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Original Article

Frequency of mycological diagnosis in a clinical laboratory in the city of Pelotas, south of Brazil, during the three-year period (2020-2023)

Frequência de diagnóstico micológico em um laboratório clínico da cidade de Pelotas, sul do Brasil, durante o período de três anos (2020-2023)

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Abstract

The increasing prevalence of immunosuppressive diseases, the increase in the elderly population, and globalization have led to the urgent need for research on the epidemiology and prevalence of fungi that cause mycoses in Brazil and worldwide. This is especially important, in the case of Brazil, for the South region, which currently lacks updated data on the subject. To this end, the objective was to investigate fungi diagnosed in mycoses by a laboratory in the city of Pelotas, South of Brazil. An observational and analytical survey was conducted for three years to analyze fungal diagnoses in the laboratory. Statistical analyses were performed by using SPPS and Joinpoint software. In total, 1220 fungal diagnoses were conducted, with 169 (13,8%) in 2020, 399 (32,7%) in 2021, 470 (38,5%) in 2022, and 182 (15,0%) in 2023, showing an increase in diagnoses over the years, with women seeking more diagnoses than men. Spring and summer were the seasons with the most diagnoses, and cutaneous and opportunistic mycoses were the most prevalent. Dermatophytes were the most diagnosed, followed by *Candida* spp. However, there was an increase in non-dermatophyte fungi diagnosed for onychomycosis. Therefore, despite the increase in the number of diagnoses over the years, it is important and necessary to establish standards for diagnosis in Brazil and worldwide for fungal diseases, especially the mandatory notification of serious mycoses. This will raise awareness in the healthcare sector and the public about the importance of diagnosing and treating fungal infections, which cause many fatalities every day.

Keywords: fungal diseases, epidemiology, fungi, mycoses.

Resumo

A globalização, o aumento das doenças de caráter imunossupressor e da população de idosos fazem com que haja necessidade de estudos sobre a epidemiologia e prevalência dos fungos que causam micoses no Brasil e no mundo. No Brasil, é de suma importância, principalmente na região Sul, a qual não possui dados atualizados a respeito. Logo o objetivo foi investigar amostras com solicitação para diagnóstico fúngico em um laboratório da cidade de Pelotas, no sul do Brasil. Foi realizada um estudo observacional e analítico durante três anos para analisar todas as solicitações para suspeitas de micoses. As análises estatísticas foram realizadas nos softwares Joinpoint e SPSS. Ao todo foram realizados 1220 diagnósticos fúngicos, sendo 169 (13,8%) em 2020, 399 (32,7%) em 2021, 470 (38,5%) em 2022 e 182 (15,0%) em 2023, mostrando um aumento no número de diagnósticos ao longo dos anos, as mulheres buscaram mais atendimentos que os homens. A primavera e o verão foram as estações com maior número de diagnósticos e as micoses cutâneas e oportunistas foram as mais prevalentes. Os dermatófitos foram os fungos mais diagnosticados, seguido por *Candida* spp., além disso houve um aumento de outras espécies de fungos não dermatófitos principalmente em onicomicoses. Portanto, apesar do aumento do número de diagnósticos ao longo dos anos, se faz necessário estabelecer padrões para diagnósticos fúngicos no Brasil e no mundo, especialmente a notificação obrigatória para micoses mais graves, aumentando a conscientização no setor de saúde e no setor público sobre a importância do diagnóstico e tratamento das infecções fúngicas, as quais causam muitos óbitos todos os dias.

Palavras-chave: infecções fúngicas, epidemiologia, fungos, micoses.

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1. Introduction

Mycoses (MY) are diseases caused by fungi and predominantly observed in tropical climates. Currently, there are approximately 400 species of fungi that cause pathologies in humans. Mild fungal infections (FI) affect approximately one billion people worldwide, while millions suffer from more severe forms of FI (Chen et al., 2018; Bongomin et al., 2017; de Albuquerque Maranhão et al., 2019). In recent years, there has been a notable increase in the prevalence of FI. This can be attributed to several factors, including the increase in the incidence of immunosuppressive diseases such as diabetes, cancer, human immunodeficiency virus (HIV), hepatitis C, tuberculosis and coronavirus disease (SARS-CoV). Other factors such as global warming, poor socioeconomic conditions, increase in the number of domestic animals and the aging global population have also contributed to this increase (Melo et al., 2020; Yoon et al., 2014; Oliveira et al., 2023; Reiss et al., 2011).

MY can be categorized as superficial (SM), cutaneous (CM), subcutaneous (SCM), systemic (SYM), and opportunistic (OM). The classification of these diseases depends on the type of fungus causing the infection, the site of infection, and the status of the patient's immune system (Howell, 2023; Gnat et al., 2021). The main fungi that cause SM are the genera *Hortae werneckii*, *Malassezia* spp., *Piedra hortae*, and *Trichosporon* spp. (Veasey et al., 2017). For CM the main agents are of the genus *Trichophyton* spp., *Nanizzia gypsea*, *Microsporon canis*, and *Epidermophyton floccosum* (Deng et al., 2023; Lombardi et al., 2020). SM and CM affect the outermost layer of the skin, mainly the epidermis, hair, and nails (Melo et al., 2020; Howell, 2023). These types of MY are mainly caused by dermatophyte fungi (Veasey et al., 2017; Segal and Frenkel, 2015).

The main agents for SCM are *Sporothrix* spp., *Fonsecae pedrosoi*, *Cladophialophora carrionii*, and *Madurella* spp. (Bertin et al., 2024; Valentin et al., 2024), are caused by the implantation of fungi, requiring an entry point, and the lesions typically occur in the dermis and subcutaneous tissues (Lombardi et al., 2020; Jahr et al., 2022; Sendrasoa et al., 2023). For SYM, the main agents are *Histoplasma* spp., *Paracoccidioides* spp., *Blastomyces* spp. and *Coccidioides* spp. (Abad and Razonable, 2024), are caused by saprophytic and dimorphic fungi that take advantage of the host's compromised immune system to develop. These types of MY are restricted to certain geographic areas, though globalization is facilitating their spread to other environments (Salzer et al., 2018; Lockhart et al., 2021; Caceres et al., 2022).

Finally, OM, which depend exclusively on the patient's immunosuppression state to manifest themselves, are caused by different fungal genera and can range from mild to invasive and disseminated MY, which are extremely lethal (Spallone and Schwartz, 2021; Tufa et al., 2023). The main ones described are *Candida* spp., *Cryptococcus* spp., *Aspergillus* spp., *Fusarium* spp., *Penicillium* spp., *Mucorales* spp., *Pneumocystis jirovecii*, among other dematiaceous and hyaline fungi that may cause MY of this type (Magda, 2024; Rodríguez-Vargas et al., 2024). It is well known that the great challenge of OM is to differentiate them as a possible contaminant from a pathogen, as is the case with Aspergillus spp. and Penicillium spp. that live dispersed in the air and in the environment, and Candida that is part of our microbiome, but we also know their pathogenic potential, thus requiring qualified professionals to make an adequate diagnosis (Naik et al., 2024; Suehara and Silva, 2023).

Currently, there is a lack of accurate data on the diagnosis of fungal diseases in Brazil and worldwide. Although several studies indicate a global increase in the incidence of MY, these statistics are inadequate, mainly because MY is often neglected and underreported, particularly in cases of severe CM, SYM, and OM (Huh et al., 2017; Milani et al., 2023).The lack of accurate diagnosis and appropriate

treatment of FI has resulted in over 1.5 million fatalities annually due to the disease (Ahmed et al., 2023). According to Bongomin et al. (2017), 80% of severe FI patients could be saved if adequate fungal diagnostic (FD) and potent antifungals were available to the public. Therefore, an epidemiological investigation into fungal diseases is crucial in the present scenario, where the incidence of FI is growing every day. The inaccuracy of statistics is primarily due to the lack of trained professionals for diagnosis and non-mandatory reporting for severe MY. Moreover, there is a possibility that a larger number of fungi, which have not yet been identified, cause FI, necessitating a comprehensive and specific study for each country, state, and city to determine the prevalence of MY in each population. Thus, the present study aims to study the frequency of FI in the city of Pelotas, RS, Brazil, to fill the lack of data in the region and enable the identification of the main MY and fungal agents that cause diseases in this population.

2. Methods

From May 2020 to May 2023, an observational and analytical study of fungal culture test requests performed in a clinical analysis laboratory was carried out in a private clinical analysis laboratory in the city of Pelotas, state of Rio Grande do Sul, Brazil. To characterize the place of study, there are two types of health systems in Brazil: a public one, called the Unified Health System (SUS), which is paid in full by the country's government for all Brazilians, and the private system, which is part of our study environment, made up of health plans and insurance companies that offer medical care and hospitalization to their users, and private care, where the patient pays for his or her treatment or hospitalization in its totality (Silva et al., 2020; *A saúde no Brasil em 2030: estrutura do financiamento e do gasto setorial - Vol. 4* 2013).

These diagnoses are performed by seeding the collected materials in two culture media, sabouraud and mycosel, with the possibility of also using Brain Heart Infusion (BHI) medium for incubation at 27ºC for 7-15 days, where the growth or absence of fungi is observed.

2.1. Scope and spatial characteristics of the study

This laboratory serves outpatients and inpatients from the city of Pelotas and nearby smaller cities, such as Arroio do Padre, Capão do Leão, Canguçu, Morro Redondo, Rio Grande, São Lourenço do Sul and Turuçu. Pelotas is the fourth most populous city in the state, with 325,689 inhabitants (IBGE, Pelotas (RS): IBGE, 2022). It has an area of 1,610.091 km^2 and a population density of 203.89 inhabitants per km^2. Located at the coordinates: Latitude 31º 46' 10" S and longitude 52º 20' 33" W, 250 km from the state capital. The city's climate is classified as humid subtropical, with rainfall well distributed throughout the year. Summers are warm. The absolute maximum temperatures of the year are between 35°C and 36°C. Winters are relatively cold, with frequent frosts (an average of 20 per year) and dense fog from May to August, with absolute minimum temperatures of the year between -1°C and 0°C. The annual averages are: Temperature: 17.5°C, Relative humidity: 80%, Rainfall: 1,379 mm and Winds: 3.2 m/s (IBGE. Censo Brasileiro de 2022. Pelotas (RS): IBGE, 2022).

The life expectancy in the city is 72.6 years (70.1 for men and 74.5 for women). The ethnic distribution is 80.25% white, 10.68% black, 8.6% mixed race, 0.15% indigenous and 0.32 yellow. Distribution by gender: 53% women and 47% men (Viva o Charque, 2025).

2.2. Data collection

For the period in question, the search for diagnostic results for fungal cultures was carried out using the Tasy Philips® program, which is utilized by the laboratory to release patient reports. The initial search was performed using the Microsoft Excel (2021) program, and data was entered, including the patient's prescription number (used to identify the patient in the laboratory), the date of result release, the patient's sex and age, the type of material collected, and the result of the FD as reported. A second spreadsheet was created to facilitate statistical analysis, adding information regarding season, age group, and classification of the type of mycosis for each patient.

2.3. Statistical analysis

Statistical analysis was performed using the data collected in the Tasy Philips® program and spreadsheeted in the Microsoft Excel 2021 program. The variables analyzed included the date and year of diagnosis, season, sex and age group of patients, types of materials collected, diagnoses, and classification of MY. The association between the independent variables in the study, including sex, age, age group, and season, and the dependent variables, including type of material collected and diagnosis, was assessed using chi-square tests, odds ratios, and the calculation of their 95% confidence intervals (95% CI). Trend assessment was performed using the linear regression test, and prevalence analyses were conducted regarding the classification of MY.

Two programs were used for statistical analysis, including Joinpoint Regression 5.0.2 for the time trend test, which analyzed the number of fungal diagnoses over the three years, and IBM® SPSS 20.0 for the chi-square test, odds ratio calculation, and prevalence rates for CM and OM.

2.4. Ethics statement

The authors confirm that the ethical policies of the journal, as noted on the journal's author guidelines page, have been adhered to and the appropriate ethical review committee approval has been received. The US National Research Council's guidelines for the Care and Use of Laboratory Animals were followed. The project was granted approval by the Research Ethics Committee of the Faculty of Medicine affiliated with the Federal University of Pelotas under the registration number 6.504.927.

3. Results

Over the course of the study, there were 1220 requests for FD, of which 900 (73.8%) had fungal growth (FG) and 320 (26.2%) did not have FG. Of these requests, 98.2% were from outpatients and 2% from inpatients. The linear regression test clearly shows an increase in the number of fungal diagnoses over the years of the study ($p < 0.05$) (Figure 1).

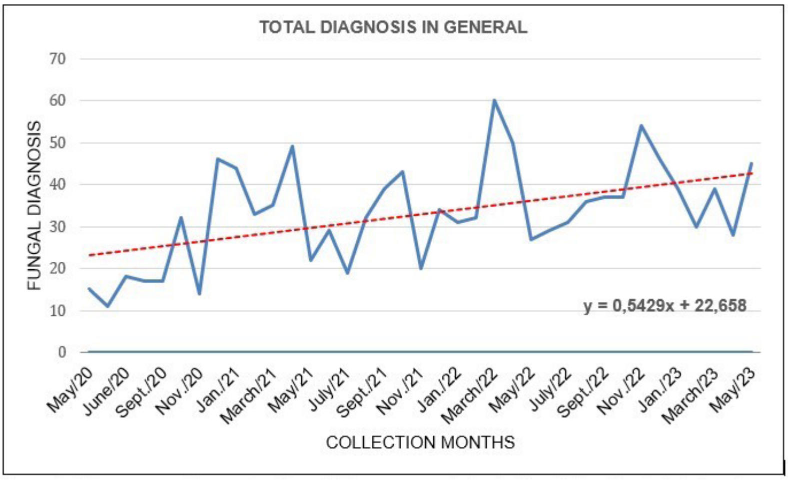

Figure 1. Linear regression graph of fungal diagnoses carried out at the Clinical Analysis Laboratory in the city of Pelotas - RS - Brazil over three year (2020-2023).

According to the months, it is possible to visualize those with the highest and lowest number of collections over the course of each year. Concerning the seasons, we can see that the seasons with the lowest number of diagnoses were between autumn and winter, and those with the highest number of diagnoses had greater variations between the warmer and less warm seasons. When analyzing the general diagnoses in relation to the three years of study, it can be seen that March (134/11.0%) was the month with the highest number of fungal diseases, and June (69/5.7%) with the lowest number. For the seasons, we had 310 (25.4%) diagnoses in autumn, 247 (20.2%) in winter, 336 (27.5%) in spring and 327 (26.8%) in summer.

Concerning the sex of the patients, 728 (59.7%) were female and 492 (40.3%) male, and these figures showed a significant difference ($p < 0.05$). Ages ranged from 0 to 95 years with an average of 46.85 years. Figure 2 shows the distribution of age groups according to sex.

As for the age groups and seasons, there was no significant seasonal relationship, but for children, adolescents, and the elderly there were more diagnoses in the fall and for adults in the spring (Table 1).

As for the site of the material collected, the results are shown in Figure 3, for the skin material, the samples were obtained by scraping the edge of the lesions, and in cases where there were wounds, in addition to scraping the skin, they were also collected concomitantly with a swab for transporting the exudates, but we do not have data on how many materials these two types of collection were carried out, since this is a survey of data from reports and only one of the skin samples was collected surgically, resulting, after the incubation time of the culture, in the growth of the fungus *Sporothrix* spp.. There was a significant difference ($p<0.05$) for nail scrapings, which showed more diagnoses in women than in men, with an OR value of 2.247 (CI 1.773 – 2.848). For skin scrapings, there was an OR of 0.720 (CI 0.544 – 0.952) with a higher frequency of diagnoses for men, for genital secretions OR of 0.125 (CI 0.060 – 0.258), for bronchoalveolar lavage or sputum OR 0.524 (CI 0.327 – 0.842) and finally for secretions in general OR of 0.381 (CI 0.158 – 0.914). The relationship between the type of material collected and the sex of the patients can be seen in Figure 4.

The relationship between age groups and types of materials is shown in Table 2. In the chi-square test, we obtained significant values ($p<0.05$) for children's collection materials for nails, skin scrapings with an OR of 5.403 (CI 3.066 – 9.520), eye secretions with an OR of 3.987 (CI 1.302 – 9.921) and feces with an OR of 46.680 (CI 4.163 – 523.430). For adolescents, we obtained significant values ($p<0.05$) for nail collection and skin scraping with an OR of 2.147 (CI 1.129 – 4.086) and bronchoalveolar

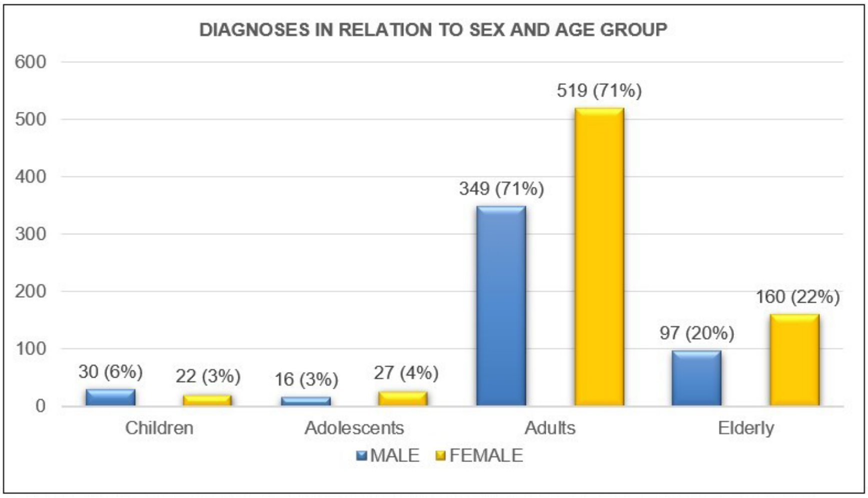

Figure 2. Distribution of requests for fungal diagnosis carried out in a Clinical Analysis Laboratory in the city of Pelotas - RS - Brazil over three years (2020-2023) for males and females according to the age group of the patients.

Table 1. Distribution of requests for fungal diagnosis carried out in a Clinical Analysis Laboratory in the city of Pelotas – RS – Brazil over three years (2020-2023) in relation to age group and seasons.

	Autumn	**%**	**Winter**	**%**	**Spring**	**%**	**Summer**	**%**
Children	17	5.5%	7	2.8%	12	3.6%	16	4.89%
Adolescents	14	4.5%	8	3.2%	8	2.4%	13	3.98%
Adults	219	70.6%	186	75.3%	243	72.3%	220	67.28%
Elderly	60	19.4%	46	18.6%	73	21.7%	78	23.85%
Total	310		247		336		327	

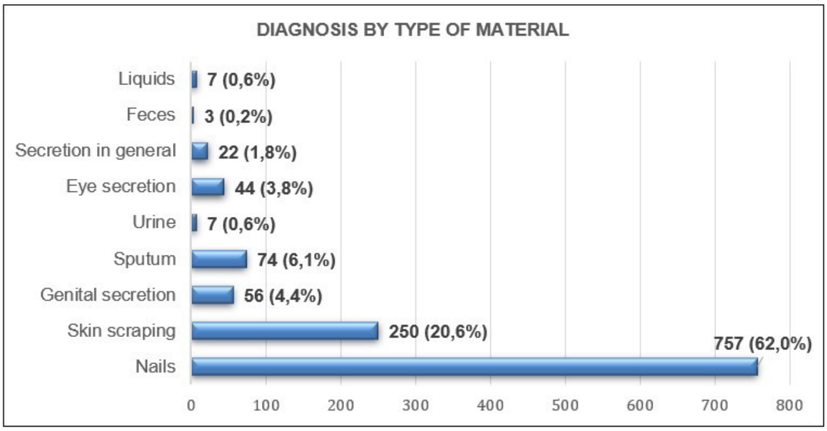

Figure 3. Number of requests for fungal diagnosis carried out in a Clinical Analysis Laboratory in the city of Pelotas - RS - Brazil over three years (2020-2023) according to the type of material sent for cultivation.

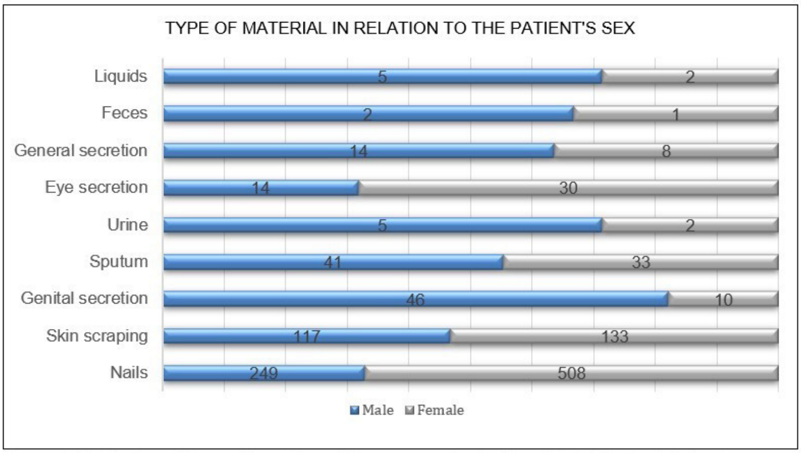

Figure 4. Distribution of the number of fungal diagnoses carried out in a Clinical Analysis Laboratory in the city of Pelotas - RS - Brazil over three years (2020-2023) according to the patient's sex and type of material sent.

Table 2. Material collected for fungal diagnosis carried out in a Clinical Analysis Laboratory in the city of Pelotas – RS – Brazil over three years (2020-2023) according to the age group of the patients.

Type of Material	Children (0-12 years old)	%	Adolescents (13-18 years old)	%	Adults (19- 65 years old)	%	Elderly (From 66 years old)	%
Nails	9	17.3	19	44.2	573	66.1	156	60.7
Skin scraping	29	55.8	15	34.9	167	19.3	39	15.2
Genital secretion	2	3.8	0	0.0	54	6.0	0	0.0
Bronchoalveolar lavage or sputum	3	5.8	6	14.0	30	3.5	35	13.6
Urine	0	0.0	1	2.3	5	0.6	1	0.4
Eye secretion	6	11.5	0	0.0	20	2.3	18	7.0
Secretions in general	1	1.9	2	4.7	15	1.7	4	1.6
Feces	2	3.8	0	0.0	0	0.0	1	0.4
Liquids in general	0	0.0	0	0.0	4	0.5	3	1.2
Total	52	100	43	100	868	100	257	100

lavage or sputum with an OR of 2.645 (CI 1.079 – 6.483). For adults, the significant values ($p<0.05$) regarding the type of material were for fingernails with an OR of 1.763 (CI 1.370 – 2.268), genital secretion with an OR of 11.061 (CI 2.679 – 45.664), for bronchoalveolar lavage or sputum, for eye secretion and feces and for the elderly, the significant values ($p<0.05$) were significant for skin scrapings, genital secretions, bronchoalveolar lavage with OR 3.714 (CI 2.300 – 5.998) and eye secretions with OR 2.811 (CI 1.509 – 5.237).

Concerning the FD during the study, of the 900 results with FG we had 33 different fungal genera (Table 3), the most prevalent being, in order of citation, *Trichophyton rubrum* (n= 319/18.9%) is a dermatophyte, *Candida* spp. (n= 185/15.2%) an OF that can also cause skin MY, and *Trichophyton mentagrophytes* (n= 184/15.1%) another dermatophyte with fewer diagnoses. However, opportunistic fungi (OF) such as *Curvularia* spp. (n= 43/3.5%), *Aspergillus* spp. (n= 37/3.0%), *Cladosporium* (n=26/2.1%), *Penicillium* spp. (n=22/1.8%), and *Fusarium* (n= 21/1.7%) also show significant numbers.

As for the classification of types of MY, of the 1220 (100%) FD, 08 (0.7%) were classified as SM, 444 (36.4%) CM, 14 (1.1%) SCM, 02 (0.2%) SYM and 432 (35.4%) OM. Of these classifications, it was possible to calculate the prevalence only for CM and OM; for the others, due to the low number of diagnoses, the calculation became irrelevant. There were 343 CM and 278 OM in nail scrapings, 97 CM and 66 OM in skin scrapings, 03 CM and 19 OM in genital secretions, 45 OM in bronchoalveolar lavage or sputum, 14 OM in eye secretions, 01 CM and 08 OM in secretions in general, 02 OM in feces and no MY.

The main fungi diagnosed according to the type of material and the classification of MY are shown in Table 4. For fungi that cause SM, the most diagnosed agent was *Malassezia* spp. in skin scrapings, for CM the most diagnosed were *Trichophyton rubrum* on skin and nails, followed by *Trichophyton mentagrophytes* and in smaller numbers *Epidermophyton flocossum*, for SCM, *Cladophialophora carrionii* causing chromoblastomycosis was the most diagnosed fungus followed by *Sporothrix* spp. causing sporotrichosis, both in skin material. For SMI, there were only two diagnoses, both for *Paracoccidioides* spp. in bronchoalveolar lavage. For OM, the yeast *Candida* spp. predominated in the diagnoses for bronchoalveolar lavage and sputum, skin, genital secretion, and nails, for ocular and general secretion materials Aspergillus spp. was the most prevalent, and for nail material, there was also a significant number of diagnoses of *Curvularia* spp., *Cladosporium* spp., *Aspergillus* spp., *Neocytalidium dimidiatum*, *Fusarium* spp., and *Trichosporon* spp.

For the chi-square test, the following materials were significant ($p<0.05$) for the diagnosis of CM: nail scrapings with an OR of 2.864 (CI 2.209 – 3.714), genital secretions, eye secretions, secretions in general and liquids in general, while for OM, skin scrapings and broncho-alveolar lavage or sputum were significant ($p<0.05$) with an OR of 3.091 (CI 1.908 – 5.009).

As for the classification of MY and the seasons, 103 (23.2%) CM and 97 (22.7%) OM were diagnosed in the fall, 74 (16.4%) CM and 96 (29.2%) OM in the winter,

Table 3. Fungal genera diagnosed over the three years (2020-2023) in a Clinical Analysis Laboratory in the city of Pelotas – RS – Brazil according to the different types of materials analyzed (continues).

Diagnosis	Number of diagnosis	%
Negative *(no growth)*	320	26.2
Trichophyton rubrum	231	18.9
Candida spp.	185	15.2
Trichophyton mentagrophytes	184	15.1
Curvularia spp.	43	3.5
Aspergillus spp.	37	3.0
Cladosporium spp.	26	2.1
Rhodoturola spp.	22	1.8
Penicillium spp.	22	1.8
Fusarium spp.	21	1.7
Epidermophyton floccosum	20	1.6
Trichosporum spp.	20	1.6
Neoscytalidium dimidiatum	19	1.6
Cladophialophora carrionii	10	0.8
Scytalidium hyalinum	10	0.8
Acremonium spp.	9	0.7
Malassezia spp.	6	0.5
Alternaria spp.	5	0.4
Trichophyton tonsurans	4	0.3
Scopulariopsis spp	3	0.2
Aureobasidium pullulans	2	0.2
Bipolaris spp.	2	0.2
Geotrichum spp.	2	0.2
Hortaea werneckii	2	0.2
Paracoccidioides spp.	2	0.2
Rhizopus spp.	2	0.2
Scedosporium spp	2	0.2
Sporothrix spp	2	0.2
Trichophyton violaceum	2	0.2
Phialophora verrucosa	1	0.1
Fonsecaea pedrosoi	1	0.1
Microsporum canis	1	0.1
Nanizzia gypsea	1	0.1
Trichophyton schoenleinii	1	0.1
TOTAL	1220	100

Table 4. Fungi diagnosed in a Clinical Analysis Laboratory in the city of Pelotas – RS – Brazil over three years (2020-2023) according to type of material and classification of mycoses (continues).

Type of Mycoses	Material	Fungus diagnosed	Number of diagnoses	%
Superficial	Skin Scraping	*Malassezia* spp.	6	75
Superficial	Skin Scraping	*Hortaea werneckii*	2	25
		Total	8	100
Cutaneous	Skin Scraping	*Trichophyton rubrum*	53	54.6
Cutaneous	Skin Scraping	*Trichophyton mentagrophytes*	32	33
Cutaneous	Skin Scraping	*Epidermophyton floccosum*	8	8.2
Cutaneous	Skin Scraping	*Microsporum canis*	1	1
Cutaneous	Skin Scraping	*Nannizzia gypsea*	1	1
Cutaneous	Skin Scraping	*Trichophyton schoenleinii*	1	0.8
Cutaneous	Skin Scraping	*Trichophyton tonsurans*	1	0.8
		Total	97	100
Cutaneous	Secretion in General	*Trichophyton mentagrophytes*	1	100
		Total	1	100
Cutaneous	Genital Secretion	*Trichophyton mentagrophytes*	3	100
		Total	3	100
Cutaneous	Nails	*Trichophyton rubrum*	178	51.9
Cutaneous	Nails	*Trichophyton mentagrophytes*	148	43.1
Cutaneous	Nails	*Epidermophyton floccosum*	12	3.5
Cutaneous	Nails	*Trichophyton tonsurans*	3	0.9
Cutaneous	Nails	*Trichophyton violaceum*	2	0.6
		Total	343	100
Subcutaneous	Skin scraping	*Cladophialophora carrionii*	10	71.4
Subcutaneous	Skin scraping	*Sporothrix* spp.	2	14.3
Subcutaneous	Skin scraping	*Phialophora verrucosa*	1	7.1
Subcutaneous	Skin scraping	*Fonsecaea pedrosoi*	1	7.1
		Total	14	100
Systemic	Sputum	*Paracoccidioides* spp.	2	100
		Total	2	100
Opportunistic	Feces	*Candida* spp.	2	100
		Total	2	100
Opportunistic	Sputum	*Candida* spp.	31	68.9
Opportunistic	Sputum	*Penicillium* spp.	8	17.8
Opportunistic	Sputum	*Aspergillus* spp.	3	6.7
Opportunistic	Sputum	*Cladosporium* spp.	1	2.2
Opportunistic	Sputum	*Fusarium* spp.	1	2.2
Opportunistic	Sputum	*Rhizopus* spp.	1	2.2
		Total	45	100.00
Opportunistic	Skin scraping	*Candida* spp.	35	53
Opportunistic	Skin scraping	*Curvularia* spp.	5	7.6
Opportunistic	Skin scraping	*Aspergillus* spp.	4	6.1
Opportunistic	Skin scraping	*Cladosporium* spp.	3	4.5

Table 4. Continued...

Type of Mycoses	Material	Fungus diagnosed	Number of diagnoses	%
Opportunistic	Skin scraping	*Fusarium* spp.	3	4.5
Opportunistic	Skin scraping	*Scytalidium hyalinum*	3	4.5
Opportunistic	Skin scraping	*Trichosporon* spp.	3	4.5
Opportunistic	Skin scraping	*Rhodotorula* spp.	2	3
Opportunistic	Skin scraping	*Penicillium* spp.	2	3
Opportunistic	Skin scraping	*Scedosporium* spp.	2	3
Opportunistic	Skin scraping	*Scopulariopsis* spp	2	3
Opportunistic	Skin scraping	*Neocytalidium dimidiatum*	1	1.5
Opportunistic	Skin scraping	*Rhizopus* spp.	1	1.5
		Total	66	100
Opportunistic	Genital secretion	*Candida* spp.	16	84.2
Opportunistic	Genital secretion	*Rhodotorula* spp.	3	15.8
		Total	19	100
Opportunistic	Eye secretion	*Aspergillus* spp.	8	57.1
Opportunistic	Eye secretion	*Candida* spp.	3	21.4
Opportunistic	Eye secretion	*Alternaria* spp.	1	7.1
Opportunistic	Eye secretion	*Cladosporium* spp.	1	7.1
Opportunistic	Eye secretion	*Rhodotorula* spp.	1	7.1
		Total	14	100
Opportunistic	Secretions in general	*Aspergillus* spp.	4	50
Opportunistic	Secretions in general	*Candida* spp.	2	25
Opportunistic	Secretions in general	*Cladosporium* spp.	1	12.5
Opportunistic	Secretions in general	*Geotrichum* spp.	1	12.5
		Total	8	100
Opportunistic	Nails	*Candida* spp.	95	34.3
Opportunistic	Nails	*Curvularia* spp.	38	13.7
Opportunistic	Nails	*Cladosporium* spp.	20	7.2
Opportunistic	Nails	*Aspergillus* spp.	18	6.5
Opportunistic	Nails	*Neocytalidium dimidiatum*	18	6.5
Opportunistic	Nails	*Fusarium* spp.	17	6.1
Opportunistic	Nails	*Trichosporon* spp.	17	6.1
Opportunistic	Nails	*Rhodotorula* spp.	16	5.8
Opportunistic	Nails	*Penicillium* spp.	12	4.3
Opportunistic	Nails	*Acremonium* spp.	9	3.2
Opportunistic	Nails	*Scytalidium hyalinum*	7	2.5
Opportunistic	Nails	*Alternaria* spp.	4	1.4
Opportunistic	Nails	*Aureobasidium pullulans*	2	0.7
Opportunistic	Nails	*Bipolaris* spp.	2	0.7
Opportunistic	Nails	*Geotrichum* spp.	1	0.4
Opportunistic	Nails	*Scopulariopsis* spp.	1	0.4
		Total	277	100

Table 5. Distribution of diagnoses and types of mycoses according to the gender of the patients, age groups, and type of material collected over the three years of research at the Clinical Analysis Laboratory in the city of Pelotas, Rio Grande do Sul, Brazil.

Year	Sex	Age group	Type of material	Diagnosis	Type of mycoses
2020	Female	Elderly	Liquids in general	*Candida* spp.	Opportunistic
2021	Male	Adults	Secretions in general	*Aspergillus* spp.	Opportunistic
2022	Male	Adults	Secretions in general	*Aspergillus* spp.	Opportunistic
	Female	Children	Secretions in general	*Candida* spp.	Opportunistic
	Male	Adolescents	Secretions in general	*Penicillium* spp.	Opportunistic
	Male	Adults	Skin scraping	*Sporothrix* spp.	Subcutaneous
2023	Male	Elderly	Liquids in general	*Candida* spp.	Opportunistic
	Female	Elderly	Secretions in general	*Candida* spp.	Opportunistic

126 (28.2%) CM and 125 (29.2%) in the spring and 141 (32.2%) CM and 114 (25.76%) OM in the summer. The chi-square test proved to be significant ($p< 0.05$) for CM diagnosed in the winter with an OR of 0.679 (CI 0.502 – 0.918) and for CM in the summer with an OR of 1.536 (CI 1.186 – 1.989); however, in the seasonality test, there was no relationship between seasonality and types of MY.

Regarding the age groups and the classification into CM and OM in relation to the other classifications; it was not possible to calculate the other classifications due to the low number of diagnoses. For the age groups in relation to the types of MY, the chi-square test proved to be significant ($p< 0.05$) for the diagnosis of CM and OM, 12 (23.1%) children were diagnosed with CM with an OR of 0.500 (CI:0.259-0.963) and 18 (34.6%) were diagnosed with OM, 14 (32.6%) adolescents were diagnosed with CM and 15 (34.9%) with OM, 331 (38.4%) adults were diagnosed with CM and 287 (33.0%) with OM with OR 0.717 (CI 0.555 – 0.927) and for the elderly, 87 (33.7%) were diagnosed with CM and 112 (43.4%) with OM with OR 1.536 (CI 1.160 – 2.034).

And finally, regarding the hospitalized patients served by the laboratory, we had 22 (2,15%) requests for fungal diagnoses, with two (0,2%) in 2020, three (0,3%) in 2021, eleven (1,1%) in 2022, and six (0,6%) in 2023. Out of these, fourteen showed no FG during the incubation period, while eight did. Similar to the total diagnoses in the study, there was also an increase in requests for FDs for hospitalized patients over the years. As for the diagnosed fungi, we had four *Candida* spp diagnoses, two *Aspergillus* spp., and one each for *Penicillium* spp. and *Sporothrix* spp. other details are described in Table 5.

4. Discussion

This study provided unprecedented data on the frequency of FD in a private Clinical Analysis Laboratory in the city of Pelotas, southern Brazil. Private sector clinical laboratories are often well-equipped and state-of-the-art, offering a wide range of tests quickly and accurately. Access is faster, with generally shorter waiting times than in the SUS. However, the cost of testing can be significant without health insurance coverage (Silva et al., 2020; Santos et al., 2013). Private laboratories generally offer faster and more convenient services, but this depends on the patient's purchasing power. Despite its limitations, the SUS plays a crucial role in guaranteeing universal access to health care. Both sectors are regulated by the National Health Surveillance Agency (ANVISA) and follow established quality standards (RESOLUÇÃO RDC No 512, DE 27 DE MAIO DE 2021 - RESOLUÇÃO RDC No 512, DE 27 DE MAIO DE 2021 - DOU - Imprensa Nacional)

During the research period, there was an increase in the number of requests for fungal diseases over the years, as in other studies, which cite the high number of immunosuppressive diseases, globalization and global warming as drivers of this problem (Wang et al., 2020; Ma et al., 2021; Vallabhaneni et al., 2016; Almeida et al., 2019).

Regarding the prevalence of diagnoses, seasons of the year and classification of MY, there was no statistically proven seasonality relationship. According to Brito *et al.*, 2018 (Brito et al., 2018), who analyzed seasonality in relation to hospitalizations for dermatological diseases in a public hospital in Brazil, they show that hospitalizations for MI were also higher in summer and spring.

It is believed that the increase in diagnoses in warmer climates occurs because patients seek solutions for the appearance of lesions, especially on the skin and nails. Another factor that possibly contributes is that fungi proliferate and grow better in warmer climates, which could influence the greater number of fungal diseases (Fisher et al., 2020; Sharma and Nonzom, 2021). Regarding the classification into CM and OM, it was observed that CM occurs in a smaller proportion of 1:1.47 in winter and 1:1.54 more in summer for OM, probably occurring due to the variation in climate in our state, which has four different seasons (Veasey et al., 2017; Deng et al., 2023; Abad and Razonable, 2024; Tiwari et al., 2023).

Regarding FD and the patient's gender, we observed a greater demand for diagnosis by females. The same occurs in other studies carried out in Brazil, South Korea and in another that analyzed 195 countries and territories in 2017 (de Albuquerque Maranhão et al., 2019; Yoon et al., 2014; Martinez et al., 2020; Urban et al., 2021). This greater demand by women than by men probably occurs, as explained by Bidmon;Terlutter, 2015 (Bidmon and Terlutter, 2015) and Levorato *et al*., 2014 (Levorato et al., 2014) who analyzed factors associated with the demand for health services, showing that women take more care of their health, and men seek care in more extreme cases.

With regard to age demographics, adults and the elderly constituted the demographic with the highest demand for diagnostic assessments. Research conducted in Brazil, in the states of Alagoas and Rio Grande do Sul, also demonstrated a higher prevalence of diagnostic assessments within this age group. (de Albuquerque Maranhão et al., 2019; Pigatto, et al., 2019).

Regarding the materials analyzed in the research, the most requested in the study were nails, skin, sputum and vaginal secretion. In other studies, in Brazil on MY, such as the one in Alagoas, the most requested material was genital secretion, followed by nails and skin and, to a lesser extent, sputum (de Albuquerque Maranhão et al., 2019) .The lower number of requests for genital secretion in this study is probably due to the fact that yeast research in vaginal secretion is performed by fresh examination using Gram staining. In this case, it is not added to the data for fungal culture. For sputum materials, the highest rate observed is probably due to the fact that it is a private laboratory, where elderly patients tend to undergo more FDs for this type of disease (de Albuquerque Maranhão et al., 2019).In this case, there is no possibility of comparisons in relation to the different materials analyzed, as there are no other studies that encompass the research of different types of materials for fungal analysis.

Regarding sex and the type of material analyzed, it was observed that women were more likely to request nail scrapings, showing a 1:2.5 higher probability than men of performing this type of collection for fungal tests. The same occurred in another 30-year study on onychomycosis in mainland China, where women were 1:1.32 more likely to perform nail diagnoses (Song et al., 2022), as well as in another in Ireland in which women had 63% more nail collections compared to men (Powell et al., 2022). This higher frequency possibly occurs once again because women seek more health care and also because they are more predisposed to immunosuppressive diseases, especially those of autoimmune origin, which favors the emergence and proliferation of fungal diseases (Song et al., 2022; Desai and Brinton, 2019; Gutmann et al., 2022).

For males, in relation to the material analyzed, there is a probability of 1:1.39 compared to women, of diagnosis in the skin and, 1:1.91 for diagnosis in sputum material, possibly because men work more exposed to the environment, such as rural workers, gardeners, veterinarians and in civil construction, which favors contact with saprophytic fungi (Parija, 2023). For genital secretion, the ratio is 1:8 higher in men compared to women, as they use another type of diagnosis in the laboratory to search for yeast in this material (Sharma and Nonzom, 2021).

Children are more likely to collect skin, ocular secretion, and feces than other age groups. Children tend to have more MY caused by dermatophyte and OF (Aissat and Denning, 2023; Alvarado and Pereira, 2018), dermatophytes due to greater exposure and contact with domestic animals and soil, and OF due to the still-forming immune system (Vallabhaneni et al., 2016; Almeida et al., 2019; Peixoto et al., 2023; Liu et al., 2023; Haro et al., 2023). Skin MY in children, according to Urban et al. (2021), occur between 1 to 5 years old, for ocular secretions and feces in the OR test has shown to be significant, indicating a higher chance of FD in these types of materials, probably due to the fact that MO are more isolated in these materials, which are more likely to develop. For ocular secretions, according to Chandratreya *et al*., 2023 (Chandratreya et al., 2023), fungal keratitis in children represented 48.7% of diagnoses, which also explains the results found in our research. The highest probability of skin diagnosis was 2.14 times higher in adolescents compared to other age groups, possibly because skin mycosis agents are present in the environment and with domestic animals, which facilitates contagion in this group (Haro et al., 2023). For bronchoalveolar lavage or sputum, the rate was 2.64 times higher in this group compared to the others. In a study carried out in Romania, it was observed that children and adolescents showed a reduction in immunity after the COVID pandemic, since exposure to the environments was limited during that period. In the same study, co-infections of severe forms of influenza were diseases caused by bacteria and fungi (Merișescu et al., 2023), which could explain this result (Giacomazzi et al., 2016; Rodrigues and Nosanchuk, 2020).

For adults, the probability rate for diagnosis in nails was 1.76 times higher compared to other age groups and for genital secretion it was 11.06 times higher. The results are in agreement with studies carried out in Tehran (Iran) and Ireland, where there is a higher prevalence of fungal diseases in nails for adults (Powell et al., 2022; Zamani et al., 2016) and for genital secretion. In the study carried out in Alagoas (Brazil), adults also presented more diagnoses for this type of material (de Albuquerque Maranhão et al., 2019). In Spain, province of Granada, also where there was a high prevalence of requests for diagnosis for genital secretion in adults (Martínez-García et al., 2023).

For the elderly, there were 3.71 more requests for diagnosis for sputum materials and 2.81 for ocular secretion compared to the other age groups. These rates possibly occur because this group has a deficient immune system, with a greater chance of developing OM, which depend on a state of immunosuppression (Yoon et al., 2014; Caceres et al., 2022; Tufa et al., 2023).

When analyzing the results in Table 3, regarding the main fungi diagnosed in our research, we observed the predominance of the dermatophyte fungi *Trichophyton rubrum* (18.9%) and *Trichophyton mentagrophytes* (15.1%), and the yeast *Candida* spp. (15.2%), but also with an increase in the number of diagnoses of other OF and the dermatophyte *Epidermophyton flocosum*.

For fungi that cause CM, this result is expected, since dermatophytes are the most common fungi in these diseases worldwide, especially in places with tropical and subtropical climates, such as the place under study (Deng et al., 2023; Lombardi et al., 2020). This is in agreement with other studies, such as that carried out at Melaka Hospital, Malaysia, where dermatophyte fungi predominated in skin and nail materials (Ng et al., 2023). In Japan, *Trichophyton rubrum* was the most frequent agent in onychomycosis (Nakamura and Fukuda, 2023) and in Cambodia, *T. rubrum* and *T. mentagrophytes* predominated in dermatophytosis (Uhrlaß et al., 2024). In Brazil, a specific study on dermatophytoses shows *T. rubrum* as the most prevalent, followed by *T. mentragrophytes* and *T. interdigitale* (Martinez et al., 2020; De Oliveira Pereira et al., 2021).

For the genera causing OM, in our study, *Candida* spp. was the most diagnosed fungus, mainly in nails, skin and genital secretion. The same has been reported in other studies around the world, such as one that occurred in South America, where *Candida* ssp. was observed in a greater number of diagnoses compared to North America and Europe (Nucci et al., 2010). In a study carried out in Korea, Asia, it was observed that the diagnoses of *Candida* spp. increased over the years, as in the present study (Levorato et al., 2014; Park et al., 2016). In contrast, other OF appeared in our study with considerable numbers of diagnoses. This was expected, given the fact that OM are increasing every year, mainly due to the increase in people with some state of immunosuppression (Miceli et al., 2011) and the increase in onychomycosis caused by OF in other parts of the world (Agrawal et al., 2023; Gupta et al., 2024). The study on OM in Latin America also shows this increase in MY caused by other OF other than *Candida* spp., such as *Aspergillus* spp. and *Fusarium* spp., which are also described in this study (Nucci et al., 2010). Another study, carried out in France, on SM, shows Fusarium spp. as an emerging fungus with 5.1% of all diagnoses (Landreau et al., 2023). In our work, *Curvularia* spp. was the most prevalent fungus after *Candida* spp., probably due to the climatic characteristics in relation to the location of other countries. However, we must always be aware of possible cases of contamination for OM, and we know how difficult it is to differentiate a contaminant from a possible cause of disease, since the species in this group may be commensal or dispersed in the environment, but we cannot exclude them from their pathogenic potential, the inhalation route is the main entry point, which can cause severe MY in immunosuppressed patients, but today also causes skin and nail diseases, thus the importance of good quality control at the collection site and during the preparation of fungal cultures, and a trained professional to allow quality and accurate diagnoses, as is done at the study site (Egbuta et al., 2017; Nnadi and Carter, 2021).

Regarding the classification of MY, in the present study there was little diagnosis for SM, SCM and SYM. For SM, the most diagnosed genus was *Malassezia* spp., however, the low number of diagnoses probably occurred because this fungus is not normally identified in the laboratory, being most often underdiagnosed by doctors, who take into account the appearance of the lesions to make the diagnosis and recommend treatment (Nakamura and Fukuda, 2023).

The most frequently identified species in the research for SCM was Cladophialophora carrioni, which is one of the agents of chromoblastomycosis. This is in contrast to research carried out in Brazil in the state of Rio Grande do Sul and in Peru, a Latin American country, which states that sporotrichosis is the most common form of SCM (Ramírez Soto and Malaga, 2017; Xavier et al., 2023). In Ethiopia, mycetoma is caused mainly by *Madurella* spp., followed by Chromablastomycosis (Enbiale et al., 2023). This variation in species of SCM probably occurs according to the type of climate and terrain of each part of the world, and by socio-economic conditions. In addition, the lack of mandatory notification for this type of MY masks the numbers of diagnoses, considered by the World Health Organization (WHO) as neglected tropical diseases, which contributes to the inaccuracy of prevalence. Only two cases of MSI were observed in this study and these were caused by the genus *Paracoccidioide* spp., which made it difficult to compare the prevalence in relation to other locations.

CM and OM were the most prevalent. In other studies, such as in Korea, China and Brazil (Veranópolis), CM were more numerous than OM (Yoon et al., 2014; Pigatto et al., 2019; Cai et al., 2016) unlike in Alagoas (Brazil) where there was more OM, probably because a greater number of materials from vaginal secretions was analyzed in fungal culture. And thus it differed in relation to the others, which predominated nail and skin scrapings (de Albuquerque Maranhão et al., 2019).

Regarding the type of collection material and the classification of the mycosis, for nails in our study there is a 1.28 times greater chance of being diagnosed as CM. The same occurred in the study in Shiraz (Iran) and in the study of the prevalence of dermatophytosis carried out in Brazil, where nail material was the most prevalent in CM (De Oliveira Pereira et al., 2021; Khodadadi et al., 2021).

For OM, a rate of 1:3,09 of diagnoses of opportunistic agents for sputum materials was observed. According to Miller; Wilmott, 2019 (Miller and Wilmott, 2019), a deficient immune system is one of the causes of pulmonary fungal diseases caused by OF, being one of the main causes of mortality in immunocompromised patients, which explains our study that has a greater number of elderly people with this type of diagnosis in sputum and bronchoalveolar lavage (Zuzarte and Salgueiro, 2022).

Regarding sex, men had a higher prevalence of CM than women, on the other hand, women had more OM. In Japan, in a study that evaluated dermatomycoses, CM was also prevalent in men (Nakamura and Fukuda, 2023), as was the case in another study conducted in South Korea, which showed that obese men, consumers of alcoholic beverages and who do not exercise are more predisposed to CM (Son et al., 2022). For OM in China, in a study of onychomycosis, it was observed that the number of women affected by opportunistic onychomycosis was higher in relation to men, the same occurred in a study in Southern Brazil, where women were more affected by OM than men (Martinez et al., 2020; Song et al., 2022). Children, adolescents and the elderly developed more OM in our study and adults more CM. Furthermore, adults are 1.39 times less likely to develop OM compared to others. Elderly people have a higher probability, 1:1,54, of developing OM, an expected result, since we know that OM depends on a state of immunosuppression to develop, with elderly people being more prone, as their immune system is naturally more deficient and many still use immunosuppressive drugs (Chen et al., 2018; Song et al., 2022; Aissat and Denning, 2023).

And finally, regarding the MY diagnosed in hospitalized patients, despite the small number of diagnoses, all were caused by OF, which was expected due to the fact that hospital patients are more susceptible due to their weakened immune state. Those who are less weakened end up being prone to MY in these environments, with other factors also influencing, such as diabetes, lung disease and, in current times, H1N1 flu (Chakrabarti, 2020; Parums, 2022). The low FD index at the study site demonstrates how fungal diseases are still neglected in hospital environments, even with public organizations like the World Health Organization (WHO) currently promoting FD, the WHO fungal priority pathogens list to guide research, development, and public health action (2022) (WHO, 2022) describes FI as neglected diseases, with little investment allocated to research for diagnosis and treatment (Bongomin et al., 2017; Giacomazzi et al., 2016; Martínez-García et al., 2023; Inácio et al., 2022).

Considering all the publications related to the study of the frequency of MI in Brazil and worldwide, there is a lack of reports and records regarding MY. This is due to the negligence of the health sector in relation to the search for a diagnosis of MY, which is often due to a lack of requests or the lack of professionals qualified to perform fungal identification.

This lack of diagnosis makes it impossible for many patients to be treated appropriately, leading many to death or some type of disability. In addition, the lack of mandatory reporting of serious MY contributes to these being treated with indifference. It is necessary for public health agencies to create standards and also to raise awareness among the medical sector and the population about the importance of diagnosis and fungal diseases.

5. Conclusion

The study indicates that there has been an increase in the number of fungal diagnoses over the years, with the highest demand for fungal diagnoses in the laboratory in the city of Pelotas being observed during the hottest seasons of the year. It was observed that women sought more fungal diagnoses than men, and adults followed by the elderly were the groups with the highest demand for diagnoses. Although no evidence of seasonality was found, children, adolescents, and the elderly with more OM were diagnosed more in the fall, while adults with more prevalence of CM in the spring. Dermatophytes were found to be more prevalent in nail and skin scrapings, followed by *Candida* spp. species, with an increase in the number of diagnoses for non-dermatophyte fungi in onychomycoses. The diagnoses for CM and OM were higher, while the number of diagnoses for the other classifications was lower. It was expected that there would be a lower percentage of requests for diagnoses for hospitalized patients than for outpatients, due to the negligence of the health sector concerning fungal diseases, the lack of qualified professionals to carry out fungal identification, and the absence of mandatory reporting for serious mycoses. All of these factors contribute to these diseases being treated with indifference, and it is crucial for public bodies to create standards and raise awareness among the health sector and the population about the importance of diagnosis and fungal diseases.

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