial candidosis was most frequently expressed as onychomycosis of fingernails. Only 14% of patients were professionally engaged in any activity known for predisposing patients to the development of this infection.

As in other forms of this fungal infection, *C. albicans* (61.1%) was the most common cause of superficial canddosis, followed by *C. krusei.* A much smaller number of *C. parapsilosis* and *C. tropicalis* have been identified as the cause of superficial infections.

5. Conclusion

Based on the results of this study in the South Serbian region we can say that the prevalence (251%) of superficial mycosis is similar to the percentage of infected people worldwide. It can be concluded that dermatophytes are the most common cause of dermatomycosis; however, the *Candida spp*. has become a more frequent cause of superficial mycoses recently.

Prevention of dermatomycoses caused by zoophilic dermatophytes includes education of the population, cooperation with veterinary services, the institutions that care for abandoned dogs and cats, regular monitoring and reporting of these infections.

A significant increase in the prevalence of superficial candidosis among our patients referred for further monitoring and epidemiological investigation aimed at determining risk factors for the increasing number of infected and establishing preventive measures.

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