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PREVALENCE AND SPECIES DISTRIBUTION PATTERN OF SUPERFICIAL FUNGAL INFECTIONS IN THE NIŠAVA DISTRICT, SERBIA

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An assessment of distribution patterns of infectious disease causative agents is crucial in cases of infections managed empirically, without laboratory-based evidence.

The study aimed to explore the prevalence of superficial fungal infections (SFI) and identify the most common causative agents.

This study included a mycological examination of skin and adnexa samples of patients with clinical symptoms and signs of SFI. Standard mycological methods, microscopy and cultivation, were used for the detection and identification of causative agents. Dermatophytes were determined based on macroscopic and microscopic morphological characteristics to genus or species level, while the identification of yeast species was done by using commercial Integral System YEASTS plus (Liofilchem®, Italy) tests. The results were elaborated with the statistical method of descriptive and quantitative analysis (SPSS 14.0 for Windows 2003).

Statistical analysis revealed a high prevalence of SFI (30.2%), with a significant difference observed concerning patients' age ($p < 0.001$), while no significant difference was noted regarding patients' gender ($p = 0.504$). SFI did not exhibit a seasonal pattern ($p = 0.783$). Superficial fungal infection was confirmed by isolating and identifying fungi in 188 patients (15.1%). *Candida* spp. were isolated from 113 patients (60.1%), with *Candida albicans* identified as the causative agent of superficial candidosis in 46 patients (40.7%), while non-*albicans Candida* (NAC) species were detected in significantly more patients (59.3%). Dermatophytoses were diagnosed in 75 patients (39.9%), with *Microsporum canis* being the predominant species (38.7%).

The increasing incidence of superficial yeast infections caused by previously classified NAC species underscores the necessity for mycological analyses to determine the etiology of SFI and evaluate the *in vitro* effectiveness of antimycotics. The notable prevalence of zoophilic dermatophyte species highlights the imperative for epidemic and epizootic preventive measures.

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Key words: *superficial fungal infections, prevalence, dermatophytes, Candida* spp.

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Introduction

Superficial fungal infections (SFI) are among the most common human infections in the world with incidence rates from 20–25%, and present a significant public health problem (1). The high incidence and prevalence of dermatological fungal diseases can be attributed to various socio-economic factors, lifestyle choices, continuous migrations, and climate changes, particularly global warming.

Dermatophytes are the predominant causative agents of SFI. According to the latest suggested classification, based on molecular analyses, this group includes numerous genera, namely *Trichophyton* spp., *Epidermophyton* spp., *Nannizzia* spp., *Microsporum* spp., *Lophophyton* spp., *Arthroderma* spp., *Ctenomyces* spp., *Guarromyces* spp. and *Paraphyton* spp. The most medically significant species belong to the *Trichophyton, Epidermophyton*, and *Microsporum* genera (2). In addition to the aforementioned classification, it is essential to differentiate these species based on their natural habitat, into

antropophiles, zoophiles, and geophiles (1). Superficial fungal infections are frequently caused by yeasts of the genera *Malassezia* (3) and *Candida*. Based on the new taxonomy, which led to the exclusion of many medically significant *Candida* species from this genus, it can be highlighted that yeasts *Nakaseomyces* spp., *Pichia* spp., *Meyerozyma* spp. and *Kluyveromyces* spp. (4–6) are also causative agents of this infection. Even though species, previously classified within the genus *Candida* have been regrouped, in this article we will continue to use the old terms because this taxonomic revision could be overlooked or misinterpreted among clinicians. Furthermore, *Cryptococcus* spp. (7) and *Trichosporon* spp. can cause this form of fungal infection, primarily in immunocompromised patients. In recent years, there has been increasing data on non-dermatophyte molds as causative agents of SFI. These include molds of the genus *Aspergillus*, fungi of the Mucorales order, and members of hyaline and dermateaceae molds, which can cause infections not only in immunocompromised but also in immunocompetent individuals (4).

Despite the widespread occurrence of SFI, there are still no rapid diagnostic tests with adequate sensitivity and specificity. Consequently, routine mycological analyses for determining causative agents still need to be improved (8). The prevalence of certain species causing SFI and the localization of these infections vary depending on geographic, socio-economic, and ecological characteristics. Continuous monitoring of specific species' incidence in a particular region can help identify changes in the spectrum of the SFI causative agents. These findings can significantly impact the selection of the most effective therapy regimen, often based on empirical approaches. This retrospective study aimed to determine the prevalence of SFI, as well as the distribution of genera and species which had caused those infections from 2019 to 2022 in patients from the Nišava district in Southeast Serbia.

Material and Methods

The study entailed an analysis of data derived from mycological examination of skin and adnexa samples collected from 1243 patients suspected of having superficial fungal infections between the beginning of 2019 and the end of 2022. Only patients from the Nišava district were included with the aim of determining the incidence of SFI. The research was conducted at the laboratories of the Department of Parasitology and Mycology at the Institute of Public Health in Niš. The research was approved by the Ethic Committee of the Faculty of Medicine of the University of Niš (Decision No. 12-6316-2/1-2014) and the Ethic Committee of the Institute of Public Health in Niš (Decision No. 07-4665/2014).

The standard mycological analysis included microscopic examination using 10% potassium hydroxide and chlorine lactophenol to identify fungal elements in active reproduction (conidia, germination, pseudohyphae, and hyphae) in patients' material, along with cultivation to obtain fungal growth. Positive findings of one of those two methods, along with characteristic clinical findings, were considered as confirmation of SFI. Cultivation of fungi was done on selective media (Liofichem®, Italy) for dermatophyte and yeast isolation: Sabouraud dextrose agar (SDA); SDA with the addition of chloramphenicol and cyclohexamide; Dermatophyte test medium agar (DTM). Genera and species of dermatophytes were identified based on macroscopic and microscopic morphological characteristics (9), while the yeast species were identified using CHROMagar Candida and commercial Integral System YEASTS Plus (Liofichem®, Italy) test.

Statistical analysis

The interpretation of obtained, systematized, and encrypted data was done using a statistics calculator within the Epi Info program (Version 6.04) and statistical package SPSS (16.0 for Windows). The data were presented as arithmetic mean, standard deviation, and absolute and relative numbers. A T-test was used for the comparison of values between two groups of examinees. Fisher's test of exact probability, as well as the $\chi 2$ test with or without Yates' correction, were used for the comparison of different frequency distributions. The hypothesis was tested with a level of significance of $p < 0.05$.

Results

The research included 1243 patients: I) 456 men and 787 women; II) with an average age of 39.72 ± 23.09 years (min 1 year, max 86 years). Among them, 256 patients (20.9%) were referred with a diagnosis of tinea (t.) capitis; 117 (9.4%) with t. faciei; 171 (13.8%) with t. pedis; 111 (8.9%) with t. manuum; 19 (1.5%) with t. inguinalis; and 569 (45.7%) patients were referred due to onychomycosis, with 264 (21.2%) having changes in fingernails and 305 (24.5%) with changes in toenails.

Using the standard procedure, positive mycological findings were recorded in 375 patients, and among them, fungal isolation and identification confirmed the findings in 188 patients (15.1%). During the examined time frame, a high prevalence of fungal infections was noted (30.2%), exhibiting statistically significant variations across the years (25.6% in 2019; 43.5% in 2020; 33.3% in 2021; 24.1% in 2022; $p < 0.001$; Figure 1).

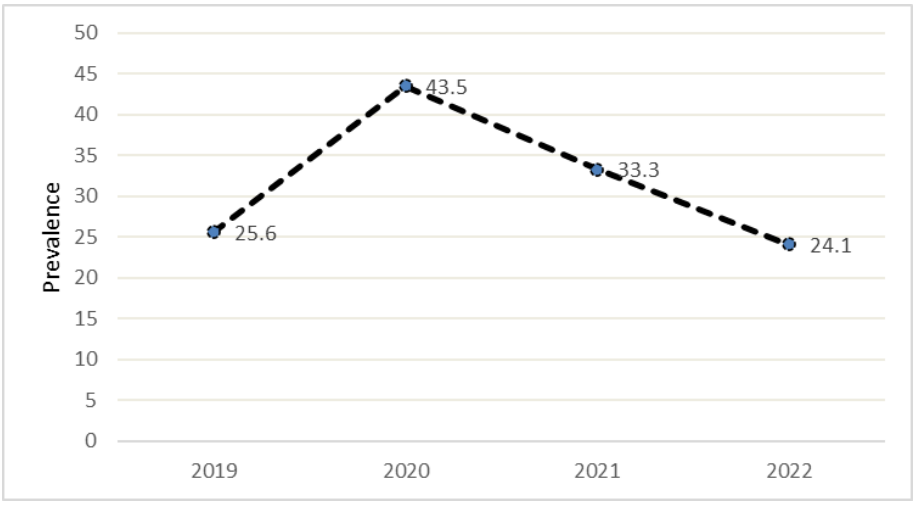

Figure 1. Trend of prevalence of superficial fungal infections from 2019 to 2022

Furthermore, a high cumulative incidence (26/105 inhabitants) was determined and calculated based on the assessment of inhabitants of the Nišava district in 2022 (343,950 inhabitants). Statistical analysis showed no statistically significant difference regarding gender ($p = 0.504$) and season ($p = 0.783$). Patients with fungal infections were statistically significantly younger than those without infection (35.66 ± 24.07 vs. 41.38 ± 22.53, $p < 0.001$; Table 1).

Table 1. Demographic characteristics of patients and season of superficial fungal infections occurrence

	Patients without SFI		Patients with SFI		p[1]
Age (years)	41.38 ± 22.53		35.66 ± 24.07		< 0.001[2]
Age categories	Examinee number	Percentage (%)	Examinee number	Percentage (%)	
< 18	189	22.0	130	34.9	< 0.001
18–65	507	58.9	192	51.5	
65 or older	165	19.2	51	13.7	
Sex					
Male	311	36.0	143	38.1	0.504
Female	554	64.0	232	61.9	
Season					
Winter	200	23.2	91	24.5	0.783
Spring	181	21.0	73	19.7	
Summer	244	28.3	98	26.4	
Autumn	237	27.5	109	29.4	

SFI—superficial fungal infections

Candida spp. were isolated from the materials of 113 patients (60.1%), among which, as a causative agent of superficial candidosis, *Candida* (*C.*) *albicans* species was confirmed in 46 patients (40.7%), while non-*albicans Candida* (NAC) species were found in a significantly more patients (59.3%). Among the NAC species, 15 isolates were not identified at the species level. In the identified NAC species group, *C. parapsilosis* was isolated as the dominant species (32; 47.8%), followed by *C. guilliermondi* (11; 16.4%). Other NAC species were found in significantly fewer patients (*C. glabrata* in 4; *C. tropicalis* in 3; *C. kefyr* in 1; *C. krusei* in 1) (Figure 2).

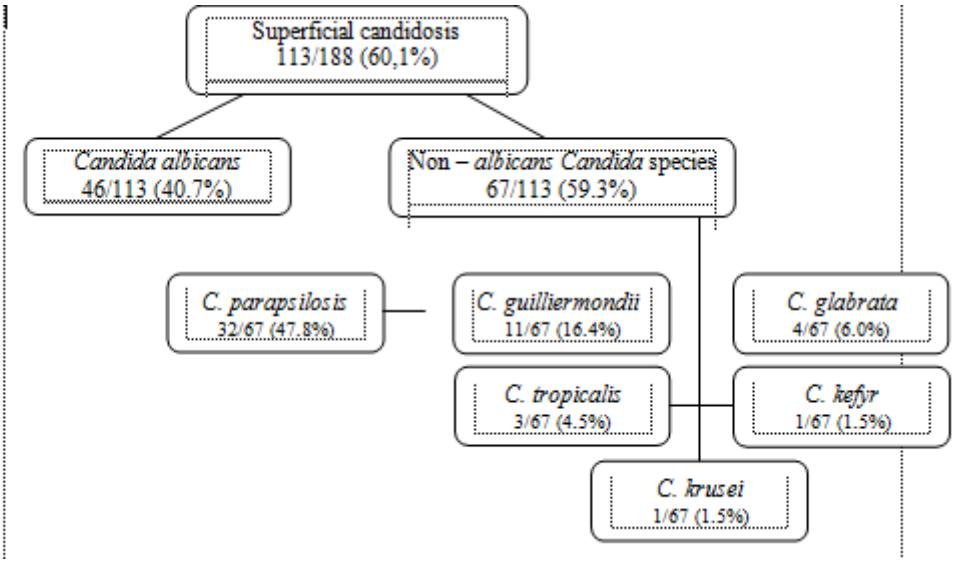

Figure 2. Yeast species isolated from materials of patients with SFI

Dermatophytoses were confirmed in a smaller number of patients (75; 39.9%). In this group, causative agents were *Trichophyton* species in 58.7% of patients, while species from the genus *Microsporum* were isolated from the materials of 41.3% of patients with dermatophytosis. The most prevalent dermatophyte species identified was *Microsporum (M.) canis* (38.7%), followed by the most prevalent species from the genus *Trichophyton*, *Trichophyton* (*T.*) *mentagrophytes* (12.0%). Additionally, species *T. interdigitale* and *T. tonsurans* each accounted for 8.0% of cases. Other species in this genus, *T. rubrum*, and *T. verrucosum*, comprised a smaller percentage of isolates (Figure 3).

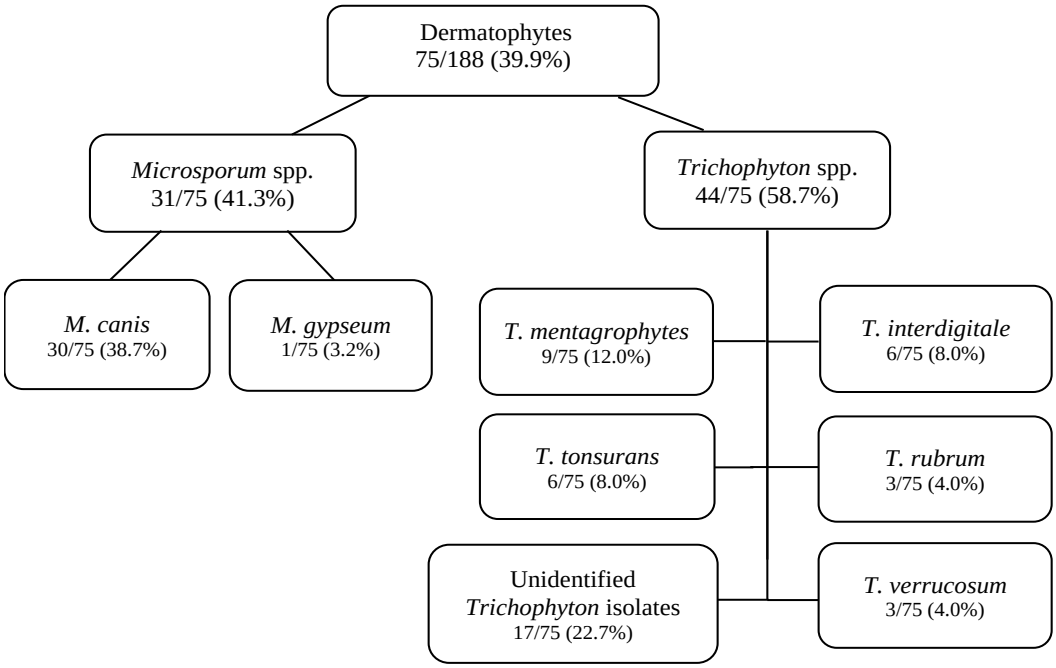

Figure 3. Dermatophyte species isolated from materials of patients with SFI

Discussion

The prevalence of fungal species causing SFI, as well as the localization of fungal infections, varies depending on the geographic, socio-economic, ecological, and climatic characteristics of a particular region, alongside the habits and traditions of its inhabitants. Given the lack of rapid fungal diagnostic tests, diagnoses are primarily based on clinical findings, while therapy is empirical, and chosen based on recommended treatments (8–12). Monitoring the incidence and prevalence of the most common causes of SFI could assist dermatologists in selecting the most effective, primarily empirically-based therapy, as well as in implementing appropriate preventive measures to halt the spread of infectious diseases, including SFI.

The results of this study show a high cumulative incidence of SFI over the examined period, consistent with the incidence levels reported in earlier studies (13, 14). The prevalence of SFI is also notably high, with variations observed depending on the year of research, particularly evident in 2020 and 2021. This is understandable, as these two years coincided with the COVID-19 pandemic, during which only patients with more intense, severe symptoms and indicative clinical findings were referred for mycological examination. Statistical analysis did not reveal a significant difference regarding gender, nor did the infections demonstrate seasonal patterns. However, a statistically significant age difference was observed, with a higher frequency of infections recorded in younger individuals. This can be attributed to the high number of children diagnosed with t. capitis among the study participants (15, 16).

Additionally, this study showed that SFI caused by yeasts were present in significantly more patients compared to dermatophytoses. This reflects a consistent upward trend in yeast SFI prevalence, previously recorded only as sporadic infections, increasing to 30%, then to 50%, and now up to 60% (13, 14). Dermatophytes, formerly the dominant causative agents accounting for over 90% of cases, were identified as causes in approximately 40% of our study participants. Based on the results of the recent studies, it can be emphasized that the most common causes of SFI in European countries are species *T. rubrum, M. canis, T. mentagrophytes*, and *T. verrucosum* (1, 14). *Species M. audouinii, T. soudanense*, and *T. violaceum*, known as causes of so-called immigrational SFI, are rarely detected in this region, as their endemic zones are in Africa and some parts of Asia (16). However, it can be assumed that the incidence of mycoses caused by these species could increase due to ever-increasing migration and tourism.

The dynamic nature of the spectrum of dermatophyte fungi within a specific area is evidenced by the long-term monitoring of dermatophytoses in the Nišava district, which commenced in the 1950s. Over this extended period, the incidence of species causing dermatophytoses has changed. It evolved from epidemic occurrences of SFI caused by anthropophilic species (17) to a plethora of species including *T. verrucosum, T. violaceum, T. tonsurans, T. quinceaneum, T. rubrum, M. gypseum* and *M. ferrugineum* (18, 19), then to a significant increase in the prevalence of species *M. Canis*, which emerged as the predominant species in the 1990s, persisting until today. Until 2011, this species was responsible for causing SFI in more than half of the infected patients (13).

This trend continued in the later years (14), with zoophilic species *M. canis* and *T. mentagrophytes* being the most common causes of dermatophytoses in patients residing in the Nišava district, accounting for 60.1% of cases. *M. canis* has been recognized as the most common cause of dermatophytoses for quite some time, not only in this region but also in the former Yugoslavia countries, southeast European countries, and rural areas of economically developed European countries (10, 20, 21). The results of this study indicate that the prevalence of these species remains relatively stable, with both species still dominating as causative agents, accounting for as much as 50.7% of identified dermatophyte isolates. However, unlike the previous study covering the period from 2012 to 2017, species *T. tonsurans* and *T. verrucosum* were isolated among dermatophytes in this region, which had not occurred since the 1990s. Although the number of patients infected with these species is currently low, an increase in prevalence can be anticipated in the future, particularly among those infected by *T. tonsurans*, given its anthropophilic nature and the possibility of interhuman transmission (22, 23).

In addition to dermatophytes, SFI can be caused by yeasts. Previous studies involving patients from our region have indicated a decreasing prevalence of dermatophytoses relative to superficial candidosis, with nearly equivalent prevalence of SFI. However, contrary to previous findings, this study reveals a lower prevalence of *C. albicans* alongside an increasing prevalence of other yeasts. In previous research, *C. albicans* was identified as the causative agent in 55.6% of patients, whereas in the current study, this number has declined to 40.7%. Besides epidemiological significance, this information can be important for devising appropriate therapy regimens. While previous research on yeasts causing SFI did not demonstrate resistance of NAC species and newly classified yeasts to antifungal medicines (4), the prevailing opinion is that NAC species exibit a more frequent occurrence of resistance, lower sensitivity, as well as dose-dependent sensitivity to antifungal medicines. The widely accepted division of the *Candida* genus into *C. albicans* and NAC species may soon be discarded as recent extensive phylogenetic studies on its members have prompted reclassification. As a result, the mentioned NAC species no longer

belong to this genus, which explains their lower sensitivity to antifungal agents and discrepancies in sensitivity/resistance. Species *C. albicans*, *C. parapsilosis,* and *C. tropicalis* remain classified in the *Candida* genus (6), while others, due to their characteristics, have been assigned to different or newly created genera. For example, the widespread species C. glabrata was added to the *Nakaseomyces (N.)* genus, and its name is now changed to *N. glabrata*. Similarly, one of the most prevalent species for human pathology, *C. krusei*, was reclassified into the *Pichia (P.*) genus, so now it is referred to as *P. kudriavzevii*. Species *C. guilliermondii* and *C. kefyr* have also been reassigned and are now members of the genera *Meyerozyma (M. guilliermondii*) and *Kluyveromyces (K. marxianus*) (5). However, the newly determined classification of yeasts poses a new challenge in medical mycology. Adopting and defining isolates according to the new taxonomy could confuse clinicians when interpreting mycological analyses. Many authorities argue against changing the old names of yeasts, now classified into separate genera. Alternatively, at this point, retaining the old nomenclature while writing the new names in parentheses is suggested. Additionally, the clinically recognizable entity of superficial candidosis, recognized for many years, will also change, whether using "superficial fungal infection" or, more precisely, "superficial yeast infection".

Considering that conventional diagnosis of SFI often requires a long time, there is an effort to introduce and develop new, rapid molecular methods, such as multiplex PCR, to detect fungi directly in patient samples (24, 25). Also, for more accurate identification of the causative agent, in recent years, the MALDI TOF (matrix-assisted laser desorption/ionization-time of flight) method has become very useful in diagnostics since it identifies yeasts and molds at the species level based on the detection of specific fungal proteins (26). All these methods aim to contribute to the rapid diagnosis of SGI as well as the adequate treatment of these infections.

Conclusion

Results of this retrospective study revealed a significantly high prevalence (30.2%) and cumulative incidence (26/105 inhabitants) of SFI. The high prevalence of zoophilic species, *M. canis* and *T. mentagrophytes*, emphasize the need for epidemic and epizootic preventive measures in order to stop the spread of dermatophytoses through diagnostics and treatment of not only people but infected animals as well. The emergence of anthropophilic species, not documented in the past 30 years, calls for constant monitoring of these SFI due to their potential to cause infections with epidemic characteristics. Ever-higher numbers of patients with superficial candidosis caused by NAC species demand mycological analyses in order to determine the cause and examine the *in vitro* effectiveness of antimycotics.

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PREVALENCIJA I DISTRIBUCIJA VRSTA UZROČNIKA SUPERFICIJALNIH GLJIVIČNIH INFEKCIJA U NIŠAVSKOM OKRUGU U SRBIJI

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Procena distribucije vrsta uzročnika infektivnih bolesti ključna je u slučajevima infekcija čije se lečenje sprovodi empirijski, bez laboratorijskih dokaza.

Cilj ovog rada bio je da utvrdi prevalenciju superficijalnih gljivičnih infekcija (SGI) i da identifikuje njihove najčešće uzročnike.

Za potrebe ove studije sproveden je mikološki pregled uzoraka kože i adneksa bolesnika sa kliničkim simptomima i znacima SGI-ja. Za detekciju i identifikaciju uzročnika korišćene su standardne mikološke metode – mikroskopija i kultivacija. Dermatofiti su identifikovani na osnovu makroskopskih i mikroskopskih morfoloških karakteristika do nivoa roda ili vrste, dok je identifikacija vrsta kvasnica obavljena komercijalnim testom *Integral System Yeasts plus* (Liofilchem®, Italija). Pri statističkoj obradi podataka korišćene su metode deskriptivne i kvantitativne analize (*SPSS* 14.0 za *Windows* 2003).

Statističkom analizom utvrđena je visoka prevalencija SGI-ja (30,2%); pritom, uočena je značajna razlika u pogledu starosti bolesnika ($p < 0,001$), ali nije zabeležena značajna razlika kada je reč o polu bolesnika ($p = 0,504$). Osim toga, SGI nije pokazao sezonski obrazac ($p = 0,783$). Superficijalne gljivične infekcije potvrđene su izolacijom i identifikacijom kvasnica kod 188 bolesnika (15,1%). *Candida* spp. izolovana je kod 113 bolesnika (60,1%); *Candida albicans* identifikovana je kao uzročnik superficijalne kandidijaze kod 46 bolesnika (40,7%), a ne-*albicans Candida* (NAC) vrste otkrivene su kod značajno većeg broja bolesnika (59,3%). Dermatofitoze su dijagnostikovane kod 75 bolesnika (39,9%), a dominantna vrsta bila je *Microsporum canis* (38,7%).

Viša incidencija SGI-ja čiji su uzrok NAC vrste ukazuje na to da su mikološke analize potrebne da bi se utvrdila etiologija SGI-ja i procenila *in vitro* efikasnost antigljivičnih lekova. Značajna zastupljenost zoofilnih vrsta dermatofita ukazuje na potrebu za osmišljavanjem strategije sprovođenja epidemioloških i epizootioloških preventivnih mera.

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***Ključne reči**: superficijalne gljivične infekcije, prevalencija, dermatofiti, Candida* spp.