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ORIGINAL ARTICLE

Serious fungal infections in Portugal

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Abstract There is a lack of knowledge on the epidemiology of fungal infections worldwide because there are no reporting obligations. The aim of this study was to estimate the burden of fungal disease in Portugal as part of a global fungal burden project. Most published epidemiology papers reporting fungal infection rates from Portugal were identified. Where no data existed, specific populations at risk and fungal infection frequencies in those populations were used in order to estimate national incidence or prevalence, depending on the condition. An estimated 1,510,391 persons develop a skin or nail fungal infection each year. The second most common fungal infection in Portugal is recurrent vulvovaginal candidiasis, with an estimated 150,700 women (15–50 years of age) suffering from it every year. In human immunodeficiency virus (HIV)-infected people, oral or oesophageal candidiasis rates were estimated to be 19.5 and 16.8/100,000, respectively. Candidaemia affects 2.19/100,000 patients, in a total of 231 cases nationally. Invasive aspergillosis is less common than in other countries as chronic obstructive pulmonary disease (COPD) is uncommon in Portugal, a total of 240 cases annually. The estimated prevalence of chronic pulmonary aspergillosis after tuberculosis (TB) is 194 cases, whereas its prevalence for all underlying pulmonary conditions was 776 patients. Asthma is common (10% in adults) and we estimate 16,614 and 12,600 people with severe asthma with fungal sensitisation and allergic bronchopulmonary aspergillosis, respectively. Sixty-five patients develop *Pneumocystis* pneumonia in acquired immune deficiency syndrome (AIDS) and 13 develop cryptococcosis. Overall, we estimate a total number of 1,695,514 fungal infections starting each year in Portugal.

Introduction

Despite their growing importance, many fungal infections have been neglected all over the world until recently. More fungal diseases than ever before are now being encountered in a wider range of human hosts and caused by a large array of fungal species. Prevalence depends on many factors, such as the number of persons with underlying diseases associated with an increased risk for fungal infection, invasive procedures and antifungal therapies used. The prevalence of such infections thus varies greatly across different countries, and it is, therefore, mandatory to estimate the actual fungal burden in each country, in order to fully grasp the current scenario of these emergent infections.

Portugal is geographically situated on the west coast of Europe and includes the archipelagos of the Azores and Madeira. The estimated population is over 10,500 million people [1]. Portugal has also accepted large inflows of immigrants from its former colonies in Africa, from Central and Eastern Europe and from Brazil.

According to the European Centre for Disease Prevention and Control (ECDC) [2], Portugal is the Western European country with comparatively higher estimates of human immunodeficiency virus (HIV) and tuberculosis (TB) proportions,

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having also the highest proportion of co-infected cases. Furthermore, the incidence of cancer has also been noticeably increasing in this country in recent years [3].

Until a few decades ago, the prevalence of fungal infections was low or unknown in Portugal, which may be explained by the lack of regular and national surveillance and the lack of obligatory reporting of the occurrence of these infections. The ones with highest prevalence were (and still are) those with very low mortality and morbidity (e.g. dermatomycosis and vaginal candidiasis). As yet, national statistics of the majority of invasive infections have not been generated and the information available is very scarce or non-existent.

As a consequence, until recently, the public health importance of mycology has been underestimated by the national scientific community.

The aim of this work is to estimate the burden of serious fungal disease in Portugal using deterministic scenario modelling and published incidence and prevalence data.

Methodology

Relevant literature was searched via PubMed and several other search tools using different sets of key words (in Portuguese and English): ([Candidemia Portugal]; [Aspergilose Portugal]; [Infeções Fúngicas Portugal]; [Mucormicose Portugal]; [Mucormicose Portugal]; [Candidose vaginal Portugal]; [Infeções hospitalares Portugal]; [Infeções oculares Portugal]; [Infeções fúngicas olhos Portugal]; [Histoplasmose Portugal]. A simple deterministic model that was created in Excel and has consistently been applied in many countries by the LIFE program was used [4, 5]. For the purpose of creating a comparable dataset for each country, a template was provided to all participants that included demographic data, and specific disease-related information was gathered. The template used across the countries involved extrapolated estimates of fungal infection from published data. Standardised assumptions, which allow directly observed incidence of fungal infection to be used in conjunction with surrogate markers, enabled an estimate of the total national burden to be derived. Thus, where no data existed, specific populations at risk were used and the frequency of fungal infections on those populations was applied to estimate the national incidence or prevalence, depending on the condition. In the absence of local data, published estimates from neighbour countries were used.

Population statistics were obtained from the National Statistics Institute – Statistics Portugal [6] and demographic data collected from the census performed in 2011 [1]. Data regarding underlying diseases and risk factors [HIV/acquired immune deficiency syndrome (AIDS), pulmonary tuberculosis (PTB), oncological diseases, respiratory diseases and the number of solid organ transplants] were collected from different national and international studies [2, 3, 7–18].

The number of chronic pulmonary aspergillosis (CPA) cases was estimated using the previously described approach taken by Denning et al. [19] in their global estimates, where the number of annual PTB cases with cavities (12%) was multiplied by the incidence of CPA in cavities (22%) and the number of PTB cases without cavities (88%) was multiplied by CPA incidence (2%). The number of histoplasmosis episodes occurred during 2015 were obtained from the Diagnostic Homogeneous Groups database, made available by the Central Administration of the Health System [12]. The number of critical care beds in Portugal (data from 2013) was also obtained from the Central Administration of the Health System [12].

Data from skin and nail infections were collected from different local studies [20, 21].

Results and discussion

Country profile

In 2011, the Portuguese total population was estimated to be 10.6 million (Table 1), with 47.8% being males and 52.2% being females. 14.9% of the individuals were estimated to be below 15 years old, whereas 25.0% were estimated to be 60 years old or more. 23.8% of the population is constituted by women aged from 15 to 49 years old [1]. In 2013, the gross domestic product per capita was $21,733 [22]. All residents in Portugal have access to healthcare provided by the National Health Service, financed mainly through taxation. Approximately one-fifth to a quarter of the population enjoys a second (or more) layer of health insurance coverage, through health subsystems and voluntary health insurance. Healthcare delivery is based on both public and private providers. The primary care system in Portugal performs well, with rates of avoidable hospitalisation amongst the best in the Organisation for Economic Co-operation and Development (OECD) for asthma and chronic obstructive pulmonary disease (COPD)

Table 1 Portuguese population profile

Population data	Number (%)
Population in Portugal	10,562,178 (100%)
Population over 40 years old	5,588,208 (52.9%)
Children (0–14 years old)	1,572,329 (14.8%)
Adults (≥15 years old)	8,989,849 (85.2%)
Adult women	4,747,248 (44.9%)
Women over 60 years old	1,503,935 (31.6% adult women)
Women aged 15–50 years	2,511,645 (52.9% adult women)

[23]. The number of critical care beds in continental Portugal was estimated as 666 [12].

Due to the scarcity of national epidemiological data, almost every estimate was based on modelling combined with the few epidemiology papers found.

Pneumocystis pneumonia and cryptococcosis

In the period from 1983 to 2015, the total number of fungal infections in HIV+/AIDS patients is reported in Table 2 (data not published: cases notified until 31 December 2015, database HIV, Surveillance on HIV-AIDS infection – National Institute of Health Dr. Ricardo Jorge) [7]. The incidence of HIV was estimated to be 14.0 cases/100,000 inhabitants in 2013 [8]. Of the 30,956 HIV cases reported by the end of 2014, data regarding antiretroviral therapy (ART) were available for 26,580. Overall, 22,005 (82.8%) of the patients were prescribed ART, while 4575 (17.2%) were not [8] (Table 2). In 2014, 920 new HIV cases were reported. 914 (99%) of these cases were diagnosed in adults over 15 years old; in 51.2%, the $CD4^+$ count was <350 cells/mm^3 and in 33.1% of cases it was <200 cells/m^3. During 2014, 249 new cases of AIDS were diagnosed. In 65 cases (26.2%), pneumonia caused by *Pneumocystis jirovecii* (PCP) was diagnosed and 13 (5.2%) patients were diagnosed with extrapulmonary cryptococcosis. The annual incidence of PCP was calculated to be 0.61/100,000 inhabitants and of extrapulmonary cryptococcosis to be 0.12/100,000 inhabitants [8].

When compared to other similar European studies [5, 24, 25], Portugal shows the highest annual incidence of cryptococcal meningitis but one of the lowest incidences of *Pneumocystis* pneumonia. The high number of HIV-infected patients without antiretroviral treatment (17%) together with the very easy diagnosis of cryptococcosis (in contrast to PCP) may explain this apparent disparity in incidence rates.

Aspergillus infections

In 2014, the incidence of tuberculosis in Portugal was 25/100,000 inhabitants (Table 3). During this year, 2169 TB cases were notified and PTB was the most prevalent manifestation, in 1558 cases [11]. The number of deaths attributed to PTB was 160. Treated PTB can lead to complications, including progressive loss of lung function, persistent pulmonary symptoms and CPA [19] (Table 3). Therefore, and following previous assumptions, the annual CPA incidence is estimated to be 62 patients, with a 5-year period prevalence of 194 people with CPA following PTB [19]. Assuming that the PTB patients represent only a quarter of CPA cases in a year [5], and that the other three-quarters are due to COPD, sarcoidosis, allergic bronchopulmonary aspergillosis (ABPA), prior pneumothorax, rheumatoid arthritis and non-tuberculosis mycobacterial infection, it is inferred that there is a prevalence of ~776 cases of CPA in Portugal.

The total number of cystic fibrosis (CF) cases was 320 in 2015, with 79 individuals aged more than 18 years old [26]. Only two adult CF patients are recorded as having ABPA, but an anticipated seven have ABPA and another 11 *Aspergillus* bronchitis.

It is estimated that about 10% of the Portuguese adult population suffers from asthma, with a total number of patients that varies between 700,000 and 1,000,000 in 2014. We

Table 2 Portuguese human immunodeficiency virus (HIV)-related data

HIV-related data	Patient numbers (actual)	References
Current total HIV/AIDS	30,956	[7, 8]
Proportion of diagnosed cases on ARVs	22,005	
Number of diagnosed cases not receiving ARVs	4575	
Annual new AIDS cases (at risk of OIs)	249	
Proportion of AIDS patients presenting with PCP	26%	
Proportion of AIDS patients presenting with cryptococcal meningitis	5%	
HIV patients who died in 2014	196	
AIDS-related deaths in 2011	126	
	Total no. of cases (1983–2015)	
Pneumocystis pneumonia	4418	[7]
Oesophageal candidiasis	1777	
Cryptococcosis	1028	
Tracheal/pulmonary/bronchic candidosis	32	
Histoplasmosis	8	
Coccidioidomycosis	4	

Table 3 Portuguese profile of respiratory diseases

Respiratory diseases	Number	References
Pulmonary tuberculosis annual incidence HIV+ Pulmonary tuberculosis annual incidence HIV−	3,5/100,000 inhabitants 18/100,000 inhabitants	[2, 8–10]
Pulmonary tuberculosis annual incidence (total numbers)	1398	
COPD admissions to hospital per year (number) COPD admissions to hospital per year (prevalence)	6383 71/100,000 adults	[13, 15–17]
Asthma rate in adults Asthma numbers in adults	10% 503,459	[13, 14]
Cystic fibrosis total Cystic fibrosis adults	320 79	[13, 18]

decided to choose the lowest estimate [27]. It is also estimated that about 12.5% of Portuguese children suffer from asthma. ABPA numbers were determined by assuming rates of 2.5% among adult asthmatics, totalling ~12,600 patients [26, 28]. For severe asthma with fungal sensitisation (SAFS), the assumption is that, among asthmatics with the most severe disease (10% of adult asthma patients), a third will be affected (16,614 patients) [29] (Table 3). There is likely some overlap between ABPA and SAFS estimates depending on the proportions of ABPA patients with severe asthma and SAFS patients who are sensitised to *Aspergillus*. The estimated annual incidence of ABPA and SAFS is lower than in other European countries, whereas the annual incidence of CPA is higher in our country than in Germany, Ireland and Denmark. Our estimated values need to be confirmed and improved diagnostic testing such as *Aspergillus* IgE and IgG testing are needed to further validate these data and should be used routinely as diagnosis tools [30].

A 1.1% prevalence of COPD in the Portuguese population was estimated nationally [13]. This percentage is probably very low due to the under-diagnosis of this disease. In fact, Bárbara et al. [17] performed a study on the prevalence of COPD in adults aged ≥40 years old in the Lisbon area and found a much higher level of prevalence (14.2%). The total number of COPD admissions to hospital per year totalled 6383, according to the OECD report [16] (Table 3). The rate of invasive aspergillosis (IA) attributable to COPD patients assumes that 1.3% of COPD admissions developed IA: a total of 83 cases of COPD will develop IA every year. According to Yan et al. [31], 2.63% of the patients diagnosed with lung cancer develop IA. Portugal has a high incidence of lung cancer and about 3500 new cases are diagnosed every year [32], which corresponds to an estimate that 92 patients with lung cancer will develop IA.

In a one-year study (2015) on invasive fungal infections (IFI) in haematological patients in one of the major central hospitals of Lisbon, 17 cases of IA were diagnosed. Among those, eight were allogeneic stem cell transplanted (HSCT) patients. The number of HSCT in this hospital during 2015 was 26. These data generate an estimate of 31% IA incidence in HSCT patients in this hospital and we used this value to extrapolate to the total number of 46 cases per year in our country, since we had 147 HSCT during 2015. Therefore, 65 cases of IA were estimated annually in HSCT and solid organ-transplanted patients, assuming that IA (proven and probable) occurred in 6% of heart, 4% of lung and liver and 1% of kidney-transplanted patients [33] (Table 4). The estimated IA rate is lower than other European countries but similar to those estimated by Spain [5] or the Czech Republic [25].

Table 4 Number of transplants in 2015 and estimated number of cases of invasive aspergillosis (IA)

	Number	Number of estimated cases of IA	References
AML population frequency x/100,000 AML patients per year	8.1 852		[3]
Allogeneic HSCT per year	147	46	[3, 12]
Renal Tx per year Lung Tx per year	483 15	5 1	[3, 11]
Heart Tx per year	50	3	
Liver Tx per year	249	10	
Lung cancer	3500	92	
Total	4444	157	

Candida infection

Based on a one-year multicentre study conducted in 2010–2011 involving ten district hospitals, the candidaemia rate was found to be 2.19 per 100,000 inhabitants based on 240 episodes [34]. Despite the scarcity of systematic studies in our country, the values found are more similar to the ones reported in other countries in the north of Europe, such as Norway or Finland (3–5 cases per 100,000 inhabitants). We, therefore, anticipate a total of 231 cases of candidaemia per year. In a multicentre study, 15 different *Candida* species were found, with *C. albicans* (40%) being the most prevalent, followed by *C. parapsilosis* (23%) and *C. glabrata* (13%) [34].

The cases of *Candida* peritonitis were estimated as the proportion of candidaemia cases in the intensive care unit (ICU), while there was one case of peritonitis per every two cases of candidaemia and, therefore, 40 cases of post-surgical *Candida* peritonitis were estimated to occur annually. The sensitivity of *Candida* detection in blood cultures is about 40% [35], so invasive candidiasis is certainly under-represented.

According to previous studies [36], the ratio of HIV patients without antiretroviral therapy and CD4 counts below $200 \times 10^6/\mu L$ affected with oral candidiasis is 90%. Therefore, we estimate a number of 2059 HIV patients with oral candidiasis during 2014, assuming that half of the patients without antiretroviral therapy presented CD4 counts below $200 \times 10^6/\mu L$. We have found neither official records nor studies on the prevalence of oral candidiasis in patients with cancer or after transplant nor the number of patients who are denture wearers, as these are other conditions commonly associated with oral candidiasis. The same applies to people living with diabetes mellitus. The number of oesophageal candidiasis in HIV patients was estimated assuming 20% of patients with HIV not on ARVs and with CD4 counts below $200 \times 10^6/\mu L$ and 5% of those on ARVs (Table 2).

Recurrent vaginal thrush (rVVC) is defined as at least four episodes every year. The self-reported rate of rVVC in five European countries and the USA was 9%, with some variation between age groups. However, this is probably an over-estimate, as it is based on self-diagnosis, so we have applied a 6% rate. As many as 150,699 Portuguese women between 15 and 50 years of age get recurrent vaginal thrush every year. This translates into an annual incidence of approximately 2854 cases per 100,000 females. The incidence of recurrent *Candida* vaginitis is lower than our neighbour country [5] but similar to other European studies [24, 25].

Dermatophyte infections

Regarding superficial mycoses, and using the global prevalence of 14.3% estimated by Vos et al. [37], 1,510,391 Portuguese inhabitants should have a skin fungal infection, corresponding to an incidence of 14,300/1,000,000 inhabitants, values similar to those obtained in other countries [5, 25].

In a study by Valdigem et al. [21], during the period 1983–2002, 10,003 samples from the North of Portugal were analysed and the frequency of dermatophytes was 23.6%, with a prevalence of tinea capitis of 4.9%. A more recent retrospective study, performed by the Portuguese National Institute of Health [20], included 4193 biological samples from patients within the NUTSII region of Lisbon and Tagus Valley with medical suspicion of fungal infection, collected from 2004 to 2013. The average frequency of dermatophyte infection was 21%, ranging from 18 to 26%. Tinea capitis was confirmed in 236 (28%) patients and was more frequent in children from the age group 1–9 years old. In scalp dermatomycosis, *Microsporum audouinii* was the most frequently isolated species ($n = 120$, 51%), followed by *Trichophyton soudanense* ($n = 44$, 19%). Onychomycosis caused by dermatophytes was confirmed in 385 cases (46%). Other fungi recognised as causes of onychomycosis were not considered for this report. Skin samples were positive in 220 cases (26%). The most prevalent dermatophytes isolated in nail and skin samples were *T. rubrum* (206 and 93 isolates, respectively) and *T. mentagrophytes* (49 and 39 isolates respectively). *Epidermophyton floccosum* was the species less frequently found in skin samples (1%). Our national data shows a high prevalence of anthropophilic species (*M. audouinii* and *T. soudanense*) in the region of Lisbon and Tagus Valley, where the number of foreign citizens from African countries is higher.

Other fungal infections

– Mucormycosis

In Spain, the estimated incidence was 0.43 cases/1 million inhabitants [38], whereas this incidence in France was higher, at 0.9 cases/1 million inhabitants [39]. Portugal has one of the highest incidences of diabetes mellitus in Europe (13% of the total population aged between 20 and 79 years old) [40]. We, therefore, adopted the value of 0.9 cases/1 million inhabitants, which translates into an annual incidence of ten cases. Among the clinical reports published in Portugal between 2001 and 2015, 19 cases of mucormycosis were found.

– Histoplasmosis

Portugal receives many people from South America, especially from Brazil and also from Portuguese language African countries. After the Independence of the African countries in 1974–1980, more than 1 million Portuguese people returned to Portugal, and many of them are now over 60 years old. Thus, we anticipate many individuals to have been exposed to endemic fungi such as *Histoplasma*. Furthermore, Gascón

Table 5 Estimated annual case load of serious fungal infections in Portugal

Serious fungal infection	No underlying disease	HIV/AIDS	Respiratory disease	Cancer + immunocompromised	Critical care + surgery	Totals	Rate/100,000
Cryptococcal meningitis	–	13	–	–	–	13	0.12
Pneumocystis pneumonia	–	65	–	–	–	65	0.62
Invasive aspergillosis	–	–	–	157	83	240	2.3
Chronic pulmonary aspergillosis	–	–	784	–	–	776	31
Allergic bronchopulmonary aspergillosis (ABPA)	–	–	12,600	–	–	12,600	119
Severe asthma with fungal sensitisation (SAFS)	–	–	16,782	–	–	16,614	159
Candidaemia	–	–	–	152	79	231	2.2
Candida peritonitis	–	–	–	–	40	40	0.37
Oral candidiasis	–	2059	–	–	–	2059	20
Oesophageal candidiasis	–	1777	–	–	–	1777	17
Recurrent *Candida* vaginitis (>4×/year)*	150,699	–	–	–	–	150,699	2853
Mucormycosis	–	–	–	10	–	10	9.5
Skin and nail infection	359,165	–	–	–	–	1,510,391	14,300
Total serious fungal infection burden						1,695,514	

*Females only

et al. [41] estimates that the incidence of histoplasmosis is 20% for individuals who had travelled to Latin America for the first time. According to the report of the Portuguese Tourism Office in 2011, 2,458,900 Portuguese residents travelled to the Americas and 1,154,000 travelled to Africa. Bearing this in mind, we can estimate that 491,780 Portuguese travellers could have been infected by *H. capsulatum*. However, our literature review found just ten published cases of histoplasmosis between 2009 and 2015 (five var. *capsulatum*, five var. *duboisii*), with the majority of them from Lisbon and Tagus Valley. Interestingly, some of those cases refer manifestation of disease only after 40 years after exposure [42]. According to the Diagnostic Homogeneous Groups database [12], 16 episodes of histoplasmosis occurred in Portugal during 2014 (six in the North Region, three in the centre and seven in Lisbon and Tagus Valley).

– Paracoccidioidomycosis (PCM)

Paracoccidioidomycosis is an endemic systemic fungal infection caused by the thermally dimorphic fungus *Paracoccidioides brasiliensis*. Approximately 80% of PCM cases in Latin America have been reported in Brazil. Since Portugal has 165,530 Brazilian inhabitants [1], we believe that this mycosis can be under-diagnosed in our country. Few case reports diagnosed in Portugal are published.

Conclusion

This is a preliminary study but constitutes the first report of the global burden of fungal infections in Portugal. Using published and estimated data, we were able to estimate the incidence or prevalence of the above referred fungal infections and ~1,695,514 (16%) people in Portugal suffer from those fungal infections each year (Table 5). Skin and nail infections were the most frequent fungal infection detected in this study, followed by vaginal candidiasis. Additional studies are required, especially for diseases such as allergic bronchopulmonary aspergillosis (ABPA) and severe asthma with fungal sensitisation (SAFS).

Diagnosing infections remains a problem in the management of fungal diseases, particularly in the immunocompromised host. Signs and symptoms are non-specific, patients are often unable to undergo invasive diagnostic procedures, colonisation is difficult to distinguish from invasive disease and blood cultures are commonly negative. Patients who have thrombocytopaenia, severe bleeding disorders or other complications cannot be subjected to biopsies. For this reason, the autopsy is an important key to the understanding of the epidemiology of invasive fungal infections, especially in the immunocompromised host. The overall rates of autopsies decreased

in our country, which underscores the difficulty of obtaining more precise data on the incidence and prevalence of invasive fungal infections. Even bearing in mind the lack of sensitivity of some methods, the available conventional diagnostic tools in our country (cultural and serological methods) allow the diagnosis of the most frequent fungal diseases. The implementation of molecular methods is also ongoing and is being currently done by specific Portuguese labs either for panfungal DNA detection or to genus/species targeted detection. Since *Aspergillus* resistance to azoles is becoming an emergent public health concern in Europe, a new multiplex real-time polymerase chain reaction (PCR) assay that differentiates susceptible from resistant *A. fumigatus* strains directly in clinical samples is also available.

With the increase of individuals at risk for fungal infection and the change in fungal epidemiology, clinicians and microbiologists must develop more expertise in the epidemiology, pathogenicity and diagnostic methods of fungal infections in order to address these challenges more adequately.

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